Graduate School of
Systemic Neurosciences
LMU Munich

Aspects of spatiotemporal integration in bat sonar

Dissertation of the
Graduate School of Systemic Neurosciences
Ludwig-Maximilians-Universität
München

Submitted by
Melina Heinrich
Munich, 22nd of December 2014

First Reviewer/Supervisor
Prof. Dr. Lutz Wiegrebe

Second Reviewer/Supervisor
Prof. Dr. Harald Luksch

Date of oral defense
Munich, 29th of April 2015

This thesis was funded and supported by:

- The Volkswagenstiftung (I/83838)
- The Human Frontier Science Program (HFSP RGP 0062/2009)
- The Graduate School of Systemic Neurosciences (GSN-LMU, DFG-GSC 82/1)

To My Family:

Future,

Present,

And Past

Index

Appendix

Abstract

Bat sonar is an active sense that is based on the common mammalian auditory system. Bats emit echolocation calls in the high frequency range and extract information about their surroundings by listening to the returning echoes. These echoes carry information, like spatial cues, about object location in the three-dimensional space (azimuth, elevation, and distance). Distance information, for example, is obtained from temporal cues as the interval between the emission of an echolocation call and the returning echo (echo delay). But echoes also carry information about spatial object properties like shape, orientation, or size (in terms of its height, width, and depth). To achieve a reliable internal representation of the environment, bats need to integrate spatial and temporal echo information.

In this cumulative thesis different aspects of spatiotemporal integration in bat sonar were addressed, beginning with the perception and neural encoding of object size. Object width as size relevant dimension is encoded by the intensity of its echo. Additionally, the sonar aperture (the naturally co-varying spread of angles of incidence from which the echoes impinge on the ears) co-varies proportionally.

In the first study, using a combined psychophysical and electrophysical approach (including the presentation of virtual objects), it was investigated which of both acoustic cues echolocating bats (*Phyllostomus discolor*) employ for the estimation of object width. Interestingly, the results showed that bats can discriminate object width by only using sonar-aperture information. This was reflected in the responses of a population of units in the auditory midbrain and cortex that responded strongest to echoes from objects with a specific sonar aperture, independent of variations in echo intensity. The study revealed that the sonar aperture is a behaviorally relevant and reliably encoded spatial perceptual cue for object size. It furthermore supported the theory that the mammalian central nervous system is principally aiming to find modality independent representation of spatial object properties. We therefore suggested that the sonar aperture, as an echo acoustic equivalent of the visual aperture (also referred to as the visual angle), could be one of these object properties.

In the visual system object size is encoded by the visual aperture as the extent of the image on the retina. It depends on object distance that is not explicitly encoded. Thus, for reliable size perception at different distances, higher computational mechanisms are needed. This phenomenon is termed ‘size constancy’ or ‘size-distance invariance’ and is assumed to reflect an automatic re-scaling of visual aperture with perceived object distance. But in echolocating bats object width (sonar aperture) and object distance (echo delay) are accurately perceived and explicitly neurally encoded.

In the second study we investigated whether bats show the ability to spontaneously combine spatial and temporal cues to determine absolute width information in terms of sonar size constancy (SSC). This was addressed by using the same setup and species as in the psychophysical approach of the first study. As a result SSC could not be verified as an important feature of sonar perception in bats.

This lack of SSC could result from the bats relying on different modalities to extract size information at different distances. Alternatively, it is thinkable that familiarity with a behaviorally relevant, conspicuous object is required, as it was discussed for visual size constancy. But size constancy is found in many sensory modalities and more importantly, SSC was recently found in a blind human echolocator. It was discussed to be based on the same spatial and temporal cues as presented in our study. Thus, this topic should be readdressed in bats in a more natural context as size constancy could be a general mechanism for object normalization.

As the spatiotemporal layout of the environment and the objects within changes with locomotion, in the third study the spatiotemporal integration in bat biosonar in a natural and naturalistic context was addressed. Trawling bats species hunt above water and capture fish or insects directly from or close to the surface. Here water acts as an acoustic mirror that can reduce clutter by reflecting sonar emissions away from the bats. However, objects on the water lead to echo enhancement. In a combined laboratory and field study we tested and quantified the effect of different surface types with different reflection properties (smooth and clutter surface) and object height on object detection and discrimination in the trawling bat species, *Myotis daubentonii.* The bats had to detect a mealworm presented above these different surfaces and discriminate it from an inedible PVC disk. At low heights above the clutter surface, the bats' detection performance was worse than above a smooth surface. At a height of 50 cm, the surface structure had no influence on target detection. Above the clutter surface, object discrimination decreased with decreasing height.

The study revealed different perceptual strategies that could allow efficient object detection and discrimination. When approaching objects above clutter, echolocation calls showed a significantly higher peak frequency, eventually suggesting a strategy for temporal separation of object echoes from clutter. Flight-path reconstruction showed that the bats attacked objects from below over water but from above over clutter. These results are consistent with the hypothesis that trawling bats exploit an echo-acoustic ground effect, in terms of a spatiotemporal integration of direct object reflections with indirect reflections from the water surface. It could lead to optimized prey-detection and discrimination not only for prey on the water but also above. Additionally, the bats could employ a precedence-like strategy to avoid misleading spatial cues that signal the wrong object elevation by using only the first and therewith direct echo for object localization.

1 General Introduction

Sensory systems and stimulus processing

Environments are defined by the sum of biotic (animate) and abiotic (inanimate) factors that surround and act on an organism (e.g. defined in Begon, Harper *et al*. 1996) and can influence its survival and development. The perception of environments is crucial for organisms to optimize their fitness. Since the environment delivers information that is encoded by different signals (e.g. diverse physical parameters), a multitude of specialized accessory structures evolved.

The development, adaptation and specialization of animal morphology, physiology as well as animal behavior results from selective pressure. It depends on the environmental conditions that are found in the habitat or the ecological niche organisms occupy (e.g. reviewed in Niven and Laughlin 2008, Dangles, Irschick *et al*. 2009).

Consequently in course of evolution multiple sensory systems (e.g. the auditory, visual, gustatory, vestibular or the somatosensory system) of different specialization, complexity, size, and efficiency developed across species.

Basically sensory systems consist of sensory receptors (for example photoreceptors, mechanoreceptors, thermoreceptors, chemoreceptors or nociceptors) forming accessory structures of sensory organs (like the retina), neural pathways and the corresponding information processing areas (Niven and Laughlin 2008).

Everything that can evoke a physiological or behavioral response, for example, in cells, tissue or the behavior of an organism is called a stimulus. Stimuli can be differentiated in external stimuli (e.g. sound, light, temperature, pressure etc.) and internal stimuli (e.g. a reflex that is triggered by an external stimulus).

In vertebrates all sensory organs (and accordingly sensory receptors) receive stimulus energy und transduce it to electric signals (sensory transduction) that are subsequently conducted via neural afferent pathways to the brain and the corresponding specialized areas where the information is processed. Thus, sensory systems allow organisms to perceive (sensory input), interpret and consequently behaviorally respond (motor output) to the components of their internal and external environment (e.g. Jacobs, Nakanishi *et al*. 2007, Dangles, Irschick *et al*. 2009, Nilsson 2009).

Limitations of sensory systems

Sensory systems underlie a trade-off between the costs of a sensory structure that encodes a specific sensory modality (e.g. heat, the light spectrum, or acoustic frequency range) and the quantity of reliable information acquired (Niven and Laughlin 2008). Therefore, they only work within the boundaries of certain physical parameters and do not represent all information provided by the internal or external environment.

For instance the human eye does only perceive a certain portion of the electromagnetic spectrum that provides wavelengths from circa 400 nanometre (nm) for violet light to 800 nm for red light (e.g. reviewed in Dusenbery 1992). Shorter wavelength is ultraviolet light (UV) whereas longer wavelength is in the infrared range. In contrast to humans, animals e.g. birds and some bat species can perceive wavelengths between 300-400 nm that lie in the ultraviolet range (Winter, Lopez *et al*. 2003, Muller, Glosmann *et al*. 2009, Werner, Tupper *et al*. 2012). This can be e.g. advantageous for foraging as certain plants are very conspicuous only in this spectrum. This specialization was probably the result of a co-evolution between the plant and its pollinating species. In birds certain parts of the plumage are only visible in that range and are suggested to be attractive on females due to sexual selection (Werner, Tupper *et al*. 2012). Some snake species such as pit vipers even perceive wavelength in the infrared spectrum (Chen, Deng *et al*. 2012). For humans this kind of information is ecologically not relevant and thus this spectral information cannot be perceived without technical support.

Passive and active sensory systems

The sensory receptors of the sensory systems described above are all activated by the input of external energy/stimuli of the environment (e.g. the electromagnetic spectrum of light, sound etc.). These systems cannot actively control the physical parameters of the external stimuli. In contrast to these so called ‘passive sensory systems’, ‘active sensory systems’ use self-generated energy to probe their environment and allow full control over these probing signals. For example, signal intensity, timing, spectrum or directionality can be modified (reviewed in Nelson and MacIver 2006).

Examples of active sensory systems are bioluminescence in deep-sea fish that create their own light for using vision in a spectral waveband that can only be perceived by themselves and conspecifics (Denton, Gilpin-Brown *et al*. 1970, O'Day and Fernandez 1974, Fernandez 1979, reviewed by Douglas, Partridge *et al*. 1998, Douglas and Partridge 2011), or the electric field of weakly electric fish allowing navigation, object detection (electrolocation) and communication with other electric fish (electrocommunication) (von der Emde, Schwarz et al. 1998, Nelson and MacIver 2006, von der Emde, Behr et al. 2010, Nelson 2011). The most prominent example for an active sense is animal biosonar or ‘echolocation’.

Animal biosonar; vision independent representation of space

In mammals, bats and toothed whales (dolphins, porpoises, river dolphins, killer whales and also sperm whales) use a sophisticated and very powerful imaging system that is based on the auditory analysis of the returning echoes of self-generated ultrasonic sounds (e.g. Griffin 1958, Simmons and Stein 1980, Au 2004, Au and Simmons 2007). This animal biosonar in bats and whales allows life in environments where visual information is scarce or not available. Here it facilitates detailed imaging of the environment within its range limits.

Note that there are other species that use echolocation. For example, studies in the ground-living shrews (Tomasi 1979, Forsman and Malmquist 1988) and tenrecs (Gould 1965) suggest that some species of these families also possess a primitive sense of animal sonar. Simulations of shrew-like calls revealed that the twittering calls of shrews cover a short range behind the animals’ vibrissae (Siemers, Schauermann *et al*. 2009), not allowing detailed imaging of the surroundings.

Humans are also able to extract information about environments via echolocation e.g. by producing clicks with their tongue and listening to the returning echoes (Kellogg 1962). Human echolocation allows, for example, the assessment and discrimination of object position, distance, size, shape, material, texture and changes in room-sizes (e.g. Rice and Feinstein 1965 A, Rice, Feinstein *et al*. 1965 B, Schenkman and Nilsson 2010, Schenkman and Nilsson 2011, Teng and Whitney 2011, Schornich, Wallmeier *et al*. 2013, Wallmeier, Gessele *et al*. 2013, Kolarik, Cirstea *et al*. 2014). Especially some blind people have developed echolocation abilities allowing remarkable spatial perception and resolution (Teng, Puri *et al*. 2012).

In birds, for example, the oilbird (*Steatornis caripensis*) and different species of swiftlets employ a sonar system (Price, Johnson et al. 2004, Thomassen and Povel 2006, Brinkløv, Fenton et al. 2013) that is used for navigation in darkness (e.g. in caves). These species produce relatively stereotyped low frequency click trains that are still in the audible range of humans (below 20 kHz) (Griffin and Thompson 1982, Brinkløv, Fenton et al. 2013). Until today avian sonar was discovered in 16 species and evolved several times independently.

Taken together, biosonar is a mode of spatial perception that can be employed especially when there is only scarce visual information available (Simmons and Grinnell 1988) . In the following pages I will focus on bats and consequently on bat sonar.

Bats; an introduction

In the past decades, bats were described phylogenetically as the order Chiroptera that is the second largest mammalian order besides the rodents. The Chiroptera are divided in two suborders: the Microchiroptera (‘true echolocating bats’ or ‘microbats’, >800 species) and the relatively large Megachiroptera (the ‘flying foxes’ or ‘megabats’, >175 species) (Suga 1990, Neuweiler 2000). Until the year 2005, 1116 bat species have been described (Simmons, Wilson *et al.* 2005). Due to new molecular techniques for species identification our knowledge of bat biodiversity and hence the number of species is still growing (Mayer, Dietz *et al*. 2007). Additionally, due to new results from taxonomic DNA analysis as well as comparative morphological analysis, the phylogeny of the Chiroptera is still subject of debate. Especially the origin of bat sonar is not completely answered yet (Jones and Teeling 2006, Veselka, McErlain *et al*. 2010, Davies, Maryanto *et al*. 2013, Boonman, Bumrungsri *et al*. 2014).

All microchiropteran bat species have a highly developed sonar system. The echolocation calls are produced by the larynx (Galambos and Griffin 1942, Griffin 1958). The dietary spectrum of the microbats is very diverse and depends strongly on the ecological niche they occupy. Especially in the northern hemisphere most of the microchiropteran bat species are insectivorous (even though some species catch small fish) (Siemers, Dietz *et al*. 2001, Aizpurua, Aihartza *et al*. 2014). In the tropics and neotropics a broader dietary spectrum appears. Hence additional dietary feeding strategies and specializations in bats developed (e.g. frugivory, pollinivory, carnivory, piscivory, omnivory, sanguinivory, or hematophagy) (see for example Norberg and Fenton 1988, Arita and Fenton 1997, Freeman 2000, Schnitzler and Kalko 2001, Denzinger and Schnitzler 2013).

In the Megachiroptera (family: Pteropodidae, 'Old World fruit bats') only in the rousette bats *(Rousettus aegyptiacus, Rousettus leschenaulti*, as well as *Rousettus amplexicaudatus*) another sophisticated type of bat sonar has developed. In contrast to the microbat sonar it is based on double clicks performed with the tongue (Kulzer 1956, Möhres and Kulzer 1956, Griffin, Novick *et al*. 1958, Holland, Waters *et al*. 2004). A recent study of Boonman *et al*. (2014) suggests that several Old World fruit bat species also use clicks, that are generated by their wings, as another functional but rudimentary form of echolocation.

Megachiroptera are majorly frugi- and nectarivorous and are described to rely on vision for orientation, even at night (e.g. Möhres and Kulzer 1956, Neuweiler 2000).

General principles of bat sonar

Bat sonar enables life in complexly structured environments in darkness. This includes: navigation, orientation, object detection, localization, identification, discrimination, tracking, and interception (e.g. of prey). Hence bats perceive multidimensional images with explicit information about object location in three-dimensional space (horizontal plane, vertical plane, and object distance) including velocity of moving objects (Simmons and Grinnell 1988, Moss and Schnitzler 1995, Schnitzler and Kalko 2001). The images also carry information about object properties like shape, surface structure (texture), orientation, and size (for example Simmons and Vernon 1971, Schnitzler and Henson 1980, Schmidt 1988, Simmons and Grinnell 1988, Schmidt 1992, Grunwald, Schoernich *et al*. 2004, von Helversen 2004, Holderied and von Helversen 2006, Simon, Holderied *et al*. 2006, Firzlaff, Schuchmann *et al*. 2007, Falk, Williams *et al*. 2011, Simon, Knornschild *et al*. 2014) (cf. chapter 1, figure 1).

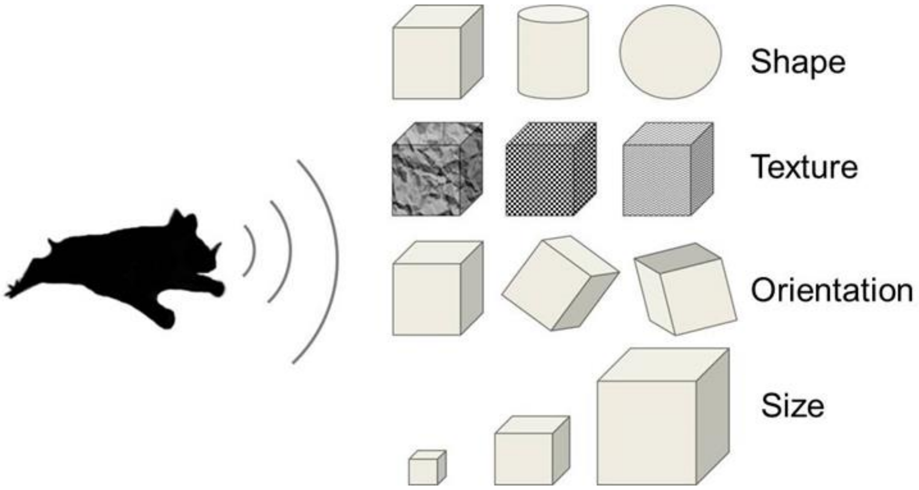

Figure 1: Examples of object properties that bats extract from echo information.

These acoustic images derive from acoustic information that is carried by the echoes of reflected echolocation calls reaching the bats ears.

From the physical point of view, each object is acoustically defined by its impulse response (IR), which is the sum of its reflections when it is ensonified with an impulse of infinite shortness and amplitude (Dirac impulse) (Blauert 1997). When the impulse meets a surface it is reflected. Due to constructive and destructive interference spectral peaks and notches are created in the magnitude spectrum of the IR. Hence the spectral reflection pattern is object-specific and depends, for example, on the number and spatial arrangement of reflective surfaces, the ensonification angle, material etc. Echolocating bats do not perceive the IRs of objects per se, but their emitted echolocation call convolved with the IR of the ensonified object (Weissenbacher and Wiegrebe 2003). This means that bats gather information about objects by comparing the emitted calls with the returning echoes.

Acoustic researchers and neurobiologists are especially interested in bats due to the fact that their highly developed echolocation system is based on the building blocks of the common mammalian auditory system that is working in air (Suga 1990, Covey and Casseday 1999).

The bat sonar (here with focus on the Microchiroptera) acts as an active and flexible sender-receiver system (Simmons 1971, Kick and Simmons 1984, Neuweiler 2000). I will first briefly describe the emitting system of bats with a focus on the different echo-acoustic signals. Then I will explain the receiver system in terms of the ascending mammalian auditory system with emphasizing on the processing of spatial and temporal information.

The emitter system

Bats produce complex echolocation calls with their larynx. From here they are projected through the vocal tract and are emitted through the nose or the mouth (cf. chapter 1, figure 2a, c). Species that emit echolocation calls through their nose like the ‘Phyllostomidae’ or ‘leaf-nosed bats’, have often complexly formed nose structures that can influence the characteristics of the emitted call, for instance frequency, and sonar-beam width (e.g. Simmons 1969, Simmons and Grinnell 1988, Zhuang and Müller 2006, Vanderelst, De Mey *et al*. 2010).

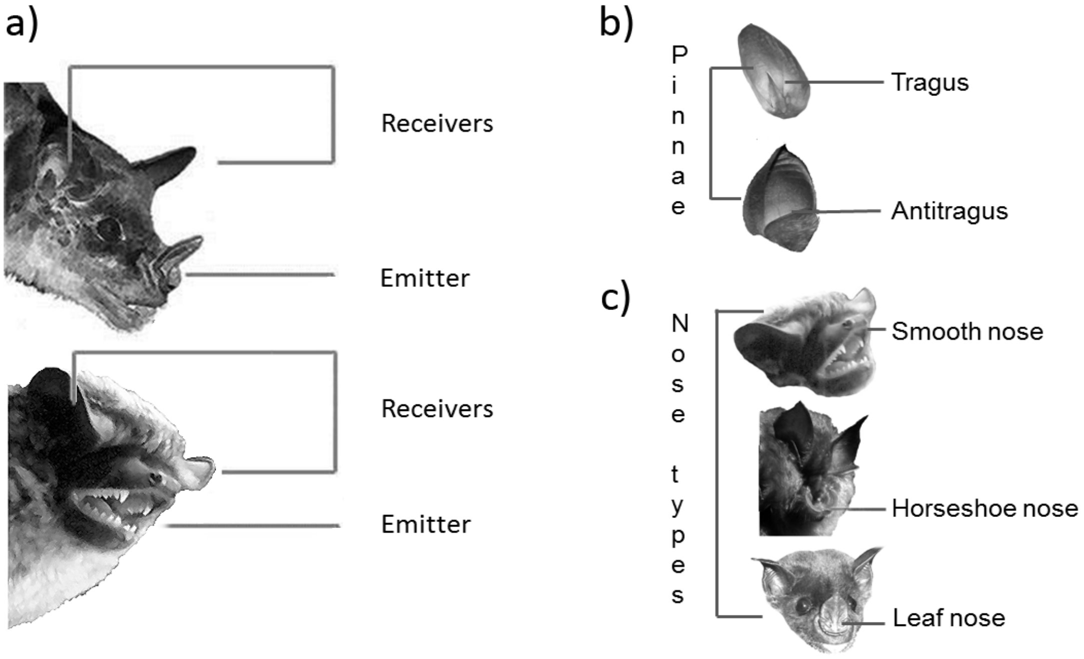

Figure 2: The external bat emitter and receiver system: a) The receiver structures (external ears) and emitter structures in ‘leaf-nosed’, or ‘phyllostomid bats’ and in ‘smooth-nosed’ or ‘vespertilionid bats’. b) Bat pinnae and example of tragus and antitragus c) Examples of the most common nose types/structures in microbats (here: smooth nose in the Vespertilionidae, the horseshoe nose in the Rhinolophidae, and the leaf nose in the Phyllostomidae).

Acoustic signals in echolocating bats

In echolocating bats one has to differentiate between social calls that mainly serve communication (mother-infant calls, foraging in groups, mate attraction, warning of conspecifics) (e.g. Esser and Schmidt 1989, Wilkinson and Boughman 1998, Fenton

2003, Bohn, Boughman *et al*. 2004, Knörnschild and von Helversen 2008) and echolocation calls. Social calls are relatively long compared to echolocation calls that only last a few milliseconds. In many species social calls are in the frequency range of human hearing (e.g. in *Phyllostomus discolor*) (Esser and Daucher 1996).

Echolocation calls can widely differ between species and are, like social calls, species specific (Neuweiler and Schmidt 1993, Schnitzler and Kalko 2001). The frequency range in bats covers frequencies from 8-210 kHz (Fenton and Bell 1981, Au and Simmons 2007). Echolocation calls consist of multiple harmonics (e.g. Suga 1990, Neuweiler 2000, Schnitzler and Kalko 2001, Vanderelst, Reijniers *et al*. 2011). This means that these calls contain a certain number of different frequencies that are multiple integers of a fundamental frequency (or first harmonic) (Griffin and Novick 1955, Suga 1990). In case that the fundamental frequency is 20 kHz, its multiple integers are 40 (2nd harmonic), 60 (3rd harmonic), and 80 kHz (4th harmonic) etc. The bat echolocation calls normally show the largest amount of energy in the 2nd or 3rd harmonic (Neuweiler 2000). Echolocation calls comprise of up to three elements. These elements are termed downward-frequency modulated, constant-frequency and upward-frequency modulated. The most common type of echolocation signals is the downward frequency-modulated (FM) call. Here calls are broadband and arranged in one or more downward sweeping harmonics (from high to low frequencies) and last between 0.5 and 20 ms. Constant frequency (CF) calls are pure tone and only lightly frequency modulated. Their duration is either short (between 1 and 10 ms) or long lasting (between 10-100, sometimes even 300 ms) (Au 2004). They often serve as search signals. The echolocation calls of the generea 'round-leaf bats' and 'horseshoe bats' always contain CF components. The rarest elements are upward-frequency modulated and only precedes CF components of a call (Schnitzler and Henson 1980, Suga 1990, Neuweiler and Schmidt 1993, Grinnell 1995, Neuweiler 2000, Schnitzler and Kalko 2001, Ulanovsky and Moss 2008).

As indicated above, bats are able to adapt call parameters like frequency, call modulation, signal duration and timing, call intensity etc. to solve different behavioral tasks (e.g. Suga 1990, Schnitzler and Kalko 2001). The spatial characteristics of the emitting system/sonar beam depend on the spatial dimension of the emitter as well as well as frequency content of the signal (Vanderelst, De Mey *et al*. 2010). The spatial characteristics of the sonar beam have impact on the echoes received by the bat (Ghose and Moss 2003, Surlykke, Pedersen *et al*. 2009, Jakobsen and Surlykke 2010, Jakobsen, Brinkløv *et al*. 2013).

The use of echolocation also has disadvantages. Since it is not a continuous sensory systems, bats only have a stroboscopic acoustic view. This means bats perceive spatial information only call by call (Neuweiler 2000, Ulanovsky and Moss 2008). Between the emissions they do not obtain information from their sonar system. In order to overcome this disadvantage, bats increase call emission rate, for example, when catching insects (feeding buzz) (Moss and Schnitzler 1995, Nelson and MacIver 2006, Moss and Surlykke 2010), drinking on the wing (drinking buzz) (Griffiths 2013), or for landing control (landing buzz) (Russo, Jones *et al*. 2007, Melcon, Schnitzler *et al*. 2009). Another disadvantage is the limited sonar range that normally operates in ranges less than 20 m, and maximally 50-60 m (Neuweiler 2000, Schnitzler and Kalko 2001). Sonar range can be partially improved by an increase of call intensity (Jakobsen, Brinkløv *et al*. 2013). The call intensity, that is measured as dB SPL (logarithmic measure of the sound pressure of an acoustic stimulus) in bats, is relatively high and ranges between 80 dB SPL and 135 dB SPL (Neuweiler 2000, Jakobsen, Brinkløv *et al*. 2013). There are many environmental influences that can attenuate echo intensity (e.g. atmospheric and geometric spreading losses; noise, wind etc.) which makes echo intensity an interference-prone cue (Schnitzler and Kalko 2001). The limited sound field can be improved by broadening the sonar beam or scanning the environment by emitting successive calls in different directions (Surlykke, Ghose *et al*. 2009, Surlykke, Pedersen *et al*. 2009, Jakobsen and Surlykke 2010, Moss and Surlykke 2010, Jakobsen, Brinkløv *et al*. 2013). Taken together the dynamic control of the emitting system helps bats to overcome disadvantages of echo imaging and increases spatial perception. Note that the bat receiver system must be as flexible as the emitter system.

The receiver system

The echolocation calls are reflected from obstacles and are picked up by the receiver system in terms of the ears (cf. chapter 1, figure 2a, b) and the associated neuronal processing system (Griffin 1958, Henson and O'Dell 1967, Simmons 1971). In the visual system the sensory epithelium (the retina) is arranged along spatial axes. Thus, spatial information is represented as a two dimensional image that is mapped on the retina. The sensory epithelium of the auditory system (the basilar membrane, for details see below) is not arranged along spatial axes and spatial information has to be translated into frequency. Thus, spatial information must be computed in the central auditory system.

Spatial and temporal information in the bat auditory system

The periphery in the auditory system consists of the outer, middle, and inner ear. The sound evoked excitation is led via the auditory nerve to the ascending pathways of the central auditory system. In specialized brain areas the echoes are analyzed along their time and frequency domains.

The outer ear involves the pinna (cf. chapter 1, figure 2b) and the auditory canal (meatus). In bats the flexible and moveable (in terms of rotatable and bendable) pinnae act as highly adaptable directional receiver system. The directional characteristics result from modification by diffraction, reflection and resonance (Batteau 1967). Consequently it depends on pinna geometry and size, as well as signal wavelength. Note that pinna geometry differs across bat species. Pinna size is also thought to reflect an adaptation for prey detection in different habitats (e.g. Fenton 1972, Neuweiler 1989, Neuweiler 2000).

The sound waves that enter the auditory meatus can be altered by sound waves of the same signal that were reflected by the outer ear structures and therefore arrive later. As a result the sound spectrum is modified (Roffler and Butler 1968). Depending on the phase of the incoming sound the signal gets attenuated or amplified. When the incoming sound is out of phase it is modifying the sound spectrum in a way that subtraction can cause so called spectral notches. These are regions in the spectrum where definite frequencies are nearly completely attenuated or rather cancelled. When the incoming sound is in phase, this can lead to amplification of certain frequencies. Many bat species possess a so called ‘tragus’ (a robust, pointy skin flap close to the pinna opening, cf. chapter 1, figure 2b) that is important for sound localization (see below).

Sound that travels through the meatus causes a vibration of the ear drum (tympanic membrane). Three tiny bones (‘ossicles’: malleus, incus and, stapes) transmit the vibration from the middle ear through a membrane covered oval opening (oval window) in the bony walls of the inner ear into the fluid filled cochlea. The middle ear therewith transmits sounds from one medium (gas, air) to another (fluid, perilymph) and acts as a transformer between two different impedances (Moore 2013). In bats there are some morphological adaptations and modifications (e.g. Suga 1990, Neuweiler 2000) that allow very efficient transmission of high frequencies compared to other mammals. The so called ‘basilar membrane’ (BM) and the ‘Reissner’s membrane’ inside the spiral formed cochlea divide it at full length into two compartments/chambers. The base of the BM is located at the oval window. The tip of the BM is termed the apex or apical end. Here a small opening (the helicotrema) is located between the BM and the bony walls of the cochlea and connects the scala tympani and the scala vestibula. When the oval window moves inwards, a round, also membrane covered window (the round window) moves automatically outwards. The movement of the oval window creates a pressure difference which results in a movement on the BM from base to apex. Due to BM mechanical structure (base: stiff and narrow, apex: wide and more flexible) different frequencies result in different BM movements. High frequencies of incoming sound create a maximal displacement of the BM close to the base whereas low frequencies create a maximal BM displacement close to the apex (e.g. Moore 2003, Bear, Connors *et al.* 2007, Moore 2013). Each point (in terms of nerve fibers) of the BM responds best at a certain characteristic frequency (or the best frequency, BF) (e.g. Suga 1990, Neuweiler 2000, Moore 2013). This means the BM therewith acts as a low pass-filter bank that splits up incoming complex sounds in their component frequencies. Note that in acoustics a filter is defined as “…a device which passes certain frequency components, but attenuates others. They can be characterized by their cutoff frequencies usually measured at the 3-dB-down points on their response functions, and by their slopes in dB/octave. The bandwidth of a band pass filter is equal to the frequency range between the two 3-dB-points.” (Moore 2003).

The hair cells are the receptor cells (mechanoreceptors) of the auditory and vestibular system and lie in the organ of Corti on the BM. One has to discriminate between two distinct types: The outer and the inner hair cells. The outer hair cells are arranged in three rows and amplify low-level sound that enters the cochlea mechanically and contributes to the sharp tuning and high sensitivity of the BM (reviewed in Ashmore 2008). The inner hair cells are arranged in one row and are mostly connected with afferent nerve fibers. They transform the mechanical energy (sound vibrations) in the fluids of the cochlea into electrical signals. The BM movement creates a bending (‘displacement’) of the stereocilia (the upper part of the hair cells) caused by the movement towards the overlying tectorial membrane. The tufts of the stereocilia project from the apical cell surface. The structure is known as hair bundle and projects into a fluid filled tube of the cochlea, the scala media. The displacement leads to receptor potentials. When a hair bundle is displaced towards the longest cilium, membrane potential is depolarized whereas displacement in the opposite direction leads to hyperpolarization. Taken together, the inner ear serves as a frequency analyzer and translates acoustic signals into neural activity. The electric signals are relayed via the auditory nerve fibers to the ascending auditory pathways of the brain. The output of the inner ear frequency analysis is represented in tonotopic maps in terms of laminar arrangement of neurons. Within a lamina all cells have similar BFs, so that each lamina processes information from a specific frequency band (Suga 1990, Neuweiler 2000, Moore 2003, Moore 2013). The ascending auditory system includes monaural (input from one ear) and binaural (input from both ears) pathways that perform parallel information processing and end both in the auditory midbrain (‘inferior colliculus’, IC) that is very large in bats. The IC is also tonotopically organized and is thought to adjust time scale of neural response to auditory stimuli (Casseday and Covey 1996). Also the representation of auditory space information is assumed to be processed here. For spatial representation monaural and binaural pathways are involved. From monaural pathways the convergence of multiple temporally delayed inputs were found that hint on delay lines within the pathways that could be basis for the discrimination of sequential auditory input (Berkowitz and Suga 1989, Suga 1990, Kuwabara and Suga 1993, Saitoh and Suga 1995, Hattori and Suga 1997, Neuweiler 2000). For spatial perception this would be important for distance perception (e.g. the interval between a call and the returning echo, for details see below).

For binaural processing the lateral superior olive (LSO) plays an essential role since some neurons encode information about interaural intensity differences (IIDs) that are location dependent and play an important role for high frequency sounds (see below). Since the LSO is also tonotopically composed, an overall activity pattern provides the representation of the difference spectrum between both ears. This is an important factor for binaural sound localization (cf. next paragraph). Binaural and monaural information is led via the IC over the medial geniculate body of the thalamus to the auditory cortex (AC) (see Moore 2003).

Sound localization

A primary function of the auditory system is sound localization. Since auditory space cannot be directly represented in the cochlea, studies of sound localization provide insight on how the brain is able to represent auditory space in terms of the construction of computational maps (Razak 2011). Note that while in other sensory modalities like vision and touch, space is represented topographically as space maps in the cortices, no cortical space map has been found for audition in mammals (e.g. humans) so far. Instead, in the AC auditory cortical neurons providing wide spatial receptive fields were found. They form a so called ‘population rate code’ that represents sound source location in azimuth (Neuweiler 2000, Salminen, Aho *et al*. 2013). In a three dimensional coordinate system (as already indicated above) sound sources are defined by the azimuth (horizontal angle), the elevation (vertical angle) as well as distance. The head and the outer ears provide sound transforming features (see above) which provide mammals with acoustic cues that are crucial for sound localization (Moore 2013). The influence of these structures is expressed in terms of the head-related transfer function (HRTF) that describes the ratio of the sound pressure measured near the tympanum and in the free field. The HRTF is frequency dependent. It shows a complex spectral pattern of peaks and notches that varies in systematic manner with sound source direction relative to the head. Thus, for each direction in space a distinctive pattern is created. The spectral changes can be used for sound localization (Fuzessery 1996, Blauert 1997, Firzlaff and Schuller 2003, Moore 2013).

Sound localization in the vertical plane

Elevation describes the angle of a signal that is produced in the vertical plane in relation to the receiver (the head). This is especially important for bats that e.g. forage and track objects in open space. Vertical sound localization predominantly depends on spectral cues as they are created by the outer ears. The tragus of the outer ear was found to be important for sound localization in the elevation in species that use broadband echolocation calls. Here the tragus influences the spectral characteristics of incoming sounds from the elevation, creating angle dependent characteristic interference patterns (Neuweiler 2000, Wotton and Simmons 2000). Note that several other studies reported changes in the HRTF when the tragus was deflected or even completely removed (e.g. in Firzlaff and Schuller 2003, Aytekin, Grassi *et al*. 2004, Firzlaff and Schuller 2004). In a study of Firzlaff and Schuller the tragus was discussed to be strongly involved into spectral notch creation (Firzlaff and Schuller 2003). In prey capture experiments with *Eptesicus fuscus* the tragus was deflected. It was found that the prey capture success decreased but was not eliminated. The bats seemed to adapt to the new acoustic cues and even altered their flight trajectories in response to tragus manipulation (Chen and Moss 2007). This indicates that bats must be able to exploit other acoustic cues. In species that do not possess such a structure like horseshoe bats that furthermore emit narrow band calls, no such characteristics are created (Firzlaff and Schuller 2004). Instead, in some species a stereotyped vertical ear movement was found that is thought to have the same function as the tragus (Pye and Roberts 1970, Simmons, Howell *et al*. 1975, Mogdans, Ostwald *et al*. 1988, Neuweiler 2000).

Sound localization in the horizontal plane

Azimuth describes the angle of a signal that is produced in the horizontal plane in relation to the head. For example, 0° azimuth describes a sound source located in front of the listener whereas 180° is directly behind it.

For object sound source localization in azimuth binaural acoustic cues in terms of interaural time differences (ITDs) and interaural intensity differences (IIDs, sometimes also referred to as interaural level differences or ILDs) of an incoming sound are employed by the common mammalian auditory system. ITDs describe the differences in the arrival time of a sound that is created at both ears. When a sound hits one ear it needs to travel an additional distance to reach the opposing one. The difference in path length also results in a difference of sound arrival time between both ears. The IIDs are created by the head that is shadowing the nonadjacent ear from the sound source. As a result the sound intensity is attenuated. When the signal frequency is low, the wavelength can be longer than the distance between both ears. In this case the sound is not attenuated and only ITDs can be used as cue (reviewed in Bear, Connors *et al*. 2007, Moore 2013). Note that most microchiropteran bats are very small. Moreover, the echolocation calls are in the ultrasonic range (high frequency). Both together seem to preclude ITDs as major cues for sound localization. As indicated above, in the ascending auditory system information about the interaural differences are encoded as differences in excitation. The lateral superior olive (LSO) provides populations of neurons that respond to IIDs. Hence

IIDs as well as spectral cues seem to be predominantly used by microchiropteran bats for directional sound localization (Firzlaff and Schuller 2003).

In birds, barn owls also use ITDs and IIDs as cues for sound localization of passive-acoustic cues (e.g. rustling noises of mice). The cues are combined and processed in the midbrain auditory pathway. In the optic tectum (that is the superior colliculus in mammals) neurons were found that respond to explicit spatial locations and are systematically organized. This means in contrast to mammals, owls possess an auditory space map that fuses with a visual map (reviewed in Pena and DeBello 2010, Efrati and Gutfreund 2011).

Encoding of object distance

As third spatial dimension object distance or ‘target range’ in echolocating bats is encoded by the delay between the emission of a call and the returning echoes (Hartrige 1945, Simmons 1971, Simmons 1973).

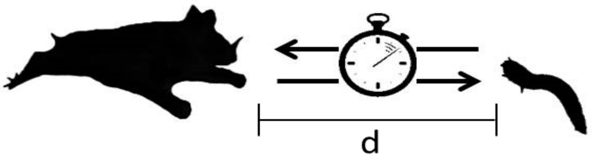

Figure 3 Encoding of object distance/target range in echolocating bats. Object distance **d** (*here*: of a mealworm) is encoded by the delay between a bat’s call emission and the returning echo.

Delay measurement takes place in the central auditory system. It needs precise start and ending signals to achieve a high resolution for distance determination. *Pteronotus parnelli* for example (O'Neill and Suga 1979, Suga and O'Neill 1979), emits complex echolocation calls (4 harmonics, CF-FM calls). A short fm-part of the call is used for the measurement of echo delay and hence distances determination. In the AC, populations of neurons were found that mainly responded to the fm-part of the calls. Note that these neurons are termed ‘combination sensitive’ and only respond in this case to a pair of sounds (call and echo). The outgoing signal must contain the correct frequency/harmonic and the echo must follow within a certain time window after the preceding signal. Distance coding neurons are chronotopically arranged in the AC according to their best delays (e.g. Hagemann, Esser *et al*. 2010). Another type of neurons important for distance determination is found in the IC.

These neurons encode arriving times and respond to the onset of a stimulus with one spike (constant latency neurons). They are thought to represent inputs into neuronal circuits for temporal analysis. The functional role of the bat auditory midbrain and cortex for distance processing (e.g. in terms of target range maps) is reviewed in detail elsewhere (Wenstrup and Portfors 2011).

Integration and resolution in the bat auditory system

The most important task of the auditory system is the internal representation of auditory space and hence auditory perceptual scenes. These are defined as ‘arrays of events arranged in time and space’ (Blauert 1997). Consequently the auditory system must be able to integrate this information in order to build up an internal representation of space. Note that the auditory system for instance, integrates localization results e.g. in order to track sound sources. For echolocating bats that are capable to fly in complete darkness, this task is probably even more demanding since during locomotion spatial and temporal information rapidly change. This likewise requires the auditory system to rapidly process and update the internal representation of space. In addition, a very good spatial as well as temporal resolution is crucial: for example, when a bat hunts an insect additional echoes from the environment like foliage appear. These masking echoes can come from different spatial positions, but also at different times. Hence the echo of the prey can be masked and the bat must be able to exploit even short differences between echo arrival times which can lead to hunting success.

Spatial integration and resolution

Generally the mammalian auditory system is able not only to sum different auditory cues that, for example, give information about object location but also decides what information is relevant for the inner representation of space. For instance, in a normal reverberant environment the sound of a sound source reaches the ears via different paths (through reflections) and thus at different times. Consequently one has to differentiate between direct and indirect incoming sounds (echoes) of the same sound source. In psychophysics it was shown that these echoes have no strong influence on sound localization. Actually these sounds are perceived as fused and the location of the perceived sound is ruled by the location of the direct sound that reaches the ears first. This phenomenon is termed the ‘precedence effect’ (Wallach, Newman *et al*. 1949) or the ‘law of the first incoming wavefront’. Spatial integration is probably comparable to this effect where high-level cognitive processes are involved to suppress the perception of irrelevant acoustic reflections, only using the first incoming sound (Blauert 1997, Keen and Freyman 2009, reviewed in Moore 2013). For the precedence effect it was (for instance) found that in experiments echoes were only suppressed when there was an overlap between the listener’s expectations of room acoustics and the sound source itself.

The spatial resolution in bat sonar is defined as the bats ability to detect an object in the presence of maskers that appear at the same time and distance but at different spatial positions (e.g. right and left from the object of interest). It depends on the directionality of the sonar beam as well as the auditory spatial selectivity for sonar emissions (Geberl 2013). Moreover, spatial resolution depends on frequency content of the sonar emissions (the higher the frequency the better the resolution, but the lesser the range of echolocation calls), beam width as well as how accurately the bats probe their environment (Neuweiler 2000, Surlykke, Ghose *et al*. 2009).

Temporal integration and resolution

As indicated above, the auditory system must be able to perform different types of temporal information processing e.g. temporal integration and temporal resolution.

The ability of a system for temporal integration is defined as a time interval in which acoustic intensity is integrated thus improving detectability of a signal with increasing duration (Zwislocki 1960, Wiegrebe and Schmidt 1996, Weissenbacher, Wiegrebe *et al.* 2002, Rimskaya-Korsakova 2004, Moore 2013). Temporal integration can be quantified with different psychophysical paradigms e.g. by recording detection thresholds of a pair of short clicks as a function of the temporal separation between the clicks (Zwislocki 1960). If the temporal separation is shorter than the integration time, the detection should improve by 3 dB (in humans) compared to the presentation of only one click. This results from the summation of the presented click intensities. Is the delay longer than the integration time, no intensity summation and threshold improvement appears. In humans integration times of 3-5 ms were found (Viemeister and Wakefield 1991). Thresholds that were obtained for bats and dolphins are much shorter (*Tursiops truncatus*: ~ 260 µs (Au, Moore *et al.* 1988), *Megaderma lyra:* ~ 200-300 µs (Wiegrebe and Schmidt 1996, Weissenbacher, Wiegrebe *et al.* 2002), *Eptesicus fuscus:* 200-400 µs (Simmons, Freedman *et al*. 1989) ~ 2.4 ms (Surlykke and Bojesen 1996)). The diverging thresholds between bat species are discussed in the background of undesired masking effects and deviating measurement paradigms elsewhere (Weissenbacher, Wiegrebe *et al*. 2002, Weißenbacher 2003).

Temporal resolution describes the capability of a system to follow quick temporal variations of a signal. In the auditory system this can be measured by the presentation of amplitude modulated stimuli and recording of the auditory evoked potentials that are recorded from brainstem activity (Mooney, Nachtigall *et al*. 2006). The shorter the integration time the better the temporal resolution of a system (Rimskaya-Korsakova 2004).

Spatiotemporal integration in bat sonar

Taken together, the neural encoding of spatiotemporal echo information is an important feature of the bat auditory system. Furthermore, the integration of spatiotemporal information leads to spatial perception (e.g. echo-acoustic scenes) and allows bats skilled navigation through space in absence of visual input. Moreover, the integration of spatial and temporal information can also be employed to predict the collision of two objects in space e.g. for obstacle avoidance or the catching of prey.

Note that the interaction of spatiotemporal acoustic information is not only important for echo-acoustic scene perception but also for object perception itself. For example, when a bat ensonifies an object from different angles, the spatiotemporal pattern of the returning echo changes systematically with object distance, shape, or surface structure (e.g. Genzel, Geberl *et al*. 2012). If a bat ensonifies an object from a fixed distance the object has a certain object size in terms of its height, width, and depth. As soon as the bat gets closer to the object, its spatial extent in terms of the naturally co-varying spread of angles of incidence from which the echoes impinge on the bat’s ears (sonar aperture) increases, whereas the time interval between call and echo decreases. How bats encode the spatial characteristics of object size as well as how bats perceive the spatiotemporal variation of object size is widely unknown. Moreover, it is widely unknown how bats are able to perform spatiotemporal integration under natural conditions, e.g. when hunting in a complex and highly cluttered environment in order to prevent masking effects.

1.1 Study Aim:

In this cumulative thesis different aspects of spatiotemporal integration in bat sonar are addressed. First, using an combined psychophysical and electrophysical approach we investigated which acoustic cue (echo intensity or sonar aperture) echolocating bats employ for the estimation of object width as a size relevant spatial object property. Second, we tested whether bats show the ability to combine spatial and temporal cues to perceive absolute width information in terms of sonar size constancy. In a final step, we studied an example of spatiotemporal integration in bat biosonar in a natural and naturalistic context.

Each of the three studies described above was published in a peer-reviewed journal and will be presented in the next three chapters of this thesis.

2 The Sonar Aperture and Its Neural Representation in Bats

This chapter was published in: The Journal of Neuroscience on October 26th; 2011 (31(43):15618 –15627) under the title: **“The Sonar Aperture and Its Neural Representation in Bats”** by Melina Heinrich (MH),* Alexander Warmbold (AW),* Susanne Hoffmann (SH), Uwe Firzlaff (UF), and Lutz Wiegrebe (LW)
(*M.H. and A.W. contributed equally to this work).

2.1 Reference

LW and UF originally designed the experiments. The electrophysical experiments were conducted by AW as part of his diploma thesis under the supervision of SH and UF. LW and MH further developed the psychophysical experiments and the experimental setup. LW and MH wrote the experimental programs and the analysis programs. MH performed the psychophysical research, collected the data of all psycho-acoustic experiments, and conducted the corresponding analysis. MH wrote the psychophysical materials and methods and the results section. AW wrote the electrophysiological materials and methods section and the results section. The other manuscript sections were written by MH, AW, SH, UF and LW. MH designed the figures 1 and 3 and delivered the psychophysical data depicted in the figures 8 and 9. The remaining figures as well as the computational models were designed and calculated by AW, SH, UF, and LW. Harald Luksch read an early version of the manuscript and made valuable comments to improve the draft.

2.2 Summary

In the first study we investigated which acoustic cue (echo intensity or sonar aperture) echolocating bats employ for the estimation of object width as a size relevant spatial object property. This was done by choosing a combined psychophysical and electrophysiological approach. In three active acoustic virtual-object playback experiments, following a two-alternative forced-choice paradigm, individuals of the neo-tropical phyllostomid bat species *Phyllostomus discolor* had to discriminate simple reflections of their own echolocation calls. The so called virtual objects were either differing in echo intensity, sonar aperture, or both. The results of the discrimination performance showed no difference when only the sonar aperture was varied compared to the experiment where sonar aperture and echo intensity were physically correct varied together (object width). As a result we concluded that in our experiments intensity cues were not needed for the discrimination of object width unlike postulated in other studies. Further it was found that *P. discolor* has a surprisingly high threshold of 5 dB (based of the mean psychometric functions of five individuals) for the discrimination of echo-intensity differences that were measured in a third experiment. The findings of the psychophysical experiments are supported by the findings of the electrophysiological approach. Here a population of units (in terms to 1-3 neurons) in the auditory midbrain and cortex was discovered that responded strongest to echoes from VOs with a specific sonar aperture, independently of variations in echo intensity. The neuronal correlate strongly supports the relevance of sonar aperture as a cue for object size perception.

Correction: Heinrich et al., “The Sonar Aperture and Its Neural Representation in Bats”

In the article “The Sonar Aperture and Its Neural Representation in Bats” by Melina Heinrich, Alexander Warmbold, Susanne Hoffmann, Uwe Firzlaff, and Lutz Wiegrebe, which appeared on pages 15618-15627 of the October 26, 2011 issue, a minor error occurred. The original sentence on page 15619: “The measured impulse response of each speaker was divided by the impulse response of an ideal bandpass filter (47th-order finite impulse response, cutoff frequencies of 15 and 94 kHz) to generate a compensatory impulse response.” should be replaced with: “The complex spectrum of an ideal bandpass filter (47th-order finite impulse response, cutoff frequencies of 15 and 94 kHz) was divided by the complex spectrum of the measured impulse response of each speaker to generate a compensatory impulse response.”
This correction does not affect any of the results, conclusions or interpretations in our paper.

Behavioral/Systems/Cognitive

The Sonar Aperture and Its Neural Representation in Bats

Melina Heinrich,[1,2]* Alexander Warmbold,[1]* Susanne Hoffmann,[1] Uwe Firzlaff,[3] and Lutz Wiegrebe[1]
[1]Division of Neurobiology, Department of Biology II, and [2]Graduate School of Systemic Neurosciences, Ludwig-Maximilians University Munich, D-82152 Planegg-Martinsried, Germany, and [3]Chair of Zoology, Technical University Munich, D-85350 Freising-Weihenstephan, Germany

As opposed to visual imaging, biosonar imaging of spatial object properties represents a challenge for the auditory system because its sensory epithelium is not arranged along space axes. For echolocating bats, object width is encoded by the amplitude of its echo (echo intensity) but also by the naturally covarying spread of angles of incidence from which the echoes impinge on the bat's ears (sonar aperture). It is unclear whether bats use the echo intensity and/or the sonar aperture to estimate an object's width. We addressed this question in a combined psychophysical and electrophysiological approach. In three virtual-object playback experiments, bats of the species *Phyllostomus discolor* had to discriminate simple reflections of their own echolocation calls differing in echo intensity, sonar aperture, or both. Discrimination performance for objects with physically correct covariation of sonar aperture and echo intensity ("object width") did not differ from discrimination performances when only the sonar aperture was varied. Thus, the bats were able to detect changes in object width in the absence of intensity cues. The psychophysical results are reflected in the responses of a population of units in the auditory midbrain and cortex that responded strongest to echoes from objects with a specific sonar aperture, regardless of variations in echo intensity. Neurometric functions obtained from cortical units encoding the sonar aperture are sufficient to explain the behavioral performance of the bats. These current data show that the sonar aperture is a behaviorally relevant and reliably encoded cue for object size in bat sonar.

Introduction

The neural encoding of object size is an important function of sensory systems. In the visual system, the spatial extent of an object, i.e., its visual aperture, is explicitly encoded in terms of the extent of the image on the retina. In the auditory system, however, frequency instead of space is explicitly encoded and auditory space information must be computed in the central auditory system. This problem becomes especially relevant for a bat, which recruits its auditory system to image its surroundings. Through echolocation, bats derive a sensory image not only about the position of an object but also about its spatial extent and 3D shape.

The ability of bats to classify complex 3D objects based on physical properties like shape, orientation, surface structure, or object size has been investigated in both psychophysical and neurophysiological studies (Habersetzer and Vogler, 1983; Schmidt, 1988, 1992; Von Helversen and Von Helversen, 1999; Sanderson and Simmons, 2002; Grunwald et al., 2004; von Helversen, 2004; Firzlaff et al., 2006; Borina et al., 2008; Falk et al., 2011). There are few studies addressing the acoustic cues that underlie size perception in the echo imaging of bats (Simmons and Vernon, 1971; Simon et al., 2006; Firzlaff and Schuller, 2007; Firzlaff et al., 2007), but it is unclear by which echo-acoustic parameters the width of an ensonified object is encoded and how these parameters are processed in the bat's auditory system.

A wider object creates a louder echo because the surface area reflecting the bat's call increases (Simmons and Vernon, 1971). Additionally, the "spread of angles of incidence" from which the echoes impinge on the bat's ears increases with increasing object width. In the present study, this spread of angles of incidence is called the sonar aperture of an object. Using real objects, as in earlier approaches, precludes an experimental isolation of these echo-acoustic cues. This problem can be overcome by the use of virtual objects allowing systematic manipulation and analysis of well isolated object properties (Schmidt, 1988; Weissenbacher and Wiegrebe, 2003). The aim of the present study is to quantify the relevance of echo intensity and sonar aperture for the perceptual evaluation of object width by bats and to find a possible neural correlate for the bat's behavioral performance.

The psychophysical results show that to discriminate objects of different width *Phyllostomus discolor* predominantly uses sonar aperture even if echo-intensity information is also available. A control experiment shows that in the absence of sonar aperture information, the bats are quite sensitive to the changes of echo intensity. The perceptual salience of the sonar aperture for object–width discrimination is supported by the electrophysiological results: both in the inferior colliculus (IC) and auditory cortex (AC), a population of units is found that responds strongest to

Received May 25, 2011; revised Aug. 5, 2011; accepted Aug. 29, 2011.

Author contributions: U.F. and L.W. designed research; M.H. and A.W. performed research; M.H. and A.W. analyzed data; M.H., A.W., S.H., U.F., and L.W. wrote the paper.

This study was funded by the Volkswagenstiftung Grant I/83838 to U.F. and L.W., by Deutsche Forschungsgemeinschaft Grant FI 1546/1 to U.F., and by the Human Frontier Science Program Grant 0062/2009 to L.W. We thank Harald Luksch for comments on an earlier draft of this paper and acknowledge the workshop crew, especially Michael Fellner, of the Biozentrum Martinsried for valuable assistance in technical issues. We also thank the two anonymous reviewers whose comments and suggestions significantly improved both clarity and precision of the paper.

*M.H. and A.W. contributed equally to this work.

Correspondence should be addressed to Dr. Lutz Wiegrebe, Division of Neurobiology, Department Biology II, Ludwig-Maximilians-Universität München, Grosshaderner Strasse 2, D-82152 Planegg-Martinsried, Germany. E-mail: lutzw@lmu.de.

DOI:10.1523/JNEUROSCI.2600-11.2011

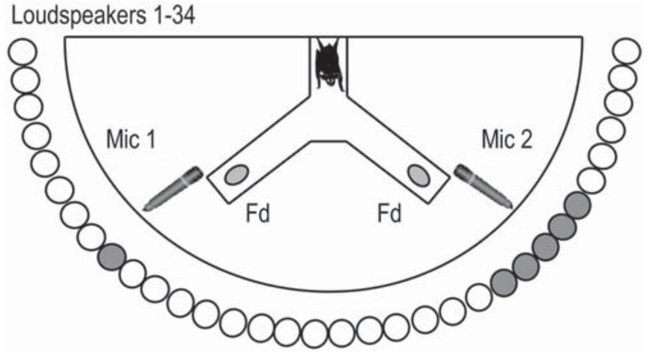

Figure 1. Psychophysical experimental setup. The loudspeaker array is depicted from the front with a bat sitting on the focal point of the Y-maze placed in a semicircular wire mesh cage. Indicated are the feeders (Fd), the ultrasonic microphones used for stimulus generation (Mic), and the 34 loudspeakers for virtual object presentation in the azimuth. The single active speaker (gray) on the left shows the position from where the rewarded object is presented, whereas the 5 adjacent active speakers on the right represent an unrewarded object of the Width and Sonar aperture experiments.

echoes of an object with a certain sonar aperture independent of echo intensity. This neural population may represent the basis for the bat's ability to deduce an object's width only through the sonar aperture of its echoes.

Materials and Methods

Experimental animals

The bat species used in this study was the neotropical phyllostomid bat, *P. discolor*. The bats came from a breeding colony in the Department of Biology II of the Ludwig-Maximilians-University in Munich. These bats emit short ($<$3 ms) broadband, downward frequency-modulated, multiharmonic echolocation calls in the frequency range between 45 and 100 kHz (Rother and Schmidt, 1982). *P. discolor* feeds mainly on fruit, pollen, and insects (Nowak, 1994).

Psychophysics

The psychophysical experiments were implemented as active-acoustic, virtual-object playback experiments in which bats had to discriminate echoes of their own echolocation calls that were presented as reflections from a virtual object extending along the azimuth.

Animals. Five adult males and one female *P. discolor* bats with a body weight ranging between 30 and 50 g participated in the psychophysical experiments. On training days, the individuals were kept in a cage (80 × 60 × 80 cm). After training sessions, the animals could fly freely in a room of 12 m^2 until the next morning. All individuals had access to water *ad libitum*. The training was realized in daily sessions of 20 min at 5 d per week, followed by a 2 d break. The bats were fed with a fruit pulp as reward during training sessions. On days without training, the animals had had access to fruit and mealworms *ad libitum* (larvae of *Tenebrio molitor*).

Experimental setup. All psychophysical experiments were conducted in complete darkness inside an echo-attenuated chamber (2.1 × 1.8 × 2.1 m). In this chamber, a Y-shaped maze (Y-maze) placed in a semicircular wire mesh cage (radius = 55 cm) was inversely mounted on a metal post at an angle of 45° (Fig. 1). The top end of the Y-maze held a starting perch, whereas a feeder was located at the end of each leg. The angle between the legs measured 90°. Two ultrasonic microphones (CO 100K, Sanken) were installed above the feeders of the maze pointing toward the bat's perch. The cage was plane-parallel arranged towards a semicircular loudspeaker array (radius = 71 cm) that consisted of 34 ultrasonic ribbon loudspeakers (NeoCD1.0, Fountek). The speakers' front plates were covered with plane acoustic foam except for each speaker's membrane (8.0 × 38.0 mm). The speaker array was subdivided into right and left hemispheres, each consisting of 17 speakers. The spatial separation between adjacent speakers in each hemisphere was 5.6°. Each of the 34 speakers was calibrated against a 1/8 inch microphone (without protective grid; Type 4138, Brüel & Kjaer) positioned at the bat's starting perch and oriented perpendicular to the speaker axes. The measured impulse response of each speaker was divided by the impulse response of an ideal bandpass filter (47th-order finite impulse response, cutoff frequencies of 15 and 94 kHz) to generate a compensatory impulse response. Every echo presented over one of the speakers was convolved in real time with this speaker's compensatory impulse response. Thus, it was ensured that all 34 speakers provided a linear frequency response between 15 and 94 kHz and a linear phase at the starting perch of the bat.

Each echolocation call emitted by a bat in the setup was picked up by the microphones and amplified (QuadMic, RME) and digitized [HD 192, MOTU; three devices with 12 analog-to-digital (AD) and digital-to-analog (DA) channels each and a 424 PCI board, MOTU] at a rate of 192 kHz. After determining the required echo level (see below, Stimuli), the calls were convolved with the compensatory impulse response of the particular speaker, DA converted, and amplified (AVR 347, Harman Kardon; five devices with seven channels each) before being sent to the speaker. The input–output delay of the system, together with the physical propagation delay from the bat to the microphones and from the speakers to the bat, added up to 6.7 ms, which corresponds to a fixed distance of the virtual object of 1.12 m.

Residual physical echoes from the experimental setup arrived much earlier: the distance between the perch and the speakers as the source of the latest physical echoes was 0.71 m. The distance difference between the physical and virtual echoes of 41 cm was much too large to create a spectral interference pattern between the physical and virtual echoes.

For acoustic monitoring during the experiments, the digitized signals from a third (central) microphone were multiplied with a 45 kHz pure tone. The resulting difference frequency was in the audible range and sent via an additional DA channel of the MOTU HD 192 and the remaining channel of one of the amplifiers to headphones (K 240 DF, AKG).

The experimenter was seated outside the chamber, observing and controlling the experimental procedure via infrared camera and computer interface. Experimental control, data acquisition, and analysis were implemented in MATLAB 7.5 (MathWorks). For the control of the MOTU system, SoundMexPro software (HörTech) was used.

Stimuli. Each microphone recorded the animal's ultrasonic calls emitted toward its corresponding hemisphere. The virtual objects were implemented as simple reflectors. Echo intensity was manipulated by setting the attenuation of the echo before the DA conversion in each channel. Sonar aperture was manipulated by changing the number of adjacent speakers presenting an echo (see Fig. 1). As a result, 2D echo patterns differing in spatial and intensity information could be presented from both hemispheres of the speaker array.

When the sonar aperture was increased, the number of adjacent speakers presenting the echo of the call picked up by a microphone increased. The number of adjacent speakers was always increased symmetrically around the central speakers of each hemisphere such that the spatial "center of gravity" remained unchanged. Complex spatial interference patterns were generated because the speakers in each hemisphere emitted the same, fully coherent sound and they were all the same distance to the bat's perch. Note, however, that the same would be true for the reflections of a real surface when it is equidistant, i.e., it is bent around the bat's perch: if one imagined the surface as consisting of a number of point reflectors, the lateral reflections from these point reflectors would interfere the same way as the sounds from the speaker membranes in the current setup. The net effect of the interference pattern is that at the bat's starting position echoes from the speakers (and from a flat surface bent in the same way as the speaker array) add up constructively to create a strong overall echo. Moving out of this "focal point" decreases echo intensity dramatically due to destructive interference. But this destructive interference is as similar to a real equidistant object as it is for the virtual object used here. It is clear that such an equidistant surface is an unnatural object for a bat; however, it is the only reasonable object to use when trying to isolate the sonar aperture and echo intensity as the parameters of interest. All other objects would introduce space-dependent changes in echo delay, a confounding parameter to which bats are very sensitive (Simmons, 1971, 1973).

Procedure. The bats were trained on three experiments. Each experiment followed a two-alternative, forced-choice paradigm with food reward. The animals were trained to discriminate a rewarded virtual object (RO) from an unrewarded virtual object (UO). They were only rewarded for correct decisions, indicating a decision by crawling toward one of the two feeders. When a bat's performance exceeded 80% correct choices on 5 consecutive training days, data acquisition was started. Here, trials were arranged according to a staircase procedure: acquisition started with a block of three to five trials with easily discriminable virtual objects. For each subsequent block, the task difficulty was increased until the bat's performance approached chance level. Psychometric functions are based on 50 trials per UO. Significance was set at $p < 0.01$ based on a binomial test. The rewarded hemisphere was selected pseudorandomly (Gellermann, 1933).

Experimental conditions. In the Width experiment, discrimination performance for object width, represented by the physically correct covariation of echo intensity and sonar aperture, was tested. Bats had to discriminate echoes transmitted by a single speaker (RO) in one hemisphere from echoes transmitted by three or more speakers (UO) in the other hemisphere. Every single speaker of the UO provided the same echo intensity as the RO; thus, the sonar aperture covaried with echo intensity. The width of the UOs measured between 11° (the angular separation of three adjacent speaker membranes) and 90° (17 adjacent speaker membranes); the corresponding echo-intensity differences, measured at the starting perch, were between 9.5 and 24.6 dB.

"Sonar aperture" experiment. This experiment was identical to the Width experiment with the following exceptions. First, the echo intensity of each speaker transmitting the UO was reduced such that the intensity of the waveforms, summed up across all speakers constituting the UO, was equal to that of the RO. Second, echo intensity was roved (±6 dB) between the RO and the UO and over trials to preclude the bat's use of residual intensity differences to solve the task.

"Intensity" experiment. In the Intensity experiment, the perceptual threshold of *P. discolor* for differences in echo intensity was tested. Virtual objects were only presented by the center speakers (45° position) in each hemisphere. Echo-intensity differences were presented in steps of 1 dB; the maximal difference was 10 dB. In each trial, bats had to decide which virtual object provided the lower echo intensity.

Electrophysiology

Subjects. For the electrophysiological experiments four *P. discolor* were used. Recording sessions lasted 4 h and were performed 4 d per week for up to 8 weeks. After experiments and on experiment-free days the bats had access to food and water *ad libitum*.

Surgery. All experiments complied with the principles of laboratory animal care and were conducted under the regulations of the current version of the German Law on Animal Protection (approval 55.2-1-54-2531-128–08, Regulation Oberbayern). The animals were initially anesthetized by subcutaneous injection of a mixture of 0.4 μg of medetomidine (Domitor, Novartis), 4 μg of midazolam (Midazolam-ratiopharm, ratiopharm GmbH), and 0.04 μg of fentanyl (Fentanyl-Janssen, Janssen-Cilag) per 1 g of body weight of the animal. The surgery was previously described in detail by Schuller et al. (1991). In short, the skin overlying the cranium was cut along the midline. The cranium was freed from remaining tissue, and a small metal tube was attached to the rostral part of the cranium using a microglass composite (GLUMA Comfort Bond, Heraeus Kulzer). To avoid inflammation, the antibiotic enrofloxacin (Baytril, 0.5 μg/g body weight; Bayer AG;) was injected subcutaneously. After surgery, the anesthesia was antagonized with a mixture of atipamezole hydrochloride (Antisedan, Novartis), flumazenil (Anexate, Hoffmann-La Roche), and naloxon (DeltaSelect GmbH), which was applied subcutaneously (2.5, 0.5, and 1.2 μg/g body weight, respectively). To reduce postoperative pain, the analgesic meloxicam (Metacam, 0.2 mg/kg body weight; Boehringer-Ingelheim) was applied subcutaneously.

Stereotaxic fitting procedure. After surgery, stereotaxic fitting according to Schuller et al. (1986) was performed to guide the access to the subsequent recording positions.

For verification of the recording sites a tracer (wheat germ agglutinin conjugated to horseradish peroxidase; Sigma) was injected at a defined position in the brain. After histological processing of the brain, recording sites were reconstructed in brain atlas coordinates (B. Schwellnuss, T. Fenzl, A. Nixdorf, unpublished data).

Acoustic stimuli and data acquisition. All stimuli were computer generated (MATLAB; MathWorks), DA converted (RX6, sampling rate 260 kHz; Tucker-Davis Technologies), and fed into a programmable attenuator (PA5, Tucker-Davis Technologies). The signal was amplified (custom-made amplifiers) and then presented to the animal via ultrasonic earphones (custom made; Schuller, 1997). The frequency response of the ultrasonic earphones was flat (±3 dB) between 20 and 100 kHz.

For measuring the frequency response area (FRA) of neurons, pure tone stimuli with different frequency–intensity combinations were used. A detailed description of the procedure was described previously by Hoffmann et al. (2008).

Spatial receptive fields of neurons were measured using a standard echolocation call of *P. discolor* that was convolved with the head-related impulse responses (HRIRs) for the left and right ear of the corresponding position in space (Firzlaff and Schuller, 2003). The receptive field was measured in steps of 15° in the frontal hemisphere ranging from ±82.5° in azimuth and elevation. Stimuli were presented in a randomized order and repeated 10 times. The intensity of the loudest echo was adjusted such that the receptive field covered a surface of at least 60° in azimuth. Usually the intensity was around 40 dB above the neuron's pure tone threshold.

To generate echoes of objects with a specific width, the echolocation call was first convolved with the HRIRs corresponding to several adjacent horizontal positions in space (see below in this section), and the resulting echoes were summed up across the positions to generate the stimuli as they would add up on the bat's eardrums. The virtual object was centered in the spatial receptive field measured earlier. This was done to ensure that echoes over the whole range of object widths were within the excitatory region of the spatial receptive field of the unit under study. In addition, it was shown that the position of the pinnae influences the position of the spatial receptive field (Sun and Jen, 1987). It is reasonable to assume that in the psychophysical experiments the bats moved their pinnae to focus on the virtual objects. Thus, the procedure of centering the virtual object in the receptive field of a unit resembles the psychophysical paradigm. As in the psychophysical experiments, wider objects were generated by adding echoes symmetrically around the center of the receptive field. Adjacent positions were separated by 7.5° resulting in virtual objects with a width of 15, 30, 45, and 60° for 3, 5, 7, and 9 adjacent echo positions (Fig. 2). Echoes from a single position in the frontal hemisphere are referred to as having a sonar aperture of 0°.

The summing of echoes generated with different HRIR sets results in complex interference patterns. Note, however, that these are the interference patterns as they would occur in the pinnae of our experimental animal as defined by the HRIRs. The application of HRIRs thus makes the bat a unique directional stereo receiver of the echoes as opposed to a single omnidirectional microphone. On such a microphone, the echoes from different horizontal directions would add up in phase resulting in a 6 dB increase in echo intensity per doubling of the number of echoes (Fig. 2*H*). After using the bat's HRIRs, the intensity does not increase monotonically with increasing object width (Fig. 2*H*) due to the destructive interference in the bat's pinnae.

The echoes were presented at different overall intensities. For one of the four experimental animals, intensities covered a range of 24 dB in steps of 6 dB; for the other three animals, intensities covered a range of 12 dB in steps of 3 dB. The lowest intensity was adjusted to the intensity used for the receptive-field measurements. The resulting five-by-five stimulus matrix was presented randomized with 40 repetitions. The repetition rate was ~2 Hz. As a monaural control, the five-by-five stimulus matrix was presented only to the contralateral ear to determine the degree to which neural responses depend on the binaural stimulation.

All experiments were conducted in an anechoic, electrically shielded, and heated (~36°C) chamber. Earphones were inserted into the animal's ear canals. Extracellular recordings were made with glass-insulated tungsten microelectrodes (2 MΩ impedance; Alpha-Omega GmbH).

The electrode signal was recorded for 450 ms starting 50 ms before stimulus presentation. The electrode signal was amplified (ExAmp-

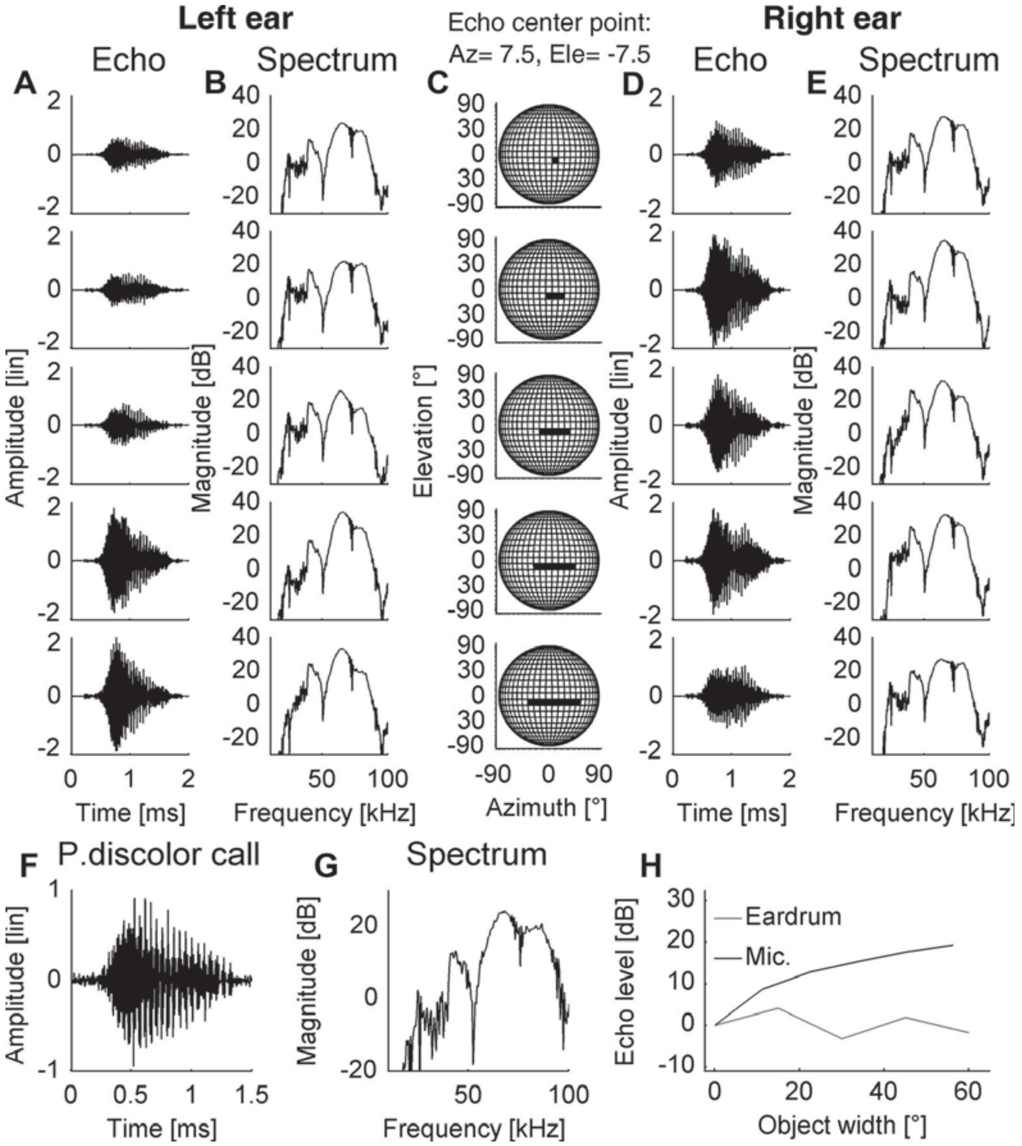

Figure 2. Spatially extended echoes at the bat's eardrums. ***A***, ***B***, ***D***, ***E***, Temporal (***A***, ***D***) and spectral (***B***, ***E***) characteristics of the virtual object stimulus for increasing width (from top to bottom). ***C***, Virtual objects were centered at 7.5° in azimuth and −7.5° in elevation and extended symmetrically. ***F***, ***G***, the original echolocation call of *P. discolor* in the temporal (***F***) and spectral domain (***G***) is depicted. ***H*** shows how identical sounds presented simultaneously from one or several speakers add up on an omnidirectional microphone (4138 1/8 inch, Bruel & Kjaer) and on the bat's ear drums. The analysis shows that due to the high directionality of the bat's pinnae, sound pressure levels do not add up systematically on the eardrums. Az, Azimuth; Ele, elevation.

20KB, M2100; Kation Scientific), bandpass filtered (300–3000 Hz, PC1; Tucker-Davis Technologies), AD converted (RP2.1, sampling rate 25 kHz; Tucker-Davis Technologies), and finally stored on a PC using Brainware (Tucker-Davis Technologies).

Since it was not possible to always analyze responses of a single neuron, the term "unit" for the responses derived from one to three neurons will further be used in this text. The number of neurons included in the term unit was estimated based on the number of different spike-waveforms that can typically be visually discriminated in terms of, e.g., positive/negative amplitudes and/or spike duration on the oscilloscope screen during recording.

Data analysis. Data were analyzed with MATLAB (MathWorks). Spikes evoked by all stimuli were displayed as peristimulus time histogram. As a large variety of response patterns across the units was observed, especially in the auditory cortex, spike responses were analyzed using a sliding window to determine the individual response duration of a unit (Schlack et al., 2005). This analysis window was set automatically by moving a 10 ms window in 1 ms steps over the time course of recorded activity. The first point at which two successive windows led to significant differences (Wilcoxon signed rank test, $p < 0.01$) in neuronal activity compared with the first 10 ms window (spontaneous activity recorded before the stimulation) was taken as the start of the analysis window. The end of the analysis window was set to the last position of two successive windows that differed significantly from spontaneous activity. For each stimulus, all spikes occurring in this analysis window were summed up.

Best frequency (BF) and threshold of a unit were determined from the FRA. The frequency where a significant response could be elicited at the pure-tone threshold was defined as the BF of a unit. Responses to different frequency-level combinations were considered to be significant if the spike count exceeded a threshold of 20% of the maximum response.

To analyze the responses to virtual objects, the number of spikes for each width–intensity combination was arranged in a five-by-five matrix. In this response matrix, the object width increases along the abscissa, whereas the echo intensity increases along the ordinate (see Fig. 4). When a Kolmogorov–Smirnov test (MATLAB Statistics Toolbox; MathWorks) revealed a significant difference ($p < 0.05$) in the response strength in one or more rows compared with all of other rows in the response matrix, the unit was categorized as an "Intensity" unit. If one or more columns were found to differ significantly, the unit was categorized as a "Sonar aperture" unit. When a unit showed significant response differences along both dimensions, it was categorized as an "Ambiguous" unit. No significant difference in any tested dimension of the matrix was the criterion for "Insensitive" units. To compare the response matrix of a Sonar aperture unit derived by normal (binaural) stimulation to the response matrix with monaural contralateral stimulation, a 2D correlation coefficient (MATLAB Image Processing Toolbox; MathWorks) was calculated (Keller et al., 1998).

To directly relate the bat's psychophysical performance in the Intensity experiment to the neural sensitivity exhibited in the extracellular recordings, a receiver operating characteristic (ROC) analysis was applied to generate neurometric functions according to Britten et al. (1992), Skottun et al. (2001), and Firzlaff et al. (2006). The neurometric function reflects the probability that an ideal observer could accurately discriminate echo-intensity differences basing his judgments on responses like those recorded from the units under study. The ROC analysis was performed by generating a so-called ROC curve for the comparison of each signal condition (reference intensity plus intensity difference) and the standard condition (reference intensity). The ROC curve shows the probability that both the rate response in a signal condition and the response in the standard condition exceed a certain threshold, e.g., one spike per stimulus. This probability was plotted as a function of the height of the threshold. From there, the (neural) percentage correct discrimination for each signal condition was generated by calculating the area under the ROC curve. When pooling across units, the spike counts across a number of randomly drawn Intensity units was aggregated to form a small population response (Britten et al., 1992).

For the comparison of the psychophysics and physiology concerning the sonar aperture, we calculated d' from the psychometric functions or from the response-strength differences in sonar aperture units. The latter was achieved, according to Rosen et al. (2010), by extracting the hit rate and the false-alarms rate across repetitions. This analysis is based on the assumption that a unit's response increases when the object width is increased from the psychophysical reference width (0° corresponding to a single speaker). The rates were transformed to z-scores (using the MATLAB "norminv" function), and the z-score of the false-alarms rate was subtracted from the z-score of the hit rate to get the value of d'.

Results

Psychophysics

Behavioral results of the Width and Sonar aperture experiments are based on a total of 2400 trials per experiment; results of the Intensity experiment are based on a total of 3000 trials. The mean performance of six bats for discriminating virtual objects of different width is shown as the solid line in Figure 3*A*. The average data show that the bats can reliably discriminate between echoes presented by a single speaker and echoes presented by seven speakers (34° object width). Data for the Sonar aperture experiment, where the spatial cues are the same as in the Width experiment but the echo-intensity cues have been removed, are shown in the same format in Figure 3*B*. Although the overall above-threshold performance of the bats is slightly inferior compared with the Width experiment, the bats solve this task with similar success.

The Width and Sonar aperture experiments were repeated while randomizing the position of the RO in one hemisphere within the spatial range of the UO presented in the opposing hemisphere. This control experiment was performed to verify that the bats attended to the difference of the object's sonar aperture as opposed to differences in the absolute azimuthal positions of the edges of the virtual objects. The RO was never presented by the two speakers next to the midline between the hemispheres to clearly separate the RO and the UO in azimuth. The control experiment showed that the RO randomization did not impair the performance in the discrimination of sonar aperture (Fig. 3, compare *C*, *D*).

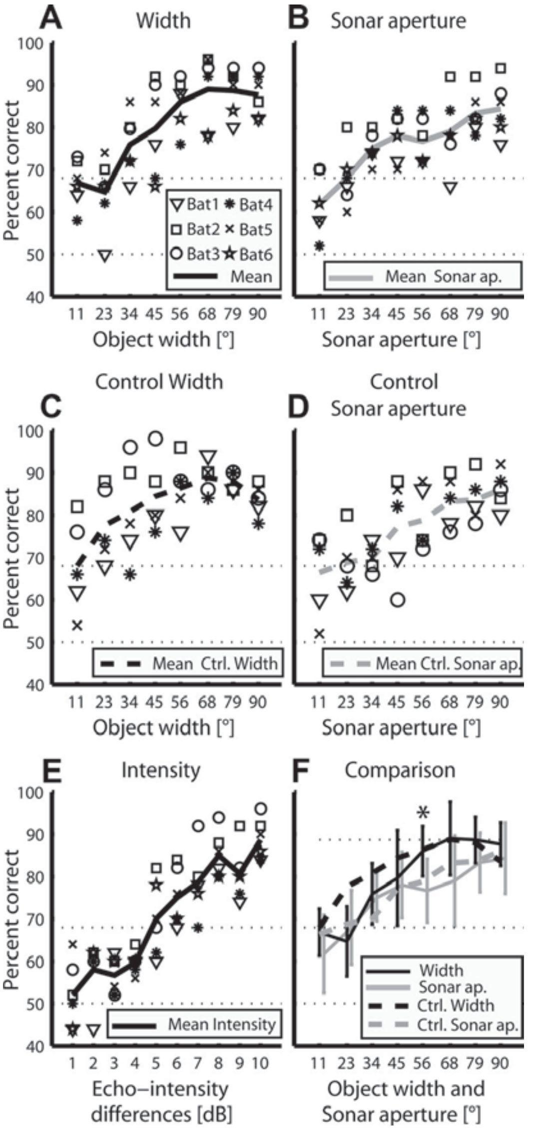

Figure 3. Psychometric functions for the discrimination of an echo presented from a single speaker (sonar aperture = 0°) from an echo presented by multiple speakers. ***A***, ***B***, In the Width experiment (***A***) both the sonar aperture and the echo intensity are covaried, whereas in the Sonar aperture experiment (***B***), echo-intensity cues are removed. ***C***, ***D***, Control versions of these experiments where the position of the RO is randomly varied within the spatial range of the UO. Psychophysical sensitivity to echo intensity with a constant sonar aperture of 0° is shown in ***E***. ***F***, The direct comparison of the Width and Sonar aperture performances shows that the bats do not significantly benefit from echo intensity variations to discriminate object width. Different symbols represent the performance of different bats: the solid lines show the average psychometric functions in the main experiments and dashed lines correspond to the control experiments. Error bars represent across-individual SEs. The horizontal dashed lines at 50 and 68% correct depict the chance and significance thresholds, respectively. The dotted line in ***F*** depicts the presumed performance in the Width experiment, if the bats had relied on an omnidirectional summation of echo-intensity cues. Ctrl., Control; ap., aperture.

Data for the Intensity experiment, where spatial cues have been removed and only echo-intensity differences are provided, are shown in the same format in Figure 3*E*. These data show that the bats require an echo-intensity difference of ~5 dB to reliably choose the fainter of two echoes. A direct comparison of the bat's performance in the Width and Sonar aperture experiments is shown in Figure 3*F*. Apart from the data for a sonar aperture of 56° (9 adjacent speakers), the performance in the Sonar aperture is not significantly lower than the performance in the Width ex-

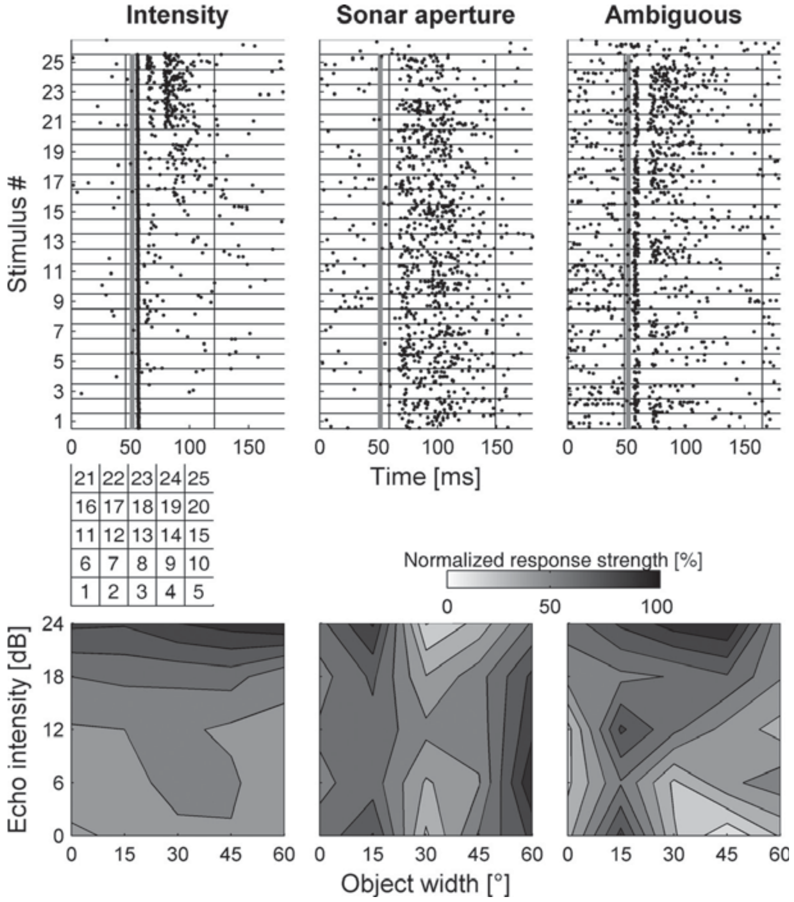

Figure 4. Examples of the different response categories to virtual object stimuli. Raster plots of the responses within the analysis window (top half) with their corresponding normalized response matrices (bottom half). The conversion of the stimulus number in the raster plots into the 2D arrangement in the response matrices is illustrated in the numbered grid in the first column. The fine vertical lines represent the beginning and the end of the analysis window; stimulus occurrence is indicated by the semitransparent, gray line. The first column shows an example for an Intensity unit. Responses change significantly with echo intensity but not with object width, reaching maximum at the highest echo intensity. The second column shows a Sonar aperture response type. This unit encodes a certain object width independent of echo intensity. The third response type is Ambiguous, which is depicted in the last column. Significant changes in the response can be observed along both the echo-intensity and the object–width axes. The contours delineate 10% decreases from the maximal response strength. Normalized response strengths are also color coded as given in the color bar.

periment (Wilcoxon rank sum test, $p > 0.05$) where intensity cues are provided together with the spatial cues.

Electrophysiology

In the AC, recordings were taken from three bats (one female and two males); in the IC, recordings were taken from two bats (one female, one male). BF tone response could be obtained from 101 and 161 units in the IC and AC, respectively. The best frequency of units ranged from 20 up to 90 kHz (IC: median 53 kHz; interquartiles, 18 kHz; AC: median 62 kHz; interquartiles, 26.5 kHz) corresponding to the power spectrum of the echolocation calls.

Recordings of virtual objects could be obtained from 74 and 84 units in the IC and AC, respectively. Subsequent analyses are restricted to these units. An example of an Intensity unit (Fig. 4, left column), a Sonar aperture unit (Fig. 4, central column), and an Ambiguous unit (Fig. 4, right column) are shown in terms of the raster plots (top) and normalized response strength (bottom). The response strength of the Intensity unit increases with increasing intensity, but it is independent of object width. The Sonar aperture unit, however, shows a very different selectivity: this unit responds to objects of a certain width regardless of overall intensity.

Additional examples of response matrices from Intensity and Sonar aperture units in the AC and IC are shown in Figure 5. Most Intensity units (Fig. 5*A*, *C*) increase their response strength monotonically with increasing echo intensity. In contrast, the Sonar aperture units in the AC and IC typically do not vary monotonically in response strength along the object width axis (Fig. 5*B*, *D*). Instead, these units show robust response-strength changes along the horizontal (object width) axis, whereas responses vary little along the vertical (intensity) axis for both the 12 and the 24 dB intensity axes.

The distribution and the numbers of units among categories are shown in Figure 6. These data show that the Sonar aperture units are almost as strongly represented in the AC as the Intensity units. In contrast, Intensity units strongly dominated the other response categories in the IC.

The most interesting, and behaviorally relevant units are in the Sonar aperture category. To assess the degree to which this conspicuous response pattern depends on a binaural stimulation, the response matrices were also obtained with monaurally contralateral stimulation. The effect of switching off the stimulation on the ipsilateral ear is shown in Figure 7. In the AC, switching off the ipsilateral input dramatically decreases the number of units in the Sonar aperture category, whereas this effect is smaller in the IC (Fig. 7*A*). Thus, it appears that binaural inputs contribute substantially to the large number of Sonar aperture units in the AC.

This population effect is also seen to some degree at the level of individual units. The 2D cross-correlation coefficient between the response matrices of the same Sonar aperture units with and without ipsilateral stimulation is shown for the AC and IC in Figure 7, *B* and *C*, respectively. The medians of cross-correlation coefficients are 0.42 for the AC and 0.60 for the IC, indicating that the IC is less influenced by the ipsilateral stimulation than the AC. Note, however, that this difference is not statistically significant.

A direct comparison between the psychometric function for echo-intensity discrimination (compare Figs. 3*C*, 8) and neurometric functions, based on populations of cortical Intensity' units (compare Figs. 4, left column, 8), is shown in Figure 8. The neurometric sensitivity improves monotonically by increasing the number of Intensity units included in the population for the ROC analysis (see Material and Methods). The analysis shows that populations of 4–8 Intensity units are sufficient to explain the psychophysical performance, whereas psychophysical performance can be exceeded by pooling across populations of 16 units.

A direct comparison between the psychophysical performance in the Width and Sonar aperture experiments and the detectability of object–width changes in cortical Sonar aperture units is shown in Figure 9. Specifically, we calculated d' from the psychometric functions and, in the physiological experiments, from the rate differences referenced against the response at object width 0′ (see Materials and Methods). As already evident from the Sonar aperture units in Figures 4 and 5, these units do not encode the sonar aperture in a rate code, i.e., as a monotonic increase in response strength with increasing sonar aperture, but more in a "labeled line" code. In the current analysis, this is reflected in the fact that there are some units that encode the width change from 0 to 15° reliably ($d' >= 1$ or $<= -1$), whereas other units encode the change from 0 to 30, 45, or 60° reliably (Fig. 9, fine black lines). The direct comparison between the psychophysical and physiological performance shows that for each object width there are some units that at least reach or even exceed the psychophysical performance.

It is suggested that in the current experiments on the sonar sensitivity to the aperture of an object, the psychophysical performance is reflected by the bats attending to the most informative units for each specific comparison in the forced-choice experiment. This would be in accordance to the lower envelope principle, which states that animals can perceptually rely on the most sensitive neurons with no interference from the less sensitive ones (Parker and Newsome, 1998).

As evident from Figure 9, we recorded from one unit that responded significantly stronger to an object width of 15° and significantly weaker to an object width of 30°. The neurometric performance for this unit is thus better than the average psychometric performance of the animals. Whereas this singular result is at variance with the "lower envelope principle," individual results in the psychophysical experiments also indicate that some bats could reliably discriminate an 11° object width (Fig. 3*C*, Bat2 in the "Control width" experiment).

Discussion

The current experiments were designed to investigate the perceptual strategy and neural representation of the sonar exploration of object width in echolocating bats. The behavioral experiments showed that while the bats were well able to discriminate differences in echo intensity, these intensity differences were not required to discriminate the width of an ensonified virtual object. Instead, the bats relied on the sonar aperture, i.e., the hor-

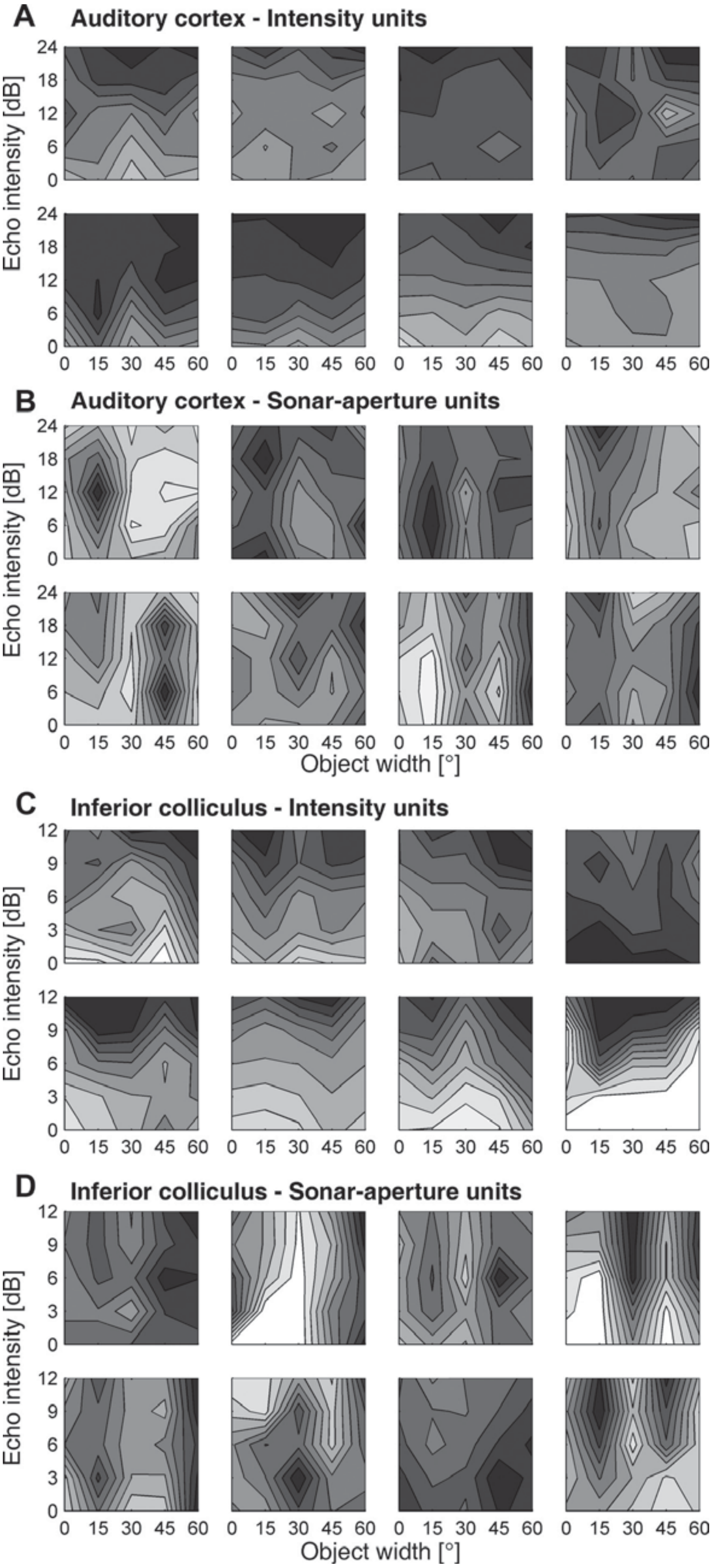

Figure 5. ***A–D***, Examples of Intensity (***A***, ***C***) and Sonar aperture units (***B***, ***D***) in the AC and IC. Note the horizontal orientation patterns of response strength for the Intensity units and the vertical orientation patterns for the Sonar-aperture units. The contours delineate 10% decreases from the maximal response strength. For color code, see Figure 4.

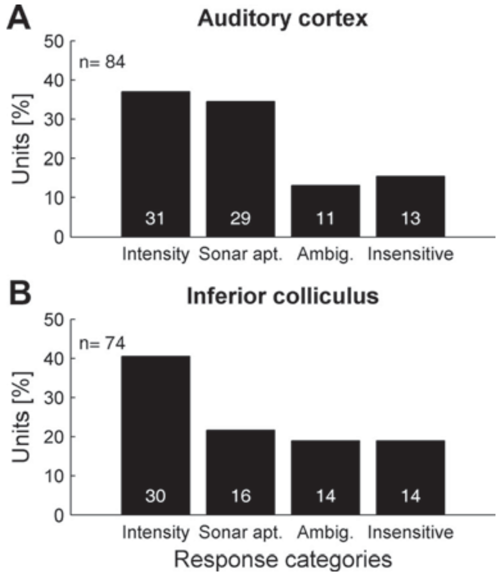

Figure 6. Distribution of response categories under binaural stimulation. ***A***, ***B***, Percentage of all recorded units that were categorized as Intensity, Sonar aperture, Ambiguous, and Insensitive in the AC (***A***) and IC (***B***) under binaural stimulation. The absolute numbers of units in each category are displayed as white numbers within the bars. apt., Aperture; Ambig., ambiguous.

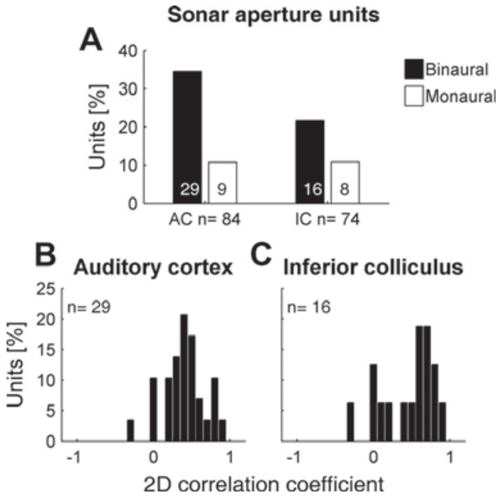

Figure 7. Effect of switching off the ipsilateral stimulation of virtual-object stimuli onto unit classification. These data depicted in ***A*** show that the percentage of Sonar aperture units in the AC decreases dramatically when the ipsilateral stimulation is switched off. This decrease is less pronounced in the IC. ***A***, ***B***, Distribution of the 2D correlation coefficients comparing response matrices of Sonar aperture units recorded during binaural stimulation to the response matrices recorded during monaural stimulation in the AC (***B***) and the IC (***C***).

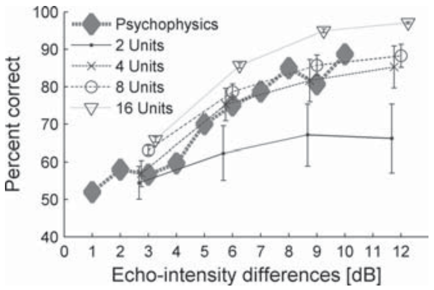

Figure 8. Comparison of psychometric functions from the Intensity experiment with neurometric functions obtained from Intensity units in the bat AC. Based on ROC analysis, the simulations show that a small population of 2–4 units is sufficient to explain the bat's psychophysical performance.

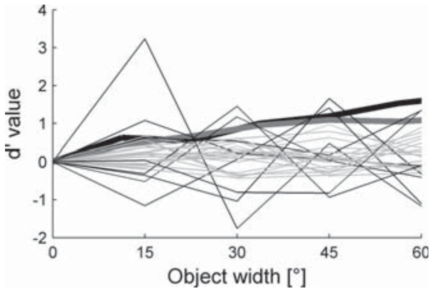

Figure 9. Comparison of psychometric performance from the Width and Sonar-aperture experiments with neurometric performance obtained from Sonar aperture units in the bat auditory cortex. Performance is quantified in terms of *d′* (see Materials and Methods). The strong solid lines show the psychophysical performances: the black line corresponds to the width experiment and the gray line corresponds to the sonar aperture experiment. The fine lines show neurometric *d′*. The fine black lines represent those cortical sonar aperture units that respond significantly different to at least one of the wider objects than to the reference object in the psychophysics (width = 0°). The fine gray lines represent the neurometric performance of those Sonar aperture units, which do not reach this criterion. Note, however, that these units also respond significantly different to other combinations of object widths. These data show that for each comparison of object width or sonar aperture, as it was done by the bats in the psychophysical experiment, there are units that encode the width difference reliably ($d' > 1$ or < -1).

izontal spread of angles of incidences of the echoes generated by the virtual objects. The psychophysical performance is reflected in the responses of a population of central-auditory units that encode changes in object width independent of echo intensity. Due to this independence, these units reflect the psychophysical performance in the behavioral Width experiment and in the Sonar aperture experiment, where intensity cues were removed.

Earlier work has addressed the acoustic parameter "echo intensity," which was considered as an important cue for object classification or discrimination in echolocating bats. Simmons and Vernon (1971) postulated that for discrimination of differently scaled triangles, differences in echo intensity were used by the bats. Processing of echo intensity is also reviewed in Yovel et al. (2011). In the present study we show that intensity is not the only cue that can be used for object–width discrimination. These current data provide psychophysical and electrophysiological evidence that bats recruit the directional characteristics of their outer ears to evaluate the sonar aperture of ensonified objects. These cues can be either monaural spectral cues or binaural echo disparities, as hypothesized by Holderied and von Helversen (2006).

It is clear that the sonar aperture cannot serve as a perceptual cue for the discrimination of the size of very small objects: for objects whose absolute sonar aperture is very small (Sümer et al., 2009), the limitations in auditory spatial directionality preclude the use of sonar aperture. For such small objects, echo intensity ("target strength") is the only available cue for object-size discrimination. The results from the Width and Sonar aperture experiments indicate that sonar aperture cues are useful for object widths larger than ~30° (58 cm at a distance of 1 m). For such large objects, echo-intensity cues become unreliable: in contrast to an omnidirectional microphone, echoes from such a large sonar aperture arrive by quite different paths at the bat's ears. Thus, while echoes add up in a coherent manner at an omnidirectional microphone, complex constructive but also destructive interference occurs at the bat's eardrums. This is seen in Figure 2*H*: although echoes are added up across the azimuth, the resulting amplitude at the bat's eardrum does not increase monotonically, because the echoes had been generated with different HRIR sets corresponding to the different azimuths. In contrast to an omnidirectional microphone, echo intensity measured at the bat's eardrums is not a good predictor of object size. Note that the width of the *P. discolor* sonar beam is wide enough to fully ensonify spatially extended objects as presented here: both simulations of sonar emission patterns based on the 3D geometry of the emitting system (Vanderelst et al., 2010) and experimental data (C. Geberl et al. unpublished data) show that the −3 dB sonar beam width is ~75° at the second harmonic (40 kHz) and 30° at the fourth harmonic (80 kHz). In flight, however, sonar-beam widths may be narrower (Brinkløv et al., 2011).

In the current psychophysical experiments, the information of the one ultrasonic microphone in each hemisphere is relayed to up to 17 adjacent speakers. Thus, the frequency content of the echo from each active speaker in that hemisphere is the same. We accepted this limitation to be able to compare the psychophysical performance to the neural performance, and the stimulus generation for which followed the exact same rules. The current experiments, however, clearly show that, even with these limitations, the spatial information provided overrules the echo-intensity information when the bats are required to estimate the size of an object.

The electrophysiology shows that the sonar aperture is reliably encoded in the auditory midbrain and cortex. The existence of this neural correlate suggests that bats may gain the information about an object's sonar aperture from the analysis of the echo of a single call. In the behavioral experiments, however, bats could emit series of calls and change their position and that of their pinnae across the series. These dynamic cues, which are no doubt used by the bats in more natural situations (Ghose and Moss, 2003; Surlykke et al., 2009), could only serve to further strengthen the spatial cues for object size.

Although we cannot exclude that the bats in the behavioral experiments sequentially scanned the virtual objects by virtue of, e.g., pinna movements across sonar sequences, the electrophysiological experiments indicate that ample information may be already gathered from the neural processing of the echoes from just one call.

The nature of the information represented by the Sonar aperture units found here is clearly not the sonar aperture per se. In the current physiological experiments, the sonar aperture was encoded in the AC mainly by units that received binaural input, making the exclusive use of monaural spectral cues unlikely. The following binaural cues may be used to encode the sonar aperture of objects.

In the frequency domain, interaural intensity differences (IIDs) change with object width. Width-dependent IID changes for the current virtual objects are shown in Figure 10*A*. IIDs provide important binaural cues in echolocating bats and are reliably encoded in the bat ascending auditory system (Park et al., 1997).

In the time domain, the interaural correlation of the echo envelope changes with object width (Shackleton et al., 2005; Aaronson and Hartmann, 2010). Width-dependent correlation changes are shown in Figure 10*B*. Such binaural echo-envelope features are reliably encoded in the IC of *P. discolor* (Borina et al., 2011).

Interestingly, although changes in the binaural envelope cross-correlation (Fig. 10*B*) are generally small, changes are nonmonotonic along the width axis, qualitatively similar to the nonmonotonic response behavior of the Sonar aperture units along the object–width axis (compare Figs. 5, 9). Thus, we suggest that the neural code of the sonar aperture is based on the binaural analysis of envelope correlations and/or IIDs.

Note that when the object is centered at 0° azimuth, none of these binaural parameters can encode changes in object width. In this case, only spectral cues generated by the bat's HRIR can be exploited (Fig. 10*C*). Perceptually, these self-generated spectral cues result in width-dependent changes of echo timbre rather than echo intensity. Monaurally, the addition of echoes from different positions in azimuth produces a complex interference pattern (Fig. 2, compare *B*, *E*), which may encode the sonar aperture in a functionally similar way as the elevation of a sound source is encoded in the human auditory system. In previous experiments, units in the AC of *P. discolor* were shown to encode spectral echo patterns independent of echo amplitude (Firzlaff and Schuller, 2007). In addition, time-variant binaural disparities introduced by ear movements may facilitate the sonar evaluation of object width.

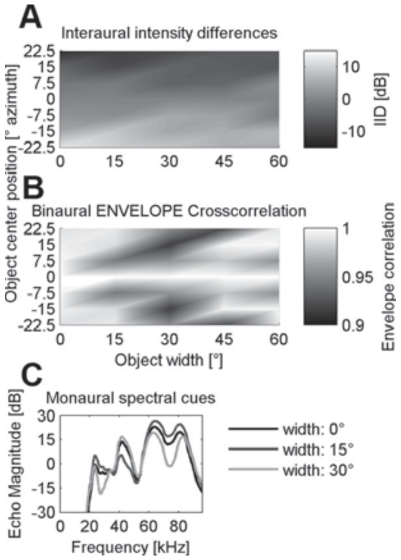

Figure 10. Binaural and monaural cues underlying the auditory analysis of Sonar aperture. ***A***, Changes of interaural intensity differences with increasing object width. ***B***, Changes of the binaural envelope cross-correlation with increasing object width. None of these binaural cues can encode changes of object width when the object is centered at 0° azimuth. However, monaural spectral cues, generated by the directionality of the bat's ears, change with object width, even when the object is centered at 0° azimuth. The width-dependent changes of echo spectra at the bat's eardrums are depicted in ***C***.

The aperture of an object increases with decreasing distance to an object. In the visual system the covariation of these object parameters is crucial. It has been shown that the size of an object's retinal image is not directly perceived. Instead, the perceived size of the object strongly depends on its perceived distance from the viewer (Gogel, 1969). The psychophysical findings are supported by an imaging study (Murray et al., 2006) that shows that the retinotopic representation of an object in primary visual cortex changes in accordance with its perceived size, which in turn depends on the perceived distance. This change of representation at early stages of the visual system is supposed to be behaviorally important as it may allow for visual scale invariance and size constancy (Richards, 1967; Murray et al., 2006). The covariation of retinal image size and object distance often necessitates specific neurocomputational mechanisms to extract size-independent information, e.g., in terms of the time-to-contact of a looming object (Sun and Frost, 1998).

In such a scenario, biosonar has a principal advantage because, through the neural analysis of call-echo delay, object distance is readily and unambiguously encoded. The current data show that the sonar aperture is also readily perceivable and neurally represented in bat biosonar. Together with the sensitivity to call-echo delay, bats may be able to implement size constancy as the physically correct covariation of sonar aperture and echo delay. This hypothesis remains to be tested experimentally.

In summary, the current data show that bats perceive and behaviorally exploit the sonar aperture of an ensonified object. A neural correlate of this percept is found in a population of midbrain and cortical units that encode the sonar aperture independent of echo intensity. These current data thus highlight the fact that based on fundamentally different peripheral representations of an object across the senses of vision and echolocation, the CNS aims to find modality-independent representations of object features. We argue that the sonar aperture, as the echo-acoustic counterpart of the visual aperture of an object, is one of these object features.

References

Aaronson NL, Hartmann WM (2010) Interaural coherence for noise bands: waveforms and envelopes. J Acoust Soc Am 127:1367–1372.

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

Borina F, Firzlaff U, Wiegrebe L (2011) Neural coding of echo-envelope disparities in echolocating bats. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 197:561–569.

Brinkløv S, Jakobsen L, Ratcliffe JM, Kalko EK, Surlykke A (2011) Echolocation call intensity and directionality in flying short-tailed fruit bats, *Carollia perspicillata* (Phyllostomidae). J Acoust Soc Am 129:427–435.

Britten KH, Shadlen MN, Newsome WT, Movshon JA (1992) The analysis of visual motion: a comparison of neuronal and psychophysical performance. J Neurosci 12:4745–4765.

Falk B, Williams T, Aytekin M, Moss CF (2011) Adaptive behavior for texture discrimination by the free-flying big brown bat, *Eptesicus fuscus*. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 197:491–503.

Firzlaff U, Schuller G (2003) Spectral directionality of the external ear of the lesser spear-nosed bat, *Phyllostomus discolor*. Hear Res 181:27–39.

Firzlaff U, Schuller G (2007) Cortical responses to object size-dependent spectral interference patterns in echolocating bats. Eur J Neurosci 26:2747–2755.

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

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

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

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

Gogel WC (1969) Sensing of retinal size. Vision Res 9:1079–1094.

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

Habersetzer J, Vogler B (1983) Discrimination of surface-structured targets by the echolocating bat *Myotis myotis* during flight. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 152:275–282.

Hoffmann S, Baier L, Borina F, Schuller G, Wiegrebe L, Firzlaff U (2008) Psychophysical and neurophysiological hearing thresholds in the bat *Phyllostomus discolor*. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 194:39–47.

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

Keller CH, Hartung K, Takahashi TT (1998) Head-related transfer functions of the barn owl: measurement and neural responses. Hear Res 118:13–34.

Murray SO, Boyaci H, Kersten D (2006) The representation of perceived angular size in human primary visual cortex. Nat Neurosci 9:429–434.

Nowak R (1994) Walker's bats of the world. Baltimore: Johns Hopkins UP.

Park TJ, Monsivais P, Pollak GD (1997) Processing of interaural intensity differences in the LSO: role of interaural threshold differences. J Neurophysiol 77:2863–2878.

Parker, AJ, Newsome WT (1998) Sense and the single neuron: probing the physiology of perception. Annu Rev Neurosci 21:227–277.

Richards W (1967) Apparent modifiability of receptive fields during accommodation and convergence and a model for size constancy. Neuropsychologia 5:63–72.

Rosen MJ, Semple MN, Sanes DH (2010) Exploiting development to evaluate auditory encoding of amplitude modulation. J Neurosci 30:15509–15520.

Rother G, Schmidt U (1982) The influence of visual information on echolocation in *Phyllostomus discolor* (Chiroptera). Int J Mammalian Biol 47:324–334.

Sanderson MI, Simmons JA (2002) Selectivity for echo spectral interference and delay in the auditory cortex of the big brown bat *Eptesicus fuscus*. J Neurophysiol 87:2823–2834.

Schlack A, Sterbing-D'Angelo SJ, Hartung K, Hoffmann KP, Bremmer F (2005) Multisensory space representations in the macaque ventral intraparietal area. J Neurosci 25:4616–4625.

Schmidt S (1988) Evidence for a spectral basis of texture perception in bat sonar. Nature 331:617–619.

Schmidt S (1992) Perception of structured phantom targets in the echolocating bat, *Megaderma lyra*. J Acoust Soc Am 91:2203–2223.

Schuller G (1997) A cheap earphone for small animals with good frequency response in the ultrasonic frequency range. J Neurosci Methods 71: 187–190.

Schuller G, Radtke-Schuller S, Betz M (1986) A stereotaxic method for small animals using experimentally determined reference profiles. J Neurosci Methods 18:339–350.

Schuller G, O'Neill WE, Radtke-Schuller S (1991) Facilitation and delay sensitivity of auditory cortex neurons in CF-FM bats, *Rhinolophus rouxi* and *Pteronotus p. parnellii*. Eur J Neurosci 3:1165–1181.

Shackleton TM, Arnott RH, Palmer AR (2005) Sensitivity to interaural correlation of single neurons in the inferior colliculus of guinea pigs. J Assoc Res Otolaryngol 6:244–259.

Simmons JA (1971) Echolocation in bats: signal processing of echoes for target range. Science 171:925–928.

Simmons JA (1973) The resolution of target range by echolocating bats. J Acoust Soc Am 54:157–173.

Simmons JA, Vernon JA (1971) Echolocation: discrimination of targets by the bat, *Eptesicus fuscus*. J Exp Zool 176:315–328.

Simon R, Holderied MW, von Helversen O (2006) Size discrimination of hollow hemispheres by echolocation in a nectar feeding bat. J Exp Biol 209:3599–3609.

Skottun BC, Shackleton TM, Arnott RH, Palmer AR (2001) The ability of inferior colliculus neurons to signal differences in interaural delay. Proc Natl Acad Sci U S A 98:14050–14054.

Sümer S, Denzinger A, Schnitzler HU (2009) Spatial unmasking in the echolocating Big Brown Bat, *Eptesicus fuscus*. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 195:463–472.

Sun H, Frost BJ (1998) Computation of different optical variables of looming objects in pigeon nucleus rotundus neurons. Nat Neurosci 1:296–303.

Sun XD, Jen PH (1987) Pinna position affects the auditory space representation in the inferior colliculus of the FM bat, *Eptesicus fuscus*. Hear Res 27:207–219.

Surlykke A, Ghose K, Moss CF (2009) Acoustic scanning of natural scenes by echolocation in the big brown bat, *Eptesicus fuscus*. J Exp Biol 212:1011–1020.

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

von Helversen D (2004) Object classification by echolocation in nectar feeding bats: size-independent generalization of shape. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 190:515–521.

Von Helversen D, Von Helversen O (1999) Acoustic guide in bat-pollinated flower. Nature 398:759–760.

Weissenbacher P, Wiegrebe L (2003) Classification of virtual objects in the echolocating bat, *Megaderma lyra*. Behav Neurosci 117:833–839.

Yovel Y, Franz MO, Stilz P, Schnitzler HU (2011) Complex echo classification by echo-locating bats: a review. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 197:475–490.

3 Size Constancy in Bat Biosonar? Perceptual Interaction of Object Aperture and Distance

This chapter was published online in: PLoS ONE on April 22nd; 2013 (8 (4); doi:10.1371/journal.pone.0061577) under the title: **"Size Constancy in Bat Biosonar? Perceptual Interaction of Object Aperture and Distance"** by Melina Heinrich (MH) and Lutz Wiegrebe (LW).

3.1 Reference

MH and LW designed the experiments, wrote the experimental programs, and the analysis programs. MH collected the data, conducted major parts of the analysis and wrote major parts of the manuscript under supervision of LW. Alexander Warmbold and Daria Genzel supported MH at times with the implementation of the analysis programs. MH designed the figures. Uwe Firzlaff read early versions of the manuscript and made valuable comments to improve the draft. Katie L. Willis did the final language grammar check.

3.2 Summary

In vision, object size is encoded by the visual angle (visual aperture) as the extent of the image on the retina. Here the visual aperture depends on object distance that is not explicitly encoded in the visual system and higher computational mechanisms are needed. This phenomenon is termed ‘size constancy’ or ‘size-distance invariance’ and is assumed to reflect an automatic re-scaling of visual aperture with perceived object distance. The first study of this thesis revealed that the sonar aperture, as size relevant spatial object property, is unambiguously perceived and neurally encoded. This is also true for object distance as temporal information (echo delay). Consequently, in the second study we investigated whether bats show the ability to combine spatial and temporal cues to determine absolute width information in terms of sonar size constancy (SSC).

Using the same setup and species as in the first study, bats were trained to reliably discriminate two simple virtual objects that differed in object width. Later test trials were randomly interspersed, using VOs that differed in aperture and distance (with the proportionally co-varying echo-intensity information). In the experiments it was tested if the bats were able to relate absolute width information by combining the spatiotemporal information provided by the presented aperture and distance. The results indicated that the bats did not combine the provided spatiotemporal cues even though they were all within the animals’ perceptual range. This non-verification of sonar size constancy was discussed in the background of the lacking ecological relevance of the presented VOs. It is also thinkable that behavioral relevance as well as object familiarity might be a basic prerequisite for SSC as it is also suggested for visual size constancy. Alternatively, the extraction of size information at different distances might be achieved by different sensory systems (the visual and the bat sonar system). Based on these behavioral results it was concluded that to our current state of knowledge SSC does not seem to be a characteristic trait of bat biosonar.

Size Constancy in Bat Biosonar? Perceptual Interaction of Object Aperture and Distance

Melina Heinrich[1,2], **Lutz Wiegrebe**[1,2,3]*

1 Department Biology II, Ludwig-Maximilians University Munich, Bavaria, Germany, **2** Graduate School of Systemic Neurosciences, Ludwig-Maximilians University Munich, Bavaria, Germany, **3** Sensory Ecology Group, Max-Planck Institute for Ornithology, Seewiesen, Germany

Abstract

Perception and encoding of object size is an important feature of sensory systems. In the visual system object size is encoded by the visual angle (visual aperture) on the retina, but the aperture depends on the distance of the object. As object distance is not unambiguously encoded in the visual system, higher computational mechanisms are needed. This phenomenon is termed "size constancy". It is assumed to reflect an automatic re-scaling of visual aperture with perceived object distance. Recently, it was found that in echolocating bats, the 'sonar aperture', i.e., the range of angles from which sound is reflected from an object back to the bat, is unambiguously perceived and neurally encoded. Moreover, it is well known that object distance is accurately perceived and explicitly encoded in bat sonar. Here, we addressed size constancy in bat biosonar, recruiting virtual-object techniques. Bats of the species *Phyllostomus discolor* learned to discriminate two simple virtual objects that only differed in sonar aperture. Upon successful discrimination, test trials were randomly interspersed using virtual objects that differed in both aperture and distance. It was tested whether the bats spontaneously assigned absolute width information to these objects by combining distance and aperture. The results showed that while the isolated perceptual cues encoding object width, aperture, and distance were all perceptually well resolved by the bats, the animals did not assign absolute width information to the test objects. This lack of sonar size constancy may result from the bats relying on different modalities to extract size information at different distances. Alternatively, it is conceivable that familiarity with a behaviorally relevant, conspicuous object is required for sonar size constancy, as it has been argued for visual size constancy. Based on the current data, it appears that size constancy is not necessarily an essential feature of sonar perception in bats.

Citation: Heinrich M, Wiegrebe L (2013) Size Constancy in Bat Biosonar? Perceptual Interaction of Object Aperture and Distance. PLoS ONE 8(4): e61577. doi:10.1371/journal.pone.0061577

Editor: Manuel S. Malmierca, University of Salamanca- Institute for Neuroscience of Castille and Leon and Medical School, Spain

Received November 30, 2012; **Accepted** March 11, 2013; **Published** April 22, 2013

Funding: This study was funded by the 'Volkswagenstiftung' (I/83838), and by the Human Frontier Science Program (HFSP RGP 0062/2009) to Lutz Wiegrebe and a scholarship by the Graduate School of Systemic Neurosciences (GSN-LMU, DFG-GSC 82/1) to Melina Heinrich. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: lutzw@lmu.de

Introduction

The representation of object size and its neural encoding is an important function of sensory systems in general. Realistic size estimation over large distances could benefit survival (e.g. in orientation, navigation, foraging, predator avoidance, or intraspecific competition). How organisms perceive the physical object size of distant objects is a fundamental question and not completely answered yet [1,2]. In vision, three-dimensional space is two-dimensionally represented on the retina along its height and width dimension. Consequently, the extent of the image on the retina in terms of its visual aperture is explicitly encoded. However, when the distance between the observer and the object changes, the visual aperture changes proportionally. "Size constancy" or "size distance invariance" [3,4,5] is assumed to reflect an automatic re-scaling of perceived object size with perceived distance. Object distance, however, is not explicitly encoded in the visual system; a mismatch of physical and perceived object distance can lead to a misinterpretation of physical object size and visual illusions [6].

Based on the evaluation of the echoes of self-produced ultrasonic sounds, bats and dolphins achieve detailed acoustic images of their surroundings [7,8,9,10,11,12,13]. But, in contrast to the retina, the sensory epithelium of the auditory system, the basilar membrane, is not arranged along spatial axes. Instead, frequency is explicitly encoded and spatial information must be computed in the central auditory system. Echolocating bats gather information about the physical properties of objects by comparing the returning echoes to their emitted calls [14,15,16]. The physical-object properties are encoded in the acoustic parameters of the returning echoes.

In contrast to the visual system where only the aperture is explicitly encoded, the bat auditory system explicitly encodes both, distance and aperture: distance information is encoded by the delay between call emission and the returning echoes [17,18,19]. Echo delay and its neural representation were addressed in several studies [20,21,22,23]. It was also shown that object distance is well represented in chronotopically arranged delay-tuned neurons in the bat auditory cortex [22,24,25,26,27,28]. The sonar aperture, as the echo-acoustic equivalent to the visual aperture, is defined as the spread of angles of incidence from which echoes impinge on the bat's ears. It was shown that bats can evaluate the sonar aperture independent of echo intensity [13]. Moreover, the sonar aperture is reliably encoded, independent of echo intensity, in the auditory midbrain and cortex [13].

Due to geometric and atmospheric attenuation, echo intensity changes with object distance [22,29,30,31] However, it is an ambiguous distance cue as it not only changes with distance, but also with object size and other physical properties (shape, orientation, texture).

The current study was designed to formally test the hypothesis that bats may combine object-distance and sonar-aperture information to explicitly encode the physical width of a sound-reflecting object independent of object distance.

Methods

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. As the experiments are neither invasive nor stressful, they do not require explicit approval. Approval to keep and breed the bats was issued by the Regierung von Oberbayern. The study is divided into a Constancy experiment and a Distance-discrimination experiment. In the Constancy experiment, bats classified the echoes of virtual objects that differed in both distance and physical width. In the Distance-discrimination experiment, the bats were trained to discriminate changes in the distance of a virtual object, while its sonar aperture remained constant. In both experiments, target strength varied proportionally with the spatio-temporal features of the presented objects, unless otherwise stated. Note that here, we use the term 'target strength' not in its original (distance-independent) definition, but as a quantification of the peak amplitude in the impulse response (see below).

Experimental setup

The experimental setup of the current study was the same as in Heinrich et al. [13]. The experiments were performed in a dark, echo-attenuated chamber. The setup inside the chamber consisted of a Y-shaped maze that was placed in a semicircular wire mesh cage (radius = 55 cm). The cage was mounted on a metal post at an angle of 45°. A starting perch was located at the top end of the Y-maze. Each of the other two legs held a feeding device. Two ultrasonic microphones (CO 100K, Sanken) were mounted above the feeders pointing towards the perch. The cage was plane-parallel arranged towards a semicircular loudspeaker array (radius = 71 cm) that consisted of 34 ultrasonic ribbon loudspeakers (NeoCD1.0, Fountek) that were also covered with plain acoustic foam except for the speaker's membrane (0.8×3.8 cm). The array was subdivided into right and left hemisphere (90°) consisting of 17 speakers, each (Fig. 1A). The angular distance between adjacent speakers was 5.6°. Each speaker was calibrated against a 1/8 inch reference ultrasonic microphone (Type 4138, Brüel & Kjær, protective grid removed) at the bat starting perch, perpendicularly oriented towards the speaker axes. To create a compensatory impulse response (IR), the complex spectrum of an ideal bandpass filter (47th-order, finite impulse response, cutoff frequencies: 15 and 94 kHz) was divided by the complex spectrum of the measured IR of each speaker .The real part of the inverse Fourier transform is the compensatory IR. During the experiments, every echo presented over a speaker was first convolved in real time with this speaker's compensatory IR. The convolution with the compensatory IRs ensured that all speakers provided a linear frequency response (between 15 and 94 kHz) as well as a linear phase at the bats starting perch. During the experiments, the bat echolocation calls were picked up by the microphones, amplified (QuadMic, RME) and digitized (HD192, MOTU; two devices with 12 analog-to-digital (AD) and digital-to-analog (DA) channels each and a 424 PCI board, MOTU) at a sampling rate of 192 kHz. To prevent bats from eventually using passive acoustic cues replayed by the speakers (e.g. rustling noises in the high frequency range caused by movements of the bat on the maze), playback was triggered when the recorded signal exceeded a defined threshold. After determining the required echo level (Fig. 2) the echolocation calls were convolved with the compensatory IR of the respective speaker, DA converted, and amplified (AVR 347, Harman Kardon; four devices with seven channels each) before being sent to the speaker. The mismatch between the number of speakers and the AD/DA channels provided by the hardware listed above resulted from the fact that in the experiments not all 34 channels were needed (Fig. 1B and C).

The input-output (I/O) delay of the system (4.3 ms), together with the physical propagation delay from the bat to the microphones (0.9 ms) and from the speakers to the bat (2 ms), added up to 7.2 ms, corresponding to a minimal virtual object (VO) distance of circa 120 cm. In order to prevent masking effects, VOs were presented well beyond the range of physical echoes (e.g.

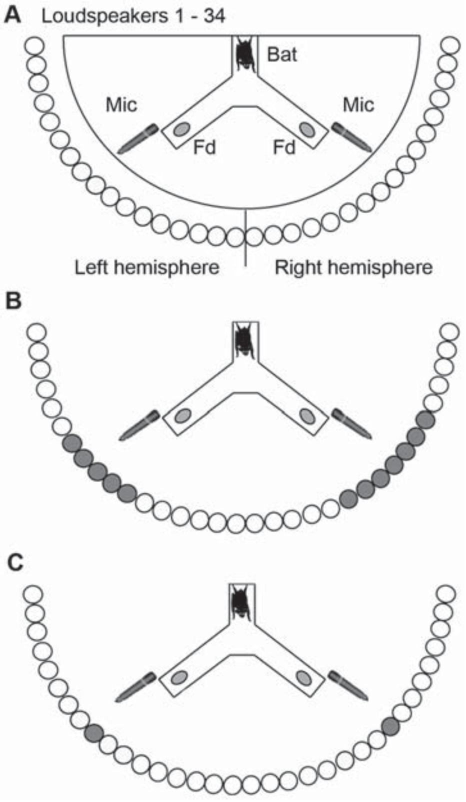

Figure 1. Experimental setup and stimulus presentation. A: The experimental setup is depicted from the front, with a bat sitting on the focal point of the y-maze placed in a semicircular wire mesh cage. Indicated are the feeders (Fd), the ultrasonic microphones (Mic), and the 34 ultrasound loudspeakers for virtual-object presentation in the azimuth (open circles). The bar in the center of the loudspeaker array indicates the division into right and left hemisphere. **B:** Presentation of the two different sonar apertures in the Constancy experiment. Left hemisphere: The aperture of the rewarded object (23°) in the trained standard condition is represented by 5 adjacent active speakers (grey). On the right hemisphere the aperture of the corresponding unrewarded object (34°) is represented by 7 adjacent active speakers. **C:** Active speakers of the control experiment on distance discrimination (grey). Here, only one speaker was active in each hemisphere, but echo delay and target strength co-varied.
doi:10.1371/journal.pone.0061577.g001

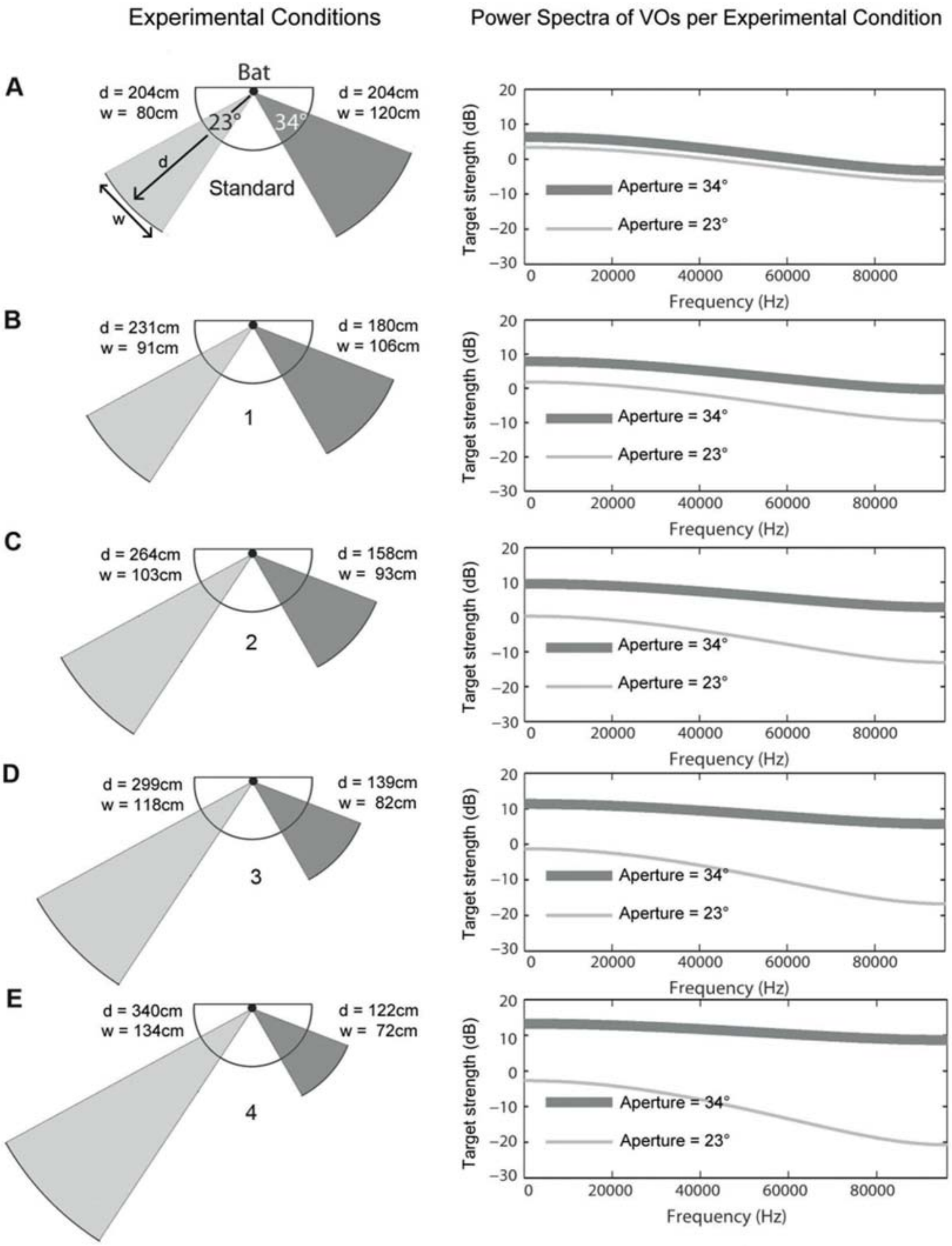

Figure 2. Stimulus presentation in the Constancy experiment. Depicted are the five different experimental conditions of the Constancy experiment (standard condition and test conditions 1–4) in the left column. The power spectra of the virtual objects (VOs) generated by the sum of the active speakers in each hemisphere are plotted on the right. Left column: The bat is indicated as a black dot on the focal point of the maze. The speaker array is indicated by the semicircular line. All VOs depicted in the left hemisphere have an aperture of 23° (light grey); those in the right hemisphere have an aperture of 34° (dark grey). In the standard condition, both VOs are presented at the same distance (d) of 204 cm. The VO with the 23° aperture also provides the smaller object width (w). In the test conditions 1–4, the object with the smaller aperture is presented progressively further away and the object with the larger aperture is presented progressively closer to the bat. As a result, the angular information provided by the aperture does no longer unambiguously code for object width: in the test conditions 2, 3, and 4, the smaller aperture of 23° represents the larger object width. The right column shows the power spectra of the VOs. The solid line shows the change of target strength (dB) with frequency of the VO with the aperture of 34° whereas the dotted line shows that of the VO providing the smaller aperture of 23°. At high frequencies target-strength difference increases even more due to the geometric and atmospheric attenuations that co-vary with distance.

doi:10.1371/journal.pone.0061577.g002

from the cage or the speakers). The experiments were monitored visually (via an infrared observation camera) and acoustically (via heterodyning the microphone signal with a 45 kHz pure tone, playing out the resulting difference frequency across two additional DA converter channels of the HD192 and a headphone amplifier (Terratec Phase 24) into AKG K240 headphones). Experiments were controlled via a graphical user interface from an experimenter seated outside the chamber. Experimental control, data acquisition, and analysis were implemented in MATLAB 7.5 (MathWorks). For the control of the MOTU system, SoundMexPro software (HörTech) was applied.

Stimuli

Each microphone recorded the animal's ultrasonic calls emitted towards its corresponding hemisphere. The VOs presented on both hemispheres were implemented as simple equidistantly arranged reflectors that could be manipulated along three different parameters: sonar aperture, distance in terms of echo delay, and, physically correct covarying with the particular spatio-temporal parameter, target strength. Sonar aperture was manipulated by changing the number of adjacent speakers presenting an echo. The number of adjacent speakers was always increased symmetrically around the center speaker of each hemisphere. For realistic simulation of object width every single speaker of a VO provided the same sound level so that target strength co-varied with the sonar aperture. Complex spatial interference patterns that could emerge using distant reflectors are discussed in detail elsewhere [13].

The simulated object distance was changed by manipulating the echo delay of the replayed echolocation calls. When object distance was manipulated, the target strength was changed proportionally by taking the atmospheric and geometric spreading losses into account. For the calculation of the atmospheric attenuation an algorithm by Stilz, 2004 [30] was used. The attenuation covered the frequency range between 10 and 96 kHz and was calculated for a relative humidity of 60% and at a temperature of 25°C. For the frequency independent, geometric attenuation the target strength was reduced by 6 dB for each doubling of distance.

General Procedure

The experimental animals participated on the main experiment ('Constancy experiment') and after successful data acquisition on its control experiment ('Distance discrimination'). Both experiments followed a two-alternative, forced-choice paradigm (2-AFC) with food reward. Before data acquisition, bats were trained to discriminate a rewarded virtual object (RO) from an unrewarded virtual object (UO). The hemisphere for the presentation of the RO was selected pseudorandomly [32]. A decision was indicated by crawling towards one of the two feeders. The test animals were only rewarded when choosing the correct VO. Data acquisition started when a bat's performance reached $\geq$80% correct choices on five consecutive training days.

Constancy experiment

For the Constancy experiment, bats were trained to discriminate between two VOs with an aperture of 23° (RO) and 34° (UO) (Figs. 1B and 2A). Both were presented at the same distance (204 cm).

Once the animals had learned this task, test trials were randomly interspersed with a probability of 25%. In these test trials, one of the four test conditions was presented and the animal's spontaneous classification of the test condition was assessed. Note that test trials were always rewarded, independent of the bat's choice. Thus, the spontaneous classification was assessed and learning was deliberately excluded. The four test conditions are illustrated in Fig. 2B–E. In the test conditions, the difference in sonar aperture remained the same, but the object distance was varied such that when the distance of the VO with the smaller aperture exceeded the distance of the VO with the larger aperture (Fig. 2B and Fig. 2C) the VO with the smaller aperture had the larger physical width. Test condition 4 provided the largest difference in object distance between both VOs: here the VO with the smaller aperture had a width that was almost twice that of the VO with the larger aperture (Fig. 2E).

We predicted that if the bats combined distance- and aperture information to get an estimate of absolute object width, they should classify the VOs with the larger aperture of 34° presented in the test conditions 2–4 as the smaller object (Fig. 3A). On the other hand, if the bats evaluated only the aperture information and did not combine it with the distance information, they should classify the VOs with the smaller aperture of 23° as the smaller VO, independent of object distance (Fig. 3B).

Data analysis

For analyzing the spontaneous classification performance of the bats in the Constancy experiment, a baseline analysis was applied to verify a reliable classification performance in the standard condition. This was needed because, while all individuals previously reached a stable performance in the standard condition, the performance in the standard condition was not always stable during data-acquisition periods where test conditions were interspersed. To this end, a sliding integration window spanning 30 consecutive trials was applied. The trials of the standard condition lying within this window were analyzed with a binomial cumulative distribution function. The significance threshold was set to $p<0.01$. When the bat's performance in the standard condition was better than threshold, the test trials within this window were accepted for the further analyses. The analysis window was shifted in one-trial steps. Duplicate test trials were excluded from performance analysis.

The last 50 test trials for each test condition, which met the above criteria, were recruited. Performance was calculated as the decisions for the smaller sonar aperture of 23° in percent. Levels of significance were based on a two-sided binomial distribution ($p<0.001$, 72%, $p<0.01$, 68%; $p<0.05$, 64%).

Control experiment 'Distance discrimination'

Echo-acoustic information about object features can only be processed when the acoustic parameters encoding that information are readily perceived. Consequently, the presented acoustic parameters must lie in the perceptual range of the test animals. To rule out the possibility that the bats cannot extract distance information of the presented VOs with sufficient fidelity, a control experiment was performed. To do so, the aperture of the two VOs was equalized and only the bat's sensitivity for differences in VO distance was tested. All three bats that successfully completed data recording in the Constancy experiment participated on the control experiment.

Initially, bats were trained to discriminate a distance difference of 17 cm centered on a reference distance of 204 cm. This corresponds to a difference in echo delay of 1 ms. The RO was always the VO presented at the shorter distance (and thus with the shorter delay). During data acquisition, the distance differences were systematically reduced (Fig. 4). Trials were presented according to a staircase procedure starting with a block of three to five trials with easily discriminable VOs. For each subsequent block, the task difficulty was increased until the bat's performance

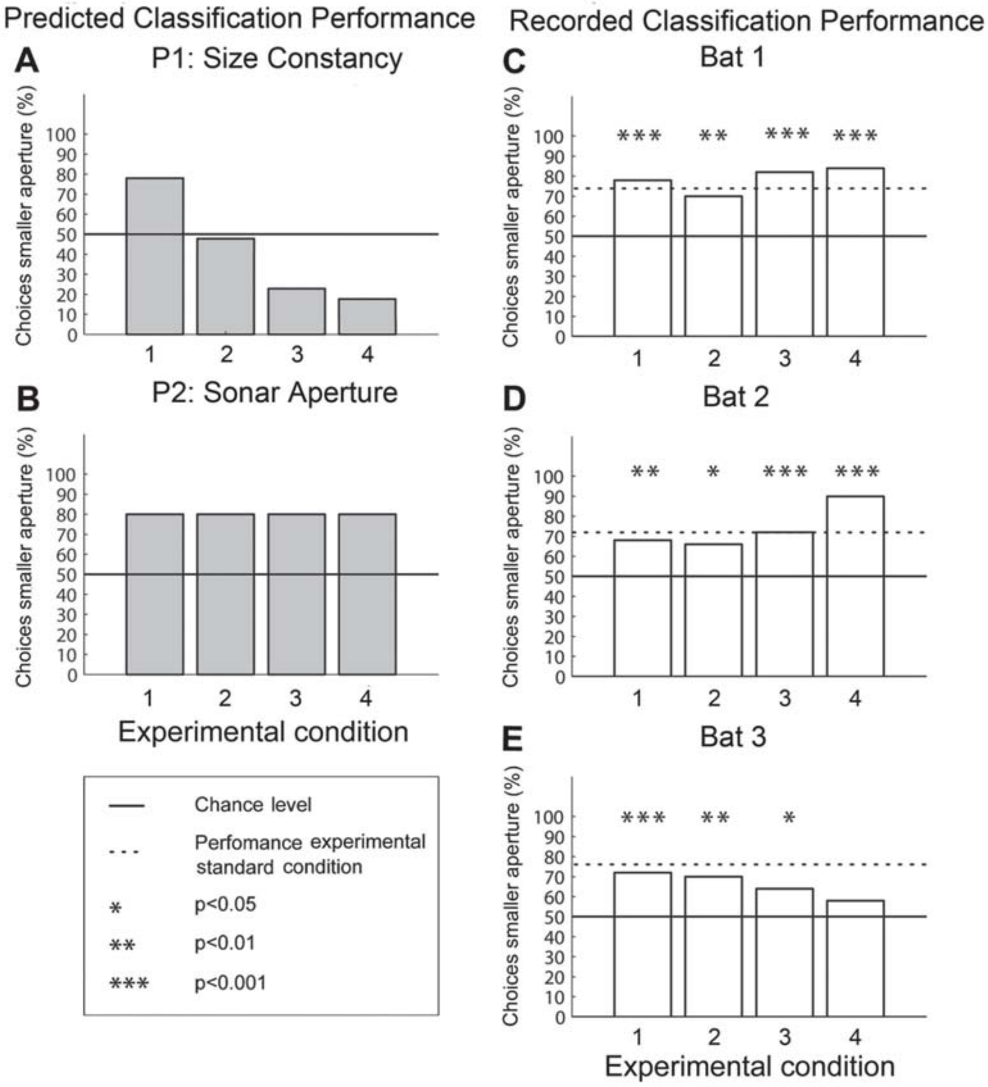

Figure 3. Results of the Constancy experiment. Plotted are the decisions in percent for the virtual object (VO) with the smaller sonar aperture of 23° in the four test conditions. Predictions and recordings of the bat's performance in the Constancy experiment are shown in the left and right column, respectively. If the bats would spontaneously show size constancy with the current paradigm, their classification of the test conditions should look as depicted in **A**. If the bats would spontaneously evaluate the aperture information independent of the accompanying distance information, their classification of the test conditions should look as depicted in **B**. Data in the right column were obtained from three bats. Classification performance is based on 50 trials per test condition. The 50 percent chance level is indicated by the solid line. The dashed line shows the bat's performance in the trained standard condition. The data show that the bats reliably chose the VO providing the smaller aperture independent of the accompanying variation in VO distance.
doi:10.1371/journal.pone.0061577.g003

approached chance level. The distance differences ranged from 17 cm to 1.2 cm (corresponding to echo-delay differences between 1.0 and 0.07 ms).

Experimental animals

The species used in this study was the Pale Spear-nosed Bat; *Phyllostomus discolor* (Wagner, 1843). This omnivorous phyllostomid bat is found in the rainforests of South-and Central America where it forages on nectar, pollen, fruits, and insects [33]. It emits short (>3 ms), broadband, downward frequency-modulated, multi-harmonic echolocation calls covering the frequency range between 45 and 100 kHz [34,35]. The individuals came from a breeding colony in the Department of Biology II of the Ludwig-Maximilians-University Munich where they were kept under inverted light/dark conditions. Five adult male *P. discolor* with a bodyweight between 32 and 45 g participated in the experiments. On training days the individuals were kept in a cage (80×60×80 cm). After training sessions the test subjects could fly freely in a room of 12 m^2 until the next morning. All bats had access to water *ad libitum*. The training was realized in daily sessions that lasted between 15–20 minutes. Five training days were followed by a two day break. The bats were fed with a fruit pulp as reward, consisting of mashed banana, melon, honey, puppy-milk powder, and safflower oil. On days without training, the bats had had access to water, fruit, and mealworms (larvae of *Tenebrio molitor*) *ad libitum*.

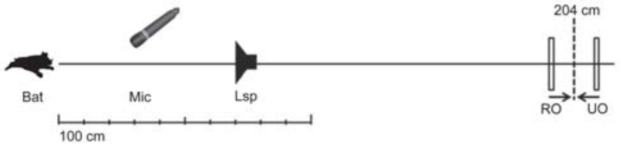

Figure 4. Illustration of real and virtual objects in the Distance-discrimination control experiment. Virtual objects were generated by delaying and attenuating the sounds picked up by the microphone and playing these from the speaker. Thus, the virtual object appears behind the speaker-microphone combination at a distance of 204 cm. The bats were trained to discriminate a distance difference of 17 cm, as indicated by the vertical bars (RO: −8.5 cm; UO: +8.5 cm). Upon successful training, the distance difference was progressively decreased until a threshold could be extracted.
doi:10.1371/journal.pone.0061577.g004

Results

Constancy experiment

In the Constancy experiment, five bats were successfully trained to discriminate the VO with a sonar aperture of 23° from the VO with an aperture of 34°, both presented at the same distance of 204 cm. Data acquisition, where bats had to classify VOs presented at different distances was successfully completed by three bats. Behavioral results of the Constancy experiment are based on a total of 5762 trials (Bat 1: 1645, Bat 2: 1490, and Bat 3: 2627). The classification performance for the four experimental test conditions is depicted in Figure 3C–E. Here, the decisions for the VO with the smaller aperture in percent are plotted as a function of test conditions 1–4. Each data point is based on the last 50 trials for each test condition (see data analysis section of methods). The solid line indicates chance level for a 2-AFC paradigm, whereas the dashed line indicates the performance level per bat, reached in the standard trials. Except for the classification performance of Bat 3 in test condition 4, the classification performances of the three individuals for all classification tasks were significantly above chance level. When comparing the actual behavioral classification performances of the three test bats to the predicted performances (Fig. 3A and B), it is obvious that none of the three animals showed a switch of the classification performance as would be expected if the animals spontaneously combined aperture- and distance-information to estimate absolute object width.

Instead, the animals' classification is consistent with the hypothesis illustrated in Figure 3B, i.e., the animals evaluated the aperture information of the VOs independent of the distance information.

Results of the Distance control experiment

All three bats were successfully trained to discriminate a distance difference of 17 cm between two VOs centered at a reference distance of 204 cm.

Individual psychometric functions for the Distance control experiment are plotted in Fig. 5. The upper x-axis shows the presented distance differences (1.2–17 cm) whereas the lower x-axis shows the corresponding echo-delay differences (0.07–1 ms). Performance is plotted as the choices for the VO simulated at shorter distance as percent correct, based on the last 30 trials per presented distance difference. The solid line indicates chance level at 50% for a 2-AFC paradigm. Level of significance was set to 70% ($p<0.01$) based on a one sided binomial distribution. The psychophysical perception threshold that derived from a sigmoidal fit function was 3.2 cm for Bat 1, 3.9 cm for Bat 2, and 5.4 cm for Bat 3, corresponding to echo-delay differences of 0.188 ms, 0.223 ms, and 0.318 ms for Bats 1–3, respectively. The mean performance of the three bats was 4.16 cm (~0.247 ms).

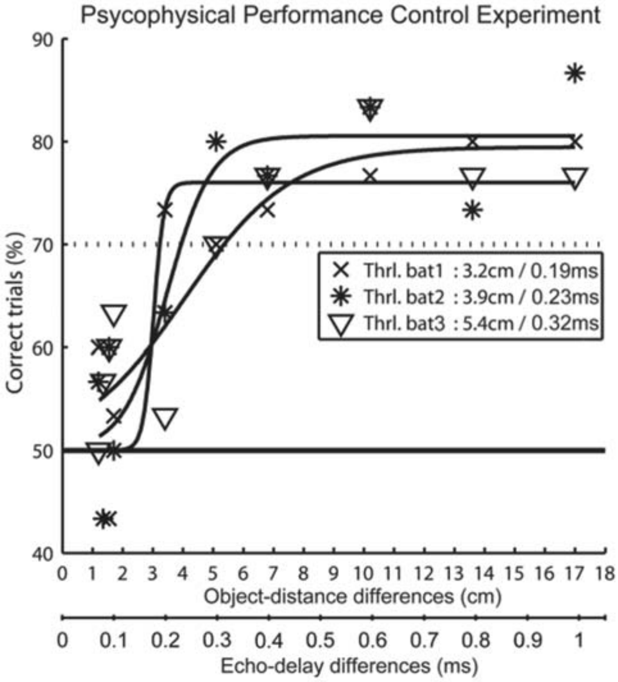

Figure 5. Psychometric functions for distance discrimination. Distance discrimination as a function of distance difference is shown for three bats, marked by different symbols. Data are based on 30 trials per presented distance difference. The upper x-axis shows the distance difference (in cm); the lower x-axis shows the corresponding echo-delay difference (in ms). All bats reliably discriminated distance differences >= 6 cm around a reference distance of 204 cm. Thresholds derived from a sigmoidal fit to the individual psychometric functions and are given in the inset. Chance level (50%) is indicated by the solid line. Level of significance (dashed line) is based on a binomial distribution function ($p<0.01$).
doi:10.1371/journal.pone.0061577.g005

The results show that in an active-acoustic virtual-object discrimination task, the sensitivity for differences in object distance of all bats was much better than the object-distance differences as presented in the test conditions 1–4 of the Constancy experiment.

General Discussion

The bat sonar system theoretically provides the explicit information for the unambiguous encoding of an objects' physical size even when presented at different distances. Therefore, this hypothesis was tested in the Constancy experiment whether echolocating bats of the species *P. discolor* spontaneously assigned absolute width information to VOs by combining distance- and aperture information. To do so, a VO setup was used that allowed for a tight control of all relevant echo parameters, delay, target strength and aperture. The stimulus pairs in the test conditions 1–4 of the Constancy experiment were chosen to create the physically and geometrically correct presentation of VOs allowing the investigation of size constancy.

The results from the Constancy experiment show that the bats significantly chose the VOs providing the smaller sonar aperture of 23° independent of the distance of the VO. This means that no spontaneous assignment of absolute object width could be observed as would be predicted by size constancy (Fig. 3A and C–E).

Sonar aperture information

For the evaluation of sonar aperture it was already psychophysically and electrophysiological indicated that bats use directional characteristics of their outer ears to evaluate the aperture of ensonified objects [13]. The resulting information can be either monaural spectral cues or binaural-echo disparities [36]. The employment of these parameters for size perception caused by sonar aperture was also supported by the behavioral results of a field study [37].

In Heinrich *et al.* 2011 [13] it was argued that differences in the sonar aperture of two ensonified objects can be monaurally encoded in terms of a difference between the excitation patterns along the tonotopic axis [38]. When variations in echo delay and the accompanying variations in target strength were introduced in the test conditions of the Constancy experiment, this difference between the excitation patterns persisted. For example, if an object with the same aperture at a larger distance was presented, only the delay of the excitation increased and magnitude decreased, but the spectral profile of the excitation pattern would remain unchanged. Thus, if the animals memorized only the excitation-pattern difference, independent of overall excitation and temporal delay, this could explain the animals' classification of the virtual objects in the test conditions.

Target strength differences

Absolute object width was simulated by combining object distance and aperture. In the current setup, sonar aperture was encoded through the number of speakers coherently radiating the echoes of the bat's emissions. Thus, the target strength of a point receiver at the bat position would increase by 6 dB per doubling of the aperture (i.e., number of radiating speakers). Therefore, the bats could base their discrimination in the standard condition and classifications in the test conditions on target-strength differences, rather than aperture or distance differences.

In the standard condition, both VOs were presented at the same distance to the bats. Consequently the geometric and atmospheric attenuation was identical for both stimuli, and only the aperture differences created target-strength differences. This difference was 3 dB (Fig. 2A, right column) which is lower than perceptual threshold for target-strength discrimination ('echo intensity') of 5 dB [13].

The target-strength differences introduced in the test conditions 1–4 were much larger due to the dependence of geometric and atmospheric attenuation on object distance (Fig. 2 B–E). Already in the first test condition (Fig. 2B) the VO with the smaller aperture had also perceivably lower target strength because of the distance difference. But given that the bats based their classifications in the test conditions on the perceptual cues used to discriminate between the VOs in the standard condition, it is unlikely that the bats spontaneously changed its perceptual cue from aperture to target strength. Note that in the test conditions, the bats were rewarded independent of their decision, and, thus, there is no advantage for the bats to switch perceptual cues. Consequently, the bats' classifications are consistent with the hypothesis that they relied exclusively on the aperture cue, and were unaffected by the target strength cues co-varying with the distance cues in the test conditions. We assume this to be true although the target-strength differences were above the perceptual threshold. Interestingly, the human auditory system also relies on perceptual cues other than target strength to evaluate the distance to an external sound source [39]. In that study, it was shown that the direct-to-reverberant ratio of the external sound contributed strongly to its perceived distance. Other potential cues are provided by the (frequency dependent) atmospheric attenuation (Fig. 2) in that more distant sound source or sound reflectors have a stronger low-pass characteristic.

Distance-discrimination thresholds

The results from the control experiment show on the one hand, that the distance differences as introduced in the Constancy experiment are well above the animals' distance-discrimination thresholds. On the other hand, these thresholds between 3.2 cm and 5.4 cm are slightly higher than those for other bat species (reviews [8,20]). The distance-discrimination threshold of the closely related bat *Phyllostomus hastatus* [40], for example, was 1.2 cm for real objects presented at a reference distance of either 60 or 120 cm [18,19]. Data from other bat species also show that thresholds were no larger than 4.1 cm (*Rhinolophus ferrumequinum*, real objects presented at a reference distance of 100 cm) [20,41].

While most studies have employed real objects for Distance discrimination; the use of VOs in the recent study should not have had negative effects on threshold determination. Using VOs presented at a reference distance of 30 cm, Simmons [19] found distance-discrimination thresholds of 1.0 cm in *Eptesicus fuscus*. These were even slightly better than the 1.2 cm threshold with real objects.

Simulation of object distance

Compared to other studies using VO setups for distance discrimination, the recent study differed in that we implemented a physically correct distance-dependent attenuation. Besides the echo-delay information, this principally introduced two additional auditory cues the bats could use for solving the task: the target-strength differences of both VOs (the closer VO creates the louder echo) and possible spectral cues. Distance differences and thus delay differences become very small around perceptual thresholds. This is also true for the other acoustic parameters: specifically, at the threshold-distance difference of 4.16 cm, the target-strength difference was only 0.32 dB, which is well below the perceptual threshold of 5 dB [13].

Multimodal object perception

Doubtlessly, the bat echolocation system is essential for orientation and foraging in complete darkness. Nevertheless, it has limitations and consequently the role of other sensory modalities in bats was also often addressed. *P. discolor* is characterized as mainly nectarivorous or frugivorous, but also feeds on insects or small vertebrates [34]. In addition to echolocation, phyllostomid bat species also rely on olfactory, visual or passive acoustic cues [42,43,44]. The fruit-eating bat *Carollia perspicillata* was shown to use primarily olfactory cues for long-range detection and switched to echolocation only to exactly localize fruit items [45]. Other studies have shown that especially frugivorous bats have higher visual acuity [46] as well as better morphological adaptations for vision at low light levels compared to strictly insectivorous bat species [42]. *P. discolor* has relatively large, well developed eyes, suggesting an important role of vision. Indeed it was shown that this species employs both vision and echolocation for object localization and obstacle avoidance [35,47,48]. In a flight tunnel experiment that was designed to evaluate the importance the two orientation systems during the object approach, it was found that *P. discolor* preferred visual information at distances larger than 40 cm [47]. This lead to the conclusion, that the use of vision could be more important for object perception at far and medium distances in this species than previously thought. When multiple cues were present, it was found that *P. discolor* chose the sensory orientation system that delivered the most conspicuous object features [34]. The natural habitats of bats are very diverse, including complex structures that cause clutter and can also mask objects of interests. Hence, the more conspicuous object information for size perception over large distances could often be delivered by vision.

Taken together, we assume that while the bats in our experiments were able to evaluate the echo-acoustic aperture- and distance parameters presented in the current experiments, the spontaneous combination of these parameters to create size invariance may lack the ecological relevance.

Independence of size- and distance-perception?

As indicated above, the discussion of how organisms perceive the physical size of distant objects by vision is still the subject of debate. Haber and Levin [1] challenge the visual size-distance invariance hypothesis in general. They argue that distance perception 'should develop and be available early in life and in tandem with maturation of locomotors abilities'. For the perception of objects, in contrast, 'familiarity with the object (acquired from the past) should be the most important variable determining the accuracy of ''perceiving'' how big the object appears to be'. In the training condition of the current experiment, the bats learned to discriminate between two objects, differing in sonar aperture. In the test trials where distance cues were varied together with aperture cues, the bats continued to choose the VO with the smaller aperture, and appeared to ignore the distance variations. Following the arguments of Haber and Levin [1], distance information in bats is used for acoustically guided locomotion (navigation and orientation) as well as the tracking of prey during pursuit [20,49,50], and serves basically the same purpose of distance perception assigned to visually guided locomotion in humans. It is conceivable that the VOs presented in the current experiments lack the behavioral significance to activate 'object-oriented' perception [12] (e.g. in terms of e.g. familiarity). This may be the reason why the bats did not show spontaneous size constancy.

We conclude that the perception of angular information as provided by the sonar aperture could be an even more persistent sonar cue than previously thought. For solving the behavioral classification task, bats seemed to ignore variations of object distance (and the covarying amplitude cues) although they were perceptually well resolved by the bats. This lack of sonar size constancy may result from the bats relying on different modalities to extract size information at different distances. An alternative explanation follows Haber and Levin [1] in that familiarity with a behaviorally relevant, conspicuous object is required for size constancy.

Acknowledgments

The authors would like to thank Uwe Firzlaff for comments on an earlier draft of this paper, as well as Alexander Warmbold and Daria Genzel for valuable advice and assistance in programming. We sincerely thank Katie L. Willis for the final language and grammar check of this paper. We would also like to thank the two anonymous referees for their useful, detailed comments, many of which we believe have significantly improved the final paper.

Author Contributions

Conceived and designed the experiments: MH LW. Performed the experiments: MH. Analyzed the data: MH LW. Contributed reagents/materials/analysis tools: MH LW. Wrote the paper: MH LW.

References

1. Haber R, Levin C (2001) The independence of size perception and distance perception. Percept Psychophys 63: 1140–1152.
2. Foley JM, Ribeiro NP, Dasilva JA (2004) Visual perception of extent and the geometry of visual space. Vision Res 44: 147–156.
3. Holway AH, Boring EG (1941) Determinants of apparent visual size with distance variant. Am J Psychol 54: 21–37.
4. McKee SP, Welch L (1992) The precision of size constancy. Vision Res 32: 1447–1460.
5. Morgan MJ (1992) On the scaling of size judgements by orientational cues. Vision Res 32: 1433–1445.
6. Murray SO, Boyaci H, Kersten D (2006) The representation of perceived angular size in human primary visual cortex. Nat Neurosci 9: 429–434.
7. Nachtigall PE (1980) Odontocete echolocation performance on object size, shape and material. In: Busnel RG, Fish JF, editors. Animal Sonar Systems. New York: Plenum Press. pp. 71–95.
8. Simmons JA, Grinnell AD (1988) The performance of echolocation: Acoustic images perceived by echolocating bats. In: Nachtigall PE, Moore PWB, editors. Animal sonar: Processes and performance. New York Plenum Press. pp. 353–385.
9. Aubauer R, Au WW, Nachtigall PE, Pawloski DA, DeLong CM (2000) Classification of electronically generated phantom targets by an Atlantic bottlenose dolphin (Tursiops truncatus). J Acoust Soc Am 107: 2750–2754.
10. Au W (2004) A comparison of the sonar capabilities of bats and dolphins. In: Thomas J, Moss C, Vater M, editors. Echolocation in Bats and Dolphins. Chicago: University of Chicago Press. pp. pp xiii–xxvii
11. DeLong CM, Au WWL, Lernonds DW, Harley HE, Roitblat HL (2006) Acoustic features of objects matched by an echolocating bottlenose dolphin. J Acoust Soc Am 119: 1867–1879.
12. Firzlaff U, Schuchmann M, Grunwald JE, Schuller G, Wiegrebe L (2007) Object-oriented echo perception and cortical representation in echolocating bats. PLoS Biol 5: 1174–1183.
13. Heinrich M, Warmbold A, Hoffmann S, Firzlaff U, Wiegrebe L (2011) The sonar aperture and its neural representation in bats. Am J Neurosci 31: 15618–15627.
14. Simmons JA, Ferragamo M, Moss CF, Stevenson SB, Altes RA (1990) Discrimination of jittered sonar echoes by the echolocating bat, Eptesicus-fuscus: The shape of target images in echolocation. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 167: 589–616.
15. Weissenbacher P, Wiegrebe L (2003) Classification of virtual objects in the echolocating bat, Megaderma lyra. Behav Neurosci 117: 833–839.
16. Schoernich S, Wiegrebe L (2008) Phase sensitivity in bat sonar revisited. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 194: 61–67.
17. Hartrige H (1945) Acoustic control in the flight of bats. Nature 156: 490–494.
18. Simmons JA (1971) Echolocation in bats - signal processing of echoes for target range. Science 171: 925–928.
19. Simmons JA (1973) Resolution of target range by echolocating bats. J Acoust Soc Am 54: 157–173.
20. Moss CF, Schnitzler HU (1995) Behavioral studies of auditory information processing. In: Popper AN, Fay RR, editors. Springer Handbook of Auditory Research; Hearing by bats. pp. 87–145.
21. Goerlitz HR, Geberl C, Wiegrebe L (2010) Sonar detection of jittering real targets in a free-flying bat. J Acoust Soc Am 128: 1467–1475.
22. Feng AS (2011) Neural mechanisms of target ranging in FM bats: physiological evidence from bats and frogs. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 197: 595–603.
23. Wenstrup JJ, Portfors CV (2011) Neural processing of target distance by echolocating bats: Functional roles of the auditory midbrain. Neurosci Biobehav Rev 35: 2073–2083.
24. Schuller G, O'Neill WE, Radtke-Schuller S (1991) Facilitation and delay Sensitivity of auditory cortex neurons in CF-FM bats, Rhinolophus rouxi and Pteronotus p.parnellii. Eur J Neurosci 3: 1165–1181.
25. Dear SP, Fritz J, Haresign T, Ferragamo M, Simmons JA (1993) Tonotopic and functional organization in the auditory cortex of the big brown bat, Eptesicus fuscus. J Neurophysiol 70: 1988–2009.
26. O'Neill WE (1995) The bat auditory cortex. In: Popper AN, R FR, editors. Springer handbook of auditory research; Hearing by bats. Berlin: Springer-Verlag. pp. 416–480.
27. Hagemann C, Esser KH, Koessl M (2010) Chronotopically organized target-distance map in the auditory cortex of the short-tailed fruit bat. J Neurophysiol 103: 322–333.
28. Hagemann C, Vater M, Koessl M (2011) Comparison of properties of cortical echo delay-tuning in the short-tailed fruit bat and the mustached bat. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 197: 605–613.
29. Lawrence BD, Simmons JA (1982) Measurements of atmospheric attenuation at ultrasonic frequencies and the significance for echolocation by bats. J Acoust Soc Am 71: 585–590.

30. Stilz W (2004) Akustische Untersuchungen zur Echoortung bei Fledermäusen. Tübingen: Eberhard Karls Universität 146 p.
31. Bates ME, Simmons JA (2011) Perception of echo delay is disrupted by small temporal misalignment of echo harmonics in bat sonar. J Exp Biol 214: 394–401.
32. Gellermann LW (1933) Chance orders of alternating stimuli in visual discrimination experiments. Journal of Genetic Psychology 42: 206–208.
33. Nowak R (1994) Walker's Bats of the World. Baltimore, Maryland: The Johns Hopkins University Press. 288 p.
34. Kwiecinski GG (2006) Phyllostomus discolor. Mammalian Species: 1–11.
35. Rother G, Schmidt U (1982) The influence of visual information on echolocation in Phyllostomus-discolor (Chiroptera). International Journal of Mammalian Biology 47: 324–334.
36. Holderied MW, von Helversen O (2006) 'Binaural echo disparity' as a potential indicator of object orientation and cue for object recognition in echolocating nectar-feeding bats. J Exp Biol 209: 3457–3468.
37. Goerlitz HR, Genzel D, Wiegrebe L (2012) Bats' avoidance of real and virtual objects: Implications for the sonar coding of object size. Behav Processes 89: 61–67.
38. Wiegrebe L (2008) An autocorrelation model of bat sonar. BiolCybern 98: 587–595.
39. Kopco N, Huang S, Belliveau JW, Raij T, Tengshe C, et al. (2012) Neuronal representations of distance in human auditory cortex. Proc Natl Acad Sci U S A 109: 11019–11024.
40. Carstens BC, Lundrigan BL, Myers P (2002) A phylogeny of the neotropical nectar-feeding bats (Chiroptera: Phyllostomidae) based on morphological and molecular data. J Mamm Evol 9: 23–53.
41. Airapetianz ES, Konstantinov AI (1974) Echolocation in Nature. Nauka, Leningrad.
42. Mistry S (1990) Characteristics of the visually guided escape response of the Mexican free-tailed bat, Tadarida brasiliensis mexicana. Anim Behav 39: 314–320.
43. Holler P, Schmidt U (1996) The orientation behaviour of the lesser spearnosed bat, Phyllostomus discolor (Chiroptera) in a model roost. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 179: 245–254.
44. Heffner RS, Koay G, Heffner HE (2007) Sound-localization acuity and its relation to vision in large and small fruit-eating bats: I. Echolocating species, Phyllostomus hastatus and Carollia perspicillata. Hear Res 234: 1–9.
45. Thies W, Kalko EKV, Schnitzler HU (1998) The roles of echolocation and olfaction in two neotropical fruit-eating bats, Carollia perspicillata and C. castanea, feeding on Piper. Behav Ecol Sociobiol 42: 397–409.
46. Suthers RA (1966) Optomotor responses by echolocating bats. Science 152: 1102–1104.
47. Joermann G, Schmidt U, Schmidt C (1988) The mode of orientation during flight and approach to landing in two phyllostomid bats. Ethology 78: 332–340.
48. Schmidt U, Joermann G, Rother G (1988) Acoustical vs. visual orientation in neotropical bats. In: Nachtigall PE, Moore PWB, editors. Animal sonar: Processes and performance. New York: Plenum Press. pp. 589–593
49. Moss CF, Bohn K, Gilkenson H, Surlykke A (2006) Active listening for spatial orientation in a complex auditory scene. PLoS Biol 4: 615–626.
50. Moss CF, Surlykke A (2010) Probing the natural scene by echolocation in bats. Front Behav Neurosci 4.

4 Trawling bats exploit an echo-acoustic ground effect

This chapter was published online in: Frontiers in Physiology: Integrative Physiology; on April 05th; 2013 (4 (65); doi: 10.3389/fphys.2013.00065) under the title: **“Trawling bats exploit an echo-acoustic ground effect”** by Sándor Zsebők (SZ)*, Ferdinand Kroll (FK)*, Melina Heinrich (MH)*, Daria Genzel (DG), Björn Siemers (BS) and Lutz Wiegrebe (LW). (* SZ, FK, and MH contributed equally to this work).

4.1 Reference

LW, DG and MH supervised the diploma thesis of FK. FK, DG, MH and LW designed the field experiments. MH wrote all proposals and reports that were required for the conduction for the field experiments and in the early phase of this project in 2010. FK, DG and LW wrote the experimental programs, and the analysis programs. FK, DG and MH collected data in the field. The data collection for the field experiments was supported at times by Barbara Pfäffl and Andrea Lingner. Data analysis of the raw data for the field experiments was done by FK with the assistance of LW and DG.

This project was conducted in collaboration with SZ and BS from the Max Planck Institute for Ornithology in Seewiesen, Germany.

A modified version of the experimental setup that was used in the field experiments was used by SZ and BS to carry out the laboratory experiments described in the published paper. The data collection and analysis of the raw data for the laboratory experiments was done by SZ.

For sound analysis Holger Görlitz provided a MATLAB based sound analysis program that was modified by FK, DG and LW. The combined data analysis and the figure design were performed by SZ, FK and LW. The spectrograms in Figure 6 were designed and measured by FK, DG, MH and LW. The manuscript was written by SZ, FK, MH and LW. MH and LW were furthermore strongly involved in the resubmission and final proof reading process.

4.2 Summary

Bats are very agile animals that can fly with high speed through the environment (e.g. during foraging). In such a scenario the spatiotemporal layout of the environment, and the objects within, changes with locomotion. Consequently, in the third study a practical example of spatiotemporal integration in foraging bats under natural and naturalistic conditions was addressed.

Smooth reflecting surfaces like water can act as optic as well as acoustic mirrors. In nature, echolocation calls are reflected away from bats that fly closely above a water surface. If a bat ensonifies a prey item that is floating on the water, the smooth surface even enhances the echo. In the experiments we tested and quantified the effect of different surfaces with different reflection properties (and thus possible enhancement or masking characteristics) on object-detection and discrimination performance of the European trawling bat species, *Myotis daubentonii*. This was done in a combined laboratory and field approach using a 2-AFC paradigm. In the laboratory and in the field bats had to detect a mealworm presented above different surfaces and discriminate it from an inedible PVC disk. The psychophysical performance was measured as a function of height above smooth surfaces (water and PVC) or a clutter surface (floating artificial grass matting). The results showed that at low heights (10, 20, or 35 cm) above the clutter surface the detection performance of the bats was worse compared to the smooth surfaces. The object detection was not influenced at object heights of 50 cm independently of the presented surface below. Object discrimination also decreased significantly with decreasing object height when presented above the clutter surface. The reconstruction of the flight paths showed that bats attacked the objects from below when flying over a smooth surface but from above when flying over the clutter surface. An increase in flight height also leads to an increase in spatial (elevation angle) and temporal difference (echo delay) between the direct echo of the ensonified object and the indirect scattered echoes of the clutter surface. This separation of direct and indirect echoes probably increased object-localization and discrimination success.

When the bats were flying at low heights over water and attacked the objects from below, this probably lead to an echo-acoustic enhancement effect. Here the elevation angle between the direct echo from the prey to the bat and the indirect echoes from the prey via the surface to the bat got smaller with decreasing flight height. Moreover, the echo-delay differences between both described echo paths

were minimized. It suggests that bats probably employed an echo acoustic ground effect by integrating spatial-and temporal cues of both, direct and indirect echoes. Since the enhancement leads to misleading spatial cues created by the indirect echoes by signaling the wrong elevation, bats could probably solve this by employing a precedence-like strategy: Exact localization could be dominated by using the first (and therewith direct) echo.

frontiers in
PHYSIOLOGY

ORIGINAL RESEARCH ARTICLE
published: 05 April 2013
doi: 10.3389/fphys.2013.00065

Trawling bats exploit an echo-acoustic ground effect

Sandor Zsebok[1,2†], Ferdinand Kroll[3†], Melina Heinrich[3,4†], Daria Genzel[3], Björn M. Siemers[1] and Lutz Wiegrebe[1,3,4]*

[1] Sensory Ecology Group, Max Planck Institute for Ornithology, Seewiesen, Germany
[2] MTA–ELTE–MTM Ecology Research Group, Budapest, Hungary
[3] Division of Neurobiology, Department Biology II, Ludwig-Maximilians-University Munich, Planegg-Martinsried, Germany
[4] Graduate School of Systemic Neurosciences, Ludwig-Maximilians-University Munich, Planegg-Martinsried, Germany

Edited by:
Mariana L. Melcón, Fundación Cethus, Argentina

Reviewed by:
Annemarie Surlykke, University of Southern Denmark, Denmark
Arjan Boonman, University of Tel Aviv, Israel

***Correspondence:**
Lutz Wiegrebe, Division of Neurobiology, Department Biology II, Ludwig-Maximilians-University Munich, Großhaderner Strasse 2, D-82152 Planegg-Martinsried, Germany.
e-mail: lutzw@lmu.de

† These authors have contributed equally to this work.

A water surface acts not only as an optic mirror but also as an acoustic mirror. Echolocation calls emitted by bats at low heights above water are reflected away from the bat, and hence the background clutter is reduced. Moreover, targets on the surface create an enhanced echo. Here, we formally quantified the effect of the surface and target height on both target detection and -discrimination in a combined laboratory and field approach with *Myotis daubentonii*. In a two-alternative, forced-choice paradigm, the bats had to detect a mealworm and discriminate it from an inedible dummy (20 mm PVC disc). Psychophysical performance was measured as a function of height above either smooth surfaces (water or PVC) or above a clutter surface (artificial grass). At low heights above the clutter surface (10, 20, or 35 cm), the bats' detection performance was worse than above a smooth surface. At a height of 50 cm, the surface structure had no influence on target detection. Above the clutter surface, also target discrimination was significantly impaired with decreasing target height. A detailed analysis of the bats' echolocation calls during target approach shows that above the clutter surface, the bats produce calls with significantly higher peak frequency. Flight-path reconstruction revealed that the bats attacked an target from below over water but from above over a clutter surface. These results are consistent with the hypothesis that trawling bats exploit an echo-acoustic ground effect, in terms of a spatio-temporal integration of direct reflections with indirect reflections from the water surface, to optimize prey detection and -discrimination not only for prey on the water but also for some range above.

Keywords: *Myotis daubentonii*, echo-acoustic mirrors, target detection, target discrimination, echo enhancement, trawling bats, ground effect

INTRODUCTION

In course of evolution, bats, as the only airborne mammals, adapted to a large variety of habitats. The species of this ecologically highly diverse group provide many morphological, physiological as well as behavioral adaptations e.g., of their sensory-motor system (Schnitzler and Kalko, 2001). Echolocating bats emit ultrasonic sounds and listen to the returning echoes reflected by objects in the environment. This enables bats to orient and hunt in complete darkness allowing prey detection, localization, and identification. But the biosonar system is prone to interferences. When bats use echolocation e.g., during foraging they have to deal with sound attenuation and masking effects. Amongst others, attenuation can be caused by atmospheric absorption losses that especially have a strong impact on high frequencies as they are used by bats (Lawrence and Simmons, 1982). Items close to the object of interest can create masking effects that impede prey detection (Fenton, 1990; Suemer et al., 2009; Bates et al., 2011). This so-called clutter interference can appear e.g., when hunting close to the ground or foliage. Hence, bats are not only morphologically adapted to their habitats (e.g., by wing shape) (Norberg and Rayner, 1987), but also by their echolocation signals (Schnitzler and Kalko, 2001; Siemers and Schnitzler, 2004; Wund, 2005). The differences in the echolocation call parameters (e.g., frequency, call duration, call intensity) are species-specific and also habitat-dependent. For example in vespertilionid bats, species that hunt in free airspace emit loud, narrowband echolocation calls to detect prey from a larger distance, whereas species that hunt near vegetation emit broadband echolocation calls to catch prey objects that are only a few centimeters in front of a clutter producing background (Schnitzler et al., 2003). Additionally the structure of echolocation signals can also differ with the behavioral task. In insectivorous bats for example, the echolocation signals during search, approach, and final buzz phase are very different (Schnitzler and Kalko, 2001).

One particularly interesting group consists of bats hunting almost exclusively above water surfaces. These so-called "trawling bats" hunt at low heights above water and capture fish or insects directly from or close to the surface. Water bodies like lakes, ponds, or streams are favorable hunting habitats for bats as the high abundance of insects provides a profitable food source (Zahn and Maier, 1997; Warren et al., 2000; Ciechanowski, 2002). In previous studies it was found that bats of this ecotype prefer to hunt over calm water compared to water e.g., covered by plants like duckweed, artificial objects, or turbulent, rippled water

(Von Frenckell and Barclay, 1987; Mackey and Barclay, 1989; Boonman et al., 1998; Rydell et al., 1999; Siemers et al., 2001b; Siemers and Schnitzler, 2004). Two laboratory studies revealed that in the three European trawling-bat species' (*Myotis capaccinii*, *M. dasycneme*, and *M. daubentonii*) capture success was increased, compared to a clutter surface, when prey was presented on a smooth surface (linoleum screen) that mimicked the reflection characteristics of calm water. It was concluded that since the water surface acts as an acoustic mirror, echolocation calls emitted by bats are reflected away in acute angles from the bat. This creates an echo-image without, or just low clutter echoes and thus increases search efficiency as the prey echo is acoustically conspicuous (Siemers et al., 2001b, 2005). The search image for these bats was defined as "small and isolated echo-reflecting objects on or above an acoustically smooth surface" (Siemers et al., 2001a,b). This theory does not exclude inedible objects e.g., small leaves or debris on a water surface. However, one would expect efficient prey discrimination during flight to be beneficial to avoid catching inedible prey. But, in actively hunting bats no discrimination between edible and inedible objects that fit the general search image could be observed so far (Barclay and Brigham, 1994; Siemers et al., 2001b). Siemers et al. (2001b) showed that under semi-natural laboratory conditions trawling bats did not discriminate between a mealworm and a dummy presented on a linoleum screen.

The trawling bat *Myotis daubentonii* often hunts over rivers and streams (Jones and Rayner, 1988) providing a unidirectional water flow that often contains inedible objects as well as drifting prey. In a field study it was shown that *M. daubentonii* switches between trawling of prey from the water surface and aerial hawking (Todd and Waters, 2007), depending on the amount of clutter on the water surface.

Since previous studies were mainly designed to investigate prey detection on acoustic mirror and clutter surfaces without testing discrimination performance in detail, this study was designed to test prey detection *and* -discrimination. As the previous studies were conducted in the field, the participating animals behaved under natural conditions, but the participating number is an unknown factor. Whereas studies conducted in the laboratory allow control over the number of animals, but are limited in their imitation of natural surroundings. To benefit from both study types we formally quantify in the current study the effect of surface structure on both prey detection and -discrimination and on the echolocation behavior in a combined laboratory and field approach.

The main objectives of our study were to investigate the effect of the surface structure on the attacking and discrimination performance of the bats as well as flight path and the sonar vocalization features. These behavioral measures are discussed with respect to echo-acoustic features of the surface structures.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

The species used in this study was the microchiropteran Daubenton's bat, *Myotis daubentonii*. It is found throughout Europe, foraging for insects above water surfaces using short (<5 ms), broadband frequency sweeps (95–25 kHz) for echolocation (Kalko and Schnitzler, 1989).

LABORATORY EXPERIMENT

Animal housing

Laboratory experiments were conducted in July and August 2011 in the Max Planck Institute of Ornithology in Seewiesen, Germany. Data from five individuals of 12 h time shifted Daubenton's bats were recorded. The experiments were conducted under license of the responsible authorities and complied with German laws (LLUR 515/5327.74.1.6).

Experimental setup

In the experiment a mealworm (larvae of *Tenebrio molitor*) and a dummy (1 mm black plastic disc with a diameter of 2 cm) were presented simultaneously. Both targets were hanging from easily exchangeable, variable-length nylon threads (Ø 0.15 mm) that were attached via small solenoids to a horizontal bar (**Figure 1**). The bar itself was suspended from the ceiling. This allowed an easy manipulation of the presented targets, e.g., target height (by variable lengths of nylon threads) and position (left or right side). The distance between the two targets was 1.2 m. The two targets were presented above either an artificial surface floating on the water or the water itself. The artificial surface measured 1.2 × 2.4 m. The targets were positioned such that each was hanging above the center of one half of a surface area with a minimum distance of 60 cm to the midline and the edges. The size of the experimental room was 3 × 7 × 3.5 m.

The experiment was monitored with synchronized normal- and high-speed video under infrared-light illumination and audio recordings. The normal-speed (25 frames/s) recordings were made by a single camera (WAT-902H2 Ultimate, Watec Co. LTD, Higashine, Japan) by means of the surveillance software (USB120 Server, Digiprotect, Frankfurt, Germany) to record the whole experimental process on the computer. The two high-speed digital video cameras (MV1-D1312I-160-CL-12, Photonfocus, Lachen, Switzerland; 100 frames/s, resolution 1312 by 1024 pixels, with specially developed software by Rauscher GmbH, Olching, Germany) recorded the last 5 s before a capture attempt. These high-speed recordings were used for reconstructing the flight path

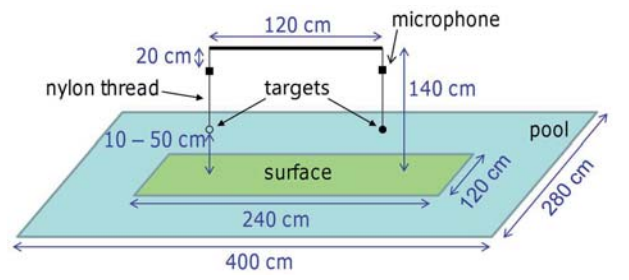

FIGURE 1 | Setup of the Field and Laboratory experiments. In the two-alternative forced-choice paradigm the bat had the opportunity to attack one of the targets (mealworm or dummy). The surface beneath the targets was covered either with artificial grass or smooth PVC, or the place was left clear for the water surface. The two targets were always presented at the same height which was 10, 20, 35, or 50 cm above the surface. The horizontal bar holding the targets and microphones was attached to the ceiling of the Laboratory or, for the Field experiment, to a fishing rod anchored to the ground.

later on. The illumination was supported by two custom made stroboscopic flash lights (Department of Animal Physiology, University of Tübingen, Germany).

Acoustic signals were picked up by two ultrasound microphones (Knowles SPM0204, Itasca, IL, USA) that were attached 20 cm below the horizontal bar, i.e., vertically above the two targets. Echolocation calls were amplified and digitized with an Ultralite-mk3 (MOTU, Cambridge, UK) at a sampling rate of 192 kHz and recorded with Adobe Audition 2.0 (Adobe Systems Inc., San Jose, CA, USA) on the computer.

Experimental procedure

In the experimental conditions target height and surface type were varied. The surface types were defined as clutter surface (artificial grass matting with a height of 3 cm) or as smooth surface (water). Above the two different surfaces the targets were presented at four different heights (10, 20, 35, and 50 cm). This resulted in eight different experimental conditions which were presented following a pseudo-random protocol. The position (left or right) of the targets was also randomized.

Before each trial, both targets were hidden by two 70 cm high paper tubes while attaching them to the setup. This prevented bats from identifying and attacking the targets before trial start. In a trial, both targets were always presented simultaneously and at the same height. As the targets were suspended from nylon threads, they were not perfectly stationary, specifically, they often rotated slowly around their vertical axis.

FIELD EXPERIMENT

The Field experiment was conducted under license of the responsible authorities (Referat für Umwelt und Gesundheit, München, 641-304/P-12/7).

Recording sites and experimental setup

The field recording site was a shallow branch of the river Würm, located in Munich-Pasing, Germany (48° 8′ 0.59″ N/11° 26′ 52.37″ E, water depth: 10–20 cm). Data recording took place on 10 evenings between April and October 2011. The experiments were performed shortly after sunset when the first bats started hunting at the recording site. Depending on bat activity, recording sessions lasted about 3 h per night. To fit the requirements of the field research site, a slightly adapted version of the laboratory setup was used in the Field experiment (**Figure 1**). The horizontal bar holding the nylon threads with the targets was suspended from a fishing rod that was anchored to the ground. For video acquisition, a single high-speed digital video camera [Basler A602f, Ahrensburg, Germany, 95 frames/s with a Pentax H612A (TH) objective lens, Pentax Ricoh Imaging Co., Ltd., Tokyo, Japan] was used. The camera was positioned about 2 m from the targets and ~50 cm above the water surface. Red light illumination (two Philips IR PAR38E 150W, Amsterdam, Netherlands) was used to supply sufficient light for the camera. The microphones and their position were exactly the same as in the laboratory. Audio and video data were recorded in a 5 s ring buffer system implemented in MATLAB 7.5 (The MathWorks, Inc., Natick, MA, USA).

Experimental procedure

In the Field experiment, an additional, smooth surface type (PVC) was used with similar acoustic reflection properties as smooth water. The PVC board (1.2 × 2.4 m) was used as a control condition to rule out the possibility of potential performance changes of the bats being merely due to the artificial surface. Moreover, the water from the river was not smooth but, due to the irregular floor beneath the shallow, flowing water, the surface had small, regular waves, and ripples.

Unlike in the Laboratory experiment, only three different target heights were applied: 20, 35, and 50 cm. The presentation of these nine different conditions (three heights times three surfaces) followed a pseudo-random protocol where in successive nine trials each condition was presented once. Like in the laboratory, the position (left or right) of the targets was also randomized.

Before each trial, the bat species hunting at the setup were identified visually and acoustically by means of their echolocation calls with a Mini-3 Bat Detector (Ultra Sound Advice, London, UK). Later, this was verified by both video and sound analyses. Data analysis (see below) was the same as for the Laboratory experiment, except that the single camera did not allow flight-path reconstruction, and acoustic data from the field was not evaluated.

DATA ANALYSIS

Attacking performance

A trial began when a bat initiated an attack or when it had circled around one or both of the targets at least three times. An executed attack was registered when the bat performed a final buzz and touched one of the targets or the threads. Later, the audio and video recordings of each trial were analyzed to correct for any wrong observations during the trials.

The data from each individual obtained in the laboratory was summarized and the attacking performance was calculated as the ratio of the number of attacks (independent of whether it was the dummy or the mealworm) divided by the number of trials where a bat initiated a trial according to the above criteria. In the water surface conditions, the attacking performance was always 100% independently of the target height (see results below), therefore it needed no statistical evaluation. For the statistical evaluation of the performance in the grass surface conditions, a General Linear Mixed Model (GLMM) was fitted on the arcsine transformed attacking performance data (as independent variable) with factors height (fixed effect) and individual (random effect).

As for the field results, it was not possible to distinguish different individuals; therefore only one performance value was calculated in each condition. In the water and PVC surface conditions, the attacking performance was maximal (100%) independently of the height (no statistics needed). To evaluate the effect of the height in the grass surface condition a Fischer's exact test was applied. All the statistical computations in this study were conducted in Statistica 8.0 (Statsoft Inc., Tulsa, OK, USA) and in MATLAB.

Discrimination performance

To calculate the discrimination performance only those trials were used in which an attack had been executed. An attack toward the mealworm was defined as a correct decision, an attack toward the dummy as a wrong decision. The discrimination performance was calculated as the ratio of correct decisions divided by all attacks in each condition.

For the laboratory results a GLMM was built on the arcsine transformed discrimination performance data (as independent variable) with the factors target height (fixed effect) and individual (random effect). This was done for the water and the grass surface conditions separately. The data obtained at the 10 cm target height conditions was omitted, as only one individual once attacked the targets offered at this height in the grass surface condition.

For the field results, the height effect was tested with the Fischer's exact test for all three surface conditions on the performance data.

A binomial test was used to test whether the probability of the mealworm choice was above 50% chance level. This was done separately for the Laboratory and the Field experiment on the pooled data.

Flight path analysis

The high-speed video recordings of the Laboratory experiment were used to reconstruct the flight paths for the trials of the 35 cm target height conditions. The calculations were made using the freely available DLTdv3 program written in MATLAB (Hedrick, 2008). After the flight path reconstruction the median and the quartiles from the water and the grass surface condition were calculated. This was done separately for each frame relative to the capture moment for the graphical presentation. The average flight height for each path was calculated and a GLMM was applied to test the effect of the individuals (random factor) and the surface (fixed factor).

Call analysis

Calls were analyzed with a custom written MATLAB program based on a program provided by Holger Görlitz. Calls were first high-pass filtered at 20 kHz. The frequency spectrum was then obtained by computing a 1024-point FFT (fast Fourier transform) over a Hanning window. Before calculating the frequency parameters the spectrum was fitted with an 18th-order polynomial to smooth out the ripples caused by constructive and destructive interferences between a call and reflections from the water surface. These interferences create higher and lower magnitudes, respectively, which are smoothed out by the polynomial fit. There was a continuous, narrow-band disturbance from a power supply in the recordings. For this narrow frequency range, the measured spectral magnitude was replaced by a linear interpolation. From the fitted spectrum, peak frequency, bandwidth and the −20 dB lower and upper cut-off frequencies were calculated. Due to reflections from the water, the analysis of the temporal call parameters was impeded. Depending on the pulse intervals (PIs), calls were separated into either Approach ($15\,\text{ms} < \text{PI} < 30\,\text{ms}$) or Buzz I phase ($6.5\,\text{ms} \leq \text{PI} \leq 15\,\text{ms}$). Kalko and Schnitzler (1989) measured a PI of 55–65 ms at the beginning of the Approach phase and 12–8 ms at the end. Here we used a rather narrow window to categorize the approach calls to ensure non-Approach calls were excluded. Buzz II calls with a PI shorter than 6.5 ms were not analyzed as the decreasing amplitude of the calls, the water reflections and the short PI impeded the analysis. In the following, Buzz I is referred to as Buzz.

To test the significance of the difference in peak frequency between the water and grass condition we applied a GLMM taking the surface as fixed factor and the identity of the individuals as random factor for each height (20, 35, and 50 cm) and phase (Approach and Buzz) separately (altogether six tests). We excluded the data from the 10 cm target height condition from this analysis, as we had only one recording in which the target was attacked. We did not analyze the echolocation calls obtained in the field, as the analysis of the laboratory data showed a highly significant individual effect for peak frequency (due to the lacking identity of the recorded bats in the field).

Ensonification and impulse response analysis

To quantify the structural properties of the surfaces, the PVC, and the grass matting were ensonified to obtain their impulse responses (IR). The IR is the echo reflected from an object when the object is ensonified with an acoustic impulse (Dirac impulse) of theoretically infinite shortness and infinite amplitude (Weissenbacher and Wiegrebe, 2003). The IR was calculated by cross-correlating the recorded echo with the original signal in the time domain.

A disc of the respective material (PVC or grass) with a diameter of 30 cm was positioned at a distance of 90 cm to an ultrasonic speaker (Matsushita EAS 10 TH 800D, Osaka, Japan), and a ¼ inch ultrasonic microphone (Brüel & Kjær 4135 with 2671 preamplifier and 2610 measuring amplifier, Nærum, Denmark) which was attached coaxially at the speaker front. The discs were ensonified from 10 different angles between 90° (sound impinging perpendicularly on the disc) and 0° (sound propagating parallel to the disc) in 10° steps. To measure the IR, white noise with a cut-off frequency of 96 kHz was created in MATLAB, sent to the DA/AD converter (MOTU Ultralite-mk3; sampling frequency 192 kHz), amplified (Toellner Toe 7606, Herdecke, Germany), and played via the ultrasonic speaker for the duration of 40 s. Simultaneously the echo was recorded by the ultrasonic microphone. Spectrograms of the IRs were calculated using a 64-point FFT over a Hanning window and an overlap of 95%.

RESULTS

ATTACKING PERFORMANCE

In the laboratory 347 trials were conducted with five individuals for eight conditions (four target heights, two surface types). For three individuals, data were obtained for four different target heights (10, 20, 35, and 50 cm). For two individuals, data were obtained for three different heights (20, 35, and 50 cm). After initiating a trial, all bats attacked one of the targets above water (**Figures 2A–E**, blue bars) independent of the target height. Above the grass surface, however, the performance deteriorated with decreasing target height (**Figures 2A–E**, green bars). The GLMM showed a significant effect of target height [$F_{(4,\ 10)} = 20.0$, $p < 0.001$] but also an effect of the individual [$F_{(3,\ 10)} = 8.4$, $p = 0.003$], meaning that the individual attacking

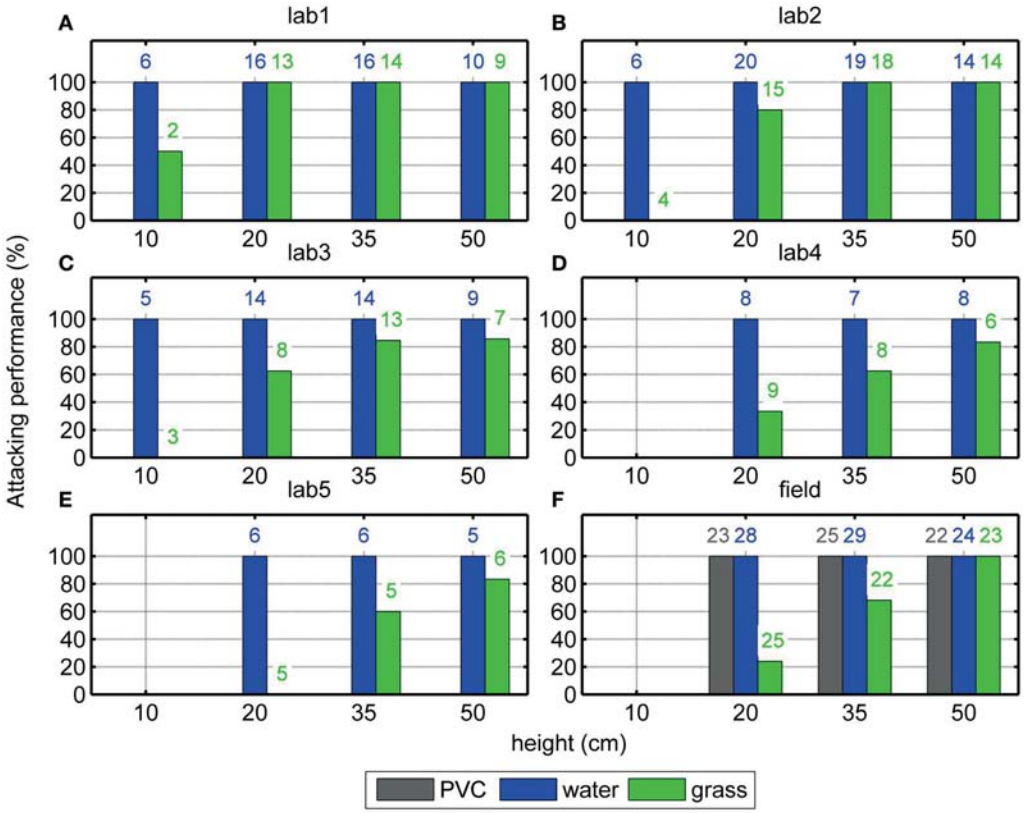

FIGURE 2 | Attacking performance above different surfaces at different heights. The results from 5 bats in the laboratory ("lab1-5", **A–E**) and from the field ("field", **F**) show that the bats always attacked one of the targets when it was above water or PVC (blue and gray bars). In contrast, above grass (green bars), the bats' attacking performance drastically diminished with decreasing height. The numbers of the successful trials are shown on the top of the bars.

performances above the grass surface differed significantly from each other.

In the Field experiment (218 trials) three different surfaces (PVC, water, or grass) and three different target heights (20, 35, or 50 cm) were presented. The same pattern of results as in the Laboratory experiment was observed: above water or PVC, the attacking performance was always 100% independently of target height (**Figure 2F**, blue and gray bars). However, above the grass surface, the attacking performance decreased monotonically with decreasing target height (green bars in **Figure 2F**, Fischer's exact test, $p < 0.001$).

DISCRIMINATION PERFORMANCE

In the Laboratory experiment, data from six different conditions [three target heights (20, 35, or 50 cm) above two surface types (water or grass)] were used to evaluate the bats' discrimination of the mealworm from the disk dummy. In general, the bats attacked the mealworm more often than the dummy, regardless of height and surfaces. While the average discrimination performance across the five bats in the laboratory was only 66% correct (206 correct trials out of 313), this performance is statistically significant because of the high number of trials (One-sided Binomial Test, $p < 0.001$). The GLMM analysis shows no significant difference in the overall (height independent) discrimination performance between water and grass surfaces [GLMM, $F_{(1,\ 27)} = 0.64$, $p = 0.43$]. Also, discrimination performance did not deteriorate significantly with decreasing height of the targets above water [blue bars in **Figures 3A–E**, $F_{(2,\ 8)} = 1.1$; $p = 0.37$]. However, discrimination performance deteriorated significantly with decreasing height of the targets above the grass surface [green bars in **Figures 3A–E**, $F_{(2,\ 7)} = 11.2$; $p = 0.007$].

In the Field experiment, data from nine different conditions [three target heights (20, 35, or 50 cm) times three surface types (PVC, water, or grass)] were used. Similar to the Laboratory experiment, the bats attacked the mealworm significantly more often regardless of height and surface (One-sided Binomial Test, $p < 0.001$, **Figure 3F**). However, in none of the surface type conditions an effect of target height was found (Fischer's exact tests, $p = 0.40$ with PVC; $p = 0.93$ with water and $p = 0.81$ with grass). There was also no significant difference between the surfaces (Fischer's exact test, $p = 0.075$).

FLIGHT PATH ANALYSIS

The bats' flight paths at the 35 cm target height conditions were reconstructed based on the laboratory video recordings of the last 4 s before capture. The median flight height above the grass surface was about 20 cm higher than above water (**Figure 4**). The

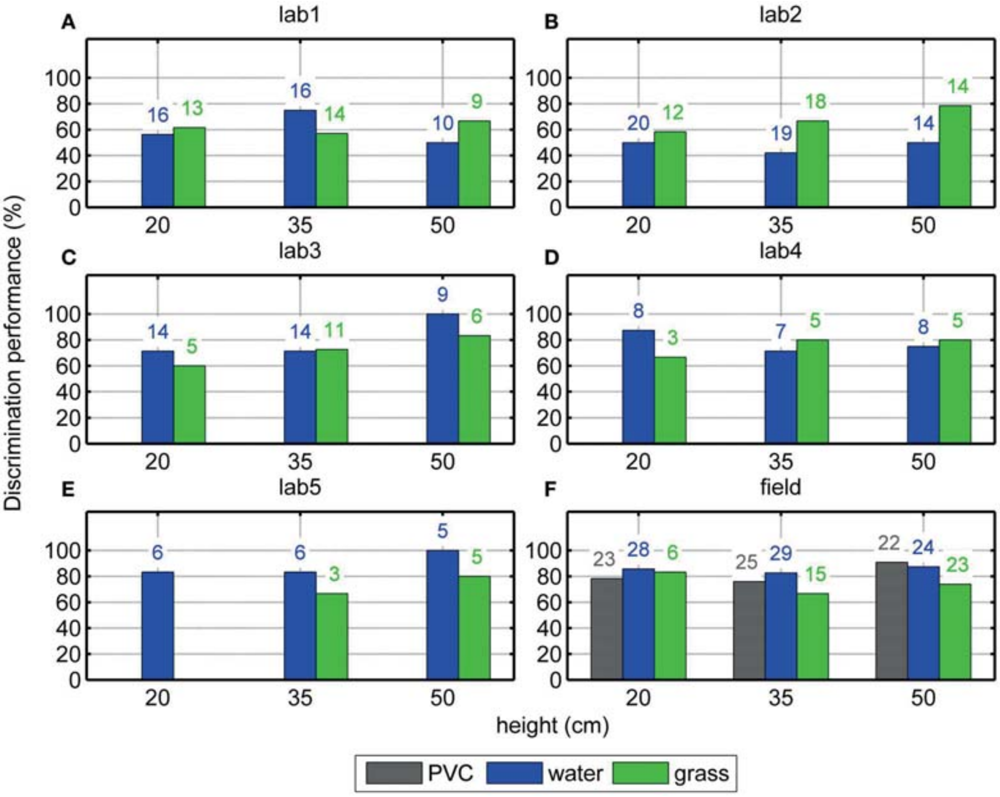

FIGURE 3 | Discrimination performance above different surfaces at different heights. Results from 5 bats in the laboratory ("lab1-5", **A–E**) and from the field ("field", **F**) are shown. There is a statistically significant decrease of the discrimination performance in the laboratory animals over grass; however we have found no such significant relationship in the field. The number of the trials in which we observed an attack are shown on the top of the bars.

median flight heights show that in the grass surface condition, the bats approached the target slightly from above, whereas in the water condition, the bats approached the target from below. The GLMM showed a significant surface effect [$F_{(1,\ 47)} = 48.9$, $p < 0.001$], but no individual effect [$F_{(4,\ 47)} = 1.26$; $p = 0.30$] on flight height.

CALL ANALYSIS

Two hundred and forty-six echolocation call sequences from Approach phases and 221 sequences from Buzz phases were analyzed in the laboratory recordings. On average, Approach phases contained 13.1 ± 0.6 calls and the Buzz phases contained 9.0 ± 0.34 calls (median ± standard error). The calls' peak frequency was analyzed for Approach and Buzz phase separately. When the targets were presented low above the grass surface, the bats increased the peak frequency of their calls significantly (**Figure 5**).

The GLMM analysis reveals significant differences in peak frequency between the water and grass surfaces conditions at a target height of 20 cm [GLMM, $F_{(1,\ 71)} = 38.5$, $p < 0.001$ in Approach and $F_{(1,\ 65)} = 12.8$; $p < 0.001$ in Buzz phase] and of 35 cm [$F_{(1,\ 82)} = 12.2$; $p < 0.001$ in Approach phase and $F_{(1,\ 68)} = 11.5$; $p = 0.001$ in Buzz phase]. No significant differences were found when the targets were 50 cm above the surfaces [$F_{(1,\ 58)} = 1.2$; $p = 0.28$ in Approach phase and $F_{(1,\ 53)} = 0.5$; $p = 0.47$ in Buzz phase].

ENSONIFICATION, IMPULSE RESPONSES

Two 30 cm discs made of either PVC or artificial grass were ensonified at different angles (**Figure 6**). At an ensonification angle of 90° (perpendicular ensonification, top row of **Figure 6**) the IR of the PVC is sharper and louder than that of the grass matting. However, at ensonification angles between 30 and 70°, the IR of PVC is weaker than that of the grass matting, especially at frequencies higher than about 50 kHz. Additionally the IR of the grass matting at these ensonification angles is temporally expanded. At a very small angle (10°) there is hardly any difference between the two surfaces.

DISCUSSION

In our study we found that for the bat *M. daubentonii*, the detection and discrimination of prey objects decreases at low heights above a clutter surface. This deterioration in psychophysical performance is accompanied by significant increases in both flight height and increases in the peak frequency of the bats' sonar emissions. The good agreement of the data from the Laboratory- and Field experiments corroborates the ecological relevance of the current tasks for the animals in the wild.

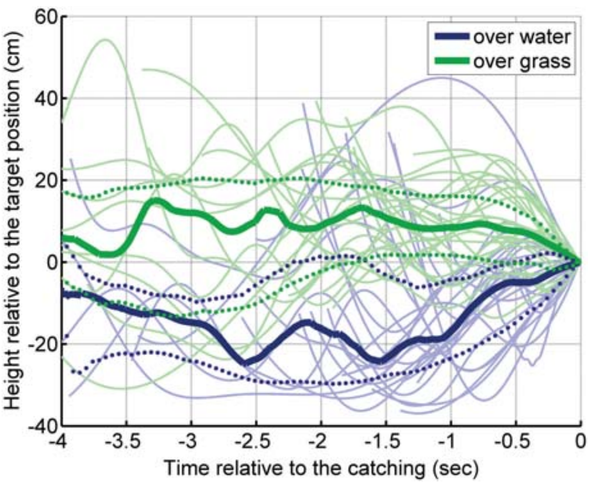

FIGURE 4 | Flight height of the bats in the last 4 s before making a capture at a target height of 35 cm. The moment of the capture is shown at 0 s (on the right side of the graph). On average, the bats flew around 10 cm above the target height when they were presented above grass (thick green line). When the targets were presented above water, the bats flew about 10–20 cm below the target height (thick blue line). The strong continuous lines show the median of the flight paths, the dotted lines show the upper and lower quartiles.

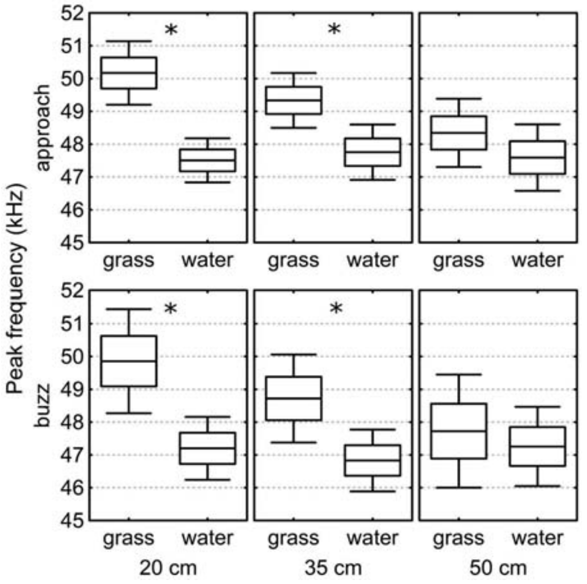

FIGURE 5 | Peak frequency of the echolocation calls above grass and water in the laboratory. In both, Buzz and Approach phase, and at target heights of both 20 and 35 cm, the peak frequency was significantly higher above grass than above water. At 50 cm height we found no significant differences in the peak frequency between the two surfaces. The box-plots show the mean, the standard error, and the confidence interval. Stars indicate significant differences ($p < 0.001$) (see call analysis section).

In the following we will discuss the data, first with respect to the performance of the bat, i.e., target detection and -discrimination, and second with respect to the behavioral adaptations of the bats, i.e., flight path and echolocation behavior.

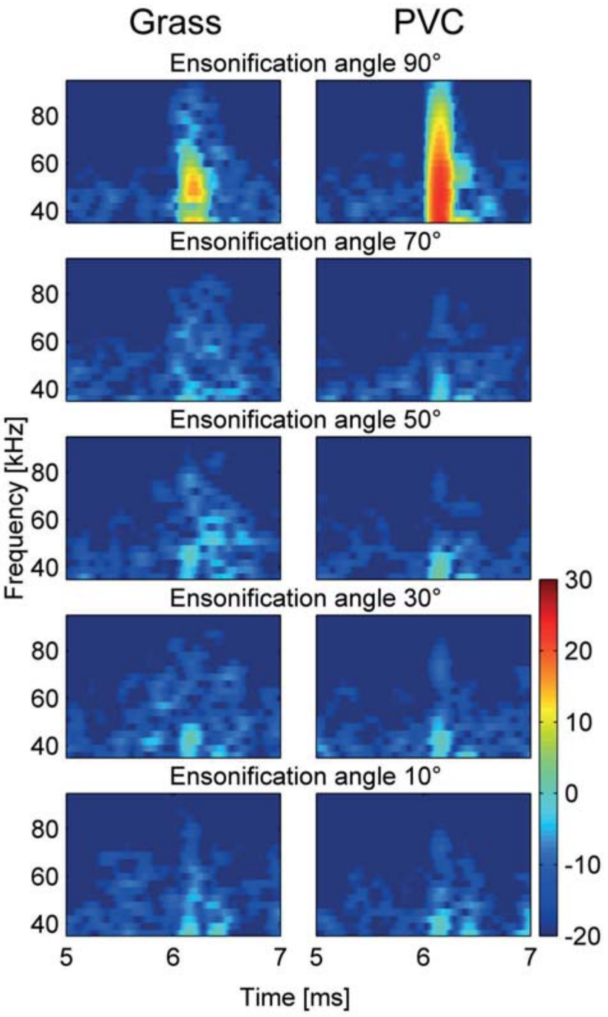

FIGURE 6 | Spectrograms of the impulse responses of the artificial grass and PVC surface at different ensonification angles. At an angle of 90° the impulse response of the PVC is stronger and smoother than that of grass (top row). At angles between 30 and 70° (second to fourth row) the impulse responses of the grass surfaces are longer and contain more high frequencies than those of the PVC surface. At 10° there is hardly any difference between the two surfaces.

TARGET DETECTION

In the Laboratory and in the Field experiments, the animals always executed an attack after they had initiated a trial when targets were presented 50 cm above any surface. However, with decreasing target height, the bats attacked less often above the grass surface (**Figures 2A–F**, green bars) while they still executed attacks above water. The current 2 AFC setup required the bats to find the one thread from which a mealworm is hanging. Unlike in a natural detection task, the general structure of the setup will indicate for the bats where to search for potential prey. Nevertheless, we observed that especially at very low heights above the grass surface, the animals attacked much less frequently.

In an experiment where *M. daubentonii* were trained to catch a mealworm suspended in front of a vertical clutter surface, Siemers and Schnitzler (2004) also found a significant decrease in attacking performance when the target distance was 25 cm or less. Thus, the attacking impairment appears independent of the absolute orientation of the surface (horizontal or vertical).

A particular case of target detection above surface is when the target is on the surface itself. Siemers et al. (2001b) found in their experiment that mealworms which were placed on smooth horizontal linoleum were readily caught, however, when mealworms were placed on the clutter screen, they were almost never caught. Accordingly, Boonman et al. (1998) found that higher duckweed density on natural water surfaces correlates with lower catching success of the bats from the surface. Moreover, bats prefer open waters against waters covered with duckweed. Rydell et al. (1999) also found reduced bat activity above rippled water. Von Frenckell and Barclay (1987) showed that bats' (*M. lucifugus*) foraging activity is higher above calm water than above turbulent water. We have found that attacking performance above the smooth water in the laboratory was the same as above rippled water in the field. These data indicate that although the water in our Field experiment was not smooth, its echo-acoustic reflection properties did not impair the bats' performance. Both literature- and our current findings thus indicate that a clearer definition of clutter is required: the surface tension of a rippled water surfaces acts as a spatial low-pass filter preventing sharp edges on the water surface. Any solid structure protruding from a water surface, however, will produce sharp edges in the surface structure. The artificial grass used in the current experiments consists mainly of such sharp edges. Also the clutter screen used by Siemers and Schnitzler (2004) and the duckweed vegetation of Boonman et al. (1998) included regular sharp edges. Thus, as soon as the background structure includes sharp edges, attacking performance of the bats is dramatically reduced. The question how sensitive the bats' sonar system is to such surface discontinuities has never been formally addressed.

The ensonification experiments showed that the grass surface created stronger echoes, especially at high frequencies, when ensonified at acute angles which represent angles used by bats hunting at low heights above a surface. It is likely that these echoes deteriorate the bats' perception of the three-dimensional shape of the target, and thus lead to the decrease in attacking and discrimination performance with decreasing target height.

Mackey and Barclay (1989) showed that both echo-acoustic clutter and the water-generated noise reduced foraging activity of the bats. By using the artificial grass, we can rule out a detrimental effect of the water-generated noise in our data. Also Siemers and Schnitzler (2004) used a "silent" clutter surface. These data indicate that echo-acoustic clutter introduced by sharp edges is much more likely to limit capture performance for most natural water surfaces.

Schnitzler and Kalko (1998) suggested that prey detection close to a clutter background is determined by the "clutter-overlap zone." This zone is defined as that prey-clutter distance at which the clutter echo overlaps with the prey echo, and thus inhibits detection. For *M. daubentonii* with a call duration of 1–1.5 ms, the clutter-overlap zone would be around 17–25 cm. Here, we show that detection performance already decreases at a distance of 35 cm to the clutter surface. Thus, a simple distinction in "Detection in the overlap free window" and "No detection in the clutter-overlap zone" is not sufficient to explain the observed hunting performance.

TARGET DISCRIMINATION

In Siemers et al. (2001b) naïve *M. daubentonii* did not spontaneously discriminate between mealworms and dummies (metal and rubber reflectors). The bats had to capture mealworms on a smooth or clutter linoleum screen. They readily captured mealworms on the smooth screen and repeatedly attacked the dummies placed in the same manner. Thus, the following search image was proposed: "small and isolated echo-reflecting objects on or above an acoustically smooth surface." Our results indicate that when challenged in a two-alternative forced-choice task bats show the ability to discriminate correctly between a mealworm and a similar-sized dummy. However, in nature bats are rarely confronted with such a defined task and it is more often the case that bats have to discriminate between different kinds of objects and prey, e.g., between leaves or little twigs and insects floating on the water surface. Thus, the suggested search image is reasonable, but not generally valid. Boonman et al. (1998) suggested that Daubenton's bats discriminate edible from inedible objects by analyzing changes in the spectrum of subsequent echoes. These changes are evoked by either moving targets, or by the bats moving around the stationary targets, when the targets have aspect-dependent reflection characteristics. In our study, both targets, the mealworm and the dummy, were moving (typically rotating slowly) and thus created changes in the spectrum over subsequent echoes. Yet the bats were still able to discriminate the mealworm from the dummy. Hence, *M. daubentonii* has to have a more sophisticated echo analysis than just analyzing a sequence of echoes which change in their spectral content over time from an echo sequence which is spectrally invariant over time.

FLIGHT PATH

Flight paths illustrated in **Figure 4** show that above water, the bats fly very close to the surface and attack the prey from below. This behavioral strategy appears to maximize the echo-acoustic enhancement effect (Siemers et al., 2001b, 2005): the lower the height of the bat above the water, the smaller the elevational angle between the direct echo from the prey to the bat and the indirect echo from the prey via the water to the bat. Moreover, when the bat flies close to the water surface, the echo-delay difference between the two echo paths is minimal. As the perceptual echo enhancement will increase with both decreasing angular difference and decreasing temporal difference, the observed flight behavior strongly supports the hypothesis that bats exploit the additional echoes from the water surface to detect and possibly also identify the prey item. As it is true for the aerodynamic ground effect, the increased acoustic impedance of the water surface facilitates the generation of additional prey echoes. Thus, the animals appear to exploit an echo-acoustic ground effect through the spatio-temporal integration of direct echoes from the prey with indirect echoes via water surface. Note, however that this enhancement comes at the expense of misleading spatial cues in the echo, because the indirect echo via the water surface signals the wrong elevation of the prey. To avoid this problem bats could resign to precedence-like auditory strategies, where accurate localization is dominated by the first sound. Precedence effects in the vertical plane have been described in human psychophysics (Litovsky et al., 1997).

Above a clutter surface, the bats flew significantly higher. Increasing the flight height will increase both the angular and temporal differences between the direct echo and the scattered indirect echo (cf. **Figure 6**) via the clutter surface. Thus, the observed increase in flight height is consistent with the hypothesis that echoes from the clutter surface are not useful for the bat and the bat tries to separate those echoes (both in terms of echo delay and elevational angle) from the direct echoes.

The bats' increased flight height could also be an indication that they fail to properly determine their height above the surface due to the increased and diffuse reflections caused by the clutter surface. As a consequence, they increase the flight height to avoid colliding with the surface as the roughness may indicate a higher likelihood of objects protruding high enough to interfere with the flight path.

Another possible explanation for this adjustment of flight height may lie in echo-acoustic flow-field information. Bhagavatula et al. (2011) showed that, based on visual flow-field information, budgerigars adjusted their flight trajectory always to be closer to that wall which evoked a smaller visual image motion. In our experimental paradigm, the echo-acoustic image motion above artificial grass would be stronger than above water. It is conceivable that such echo-acoustic flow-field information resulted in an adjustment of the flight trajectory to a larger height in the grass condition.

ECHOLOCATION BEHAVIOR

We analyzed calls from 467 sequences from the Laboratory experiment. Above grass, the bats significantly increased the peak frequency of their echolocation calls with decreasing target height. We stress that these changes in echolocation are small (~3 kHz), but due to the correlation with height and surface, are likely to be a behavioral response of the bat to the surface. Brinklov et al. (2010) showed that *Macrophyllum macrophyllum* increases its peak frequency in a cluttered environment compared to open space. Since the width of the sonar beam is mainly determined by the frequency, these changes in the bats' echolocation calls lead to narrowing of the sonar beam. Suemer et al. (2009) found that *Eptesicus fuscus* tends to increase the second harmonic of its echolocation signals when challenged with a spatial unmasking task. This suggests that a narrow sonar beam is likely to be advantageous when hunting in a cluttered environment, for it reduces the number and intensity of off-axis echoes, and thus increases signal-to-noise ratio.

Due to the downward frequency-modulated structure of the *M. daubentonii* echolocation calls, the increase of call peak frequency is likely to be correlated with decreased call duration. While, due to the strong water reflections picked up by the microphones, an analysis of temporal call parameters appears impossible in our hands, a putative decrease in call duration would further facilitate the temporal separation of prey- and clutter echoes as discussed above (see flight path section).

CONCLUSIONS

The present data provide new behavioral insight into the sophisticated hunting strategies recruited by bats hunting over water. Specifically, the data show that bats not only reliably detect targets above water but can also discriminate targets. When the water surface is covered with a clutter surface (in our case artificial grass, often vegetation in nature), the bats hunting performance, both in terms of detection and discrimination, decreased significantly with decreasing distance to the surface. Also the flight- and ensonification pattern is significantly changed: in contrast to flight over a clutter surface, the bats chose very low flight paths over water which allow for optimal spatio-temporal integration of direct echoes from the prey with indirect echoes via the water surface. This echo-acoustic strategy is analogous to trawling bats exploiting an aerodynamic ground effect (Norberg and Rayner, 1987; Aldridge, 1988; Jones and Rayner, 1991), i.e., the higher impedance of a smooth surface for the lift of an object moving above water. The suggested combination of spatio-temporal integration and precedence-like localization can be viewed as trawling bats not only exploiting an aerodynamic but also an echo-acoustic ground effect.

ACKNOWLEDGMENTS

We would like to thank Holger Görlitz for providing his MATLAB based sound analysis program. We would also like to thank Barbara Pfäffl, Andrea Lingner, Frauke Krüger, and Sara Troxell who were involved in data recording and Jupp Zimmermann who allowed us to use his property for equipment storage and provided the electricity for our technical devices in the field. We are grateful for the very constructive comments of two anonymous reviewers. This work was funded by a research grant from Human Frontiers (RGP0062/2009).

REFERENCES

Aldridge, H. D. J. N. (1988). Flight kinematics and energetics in the little brown bat, *Myotis lucifugus* (Chiroptera: Vespertilionidae), with reference to the influence of ground effect. *J. Zool.* 216, 507–517.

Barclay, R. M. R., and Brigham, R. M. (1994). Constraints on optimal foraging: a field test of prey discrimination by echolocating insectivorous bats. *Anim. Behav.* 48, 1013–1021.

Bates, M. E., Simmons, J. A., and Zorikov, T. V. (2011). Bats use echo harmonic structure to distinguish their targets from background clutter. *Science* 333, 627–630.

Bhagavatula, P. S., Claudianos, C., Ibbotson, M. R., and Srinivasan, M. V. (2011). Optic flow cues guide flight in birds. *Curr. Biol.* 21, 1794–1799.

Boonman, A. M., Boonman, M., Bretschneider, F., and Van De Grind, W. A. (1998). Prey detection in trawling insectivorous bats: duckweed affects hunting behaviour in Daubenton's bat, *Myotis daubentonii*. *Behav. Ecol. Sociobiol.* 44, 99–107.

Brinklov, S., Kalko, E. K., and Surlykke, A. (2010). Dynamic adjustment of biosonar intensity to habitat clutter in the bat *Macrophyllum macrophyllum* (Phyllostomidae). *Behav. Ecol. Sociobiol.* 64, 1867–1874.

Ciechanowski, M. (2002). Community structure and activity of bats (Chiroptera) over different water bodies. *Mamm. Biol.* 67, 276–285.

Fenton, M. B. (1990). The foraging behavior and ecology of animal-eating bats. *Can. J. Zool.* 68, 411–422.

Hedrick, T. L. (2008). Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspir. Biomim.* 3:034001. doi: 10.1088/1748-3182/3/3/034001

Jones, G., and Rayner, J. M. V. (1988). Flight performance, foraging tactics and echolocation in free-living Daubenton's bats *Myotis daubentoni* (Chiroptera: Vespertilionidae). *J. Zool.* 215, 113–132.

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

Kalko, E. K., and Schnitzler, H. U. (1989). The echolocation and hunting behavior of Daubenton's bat, *Myotis daubentoni*. *Behav. Ecol. Sociobiol.* 24, 225–238.

Lawrence, B. D., and Simmons, J. A. (1982). Measurements of atmospheric attenuation at ultrasonic frequencies and the significance for echolocation by bats. *J. Acoust. Soc. Am.* 71, 585–590.

Litovsky, R. Y., Rakerd, B., Yin, T. C. T., and Hartmann, W. M. (1997). Psychophysical and physiological evidence for a precedence effect in the median sagittal plane. *J. Neurophysiol.* 77, 2223–2226.

Mackey, R. L., and Barclay, R. M. R. (1989). The influence of physical clutter and noise on the activity of bats over water. *Can. J. Zool.* 67, 1167–1170.

Norberg, U. M., and Rayner, J. M. V. (1987). Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 316, 335–427.

Rydell, J., Miller, L. A., and Jensen, M. E. (1999). Echolocation constraints of Daubenton's Bat foraging over water. *Funct. Ecol.* 13, 247–255.

Schnitzler, H. U., and Kalko, E. K. V. (1998). "How echolocating bats search and find food," in *Bat Biology and Conservation*, eds T. H. Kunz and P. A. Racey (Washington, DC: Smithsonian Institution Press), 183–196.

Schnitzler, H. U., and Kalko, E. K. V. (2001). Echolocation by insect-eating bats. *Bioscience* 51, 557–569.

Schnitzler, H. U., Moss, C. F., and Denzinger, A. (2003). From spatial orientation to food acquisition in echolocating bats. *Trends Ecol. Evol.* 18, 386–394.

Siemers, B. M., Baur, E., and Schnitzler, H. U. (2005). Acoustic mirror effect increases prey detection distance in trawling bats. *Naturwissenschaften* 92, 272–276.

Siemers, B. M., Dietz, C., Nill, D., and Schnitzler, H. U. (2001a). *Myotis daubentonii* is able to catch small fish. *Acta Chiropterol.* 3, 71–75.

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

Siemers, B. M., and Schnitzler, H. U. (2004). Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. *Nature* 429, 657–661.

Suemer, S., Denzinger, A., and Schnitzler, H. U. (2009). Spatial unmasking in the echolocating Big Brown Bat, *Eptesicus fuscus*. *J. Comp. Physiol. A* 195, 463–472.

Todd, V. L. G., and Waters, D. A. (2007). Strategy-switching in the gaffing bat. *J. Zool.* 273, 106–113.

Von Frenckell, B., and Barclay, R. M. R. (1987). Bat actibity over calm and turbulent water. *Can. J. Zool.* 65, 219–222.

Warren, R. D., Waters, D. A., Altringham, J. D., and Bullock, D. J. (2000). The distribution of Daubenton's bats (*Myotis daubentonii*) and pipistrelle bats (*Pipistrellus pipistrellus*) (Vespertilionidae) in relation to small-scale variation in riverine habitat. *Biol. Conserv.* 92, 85–91.

Weissenbacher, P., and Wiegrebe, L. (2003). Classification of virtual objects in the echolocating bat, *Megaderma lyra*. *Behav. Neurosci.* 117, 833–839.

Wund, M. A. (2005). Learning and the development of habitat-specific bat echolocation. *Anim. Behav.* 70, 441–450.

Zahn, A., and Maier, S. (1997). Hunting activity of bats at streams and ponds. *Z. Säugetierkd.* 62, 1–11.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 14 December 2012; accepted: 16 March 2013; published online: 05 April 2013.

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

This article was submitted to Frontiers in Integrative Physiology, a specialty of Frontiers in Physiology.

5 General Discussion

This cumulative thesis aimed to provide new insights into spatiotemporal integration in bat sonar. In order to meet this objective, different aspects of spatiotemporal integration were addressed, mainly by emphasizing on behavioral studies. The integration of sensory information over time in general allows organisms to build up an internal representation of the environment. This allows more reliable object identification, object detection, localization and better estimation of other aspects within the environment (Lewicki, Olshausen *et al*. 2014). In echolocating bats, the encoding of spatiotemporal echo information is an important feature of the bat auditory system. Like in other sensory systems it leads to an internal representation of the 3-D environment and furthermore, allows skilled navigation as well as foraging under low light conditions and in complete darkness (cf. chapter one, general introduction). The interaction of spatiotemporal acoustic cues is not only crucial for echo-acoustic scene perception but is also important for object perception itself.

Object size as spatial object property

In the first study of this thesis the encoding of the spatial characteristics of object size as important physical object property was addressed. To this end, the perceptual strategy and neural representation of the bat sonar exploration of object width were investigated using a combined psychophysical and electrophysical approach. We aimed to quantify the relevance of echo intensity and sonar aperture for the perceptual evaluation of object width. Another objective was to find a possible neuronal correlate that could serve to explain the behavioral performance. In three psychophysical active acoustic virtual-object (VO) playback experiments it was tested which acoustic cue (echo intensity or sonar aperture) echolocating bats employ for the estimation of object width.

The results showed no difference when only the sonar aperture was varied compared to the experiment where echo intensity and sonar aperture were physically correctly varied together. These findings were supported by two controls that showed that the bats indeed used the differences in sonar aperture for VO discrimination and did not rely to the differences in the object's azimuthal position of the edges of the VOs.

Furthermore, we found a threshold of 5 dB for the discrimination of echo-intensity differences. It was discussed that the bats were able to discriminate echo-intensity differences in the experiments, but did not require this cue for width discrimination. The findings of the psychophysical experiments were supported by the electrophysiological results. Here a population of units in the inferior colliculus and the auditory cortex were discovered that responded strongest to echoes from VOs with a specific sonar aperture, independent of variations in overall echo intensity. These units could serve to explain the psychophysical performance in both, the Sonar aperture-and the Width experiment. Thus, the study provided psychophysical and electrophysiological evidence that, besides echo intensity, the sonar aperture is another, highly relevant perceptual cue. We proposed that bats exploit the directional characteristics of their outer ears to assess the sonar aperture of objects. The cues can be monaural spectral cues or binaural echo disparities (as suggested by Holderied and von Helversen 2006). The sonar aperture was discussed to be especially relevant for the discrimination of relatively wide objects (ø wider than 30°, ~ 58 cm at 1 m distance). Moreover, overall intensity cues were discussed to be better suited for the discrimination of smaller objects (e.g. Sumer, Denzinger *et al*. 2009) that preclude the use of the sonar aperture due to limitations of the auditory spatial directionality of the bats.

In a field study of Goerlitz *et al*. (2012) the sonar coding of object size was addressed, too. It was tested how echo intensity (echo amplitude) contributes to the perception of object size in free flying bats. In the study it was assumed that echo intensity encoded object size as declared by Simmons and Vernon (1971). They hypothesized that: “…bats use echo amplitude to perceive object size and predicted that adding an echo of larger amplitude (i.e., of an object with larger reflective area, resulting in larger target strength) to the real object will result in additional changes in the position of the flight paths compared to the real object alone.” The results showed that bats evaded a small real object (40 cm^2) but not a much larger VO (>>700 cm^2). Since the VO was only simulated with one small speaker, and not an array of speakers, the VO lacked the sonar aperture information. They consequently hypothized that:”… this mismatch of spectro-temporal and spatial echo features caused the lack of virtual object evasion and suggests that the sonar aperture of object echoscapes contributes to the sonar coding of object size.” This is in good accordance with our results. The results of Goerlitz *et al*. (2012) in combination with our findings allow further insights of how object size is encoded in bat sonar. But there are still many questions not answered yet: for example, the object height and depth dimensions were not particularly addressed in our study. Of course the employed virtual objects (VOs) had a certain object height that was determined by the emission pattern of the speakers. But object height was not addressed in terms of vertical-sonar aperture perception and its neural representation. Since we simulated only two-dimensional VOs, they completely lacked object depth. Here bats would have to perform a special case of spatiotemporal integration: surface area and echo intensity co-vary and increase with increasing object size. But object depth also leads to a temporal expansion which "… corresponds to a compression of the spectral interference patterns." (Firzlaff, Schuchmann *et al*. 2007).

Taken together, the first study provides insight into how the spatial acoustic characteristics of the width dimension are perceived, which echo acoustic cues can be exploited and how these are neurally encoded. It furthermore supports the theory that the mammalian central nervous system (CNS) is principally aiming to find modality independent representation of object properties. We therefore suggested that the sonar aperture, as an echo acoustic equivalent of the visual aperture (also referred to as the visual angle), could be one of these object properties.

Size constancy in bat sonar

The visual system must deal with the fact that a change of object distance also leads to a change of visual aperture and thus retinal image size. Due to the integration of the size and distance cues humans are able to identify the absolute object size in terms of 'size constancy' perception (cf. chapter 3, introduction). It is believed to reflect an automatic re-scaling of perceived object size with perceived distance. But, as distance information is not explicitly encoded in the visual system, higher-order computational mechanisms are needed e.g. to prevent visual illusions. In bat sonar it was found that object distance (cf. chapter 1, general introduction and chapter 3, introduction) and object width (as stated above) are accurately perceived and explicitly encoded.

Consequently, in the second study the perceptual interaction of size-and distance information (and thus a possible integration of spatial and temporal information) in bat sonar were addressed. We tested whether bats show the ability to combine spatial-and temporal cues to identify absolute object width in terms of sonar size constancy (SSC).

Again, using the same setup and species as in the first study, bats were trained to reliably discriminate two simple VOs that only differed along the width dimension (aperture and echo intensity). Later, test trials were randomly interspersed using four different experimental conditions with VOs that differed in aperture and distance to achieve SSC (cf. chapter 3, figure 2). We tested the hypothesis that bats may combine object-distance and sonar-aperture information to explicitly encode the absolute width of a sound reflecting object independent of distance. In a control experiment it was tested whether the simulated VO distances of all experiments were in the perceptual range of the bats.

As a result of this study the importance of sonar aperture as spatial cue for object perception was re-confirmed. Furthermore, it could be shown that the presented VO distances and thus the temporal information were well perceivable and discriminable by the bats. But the animals did not spontaneously assign absolute width by combining the provided spatial-and temporal cues. In the following pages I will extend the discussion of the second study (chapter 3) and readdress certain aspects as new studies provide new insights into these topics. Furthermore, I will address size constancy in other sensory modalities.

Size constancy in different sensory modalities:

Visual size constancy

Object perception and context-independent recognition in complex environments requires likewise complex neural computation. In visually oriented animals, size constancy in terms of the identification of objects irrespectively of distance dependent changes of the retinal image size, is one example. Together with other comparable abilities (e.g. rotation invariance that allows angle independent object identification (von der Emde, Behr et al. 2010, Okamura, Yamaguchi et al. 2014)) it suggests that: "…the animals establish some form of perspective-independent representation of an object's visual image in their brains, which allows object recognition even if the retinal image of the object has changed strongly". (von der Emde, Behr et al. 2010). Visual size constancy was found in different animal groups and species. For instance, in mammals it was not only shown in humans (cf. chapter 1, general introduction) but also in rhesus monkeys (*Macaca mulatta*) (Humphrey and Weiskrantz 1969, Ungerleider, Ganz *et al*. 1977, Dobbins, Jeo *et al.* 1998) and the crab-eating macaque (*Macaca fascicularis*) (Dobbins, Jeo *et al.* 1998). Dobbins *et al.* addressed in monkeys where in the visual pathway distance information and retinal size information are combined and how it is integrated. They found that: "… size and distance are combined from the outset in the visual cortex and could provide, in a distributed form, the necessary elements to achieve size constancy." (Dobbins, Jeo *et al*. 1998). Note that visual size constancy was not investigated in bats and dolphins yet. Besides mammals it was shown in fish (Douglas, Eva *et al*. 1988), amphibians (Ewert and Burghagen 1979) and birds (Pastore 1958).

But size constancy is not limited to vision alone. In the next passages I will give a short overview of the occurrence of size constancy in other sensory modalities.

Haptic size constancy

The range of the somato-sensory system is normally limited to the space we reach with our extremities. To overcome this limitation visually handicapped people often use white canes to explore their more distant environment. This can be seen as extension of the haptic sense. In this case, "…haptic parallels to visual angle and size constancy appear." (Hanley and Goff 1974). By moving the stick, one creates a haptic angle. This angle was described as direct equivalent to the visual angle/aperture changing with object distance. The study of Hanley and Goff addressed the accuracy of size perception conveyed by a stick. Within this context haptic size constancy was tested.

To do so sighted and visually impaired test subjects used a stick to haptically explore a gap that was out of their normal haptic reach. Compared to a nearby gap that was haptically explored with the index finger, gap width was likely to be underestimated. Nevertheless, size constancy was found to be relatively high using the extension. This was confirmed by gap estimation at intermediate and far distances. Note, however, that it is not clear to what extent acoustic cues were used for gap judgments.

Size constancy in electrolocation

Another example is found in weakly electric fish. They use active electrolocation for orientation and object detection even in complete darkness. The African mormyrid fish *Gnathonemus petersii* is even able to detect object properties as material, volume and 3-D shape. Furthermore, it is able to identify and discriminate object size and distance (e.g. von der Emde, Schwarz *et al*. 1998, von der Emde and Schwarz 2000, von der Emde 2004, von der Emde 2006, von der Emde and Fetz 2007, Lewicki, Olshausen *et al*. 2014) which was already described above as the prerequisite for size constancy. In an active electrolocation experiment von der Emde *et al*. (2010) could indeed show size constancy in one individual of this species. For the presented objects (metal cubes of 2 x 2 x 2 and 3 x 3 x 3 cm^3) it was significant to a distance of 4 cm. Note that the electrolocation range is normally less than 12 cm (e.g. von der Emde 1999).

Sonar size constancy

Recently a study of Milne *el al.* (2014) found that an early blind, human Expert-Echolocator (EE) showed SSC in an psycho-acoustical classification experiment with real objects. Since it was shown that human echolocators can reliably perceive and discriminate echo-acoustic object size and distance (e.g. Rice and Feinstein 1965 A, Rice, Feinstein *et al*. 1965 B, Teng and Whitney 2011, Kolarik, Cirstea *et al*. 2014), the authors also combined both, to achieve sonar size constancy. To do so real objects (circles and horizontally oriented rectangles) of three different absolute sizes were presented at varying distances in azimuth to the EE and a control group. The control group included blind and sighted blindfolded subjects with no previous echolocation experience. The differently shaped objects categorized as small (circle: 20 cm diameter, rectangle: 10.5 cm height, 30 cm width) medium (twice the size of the small object), and large (thrice the size of the small object). The distances were chosen proportionally to object-size relationships (near location: 40 cm, middle location: 80 cm, far location: 120 cm). The test subjects were allowed to explore the objects haptically but not echo-acoustically before the experiments. As control experiments, echo-acoustic size (i.e. area in degrees of the sonar aperture and echo intensity), shape and distance perception of each individual was tested. To achieve SSC only the small and large objects were presented. The small objects at the near location provided the same aperture and intensity information as the large objects that were presented at the far location. In all experiments the test subjects had to indicate their object perception verbally (e.g. size, shape, and distance). The results showed that, unlike the control group, the EE was reliably able to determine the absolute size of objects independent of the distance they were presented at. Thus, the EE was most likely able to perform spatiotemporal integration of echo information in order to solve the task, suggesting size constancy in human echolocation. Note that none of the controls was performing above chance level in any experiment.

Milne *et al.* proposed that: “The current findings suggest further parallels between vision and echolocation, in that both modalities show size constancy. This suggests that similarities in brain activity may also signify similarities in terms of behavioral principles.”(Milne, Anello *et al.* 2014) These similarities in brain activity refer to different new studies that revealed possible visual brain areas for echolocation in humans (Thaler, Arnott *et al*. 2011, Arnott, Thaler *et al*. 2013, Thaler, Milne *et al*. 2014).

Based on their recent findings Milne *et al*., recommended further that, though our study (Heinrich and Wiegrebe 2013) could not provide evidence for this phenomenon in echolocating bats, this topic should be readdressed and extended to other echolocating organisms. This is reasonable since it is always possible that applied techniques or stimuli were not optimally suited for a new scientific research topic as SSC. Furthermore, the fact that size constancy was found in different animal groups and in different sensory modalities suggests that size constancy could be a general, modality independent mechanism for object normalization that e.g. allows object recognition.

Implications for future bat studies readdressing SSC:

In order to readdress SSC in bats, future studies should take the following considerations into account:

Multimodal object perception

A recent study of Boonman *et al*. (2013) addressed the role of vision and echolocation in bats. Since many echolocating bat species start foraging on insects before darkness, it is still unclear when and to what extent vision is employed by bats. They discussed the advantages of vision compared to echolocation in terms of more spatial information per time and a higher angular resolution and hypothized that “…bats constantly integrate information acquired by the two sensory modalities”.(Boonman, Bar-On *et al.* 2013). In the study they compared visual-and echo-acoustic detection ranges of two insectivorous bat species (*Pipistrellus kuhlii* and *Rhinopoma microphyllum*) that use different foraging strategies. They found that in both species echolocation is better suited for small object detection (insects) at low to intermediate light levels (1-10 lux). Vision was suggested to be beneficial for large far away objects (landscapes, trees etc.). They proposed that: "…during evolution, echolocation was refined to detect increasingly small targets in conjunction with using vision."(Boonman, Bar-On *et al.* 2013). Taken together these results would fit into the concept of multisensory integration (e.g. Deneve and Pouget 2004) in which different bits of information are combined from different sensory modalities in order to form a reliable picture of our surroundings (environment and body). Despite the advantages of echolocation over vision at near ranges in this specific analytical approach they could not exclude that the use of vision can vary between species and foraging situation. Gutierrez *et al*. (2014) for example, recently addressed the effect of light intensity on food detection in a frugivorous phyllostomid bat (*Artibeus lituratus*). The study showed the importance of light intensity on foraging behavior, also enabling the use of visual cues for food detection at night. It furthermore revealed that visual cues were important for object discrimination. Together with the argument that the lack of SSC could result from the bats relying on different modalities to extract size information at different distances (cf. chapter 3, discussion), these results should be considered for the experimental design of future studies as well. Here objects should be presented at distances where only echolocation is employed. As these distances are probably species or family specific, one should consider this as well.

Independence of size-and distance-perception?

Alternatively, following the argumentation of Haber and Levin (2001) it is conceivable that size and distance information are perceived independently (cf. chapter 3, discussion). They argued that object perception needs object familiarity that was acquired from the past, whereas distance perception should develop early in life and come along with the improvement of locomotors abilities. We discussed that the absence of SSC in our bats could be based on the presented virtual objects, that were probably lacking a behavioral significance to activate 'object-oriented' perception (Firzlaff, Schuchmann *et al.* 2007) (in terms of familiarity). We concluded that familiarity with a behaviorally relevant, conspicuous object is required for size constancy. Taken together, the VOs lacking object familiarity and behavioral relevance could have led to the instable performance in the standard condition during data-acquisition periods in which test trials were interspersed (cf. chapter 3, results).

To prevent this in future studies, the experimenter should choose objects that meet these criteria. In addition, the attention demands of the given tasks could be increased by introducing differently shaped objects (e.g. Milne, Anello *et al.* 2014).
Note that this argumentation does principally not contradict the results of Milne *et al*.: In the study all test subjects were allowed to explore the objects haptically before the experiments. But only the EE recognized object shapes and sizes via echolocation above chance level. Whether this performance resulted from a cross-modal integration between haptics and echolocation, or simple object recognition of familiar shapes via echolocation, remains unclear. Furthermore, the EE was the only one who could significantly discriminate object distances. Comparable to bats, in daily life distance information was used by the EE for acoustically guided locomotion (e.g. navigation and orientation). Consequently he was the only test subject who met all criteria of Haber and Levin for size constancy.

Possible implications of SSC in a human expert echolocator for future studies

The verification of SSC in a human expert echolocator shows that SSC in mammals is principally possible. Nevertheless, even though one EE showed SSC, the transfer from the human percept to bats and other echolocators has still to be handled with great care. In the next passages I will revisit the work of Milne *et al.* (2014) and the possible implications for potential future studies in human echolocation and in other echolocating species. The EE already lost his eyes at early childhood and used echolocation for years when performing in the experiments. But it is not clear whether SSC was based on the experience alone or probably on subsequent extensive structural and functional changes in terms of compensatory plasticity and cross-modal reorganization in the corresponding brain areas (e.g. of the occipital cortex) (reviewed in Kolarik, Cirstea *et al*. 2014, Kupers and Ptito 2014, Lazzouni and Lepore 2014). Here the question arises of which nature that reorganization would be, to what extent visual pathways are probably exploited, and whether this indeed caused SSC. Since the control group did not show SSC, it is still unresolved if the controls (sighted and blind individuals) can show SSC e.g. with extensive training. Furthermore, additional blind human expert echolocators should participate in the experiments to exclude that this phenomenon is restricted to a single individual. Moreover, it remains unknown which acoustic cues were actually exploited by the EE.

Multimodal size-constancy perception in echolocation and vision?

With the study of Milne *et al*. (2014) another research question arises: can visual and sonar size constancy be found in one and the same individual in terms of a multimodal representation? To address this question sighted human expert echolocators (as introduced in Thaler, Milne *et al*. 2014) should be tested on SSC as well. Similar to the experiments of Thaler *et al*. the modified experiments should be accompanied by fMRI in order to reveal what regions are employed by the experimental groups.

Like bats, echolocating dolphins are capable of object-size, shape and distance discrimination in absence of visual input (e.g. Nachtigall 1980, Au and Simmons 2007). Consequently future studies could address SSC in dolphins as well.

Since bats and dolphins use vision and have a well-developed visual system (e.g. Suthers and Wallis 1970, Pettigrew 1988, Harley, Roitblat *et al*. 1996, Winter, Lopez *et al*. 2003, Tomonaga, Uwano *et al*. 2014), future studies could also address visual size constancy in both echolocators. At the current state of research, the multimodal nature of size constancy in echolocation and vision in different echolocating species cannot be excluded.

Preconditions for cross-modal size constancy between echolocation and vision

Given that multimodal size constancy would be verified in bats and/or dolphins, it is conceivable to go a step further: Recently Tomonaga *et al*. (2014) tested visual perception on a visual-matching task using two-dimensional differently shaped objects in bottlenose dolphins (*Tursiops truncatus)* and compared the results with data from chimpanzees and humans. The results clearly show that "…the visual world is perceived similarly by the three species of mammals, even though each has adapted to a different environment and has differing degrees of dependence on vision."(Tomonaga, Uwano *et al.* 2014). Moreover, it was revealed in several studies (e.g. Pack and Herman 1995, Harley, Roitblat *et al*. 1996, Herman, Pack *et al*. 1998, Pack, Herman *et al*. 2002) that bottlenose dolphins show multi-modal object representation (vision and echolocation) and cross-modal identification of familiar objects. In contrast, cross-modal object recognition in bats could not be proven so far (Grunwald 2004). Therefore, it is conceivable that humans and dolphins are probably better suited to test cross-modal size constancy in distant future studies.

Spatiotemporal integration in bat sonar in complex environments

Under natural conditions, bats (as well as other echolocators) rarely encounter such simple acoustic situations as in our two laboratory studies. Under field conditions bats cope with multiple variables that change the acoustic characteristics of objects: enhance, attenuate or mask them (chapter 1, general introduction). When hunting on the wing, the bat auditory system has to process rapid changes of spatiotemporal information of continuously changing spatial layouts. In order to achieve a proper internal representation of the surroundings the bat needs to update and integrate the information acquired from their own sensor movements and/or motor actions. In the third study we addressed an example of spatiotemporal integration in bat sonar in a natural and naturalistic context by using a combined laboratory and field approach. In this study the bats encountered a much more complex situation than in the first two studies for they were flying through a real environment. Additionally, the presented real objects were also not static as they slowly rotated along their axis. In the experiments the foraging behavior of the European trawling bat species *Myotis daubentonii* and the possible employment of the water surface as an acoustic mirror were addressed. We tested and quantified the effect of different surfaces (water, PVC, and artificial grass matting) with different reflection properties (naturally smooth, artificially smooth, and clutter surface) and therefore, possible enhancement or masking characteristics on object detection and discrimination performance. Thus, the bats had to detect a mealworm presented above these different surfaces and discriminate it from an inedible PVC disk (dummy). To test the influence of surface type on performance the objects were presented at different heights. We found that at a 50 cm object height, target detection was generally not influenced by any surface type. It was shown that bats attacked less frequently at low heights above the clutter surface while they still attacked objects above the smooth surfaces.

Object discrimination

We showed that the animals could discriminate between both objects. In contrast to the performance of the bat(s) in the field, the discrimination performance of the bats under laboratory conditions showed a significant decrease of discrimination performance above the clutter surface (grass) with decreasing object height. It is conceivable that the good discrimination performance above clutter under field conditions could indicate that the exact clutter tolerance threshold of *M. daubentonii* is also depending on the motivation of the individual, e.g. due to an increased food pressure in the field.

Since the objects were rather small but of similar size, object discrimination was unlikely to be based on sonar aperture and intensity. But, depending on aspect angle, disk and mealworm created different spectral patterns that changed over time. A study of Simmons and Chen (1989) addressed the possible acoustic cues that could underlie such a discrimination performance. They ensonified a mealworm and a slightly smaller disk than ours (ø12.5 mm, 0.5 mm thickness vs. ø20 mm,1 mm) from different angles and analyzed the returning echoes. In the spectra they found different angle dependent peaks and notches and concluded that a peripheral auditory spectral representation enables airborne target discrimination. This was based on the assumption that: ”From considerations of the tuning of peripheral channels used for echo reception (Suga and Jen 1977), the auditory system of FM bats probably first encodes the target's range profile in spectral terms if the time separation of the underlying echo components is less than about 300 µs (Beuter 1980, Simmons, Kick *et al*. 1988, Simmons, Freedman *et al*. 1989), which is the case for mealworms and disks.” Note that “range” in this citation represents the spatial object properties, not target distance. The 300 µs value is the mean integration threshold that was measured by Simmons *et al*. (1989) for *Eptesicus fuscus*. But the threshold was not obtained with the classic click pair paradigm (Zwislocki 1960) (cf. chapter 1, general introduction) which makes it hard to compare with other thresholds for temporal integration. Nevertheless, similar thresholds were found in *Megaderma lyra* (~ 200-300 µs) (Wiegrebe and Schmidt 1996, Weissenbacher, Wiegrebe *et al*. 2002). This suggests that these integration times are realistic for bats. In a doctoral thesis (Weißenbacher 2003) it was suggested that despite the presence of temporal object information, *Megaderma lyra* only employs spectral cues for object identification of small objects (less than 6.6 cm). This is (*inter alia*) due limitations of cochlea filters that preclude the temporal resolution of sounds that are separated by less than 200 µs. Note that integration times for trawling bat species were not investigated yet.

Robust behavioral solutions for optimization of spatiotemporal integration in trawling bats?

The reconstructed flight paths were discussed in the background of possible behavioral strategies in order to change and thus optimize the spatiotemporal information to obtain a better acoustic ‘view’ on the object of interest. They revealed that bats attacked objects from above when flying over the clutter surface and from below when flying over a smooth surface.

Echo acoustic ground effect

Flying close to a smooth surface seems to maximize an echo-acoustic enhancement effect described by Siemers *et al*. (Siemers, Stilz *et al*. 2001, Siemers, Baur *et al*. 2005). The closer the bat flies to a reflective surface, the smaller the elevational angle between the direct echo from the prey to the bat and the indirect echo from the prey via the water to the bat. Additionally, the echo-delay difference between the two echo paths (direct and indirect) is minimized. The perceptual enhancement increases with decreasing angular-and temporal difference. The flight performance suggested that the bats probably used the additional prey echoes reflected via the water for object detection and eventually identification. We concluded that the bats probably exploited an echo-acoustic ground effect through the spatiotemporal integration of these direct and indirect echoes.

Precedence-like strategy in trawling bats

The exploitation of the echo-acoustic ground effect alone leads to misleading spatial cues (indirect echoes signaling the wrong elevation of the prey). But the auditory system of mammals can suppress misleading spatial cues introduced by reflections. For example, in a reverberant room a sound source creates a sound that is reflected many times. Compared to the direct sound the successive reflections are (*inter alia*) temporally delayed and come from various directions when impinging on the listeners ears. The neural process that fuses the direct sound and its reflections into a single image is termed the ‘precedence effect’ or the ‘law of the first incoming wavefront’ (Wallach, Newman *et al*. 1949). It includes that the location of the perceived sound source is ruled by direct sound that reaches the ears first. It describes : “…a group of phenomena that are thought to be involved in resolving competition for perception and localization between a direct sound and a reflection.” (Litovsky, Colburn *et al*.

1999). We concluded that the bats probably employed a precedence-like strategy by using only the first (and therewith direct) echo for object localization. Note that it is unclear how this strategy could be neurally encoded and to what extent cognitive processes (cf. chapter 1, general introduction) are involved. In a combined psychophysical (human listeners) and electrophysiological approach (cats) of Litovsky *et al*. (1997) it was shown that the precedence effect works in the azimuth and the vertical (sagittal) plane. They concluded that in azimuth it is encoded by binaural localization cues whereas in the vertical plane (that was relevant in our experiments) it is based on spectral cues.

Recently a study of Weinbeer *et al*. (2013) showed similar behavioral results in the only phyllostomid trawling bat species, *Macrophyllum macrophyllum.* Under laboratory conditions bats had to detect mealworms suspended above or on water at different distances to a clutter surface (water plants). Prey detection decreased with decreasing distance to clutter, too. It was only precluded when the prey item was completely buried into clutter. The reduced prey detection and capture success was discussed to depend on echo overlap due to clutter echoes and the lack of an echo acoustic ground effect. The authors suggested a higher clutter tolerance (≥ 10 cm distance) than in *M. daubentonii* (clutter interference began at 35 cm above grass) and other species (Siemers and Schnitzler 2004). Weinbeer *et al*. pointed out that the design of the echolocation calls probably enabled the integration of echo information across several harmonics and lead to the higher clutter tolerance.

In our call analysis we found a significant increase of call peak frequency with decreasing target height and discussed it regarding a narrowing of sonar beam that can be advantageous when hunting close to clutter. The downward frequency-modulated structure of the echolocation calls together with the increase of peak frequency is likely to be correlated with the decreased call duration. This could possibly lead to an even greater separation of object-and scattered clutter echoes as described above, but could not be shown with the current data.

Flight path above clutter

To improve object detection and discrimination over the clutter surface the bats approached objects from above, presumably to increase spatial (elevation angle) and temporal differences between direct and indirect echoes. This adjustment in flight behavior was also discussed in terms of echo-acoustic flow-field information as equivalent to optic flow field, e.g. in budgerigars (Bhagavatula, Claudianos *et al*. 2011). These birds change their flight path always to be closer to that wall which evoked a smaller visual image motion. We argued that in bats the echo-acoustic image motion above grass would be stronger than above water, leading to the increased flight height.

The results of our third study hint towards robust solutions for perceptual problems that appear under natural conditions. Here we suggested that the bats exploited the reflection properties of the water surface to increase foraging efficiency. Likewise we discussed different strategies to avoid clutter interference that can lead to misleading spatial cues or the masking of objects. But in order to qualify the impact of the described strategies additional experiments should be performed. Here especially the actual effects on temporal separation for clutter avoidance should be addressed. A detailed study of the temporal call parameters and the returning echoes seem to be important to verify the temporal separation of prey and clutter echoes. Future studies could also address possible neural substrates for the exploitation of water surfaces for object detection and identification.

Neural basis of spatiotemporal integration in bat sonar

To understand how bats perform spatiotemporal integration for physically correct object and/or echo-acoustic scene perception, one also needs to understand the underlying neuronal mechanisms. A study of Hoffmann *et al*. (2013) addressed spatiotemporal contrast enhancement and feature extraction in the bat auditory midbrain and cortex. The study was carried out with *P. discolor* as it was the case in our first two studies. It investigated the interdependence of temporal-and spatial parameters and how these are neurally reflected. In the study a large echo acoustic space was simulated by presenting long sequences of random spatiotemporally arranged stimuli. The resulting spatiotemporal response properties of units in the IC and AC were recorded. Interestingly the study revealed that the processing of spatiotemporal information in the IC and AC seem to serve different purposes: in the IC spatiotemporal contrast enhancement serves echo-feature extraction whereas the AC reveals the results of this process e.g. by "…high selectivity and well as behavioral task oriented recombination of the extracted features."(Hoffmann, Warmbold *et al.* 2013). Recently another study (Bartenstein, Gerstenberg *et al*. 2014) addressed the echo-acoustic-flow field that is induced by self-motion (flight) in FM bats. To do so they presented call/echo sequences that simulated laterally object passing in the horizontal plane. They simulated three different passing distances (0.2, 0.8, and 1.4 m) to the VO and two velocities (3 and 6 ms^{-1}). Flight direction and call emission rate (12 Hz) was kept constant; excluding grouped calls (e.g. buzzes). Within the different sequences, depending on the passing distances, echo delay, sonar aperture (reflection angle), call and echo intensity (amplitude), as well as echo spectral content changed proportionally. The responses of cortical units were recorded. The results suggested that the representation of target range, in terms of the computational map for echo delays in the bat auditory cortex (Wenstrup and Portfors 2011), is dynamically modified by the echo-acoustic flow field. Bartenstein *et al*. (2014) found that: "…neurons in the auditory cortex of the bat *Phyllostomus discolor* encode echo-acoustic flow information on the geometric relation between targets and the bat's flight trajectory, rather than echo delay per se. Specifically, the cortical representation of close-range targets is enlarged when the lateral passing distance of the target decreases. This flow-dependent enlargement of target representation may trigger adaptive behaviors such as vocal control or flight manoeuvres."(Bartenstein, Gerstenberg *et al*. 2014).

Since our first two studies only addressed static spatial layouts in which the essential VO representation was limited to the starting position, the impact of the dynamic modification of the computational echo-delay map caused by an echo-acoustic flow field seems negligibly small. But it is also conceivable that in bats SSC, as mechanism for object normalization, could be evoked by creating echo-acoustic flow-field information as it appears during flight and thus locomotion.

In human vision it was found that size constancy:"… is enhanced by information from observer displacement, and, more generally, processes thought to be purely perceptual may have unexpected components related to action."(Combe and Wexler 2010). This indicates a direct impact of optic flow on size constancy.

In our third study the potential use of flow field information was discussed with respect to the adjustment of the flight trajectory to a larger height in the clutter condition. The increasing object representation, with increasing velocity, on the cortical surface, was suggested by Bartenstein *et al*. (2014) to trigger situation-dependent motor behavior, e.g. call duration, timing and flight manoeuvres. It is therefore conceivable that the increased cortical representation of the nearby clutter surface led to the increased flight height of our bats to avoid collisions and/or to separate spatial and temporal object information. For the trigger, it was suggested that:”…this could be implemented in the neural circuitry as a simple threshold mechanism, by which a specific motor pattern is initiated whenever cortical activity in the map of target range exceeds a critical amount. Such a mechanism would be somewhat similar to what has been described for visual looming (Sun and Frost 1998): neurons in the nucleus rotundus of birds start to fire if the increase of the visual angle subtended on the retina by an approaching object exceeds a certain amount within a certain time. Unlike the visual system, in the auditory system the peripheral sensors do not have a two-dimensional layout. Therefore, a threshold mechanism of this kind would necessarily have to be implemented centrally, that is, as the dynamical increase of activity in a cortical map.“ (Bartenstein, Gerstenberg et al. 2014). The studies show that the dynamic interaction of spatiotemporal echo information is neurally reflected in the bat auditory system (IC and AC), suggesting a direct impact of the perceptual or behavioral task. Future studies should consider the potential impact of an echo-acoustic flow field on different aspects of spatiotemporal integration in bat sonar.

6 Conclusions

Spatiotemporal integration of echo information in bats is crucial for the internal representation of 3-D environments and the objects within. We found that object width as important size relevant dimension is reliably perceived by its sonar aperture and neurally represented both in the auditory midbrain and cortex. This is also true for object distance as it is encoded by echo delay. Therefore, we tested if the bats could spontaneously combine this spatial and temporal object information to identify absolute object width independent of the simulated distance. As a result sonar size constancy seems to lack in bat sonar. This can be due a perceptual bias as bats could use different sensory modalities at different distances or the nature of the presented virtual objects. New scientific results strongly suggest that sonar size constancy should be readdressed in bats and other echolocators. We also addressed the dynamic interaction of spatiotemporal information in complex environments and found different behavioral strategies that could solve perceptual problems for object detection and discrimination in presence of clutter. This was reflected in condition-specific changes of flight height and sonar emission parameters. Flying closely to the water surface would allow for optimal spatiotemporal integration of direct echoes from the prey with indirect echoes via the water surface (echo acoustic ground effect) but would create misleading spatial cues. We therefore suggested the use of a second, precedence-like strategy by only using spatial information from the first (direct) echo. Flight height increased above clutter, possibly to separate direct object echoes and clutter interference, or due to echo-acoustic flow-field information. This thesis furthermore suggests that the dynamics of echo-acoustic flow-field information could be more important for spatiotemporal integration in echolocating bats than previously thought.

References

Aizpurua, O., J. Aihartza, A. Alberdi, H. J. Baagoe and I. Garin (2014). "Fine-tuned echolocation and capture-flight of Myotis capaccinii when facing different-sized insect and fish prey." Journal of Experimental Biology **217**(18): 3318-3325.

Arita, H. T. and M. B. Fenton (1997). "Flight and echlocation in the ecology and evolution of bats." Trends in Ecology & Evolution **12**(2): 53-58.

Arnott, S. R., L. Thaler, J. L. Milne, D. Kish and M. A. Goodale (2013). "Shape-specific activation of occipital cortex in an early blind echolocation expert." Neuropsychologia **51**(5): 938-949.

Ashmore, J. (2008). "Cochlear outer hair cell motility." Physiological Reviews **88**(1): 173-210.

Au, W. (2004). A comparison of the sonar capabilities of bats and dolphins. Echolocation in Bats and Dolphins. J. Thomas, C. Moss and M. Vater. Chicago, University of Chicago Press.

Au, W. W., P. W. Moore and D. A. Pawloski (1988). "Detection of complex echoes in noise by an echolocating dolphin." The Journal of the Acoustical Society of America **83**(2): 662-668.

Au, W. W. L. and J. A. Simmons (2007). "Echolocation in dolphins and bats." Physics Today **60** (9): 40-45.

Aytekin, M., E. Grassi, M. Sahota and C. F. Moss (2004). "The bat head-related transfer function reveals binaural cues for sound localization in azimuth and elevation." The Journal of the Acoustical Society of America **116**(6): 3594-3605.

Bartenstein, S. K., N. Gerstenberg, D. Vanderelst, H. Peremans and U. Firzlaff (2014). "Echo-acoustic flow dynamically modifies the cortical map of target range in bats." Nature Communications **5**: 1-11.

Batteau, D. W. (1967). "The role of the pinna in human localization." Proceedings of the Royal Society of London. Series B **168**(1011): 158-180.

Bear, M. F., B. W. Connors and M. A. Paradiso (2007). Neuroscience: exploring the brain, Lippincott Williams & Wilkins.

Begon, M. E., J. L. Harper and C. R. Townsend (1996). Ecology. Oxford, Blackwell Science Limited.

Berkowitz, A. and N. Suga (1989). "Neural mechanisms of ranging are different in two species of bats." Hearing Research **41**(2-3): 255-264.

Beuter, K. J. (1980). A new concept of echo evaluation in the auditory system of bats. Animal Sonar Systems, Springer**:** 747-761.

Bhagavatula, P. S., C. Claudianos, M. R. Ibbotson and M. V. Srinivasan (2011). "Optic flow cues guide flight in birds." Current Biology **21**(21): 1794-1799.

Blauert, J. (1997). Spatial hearing: the psychophysics of human sound localization. *Revised Edition*. Cambridge, MA, The MIT press.

Bohn, K., J. Boughman, G. Wilkinson and C. Moss (2004). "Auditory sensitivity and frequency selectivity in greater spear-nosed bats suggest specializations for acoustic communication." Journal of Comparative Physiology A **190**(3): 185-192.

Boonman, A., Y. Bar-On and Y. Yovel (2013). "It's not black or white - on the range of vision and echolocation in echolocating bats." Frontiers in Physiology **4**.

Boonman, A., S. Bumrungsri and Y. Yovel (2014). "Nonecholocating fruit bats produce biosonar clicks with their wings." Current Biology **24** (24): 1-6.

Brinkløv, S., M. B. Fenton and J. M. Ratcliffe (2013). "Echolocation in Oilbirds and swiftlets." Frontiers in Physiology **4**.

Casseday, J. H. and E. Covey (1996). "A neuroethological theory of the operation of the inferior colliculus." Brain Behavior and Evolution **47**(6): 311-336.

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

Chen, Q., H. Deng, S. E. Brauth, L. Ding and Y. Tang (2012). "Reduced performance of prey targeting in pit vipers with contralaterally occluded infrared and visual senses." PLoS ONE **7**(5): e34989.

Combe, E. and M. Wexler (2010). "Observer movement and size constancy." Psychological Science **21**(5): 667-675.

Covey, E. and J. H. Casseday (1999). "Timing in the auditory system of the bat." Annual Review of Physiology **61**: 457-476.

Dangles, O., D. Irschick, L. Chittka and J. Casas (2009). "Variability in sensory ecology: expanding the bridge between physiology and evolutionary biology." Quarterly Review of Biology **84**(1): 51-74.

Davies, K. T., I. Maryanto and S. J. Rossiter (2013). "Evolutionary origins of ultrasonic hearing and laryngeal echolocation in bats inferred from morphological analyses of the inner ear." Frontiers in Zoology **10**(1): 2.

Deneve, S. and A. Pouget (2004). "Bayesian multisensory integration and cross-modal spatial links." Journal of Physiology Paris **98**(1): 249-258.

Denton, E. J., J. B. Gilpin-Brown and P. G. Wright (1970). "On the 'filters' in the photophores of mesopelagic fish and on a fish emitting red light and especially sensitive to red light." Journal of Physiology **208**(2): 72-73.

Denzinger, A. and H.-U. Schnitzler (2013). "Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats." Frontiers in Physiology **4**.

Dobbins, A. C., R. M. Jeo, J. Fiser and J. M. Allman (1998). "Distance modulation of neural activity in the visual cortex." Science **281**(5376): 552-555.

Douglas, R. H., J. Eva and N. Guttridge (1988). "Size constancy in goldfish (Carassius auratus)." Behavioural Brain Research **30**(1): 37-42.

Douglas, R. H. and J. C. Partridge (2011). Vision | Visual Adaptations to the Deep Sea. Encyclopedia of Fish Physiology. A. P. Farrell. San Diego, Academic Press**:** 166-182.

Douglas, R. H., J. C. Partridge and N. J. Marshall (1998). "The eyes of deep-sea fish I: Lens pigmentation, tapeta and visual pigments." Progress in Retinal and Eye Research **17**(4): 597-636.

Dusenbery, D. B. (1992). Light. Sensory ecology: how organisms acquire and respond to information. New York, Freeman, W. H. & Co Ltd.**:** 1-558.

Efrati, A. and Y. Gutfreund (2011). "Early life exposure to noise alters the representation of auditory localization cues in the auditory space map of the barn owl." Journal of Neurophysiology **105**(5): 2522-2535.

Esser, K. H. and A. Daucher (1996). "Hearing in the FM-bat Phyllostomus discolor: a behavioral audiogram." Journal of Comparative Physiology A **178**(6): 779-785.

Esser, K. H. and U. Schmidt (1989). "Mother-infant communication in the Lesser Spear-nosed Bat Phyllostomus discolor (Chiroptera, Phyllostomidae) - evidence for acoustic learning." Ethology **82**(2): 156-168.

Ewert, J. P. and H. Burghagen (1979). "Ontogenetic aspects on visual "size-constancy" phenomena in the midwife toad Alytes obstetricans (Laur.)." Brain Behav Evol **16**(2): 99-112.

Falk, B., T. Williams, M. Aytekin and C. F. Moss (2011). "Adaptive behavior for texture discrimination by the free-flying big brown bat, Eptesicus fuscus." Journal of Comparative Physiology A **197**(5): 491-503.

Fenton, M. B. (1972). "The structure of aerial-feeding bat faunas as indicated by ears and wing elements." Canadian Journal of Zoology **50**(3): 287-296.

Fenton, M. B. (2003). "Eavesdropping on the echolocation and social calls of bats." Mammal Review **33**(3-4): 193-204.

Fenton, M. B. and G. P. Bell (1981). "Recognition of species of insectivorous bats by their echolocation calls." Journal of Mammalogy **62**(2): 233-243.

Fernandez, H. R. C. (1979). "Visual pigments of bioluminescent and nonbioluminescent deep-sea fishes." Vision Research **19**(5): 589-592.

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

Firzlaff, U. and G. Schuller (2003). "Spectral directionality of the external ear of the lesser spear-nosed bat, Phyllostomus discolor." Hearing Research **181**(1-2): 27-39.

Firzlaff, U. and G. Schuller (2004). "Directionality of hearing in two CF/FM bats, Pteronotus parnellii and Rhinolophus rouxi." Hearing Research **197**(1-2): 74-86.

Forsman, K. A. and M. G. Malmquist (1988). "Evidence for echolocation in the common shrew, Sorex araneus." Journal of Zoology **216**: 655-662.

Freeman, P. W. (2000). "Macroevolution in Microchiroptera: recoupling morphology and ecology with phylogeny." Mammalogy Papers: University of Nebraska State Museum: 8.

Fuzessery, Z. M. (1996). "Monaural and binaural spectral cues created by the external ears of the pallid bat." Hearing Research **95**(1): 1-17.

Galambos, R. and D. R. Griffin (1942). "Obstacle avoidance by flying bats: The cries of bats." Journal of Experimental Zoology **89**(3): 475-490.

Geberl, C. (2013). Spatial and temporal resolution of bat sonar. PhD Thesis, LMU Munich.

Genzel, D., C. Geberl, T. Dera and L. Wiegrebe (2012). "Coordination of bat sonar activity and flight for the exploration of three-dimensional objects." Journal of Experimental Biology **215**(13): 2226-2235.

Ghose, K. and C. F. Moss (2003). "The sonar beam pattern of a flying bat as it tracks tethered insects." The Journal of the Acoustical Society of America **114**(2): 1120-1131.

Goerlitz, H. R., D. Genzel and L. Wiegrebe (2012). "Bats' avoidance of real and virtual objects: implications for the sonar coding of object size." Behavioural Processes **89**(1): 61-67.

Gould, E. (1965). "Evidence for echolocation in the Tenrecidae of Madagascar." Proceedings of the American Philosophical Society **109**(6): 352-360.

Griffin, D., A. Novick and M. Kornfield (1958). "The sensitivity of echolocation in the fruit bat, Rousettus." The Biological Bulletin **115**(1): 107-113.

Griffin, D. R. (1958). Listening in the dark; the acoustic orientation of bats and men, Yale University Press, New Haven, Conneticett.

Griffin, D. R. and A. Novick (1955). "Acoustic orientation of neotropical bats." Journal of Experimental Zoology **130**(2): 251-299.

Griffin, D. R. and D. Thompson (1982). "Echolocation by cave swiftlets." Behavioral Ecology and Sociobiology **10**(2): 119-123.

Griffiths, S. R. (2013). "Echolocating bats emit terminal phase buzz calls while drinking on the wing." Behavioural Processes **98**: 58-60.

Grinnell, A. D. (1995). Hearing by bats: an overview. Springer handbook of auditory research; Hearing by bats. A. N. Popper and F. R. R. New York, Springer. **5:** 1-36.

Grunwald, J.-E. (2004). Echo-acoustic evaluation of real and phantom objects in phyllostomid bats. PhD Monograph, LMU.

Grunwald, J. E., S. Schoernich and L. Wiegrebe (2004). "Classification of natural textures in echolocation." Proceedings of the National Academy of Sciences USA **101**(15): 5670-5674.

Gutierrez, E. D., V. F. Pessoa, L. M. Aguiar and D. M. Pessoa (2014). "Effect of light intensity on food detection in captive great fruit-eating bats, Artibeus lituratus (Chiroptera: Phyllostomidae)." Behavioural Processes **109**: 64-69.

Haber, R. and C. Levin (2001). "The independence of size perception and distance perception." Perception & Psychophysics **63**(7): 1140-1152.

Hagemann, C., K. H. Esser and M. Kossl (2010). "Chronotopically organized target-distance map in the auditory cortex of the short-tailed fruit bat." Journal of Neurophysiology **103**(1): 322-333.

Hanley, C. and D. Goff (1974). "Size constancy in extended haptic space." Perception & Psychophysics **15**(1): 97-100.

Harley, H., H. Roitblat and P. Nachtigall (1996). "Object representation in the bottlenose dolphin (Tursiops truncatus): integration of visual and echoic information." Journal of Experimental Psychology: Animal Behavior Processes **22**(2): 164-174.

Hartrige, H. (1945). "Acoustic control in the flight of bats." Nature **156**: 490-494.

Hattori, T. and N. Suga (1997). "The inferior colliculus of the mustached bat has the frequency vs. latency coordinates." Journal of Comparative Physiology A **180**(3): 271-284.

Heinrich, M. and L. Wiegrebe (2013). "Size constancy in bat biosonar? Perceptual interaction of object aperture and distance." PLoS ONE **8**(4): e61577.

Henson, J. and W. O'Dell (1967). The perception and analysis of biosonar signals by bats. Animal sonar systems: biology and bionics R. G. Busnel. France. **2:** 949-1003.

Herman, L. M., A. A. Pack and M. Hoffmann-Kuhnt (1998). "Seeing through sound: dolphins (Tursiops truncatus) perceive the spatial structure of objects through echolocation." Journal of Comparative Psychology **112**(3): 292-305.

Hoffmann, S., A. Warmbold, L. Wiegrebe and U. Firzlaff (2013). "Spatiotemporal contrast enhancement and feature extraction in the bat auditory midbrain and cortex." Journal of Neurophysiology **110**(6): 1257-1268.

Holderied, M. W. and O. von Helversen (2006). "'Binaural echo disparity' as a potential indicator of object orientation and cue for object recognition in echolocating nectar-feeding bats." Journal of Experimental Biology **209**(17): 3457-3468.

Holland, R. A., D. A. Waters and J. M. Rayner (2004). "Echolocation signal structure in the Megachiropteran bat Rousettus aegyptiacus Geoffroy 1810." Journal of Experimental Biology **207**(25): 4361-4369.

Humphrey, N. and L. Weiskrantz (1969). "Size constancy in monkeys with inferotemporal lesions." The Quarterly Journal of Experimental Psychology **21**(3): 225-238.

Jacobs, D. K., N. Nakanishi, D. Yuan, A. Camara, S. A. Nichols and V. Hartenstein (2007). "Evolution of sensory structures in basal metazoa." Integrative and Comparative Biology **47**(5): 712-723.

Jakobsen, L., S. Brinkløv and A. Surlykke (2013). "Intensity and directionality of bat echolocation signals." Frontiers in Physiology **4**.

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

Jones, G. and E. C. Teeling (2006). "The evolution of echolocation in bats." Trends in Ecology & Evolution **21**(3): 149-156.

Keen, R. and R. L. Freyman (2009). "Release and re-buildup of listeners' models of auditory space." The Journal of the Acoustical Society of America **125**(5): 3243-3252.

Kellogg, W. N. (1962). "Sonar system of the blind." Science **137**(3528): 399-404.

Kick, S. A. and J. A. Simmons (1984). "Automatic gain-control in the bats sonar receiver and the neuroethology of echolocation." Journal of Neuroscience **4**(11): 2725-2737.

Knörnschild, M. and O. von Helversen (2008). "Nonmutual vocal mother-pup recognition in the greater sac-winged bat." Animal Behaviour **76**(3): 1001-1009.

Kolarik, A. J., S. Cirstea, S. Pardhan and B. C. J. Moore (2014). "A summary of research investigating echolocation abilities of blind and sighted humans." Hearing Research **310**(0): 60-68.

Kulzer, E. (1956). "Flughunde erzeugen Orientierungslaute durch Zungenschlag." Naturwissenschaften **43**(5): 117-118.

Kupers, R. and M. Ptito (2014). "Compensatory plasticity and cross-modal reorganization following early visual deprivation." Neuroscience & Biobehavioral Reviews **41**: 36-52.

Kuwabara, N. and N. Suga (1993). "Delay lines and amplitude selectivity are created in subthalamic auditory nuclei: the brachium of the inferior colliculus of the mustached bat." Journal of Neurophysiology **69**(5): 1713-1724.

Lazzouni, L. and F. Lepore (2014). "Compensatory plasticity: time matters." Frontiers in Human Neuroscience **8**.

Lewicki, M. S., B. A. Olshausen, A. Surlykke and C. F. Moss (2014). "Scene analysis in the natural environment." Frontiers in Psychology **5**.

Litovsky, R. Y., H. S. Colburn, W. A. Yost and S. J. Guzman (1999). "The precedence effect." The Journal of the Acoustical Society of America **106**(4): 1633-1654.

Litovsky, R. Y., B. Rakerd, T. C. Yin and W. M. Hartmann (1997). "Psychophysical and physiological evidence for a precedence effect in the median sagittal plane." Journal of Neurophysiology **77**(4): 2223-2226.

Mayer, F., C. Dietz and A. Kiefer (2007). "Molecular species identification boosts bat diversity." Frontiers in Zoology **4**(4).

Melcon, M. L., H. U. Schnitzler and A. Denzinger (2009). "Variability of the approach phase of landing echolocating Greater Mouse-eared bats." Journal of Comparative Physiology A **195**(1): 69-77.

Milne, J. L., M. Anello, M. A. Goodale and L. Thaler (2014). "A blind human expert echolocator shows size constancy for objects perceived by echoes." Neurocase **2014**: 1-6.

Mogdans, J., J. Ostwald and H. U. Schnitzler (1988). "The role of pinna movement for the localization of vertical and horizontal wire obstacles in the greater horseshoe bat, Rhinolopus ferrumequinum." The Journal of the Acoustical Society of America **84**(5): 1676-1679.

Möhres, F. P. and E. Kulzer (1956). "Über die Orientierung der Flughunde (Chiroptera-Pteropodidae)." Zeitschrift für vergleichende Physiologie **38**: 1-29.

Mooney, T. A., P. E. Nachtigall and M. M. Yuen (2006). "Temporal resolution of the Risso's dolphin, Grampus griseus, auditory system." Journal of Comparative Physiology A **192**(4): 373-380.

Moore, B. C. (2003). An introduction to the psychology of hearing; fourth edition, Academic press San Diego.

Moore, B. C. (2013). An introduction to the psychology of hearing; sixth edition, Brill

Moss, C. F. and H.-U. Schnitzler (1995). Behavioral studies of auditory information processing. Springer Handbook of Auditory Research; Hearing by bats. A. N. Popper and F. R. R. **5:** 87-145.

Moss, C. F. and A. Surlykke (2010). "Probing the natural scene by echolocation in bats." Frontiers in Behavioral Neuroscience **4**.

Muller, B., M. Glosmann, L. Peichl, G. C. Knop, C. Hagemann and J. Ammermuller (2009). "Bat eyes have ultraviolet-sensitive cone photoreceptors." PLoS ONE **4**(7): e6390.

Nachtigall, P. (1980). Odontocete echolocation performance on object size, shape and material. Animal Sonar Systems. R. Busnel and J. Fish**:** 71-95.

Nelson, M. E. (2011). "Electric fish." Current Biology **21**(14): 528-529.

Nelson, M. E. and M. A. MacIver (2006). "Sensory acquisition in active sensing systems." Journal of Comparative Physiology A **192**(6): 573-586.

Neuweiler, G. (1989). "Foraging ecology and audition in echolocating bats." Trends in Ecology & Evolution **4**(6): 160-166.

Neuweiler, G. (2000). The biology of bats, Oxford University Press

Neuweiler, G. and S. Schmidt (1993). "Audition in echolocating bats." Current Opinion in Neurobiology **3**: 563-569.

Nilsson, D.-E. (2009). "The evolution of eyes and visually guided behaviour." Philosophical Transactions of the Royal Society B: Biological Sciences **364**(1531): 2833-2847.

Niven, J. E. and S. B. Laughlin (2008). "Energy limitation as a selective pressure on the evolution of sensory systems." Journal of Experimental Biology **211**(11): 1792-1804.

Norberg, U. M. and M. B. Fenton (1988). "Carnivorous bats?" Biological Journal of the Linnean Society **33**(4): 383-394.

O'Day, W. T. and H. R. Fernandez (1974). "Aristostomias scintillans (Malacosteidae): A deep-sea fish with visual pigments apparently adapted to its own bioluminescence." Vision Research **14**(7): 545-550.

O'Neill, W. E. and N. Suga (1979). "Target range-sensitive neurons in the auditory cortex of the mustache bat." Science **203**(4375): 69-73.

Okamura, J. Y., R. Yamaguchi, K. Honda, G. Wang and K. Tanaka (2014). "Neural substrates of view-invariant object recognition developed without experiencing rotations of the objects." Journal of Neuroscience **34**(45): 15047-15059.

Pack, A. A. and L. M. Herman (1995). "Sensory integration in the bottlenosed dolphin: immediate recognition of complex shapes across the senses of echolocation and vision." The Journal of the Acoustical Society of America **98**(2): 722-733.

Pack, A. A., L. M. Herman, M. Hoffmann-Kuhnt and B. K. Branstetter (2002). "The object behind the echo: dolphins (Tursiops truncatus) perceive object shape globally through echolocation." Behavioural Processes **58**(1-2): 1-26.

Pastore, N. (1958). "Form perception and size constancy in the duckling." The Journal of Psychology **45**(2): 259-261.

Pena, J. L. and W. M. DeBello (2010). "Auditory processing, plasticity, and learning in the barn owl." ILAR Journal / National Research Council, Institute of Laboratory Animal Resources **51**(4): 338-352.

Pettigrew, J. D. (1988). Microbat vision and echolocation in an evolutionary context. Animal Sonar, Springer**:** 645-650.

Price, J. J., K. P. Johnson and D. H. Clayton (2004). "The evolution of echolocation in swiftlets." Journal of Avian Biology **35**(2): 135-143.

Pye, J. D. and L. H. Roberts (1970). "Ear movements in a hipposiderid bat." Nature **225**(5229): 285-286.

Razak, K. A. (2011). "Systematic representation of sound locations in the primary auditory cortex." Journal of Neuroscience **31**(39): 13848-13859.

Rice, C. E. and S. H. Feinstein (1965 A). " Sonar system of the blind: Size discrimination " Science **148**(3673): 1107-1108.

Rice, C. E., S. H. Feinstein and R. J. Schusterman (1965 B). " Echo-detection ability of the blind: Size and distance factors." Journal of Experimental Psychology **70**: 246-255.

Rimskaya-Korsakova, L. K. (2004). "Temporal resolution and temporal integration of short pulses at the auditory periphery of echolocating animals." Acoustical Physics **50**(3): 331-342.

Roffler, S. K. and R. A. Butler (1968). "Localization of tonal stimuli in the vertical plane." The Journal of the Acoustical Society of America **43**(6): 1260-1266.

Russo, D., G. Jones and R. Arlettaz (2007). "Echolocation and passive listening by foraging mouse-eared bats Myotis myotis and M. blythii." Journal of Experimental Biology **210**(1): 166-176.

Saitoh, I. and N. Suga (1995). "Long delay lines for ranging are created by inhibition in the inferior colliculus of the mustached bat." Journal of Neurophysiology **74**(1): 1-11.

Salminen, N., J. Aho and M. Sams (2013). "Visual task enhances spatial selectivity in the human auditory cortex." Frontiers in Neuroscience **7**.

Schenkman, B. N. and M. E. Nilsson (2010). "Human echolocation: Blind and sighted persons' ability to detect sounds recorded in the presence of a reflecting object." Perception **39**(4): 483-501.

Schenkman, B. N. and M. E. Nilsson (2011). "Human echolocation: pitch versus loudness information." Perception **40**(7): 840-852.

Schmidt, S. (1988). "Evidence for a spectral basis of texture perception in bat sonar." Nature **331**(6157): 617-619.

Schmidt, S. (1992). "Perception of structured phantom targets in the echolocating bat, Megaderma lyra." The Journal of the Acoustical Society of America **91**(4): 2203-2223.

Schnitzler, H.-U. and O. D. W. Henson, Jr. (1980). Performance of airborne animal sonar systems: I. Microchiroptera. Animal Sonar Systems. R.-G. Busnel and J. F. Fish, Springer US. **28:** 109-181.

Schnitzler, H.-U. and E. K. Kalko (2001). "Echolocation by insect-eating bats " Bioscience **51**(7): 557-569.

Schornich, S., L. Wallmeier, N. Gessele, A. Nagy, M. Schranner, D. Kish and L. Wiegrebe (2013). "Psychophysics of human echolocation." Advances in Experimental Medicine and Biology **787**: 311-319.

Siemers, B., G. Schauermann, H. Turni and S. von Merten (2009). "Why do shrews twitter? Communication or simple echo-based orientation." Biology Letters **5**: 593–596.

Siemers, B. M., C. Dietz, D. Nill and H.-U. Schnitzler (2001). "Myotis daubentonii is able to catch small fish." Acta Chiropterologica **3**(1): 71-75.

Siemers, B. M. and H. U. Schnitzler (2004). "Echolocation signals reflect niche differentiation in five sympatric congeneric bat species." Nature **429**(6992): 657-661.

Simmons, J. A. (1969). "Acoustic radiation patterns for the echolocating bats Chilonycteris rubiginosa and Eptesicus fuscus." The Journal of the Acoustical Society of America **46**: 1054-1056.

Simmons, J. A. (1971). "Echolocation in bats - signal processing of echoes for target range." Science **171**(3974): 925-928.

Simmons, J. A. (1971). "The sonar receiver of the bat." Annals of the New York Academy of Sciences **188**: 161-174.

Simmons, J. A. (1973). "Resolution of target range by echolocating bats." The Journal of the Acoustical Society of America **54**(1): 157-173.

Simmons, J. A. and L. Chen (1989). "The acoustic basis for target discrimination by FM echolocating bats." The Journal of the Acoustical Society of America **86**(4): 1333-1350.

Simmons, J. A., E. G. Freedman, S. B. Stevenson, L. Chen and T. J. Wohlgenant (1989). "Clutter interference and the integration time of echoes in the echolocating bat, Eptesicus fuscus." The Journal of the Acoustical Society of America **86**(4): 1318-1332.

Simmons, J. A. and A. D. Grinnell (1988). The performance of echolocation: Acoustic images perceived by echolocating bats. Animal sonar: Processes and performance. P. E. Nachtigall and P. W. B. Moore. New York Plenum Press**:** 353–385.

Simmons, J. A., D. J. Howell and N. Suga (1975). "Information content of bat sonar echoes." American Scientist **63**(2): 204-215.

Simmons, J. A., S. A. Kick, A. J. Moffat, W. M. Masters and D. Kon (1988). "Clutter interference along the target range axis in the echolocating bat, Eptesicus fuscus." The Journal of the Acoustical Society of America **84**(2): 551-559.

Simmons, J. A. and R. A. Stein (1980). "Acoustic imaging in bat sonar: echolocation signals and the evolution of echolocation." Journal of Comparative Physiology A **135**(1): 61-84.

Simmons, J. A. and J. A. Vernon (1971). "Echolocation: discrimination of targets by the bat, Eptesicus fuscus." Journal of Experimental Zoology **176**(3): 315-328.

Simmons, N. B., D. Wilson and D. Reeder (2005). "Order chiroptera." Mammal species of the world: a taxonomic and geographic reference **1**: 312-529.

Simon, R., M. W. Holderied and O. von Helversen (2006). "Size discrimination of hollow hemispheres by echolocation in a nectar feeding bat." Journal of Experimental Biology **209**(18): 3599-3609.

Simon, R., M. Knornschild, M. Tschapka, A. Schneider, N. Passauer, E. K. Kalko and O. von Helversen (2014). "Biosonar resolving power: echo-acoustic perception of surface structures in the submillimeter range." Frontiers in Physiology **5**.

Suga, N. (1990). "Biosonar and neural computation in bats." Scientific American **262**(6): 60-68.

Suga, N. and P. Jen (1977). "Further studies on the peripheral auditory system of 'CF-FM' bats specialized for fine frequency analysis of Doppler-shifted echoes." Journal of Experimental Biology **69**(1): 207-232.

Suga, N. and W. E. O'Neill (1979). "Neural axis representing target range in the auditory cortex of the mustache bat." Science **206**(4416): 351-353.

Sumer, S., A. Denzinger and H. U. Schnitzler (2009). "Spatial unmasking in the echolocating Big Brown Bat, Eptesicus fuscus." Journal of Comparative Physiology A **195**(5): 463-472.

Sun, H. J. and B. J. Frost (1998). "Computation of different optical variables of looming objects in pigeon nucleus rotundus neurons." Nature Neuroscience **1**(4): 296-303.

Surlykke, A. and O. Bojesen (1996). "Integration time for short broad band clicks in echolocating FM-bats (Eptesicus fuscus)." Journal of Comparative Physiology A **178**(2): 235-241.

Surlykke, A., K. Ghose and C. F. Moss (2009). "Acoustic scanning of natural scenes by echolocation in the big brown bat, Eptesicus fuscus." Journal of Experimental Biology **212**(7): 1011-1020.

Surlykke, A., S. B. Pedersen and L. Jakobsen (2009). "Echolocating bats emit a highly directional sonar sound beam in the field." Proceedings of the Royal Society B-Biological Sciences **276**(1658): 853-860.

Suthers, R. A. and N. E. Wallis (1970). "Optics of the eyes of echolocating bats." Vision Research **10**(11): 1165-1173.

Teng, S., A. Puri and D. Whitney (2012). "Ultrafine spatial acuity of blind expert human echolocators." Experimental Brain Research **216**(4): 483-488.

Teng, S. and D. Whitney (2011). "The acuity of echolocation: Spatial resolution in the sighted compared to expert performance." Journal of Visual Impairment and Blindness **105**(1): 20-32.

Thaler, L., S. R. Arnott and M. A. Goodale (2011). "Neural correlates of natural human echolocation in early and late blind echolocation experts." PLoS ONE **6**(5): e20162.

Thaler, L., J. L. Milne, S. R. Arnott, D. Kish and M. A. Goodale (2014). "Neural correlates of motion processing through echolocation, source hearing, and vision in blind echolocation experts and sighted echolocation novices." Journal of Neurophysiology **111**(1): 112-127.

Thomassen, H. A. and G. D. E. Povel (2006). "Comparative and phylogenetic analysis of the echo clicks and social vocalizations of swiftlets (Aves : Apodidae)." Biological Journal of the Linnean Society **88**(4): 631-643.

Tomasi, T. E. (1979). "Echolocation by the Short-Tailed Shrew Blarina brevicauda." Journal of Mammalogy **60**(4): 751.

Tomonaga, M., Y. Uwano and T. Saito (2014). "How dolphins see the world: a comparison with chimpanzees and humans." Scientific Reports **4**: 3717.

Ulanovsky, N. and C. F. Moss (2008). "What the bat's voice tells the bat's brain." Proceedings of the National Academy of Sciences USA **105**(25): 8491-8498.

Ungerleider, L., L. Ganz and K. H. Pribram (1977). "Size constancy in rhesus monkeys: effects of pulvinar, prestriate, and inferotemporal lesions." Experimental Brain Research **27**(3-4): 251-269.

Vanderelst, D., F. De Mey, H. Peremans, I. Geipel, E. Kalko and U. Firzlaff (2010). "What noseleaves do for FM bats depends on their degree of sensorial specialization." PLoS ONE **5**(8): e11893.

Vanderelst, D., J. Reijniers, J. Steckel and H. Peremans (2011). "Information generated by the moving pinnae of rhinolophus rouxi: tuning of the morphology at different harmonics." PloS ONE **6**(6): e20627.

Veselka, N., D. D. McErlain, D. W. Holdsworth, J. L. Eger, R. K. Chhem, M. J. Mason, K. L. Brain, P. A. Faure and M. B. Fenton (2010). "A bony connection signals laryngeal echolocation in bats." Nature **463**(7283): 939-942.

Viemeister, N. F. and G. H. Wakefield (1991). "Temporal integration and multiple looks." The Journal of the Acoustical Society of America **90**(2): 858-865.

von der Emde, G. (1999). "Active electrolocation of objects in weakly electric fish." Journal of Experimental Biology **202**(10): 1205-1215.

von der Emde, G. (2004). "Distance and shape: perception of the 3-dimensional world by weakly electric fish." Journal of Physiology Paris **98**(1-3): 67-80.

von der Emde, G. (2006). "Non-visual environmental imaging and object detection through active electrolocation in weakly electric fish." Journal of comparative Physiology A **192**(6): 601-612.

von der Emde, G., K. Behr, B. Bouton, J. Engelmann, S. Fetz and C. Folde (2010). "3-Dimensional scene perception during active electrolocation in a weakly electric pulse fish." Frontiers in Behavioral Neuroscience **4**.

von der Emde, G. and S. Fetz (2007). "Distance, shape and more: recognition of object features during active electrolocation in a weakly electric fish." Journal of Experimental Biology **210**(17): 3082-3095.

von der Emde, G. and S. Schwarz (2000). "Three-dimensional analysis of object properties during active electrolocation in mormyrid weakly electric fishes (Gnathonemus petersii)." Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences **355**(1401): 1143-1146.

von der Emde, G., S. Schwarz, L. Gomez, R. Budelli and K. Grant (1998). "Electric fish measure distance in the dark." Nature **395**(6705): 890-894.

von Helversen, D. (2004). "Object classification by echolocation in nectar feeding bats: size-independent generalization of shape." Journal of Comparative Physiology A **190**(7): 515-521.

Wallach, H., E. B. Newman and M. R. Rosenzweig (1949). "The precedence effect in sound localization." American Journal of Psychology **62**(3): 315-336.

Wallmeier, L., N. Gessele and L. Wiegrebe (2013). "Echolocation versus echo suppression in humans." Proceedings Biological Sciences **280**(1769): 20131428.

Weinbeer, M., E. K. Kalko and K. Jung (2013). "Behavioral flexibility of the trawling long-legged bat, Macrophyllum macrophyllum (Phyllostomidae)." Frontiers in Physiology **4**.

Weißenbacher, P. (2003). Objekterkennung durch Echoortung und der Einfluß zeitlicher Integrationsmechanismen bei der Fledermaus Megaderma lyra Dissertation, LMU München.

Weissenbacher, P. and L. Wiegrebe (2003). "Classification of virtual objects in the echolocating bat, Megaderma lyra." Behavioral Neuroscience **117**(4): 833-839.

Weissenbacher, P., L. Wiegrebe and M. Kossl (2002). "The effect of preceding sonar emission on temporal integration in the bat, Megaderma lyra." Journal of Comparative Physiology A **188**(2): 147-155.

Wenstrup, J. J. and C. V. Portfors (2011). "Neural processing of target distance by echolocating bats: functional roles of the auditory midbrain." Neuroscience and Biobehavioral Reviews **35**(10): 2073-2083.

Werner, S. J., S. K. Tupper, J. C. Carlson, S. E. Pettit, J. W. Ellis and G. M. Linz (2012). "The role of a generalized ultraviolet cue for blackbird food selection." Physiology & Behavior **106**(5): 597-601.

Wiegrebe, L. and S. Schmidt (1996). "Temporal integration in the echolocating bat, Megaderma lyra." Hearing Research **102**(1-2): 35-42.

Wilkinson, G. S. and J. W. Boughman (1998). "Social calls coordinate foraging in greater spear-nosed bats." Animal Behaviour **55**(2): 337-350.

Winter, Y., J. Lopez and O. von Helversen (2003). "Ultraviolet vision in a bat." Nature **425**(6958): 612-614.

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

Zhuang, Q. and R. Müller (2006). "Noseleaf furrows in a horseshoe bat act as resonance cavities shaping the biosonar beam." Physical Review Letters **97**(21): 218701.

Zwislocki, J. (1960). "Theory of temporal auditory summation." The Journal of the Acoustical Society of America **32**(8): 1046-1060.

Abbreviations

AC	auditory cortex
AD/DA	analogue to digital/digital to analogue
AFC	alternative-forced choice
BF	best frequency
BM	basilar membrane
CF	constant frequency
Cf-Fm	constant frequency-frequency modulated
CNS	central nervous system
d	distance
dB	decibel
dB SPL	decibel sound pressure level
EE	Expert Echolocator
Fd	feeder
FFT	Fast Fourier Transform
FM	frequency modulated
FRA	frequency-response area
GLMM	general linear mixed model
HRIR/s	head-related impulse response
HRTF/s	head-related transfer function
IC	Inferior colliculus (auditory midbrain)
IID/s	interaural-intensity difference/s
ILD/s	Interaural level difference/s
I/O	input/output
IR	impulse response

ITD/s	interaural time difference/s
kHz	kilohertz
LSO	lateral superior olive
Mic	microphone
nm	nanometre/s
P. discolor	*Phyllostomus discolor*
PI/s	pulse interval/s
PVC	polyvinyl chloride
RO	rewarded object
ROC	receiver operating characteristic
SSC	sonar size constancy
UO/s	unrewarded object/s
VO/s	virtual object/s
2D/3D	two-dimensional/three-dimensional
w	width

Figure Index

Chapter One: General Introduction

Chapter Two: The Sonar Aperture and Its Neural Representation in Bats

Chapter Three: Size constancy in bat Biosonar? Perceptual interaction of object aperture and distance

Chapter Four: Trawling bats exploit an echo-acoustic ground effect

Acknowledgements

I thank the Volkswagenstiftung (I/83838), the Human Frontier Science Program (HFSP RGP 0062/2009), and the Graduate School of Systemic Neurosciences (GSN-LMU, DFG-GSC 82/1) for supporting the different projects of my PhD thesis.

Furthermore, the current work was only possible due to many lifelines that crossed on a certain time and place. When I started my long term internship in Björn Siemers' group in Seewiesen I met and worked with 'bat, bird, and shrew people' that I will definitely never ever forget. This internship led me to the research group of Lutz Wiegrebe in Munich where I got the opportunity to work on this PhD thesis.

I especially want to thank Lutz Wiegrebe for his encouragement and support throughout these years as well as nightly toboggan rides, cheese fondues, and much more. I also want to thank Harald Luksch and Andreas Zahn for the helpful discussions during the meetings of my TAC.

Many thanks to Uwe Firzlaff, Daria Genzel, Conny Geberl, Andrea Linger, Susanne Hoffmann, Alexander Warmbold, Sven Schörnich, Ferdinand Kroll, Kathrin Kugler, Markus Drexl, Ludwig Wallmeier, Sándor Zsebők, and Holger Görlitz for the friendly atmosphere at work and beyond as well as for the personal and scientific support and cooperation. I also want to thank the GSN team for their support and advice. Here I especially thank Catherine Botheroyd who made unique adventures during 'hardship of workaday life' come true. For the accommodation of my bats I thank Sabrina, Moni, Dieter, Helga, and Anja.

I definitely want to thank my experimental animals, the bats. I am very grateful for the experience to work with these wonderful animals, each being a remarkable individual having its own character. Therefore, I find it worth listing all of them (independently of how long they took part in the experiments): Primus, Secundus, Khimari, Garfield, Goliath, Sixtus, Septimus, Darcy, Enkidu, and Keiki. I also want to underline that none of 'my bats' was ever harmed during the experiments and under my caretaking.

Special thanks to my parents and my family, as well as Vladi, Sophie, Sandra, Melanie, and Steffi for listening and supporting me throughout this long time. Furthermore, I thank my former colleagues of the MU for their support.

And last but not least I want to thank my husband and best friend Micha Steinbrück:

"Micha, I couldn't have made this journey without you!

Curriculum Vitae

PERSONAL DETAILS

Name	Melina Heinrich
E-Mail	Heinrich@bio.lmu.de/ Melina.heinrich@outlook.de
Nationality	German
Date of Birth	02.02.1983
Place of Birth	Bielefeld, Germany

EMPLOYMENTS

2015 - today	**P**roject manager for nature and species conservation; Group for Ecological Expertise (GOEG), Stuttgart, Germany.
2012 - 2014	**R**eferent for species conservation and Natura 2000 in the Environmental Ministry of Lower Saxony, Hannover, Germany.
2009 - 2012	**S**cientific employee in the Department of Biology 2 of the LMU, Munich, Germany.
2007 - 2008	**T**emporary employee in the Department of Cognitive Neuroscience, University of Bielefeld, Germany.

ADDITIONAL WORK AND RESEARCH EXPERIENCE

2008	**L**ong-term internship in the Max Planck Institute for Ornithology in Seewiesen, Germany, Research Group for Sensory Ecology, PD. Dr. B.M. Siemers, including laboratory and field work with different shrew and bat species.
2006	**V**oluntary service in the CARAPAX Center for turtles and storks, Massa Marittima, Tuscany, Italy. **I**nternship in the zoological collection of Bielefeld University, Germany. **I**nternship in the zoological garden (Stiftung Artenschutz and BioCity) of Münster, Germany.
2004	**S**tudent research assistant at Bielefeld University, Germany. Topic: Sexual selection in zebra finches (Prof. Dr. K. Witte).

AWARDS AND HONORS

2011	**G**SN-LMU: 2nd Prize for the best publication 2011.
2011	**G**SN-LMU: 1st Prize of the GSN Poster Competition 2011.

EDUCATION

2010 - 2015	**P**h.D. -student at the GSN-LMU Graduate School of Systemic Neurosciences (GSN$^{\text{LMU}}$), Ludwig-Maximilians Universität, München, Germany, Ph.D. Thesis: Aspects of spatio-temporal integration of bat sonar. Supervisor: Prof. Dr. Lutz Wiegrebe.
2009 - 2010	**P**h.D. -student (rer. nat) at the Department of Biology 2, Division of Neurobiology, LMU Munich, Germany, Supervisor: Prof. Dr. Lutz Wiegrebe.
2002 - 2008	**G**raduation in biology (diploma) at the University of Bielefeld, Germany. Major: zoology; Minors: ethology, ecology and geography. Diploma thesis: Optimal foraging in *Glossophaga soricina* – do individuals profit from eavesdropping on conspecifics? Supervisor: Prof. Dr. York Winter.
1999 - 2002	**A**cquirement of the higher education entrance qualification (Abitur) at the Friedrich-von-Bodelschwingh-Gymnasium, Bielefeld, Germany.

List of Publications

Zsebok, S., F. Kroll, M. Heinrich, D. Genzel, B. M. Siemers and L. Wiegrebe (2013). "Trawling bats exploit an echo-acoustic ground effect." Frontiers in Physiology 4.

Heinrich, M. and L. Wiegrebe (2013). "Size Constancy in Bat Biosonar? Perceptual Interaction of Object Aperture and Distance." PLoS ONE 8(4): e61577.

Heinrich, M., A. Warmbold, S. Hoffmann, U. Firzlaff and L. Wiegrebe (2011). "The Sonar Aperture and Its Neural Representation in Bats." Journal of Neuroscience 31(43): 15618-15627.

Affidavit/Eidesstattliche Erklärung

Hiermit versichere ich an Eides statt, dass ich die vorliegende Dissertation „Aspects of spatiotemporal integration in bat sonar“ selbstständig angefertigt habe, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

I hereby confirm that the dissertation „Aspects of spatiotemporal integration in bat sonar“ is the result of my own work and that I have only used sources or materials listed and specified in the dissertation.

München, den 22.12.2014

Melina Heinrich

Author Contributions

“The Sonar Aperture and Its Neural Representation in Bats”

by Melina Heinrich (MH),* Alexander Warmbold (AW),* Susanne Hoffmann (SH), Uwe Firzlaff (UF), and Lutz Wiegrebe (LW) (*M.H. and A.W. contributed equally to this work).

LW and UF originally designed the experiments. The electrophysical experiments were conducted by AW as part of his diploma thesis under the supervision of SH and UF. LW and MH further developed the psychophysical experiments and the experimental setup. LW and MH wrote the experimental programs and the analysis programs. MH performed the psychophysical research, collected the data of all psycho-acoustic experiments, and conducted the corresponding analysis. MH wrote the psychophysical materials and methods and the results section. AW wrote the electrophysiological materials and methods section and the results section. The other manuscript sections were written by MH, AW, SH, UF and LW. MH designed the figures 1 and 3 and delivered the psychophysical data depicted in the figures 8 and 9. The remaining figures as well as the computational models were designed and calculated by AW, SH, UF, and LW. Harald Luksch read an early version of the manuscript and made valuable comments to improve the draft.

Munich,	Munich,
____________________	____________________
(Alexander Warmbold)	(Lutz Wiegrebe)

“Size Constancy in Bat Biosonar? Perceptual Interaction of Object Aperture and Distance”

by Melina Heinrich (MH) and Lutz Wiegrebe (LW).

MH and LW designed the experiments, wrote the experimental programs, and the analysis programs. MH collected the data, conducted major parts of the analysis and wrote major parts of the manuscript under supervision of LW. Alexander Warmbold and Daria Genzel supported MH at times with the implementation of the analysis programs. MH designed the figures. Uwe Firzlaff read early versions of the manuscript and made valuable comments to improve the draft. Katie L. Willis did the final language grammar check.

Munich,

(Lutz Wiegrebe)

“Trawling bats exploit an echo-acoustic ground effect”
by Sándor Zsebők (SZ)*, Ferdinand Kroll (FK)*, Melina Heinrich (MH)*, Daria Genzel (DG), Björn Siemers (BS) and Lutz Wiegrebe (LW). (* SZ, FK, and MH contributed equally to this work).

LW, DG and MH supervised the diploma thesis of FK. FK, DG, MH and LW designed the field experiments. MH wrote all proposals and reports that were required for the conduction for the field experiments and in the early phase of this project in 2010. FK, DG and LW wrote the experimental programs, and the analysis programs. FK, DG and MH collected data in the field. The data collection for the field experiments was supported at times by Barbara Pfäffl and Andrea Linger. Data analysis of the raw data for the field experiments was done by FK with the assistance of LW and DG.
This project was conducted in collaboration with SZ and BS from the Max Planck Institute for Ornithology in Seewiesen, Germany.
A modified version of the experimental setup that was used in the field experiments was used by SZ and BS to carry out the laboratory experiments described in the published paper. The data collection and analysis of the raw data for the laboratory experiments was done by SZ.
For sound analysis Holger Görlitz provided a MATLAB based sound analysis program that was modified by FK, DG and LW. The combined data analysis and the figure design were performed by SZ, FK and LW. The spectrograms in Figure 6 were designed and measured by FK, DG, MH and LW. The manuscript was written by SZ, FK, MH and LW. MH and LW were furthermore strongly involved in the resubmission and final proof reading process.

Budapest,	Munich,	Munich,
______________	______________	______________
(Sándor Zsebők)	(Ferdinand Kroll)	(Lutz Wiegrebe)