Perceptual strategies in active and passive hearing of neotropical bats

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List of abbreviations

2-AFC	two-alternative, forced-choice
CF	constant frequency
dB FS	decibel Full Scale
dB/oct.	decibel per octave
dB SPL	decibel Sound Pressure Level
FM	frequency-modulated
IR	impulse response
log10M4	decadic logarithm of the 4. moment
PI	pulse interval
pp	peak-to-peak
RMS	root mean square
SCAT	spectrogram correlation and transformation (a sonar receiver model)
SEM	standard error of the mean
std	standard deviation

Zusammenfassung

Das auditorische System wertet spektrale und zeitliche Schalleigenschaften, wie z.B. Frequenzgehalt und Zeitverlauf aus, um eine interne Repräsentation der Umgebung zu erhalten und auditorisch gesteuertes Verhalten zu kontrollieren. Mit echoortenden Fledermäusen als Modellsystem habe ich die spektrale und zeitliche Verarbeitung während der Echoortung untersucht und im Vergleich zum passiven Hören betrachtet, und im Anschluss die echoakustische Erkennung virtueller Objekte untersucht.

Im ersten Projekt (Kapitel 2) wurde die spektrale Verarbeitung zwischen passivem und aktivem Hören in der Fledermaus *Phyllostomus discolor* verglichen. Schalle werden während einer Vielzahl von Verhaltensweisen verwendet, z.B. zur Kommunikation, Räuber- und Beute-Detektion, oder Echoortung. Der Frequenzgehalt eines Schalles ist eine wichtige Vorraussetzung zur Entschlüsselung der übertragenen Information, wird jedoch während der Übertragung vom Sender zum Empfänger verändert. Um den Frequenzgehalt eines Schallsignals korrekt bestimmen zu können, muss der Empfänger diese Veränderungen kompensieren. Wir haben zuerst untersucht, ob *P. discolor* während des passiven Hörens Veränderungen der spektralen Form eines Schallsignals kompensieren kann. Dazu wurden die Tiere darauf dressiert, Tiefpass- von Hochpass-gefilterten akustischen Klicks zu unterscheiden, während sie gleichzeitig ein weißes Hintergrundrauschen mit einem flachen Frequenzgang hörten. Anschließend wurde ihre spontane Klassifikation akustischer Klicks mit unterschiedlichem Frequenzgehalt in Abhängigkeit vom Frequenzgang des Hintergrundrauschens (flach oder Tiefpass-gefiltert) bestimmt. Mit Tiefpass-gefiltertem Hintergrundrauschen wurden die akustischen Klicks häufiger als Hochpass-gefiltert klassifiziert als mit weißem Hintergrundrauschen. Die Fledermäuse kompensierten somit, wie Menschen, den Frequenzgehalt des Hintergrundrauschens. In einer aktiv-akustischen Version desselben Experiments mussten die Fledermäuse gefilterte Kopien ihrer Echoortungsrufe anstelle der akustischen Klicks klassifizieren. Die Klassifikation der gefilterten Echos war unabhängig von der spektralen Form des passiv präsentierten Hintergrundrauschens; ebenso änderte sich die Rufstruktur nicht. Die auditorische Verarbeitung unterscheidet sich folglich zwischen passivem und aktivem Hören; und die Echoortung stellt einen unabhängigen Modus mit eigenen Regeln der auditorischen spektralen Verarbeitung dar.

Das zweite Projekt (Kapitel 3) befasste sich mit der Präzision der Zeitmessung eines auditorischen Signals; und somit mit der Entfernungsmessung in der Echoortung. Passives Hören stellte sich außerdem als ein weiterer wichtiger und unerwarteter Faktor heraus. Fledermäuse messen Objektentfernungen, indem sie die Laufzeit zwischen dem ausgesandten Ruf und dem reflektierten Echo bestimmen. Distanzmessung wurde Gegenstand großen Interesses in der Echortungsforschung aus mehreren Gründen: (i) Distanzmessung ist wichtig im Verhalten der Fledermäuse zur Objektlokalisation und zur Orientierung in ihrer Umgebung, (ii) die präzise Messung kurzer Zeitintervalle ist physiologisch eine Herausforderung, und (iii) der vermutete echo-akustische Empfänger der Fledermäuse ist von Interesse in der Signalverarbeitung. Ich habe die nektarivore Art *Glossophaga soricina* darauf dressiert, ein schwingendes reales Objekt zu detektieren und konnte eine biologisch plausible Distanzgenauigkeit von 4–7 mm, entsprechend einer Zeitgenauigkeit von 20–40 μs, messen. Die variable Echo-Laufzeit war jedoch nicht das auffälligste Signal, das als erstes zur Detektion verwended wurde, sondern korrelierte passiv-akustische Klicks, die nur durch das Rückspielen von Rauschen maskiert werden konnten. Dies zeigt, dass auch eine Fledermaus, die nicht zu den *gleaning bats* gerechnet wird, passiv-akustische Signale auswertet und die Messung kurzer Zeitintervalle schwierig zu sein scheint. Dieses Ergebnis erhöht die Zweifel an den publizierten *time-jitter*-Schwellen von bis zu 10 ns.

Im dritten Projekt (Kaptiel 4) wurde die Wahrnehmung echo-akustischer Stimuli mit einem korrelierten Verhalten verknüpft, namentlich den Ausweichmanövern wilder, untrainierter Fledermäuse in Reaktion auf virtuelle Objekte, die in ihrer Flugbahn präsentiert wurden. Echoortende Fledermäuse navigieren in vollkommener Dunkelheit, indem sie die Echos ihrer Rufe auswerten und Objekte anhand ihrer Reflektionsmuster detektieren, erkennen und klassifizieren. Die auditorische Objektanalyse ist jedoch schwieriger als die visuelle Objektanalyse, da das eindimensionale akustische Zeitsignal nur Distanzinformation transportiert, also die Entfernung eines Objektes und seine Ausdehnung in Längsrichtung. Alle anderen Objektdimensionen wie Höhe und Breite müssen aus dem Vergleich der Signale an beiden Ohren und über die Zeit abgeleitet werden. Das Ziel dieser Studie war es, die von wilden, experimentell naïven Fledermäusen wahrgenommene Objektausdehnung anhand ihrer Ausweichmanöver um virtuelle echo-akustische Objekte zu bestimmen und mit den akustischen Echoeigenschaften zu korrelieren. Dazu wurden die Flugbahnen vorbeifliegender Fledermäuse gefilmt und ihre Ausweichreaktion quantifiziert. Als Kontrolle haben wir ebenso Flugbahnen in Reaktion auf ein reales Objekt ausgewertet, welches von den Fledermäusen seitlich umflogen wurde. Bei Präsentation virtueller Objekte konnten wir jedoch keine Änderung der Flugbahnen feststellen; vermutlich aufgrund der fehlenden räumlichen Ausdehnung virtueller echo-akustischer Objekte. Diese Studie zeigt zum ersten Mal die Wichtigkeit der räumlichen Ausdehnung auf, die bisher vernachlässigt wurde.

Summary

Basic spectral and temporal sound properties, such as frequency content and timing, are evaluated by the auditory system to build an internal representation of the external world and to generate auditory guided behaviour. Using echolocating bats as model system, I investigated aspects of spectral and temporal processing during echolocation and in relation to passive listening, and the echo-acoustic object recognition for navigation.

In the first project (chapter 2), the spectral processing during passive and active hearing was compared in the echolocting bat *Phyllostomus discolor*. Sounds are ubiquitously used for many vital behaviours, such as communication, predator and prey detection, or echolocation. The frequency content of a sound is one major component for the correct perception of the transmitted information, but it is distorted while travelling from the sound source to the receiver. In order to correctly determine the frequency content of an acoustic signal, the receiver needs to compensate for these distortions. We first investigated whether *P. discolor* compensates for distortions of the spectral shape of transmitted sounds during passive listening. Bats were trained to discriminate lowpass filtered from highpass filtered acoustic impulses, while hearing a continuous white noise background with a flat spectral shape. We then assessed their spontaneous classification of acoustic impulses with varying spectral content depending on the background's spectral shape (flat or lowpass filtered). Lowpass filtered noise background increased the proportion of highpass classifications of the same filtered impulses, compared to white noise background. Like humans, the bats thus compensated for the background's spectral shape. In an active-acoustic version of the identical experiment, the bats had to classify filtered playbacks of their emitted echolocation calls instead of passively presented impulses. During echolocation, the classification of the filtered echoes was independent of the spectral shape of the passively presented background noise. Likewise, call structure did not change to compensate for the background's spectral shape. Hence, auditory processing differs between passive and active hearing, with echolocation representing an independent mode with its own rules of auditory spectral analysis.

The second project (chapter 3) was concerned with the accurate measurement of the time of occurrence of auditory signals, and as such also distance in echolocation. In addition, the importance of passive listening compared to echolocation turned out to be an unexpected factor in this study. To measure the distance to objects, called ranging, bats measure the time delay between an outgoing call and its returning echo. Ranging accuracy received considerable interest in echolocation research for several reasons: (i) behaviourally, it is of importance for the bat's ability to locate objects and navigate its surrounding, (ii) physiologically, the neuronal implementation of precise measurements of very short time intervals is a challenge and (iii) the conjectured echo-acoustic receiver of bats is of interest for signal processing. Here, I trained the nectarivorous bat *Glossophaga soricina* to detect a jittering real target and found a biologically plausible distance accuracy of 4–7 mm, corresponding to a temporal accuracy of 20–40 μs. However, presumably all bats did not learn to use the jittering echo delay as the first and most prominent cue, but relied on passive acoustic listening first, which could only be prevented by the playback of masking noise. This shows that even a non-gleaning bat heavily relies on passive acoustic cues and that the measuring of short time intervals is difficult. This result questions other studies reporting a sub-microsecond time jitter threshold.

The third project (chapter 4) linked the perception of echo-acoustic stimuli to the appropriate behavioural reactions, namely evasive flight manoeuvres around virtual objects presented in the flight paths of wild, untrained bats. Echolocating bats are able to orient in complete darkness only by analysing the echoes of their emitted calls. They detect, recognize and classify objects based on the spectro-temporal reflection pattern received at the two ears. Auditory object analysis, however, is inevitably more complicated than visual object analysis, because the one-dimensional acoustic time signal only transmits range information, i.e., the object's distance and its longitudinal extent. All other object dimensions like width and height have to be inferred from comparative analysis of the signals at both ears and over time. The purpose of this study was to measure perceived object dimensions in wild, experimentally naïve bats by video-recording and analysing the bats' evasive flight manoeuvres in response to the presentation of virtual echo-acoustic objects with independently manipulated acoustic parameters. Flight manoeuvres were analysed by extracting the flight paths of all passing bats. As a control to our method, we also recorded the flight paths of bats in response to a real object. Bats avoided the real object by flying around it. However, we did not find any flight path changes in response to the presentation of several virtual objects. We assume that the missing spatial extent of virtual echo-acoustic objects, due to playback from only one loudspeaker, was the main reason for the failure to evoke evasive flight manoeuvres. This study therefore emphasises for the first time the importance of the spatial dimension of virtual objects, which were up to now neglected in virtual object presentations.

This thesis begins with a general introduction into the biology of bats and echolocation. The subsequent three chapters then present the experimental work with a detailed introduction into each specific topic and a discussion of the experimental findings. A general discussion at the end will then try to incorporate these findings into a broader context of echolocation research.

1 General introduction

“Bats fly and cry.” This tells in four words everything about bats – and it explains their evolutionary success and diverse adaptive radiation, their sensory ecology and neuronal specializations, as well as their physiology and energetics. This chapter gives a short general introduction into the biology of bats, then introduces hearing in mammals and bat echolocation and will finally integrate this work into the context of sensory physiology and ecology.

1.1 Bats

Bat lifestyle Two main features distinguish the classic Microchiroptera, the echolocating bats, from the other mammals: their ability for powered flight and echolocation. Both of these features are the reason behind their evolutionary success. Other prominent life history traits of bats are their small size, long lifetime and low rates of predation, their slow growth-rates, small litters and long gestation periods and their high metabolic rates (Neuweiler, 1990; Arita & Fenton, 1997).

Flight and echolocation enabled bats to occupy the niche of nocturnal aerial space. Within this large, general niche, they went trough an adaptive radiation into a variety of ecological niches (Neuweiler, 1990, 2000; Jones *et al.*, 2002), in terms of sensory, morphological and foraging specialisations – specialisations, which go often hand in hand. Most specialisations in flight performance and echolocation signal design result from the preferred foraging habitat of bats. Therefore, the first classification of bats into functional groups is mainly based on their preferred foraging habitat and its associated sensory constraints (Schnitzler & Kalko, 2001)

The foraging habitats normally recognised are the open space, the edge space, and the closed or cluttered space (Aldridge & Rautenbach, 1987; Neuweiler, 1989; Fenton, 1990; Schnitzler *et al.*, 2003). The open space is characterized by the absence of any background echoes. In edge space, bats perceive and react to background echoes, but these are still temporally separated from the prey echoes. In cluttered space, the echoes of prey sitting on vegetation or being very close in front of it temporally overlap with the background echoes. The sensory constraints on echolocation for foraging in these diverse habitats, e.g. detecting prey either far away in open space or close to vegetation in cluttered space, are discussed in the next section on echolocation.

The foraging niches occupied by bats are extremely diverse, corresponding to the diversity of habitats they forage in. Most of them feed on insects and other arthropods by aerial catching or by gleaning from the vegetation or the ground. Many bats feed on plant materials such as fruit, nectar and pollen, forcing them to orient close to and within vegetation, and some hunt small vertebrates like lizards, frogs, even little fish protruding from the water surface or birds in open space. The three species of vampire bats lick the blood of other vertebrates leaking from small wounds they have cut before (Fenton, 1990; Neuweiler, 2000; Popa-Lisseanu *et al.*, 2007; Voigt *et al.*, 2008).

The largest impact on general bat anatomy and morphology comes from the demands of powered flight, i.e. in the first place the skeletal remodelling necessary for the development of wings, but it also puts an upper limit on maximum body size. Wing shape is influenced by the foraging niche, with open space foragers having long wings for fast flight and cluttered space foragers broad wings allowing high manoeuvrability (Norberg & Rayner, 1987). Furthermore, skull morphology is heavily influenced by the preferred prey or food item of a species. Bats like the greater mouse-eared bat *Myotis myotis* preying on carabid beetles with hard carapace have strong skulls with typical predator dentition, whereas nectarivorous bats possess slender snouts and long tongues. Vampire bats and *Mystacina tuberculata* secondarily evolved a very good walking ability in response to their foraging style (Riskin *et al.*, 2006).

Bat phylogeny The order of bats (Chiroptera) is, after the Rodentia, the second largest order within the mammals, with ~1,100 species, currently grouped in 18 families and 202 genera (Simmons, 2005b). Both the position of the Chiroptera within the mammals and the systematics of the Chiroptera themselves are under discussion.

Within the mammals, the Chiroptera were classically placed into the Archonta, together with Dermoptera, Scandentia, Primates and the extant Plesiadapiformes (Shoshani & McKenna, 1998; Springer *et al.*, 2004). The Chiroptera were then generally divided, based on morphology, lifestyle and the use of echolocation vs. vision, into the suborders Microchiroptera, the echolocating or true bats, and Megachiroptera, the Old World fruit bats or flying foxes (reviewed in Simmons, 1998; Neuweiler, 2000). Pettigrew and colleagues questioned the bats' monophyly and their position in the mammalian tree in the 1980s with the 'flying primate hypothesis'. This hypothesis grouped the Megachiroptera together with Primates and Dermoptera, instead of with the Microchiroptera (Pettigrew, 1986; Pettigrew *et al.*, 1989) – but was rejected by many authors (e.g. Adkins & Honeycutt, 1991; Bailey *et al.*, 1992).

Nowadays, the monophyly of the Chiroptera is mostly accepted. However, their position within the mammalian tree is recently being changed by molecular data, placing the Chiroptera into the Laurasiatheria (together with the Cetartiodactyla, Perissodactyla, Carnivora, Pholidota and Eulipotyphla), with the remaining former Archonta now called Euarchonta (or Euarchontaglires = Euarchonta, Lagomorpha and Rodentia; Madsen *et al.* (2001); Murphy *et al.* (2001); Springer *et al.* (2004); Bininda-Emonds *et al.* (2007)). Similarly, the classic separation in Micro- and Megachiroptera is questioned by recent molecular phylogenies. Based on those data, the Microchiroptera are paraphyletic: the former microchiropteran superfamily Rhinolophoidea is a sister group to the Pteropodidae, the former Megachiroptera. Both together are constituting the new suborder Yinpterochiroptera, and leaving the rest of the "Microchiroptera" in the redefined suborder Yangochiroptera (e.g. Hutcheon *et al.*, 1998; Teeling *et al.*, 2000; Springer *et al.*, 2001; Teeling *et al.*, 2002, 2005; Jones & Teeling, 2006).

Much of the discussion about chiropteran systematics revolves around the point of the evolution of the two main features of bats: powered flight and echolocation. Both abilities were assumed to have developed once, and the succession of these evolutionary steps was discussed by the mutual exclusive 'flight-first' (Norberg, 1994; Simmons & Geisler, 1998), 'echolocation-first' (Fenton *et al.*, 1995) and 'tandem-development' (Speakman, 1993) hypotheses. A recent paper by Simmons *et al.* (2008) now provides evidence for the flight-first hypothesis by describing the novel eocene fossil bat *Onychonycteris finneyi*, which was capable of powered flight, but not of echolocation. This is the most basal ancestor of all known extinct and extant bats. All other known bat fossils were already able to echolocate, as deduced from the size of their cochlea and larynx and the shape of their ossicles. The flight-first hypothesis originally postulated the evolution of powered flight in the common ancestor of all bats (e.g. an animal with similarities to the recent Dermoptera), and then the evolution of echolocation in the common ancestor of the "Microchiroptera". The first non-echolocating ancestor of all bats is now confirmed by the new fossil. However, as the Microchiroptera are not regarded as monophyletic anymore, the most parsimonious assumption is now a small, nocturnal ancestor for all known extant and extinct bats except for *Onychonycteris finneyi*, with a simple form of echolocation. This simple echolocation was later lost in the Pteropodidae due to their increased use of vision, whereas the Rhinolophoidea and the Yangochiroptera developed it further to the present-day known sophisticated echolocating systems.

The recent echolocation by tongue-clicks found in the genus *Rousettus* seemed to have re-evolved secondarily (Springer *et al.*, 2001; Jones & Teeling, 2006). This is not as improbable as one might assume, given a pertinent evolutionary pressure: the fruit bat *Rousettus* and

Table 1.1: The systematic classification of the two species studied in chapter 2 and 3 of this thesis, *Phyllostomus discolor* and *Glossophaga soricina*, within the mammals.

Mammalia
Theria
Eutheria
Laurasiatheria
Chiroptera
Yangochiroptera
Noctilionoidea
Phyllostomidae

Phyllostominae
Phyllostomus
Phyllostomus discolor Wagner, 1843

Glossophaginae
Glossophaga
Glossophaga soricina Pallas, 1766

the non-related birds *Steatornis* and *Collocallia* are all using echolocation with acoustic clicks to navigate within dark caves.

Systematics of the studied species The work described in this thesis was carried out with neotropic bat species, mainly of the family Phyllostomidae (New World leaf-nosed bats), one of the largest bat families (Simmons, 2005b). Chapters 2 and 3 used trained, laboratory kept individuals of the species *Phyllostomus discolor* and *Glossophaga soricina*, whereas in chapter 4 the behaviour of wild free-flying bats was investigated. The systematic classification of the Phyllostomidae and the two studied species *Phyllostomus discolor* and *Glossophaga soricina* is shown in **Tab. 1.1** (Novacek, 1992; Hutcheon *et al.*, 1998; Madsen *et al.*, 2001; Teeling *et al.*, 2002; Simmons, 2005a; Teeling *et al.*, 2005).

1.2 Hearing and echolocation

The term echolocation means nothing more than the location (of objects) with echoes (Griffin, 1944). However, bats do more with echolocation than locating objects. They are able to perceive the size, surface and shape of objects, to measure structural differences between objects, to differentiate between objects and to build size-invariant mental representations of objects. Actually, echo-imaging would thus be a better term for echolocation

(Simmons *et al.*, 1990b; Neuweiler, 2000; Firzlaff *et al.*, 2006, 2007); however, echolocation is the well-established one.

Echolocation is an active sensory system, where the animal employs self-generated acoustic signals to probe its surrounding (Nelson & MacIver, 2006). A similar active sensory system is the electrolocation employed by weakly electric fish such as the members of the order Gymnotiformes and of the family Mormyridae.

What does active sensing mean? Every sensory process can be called active, as every one involves some kind of active processes, including muscle activation for eye, ear or whisker movements or for sniffing and also neuronal processes such as attention. Similarly, the active movement of the animal in space alters its sensory input. For example, the visual flow pattern on the retina depends on the animal's movement. Thus, vision is also called active by some authors (Kern *et al.*, 2005). However, the distinctiveness of active sensing systems such as echolocation and electrolocation is that the animal is actively generating a signal which is used to probe its surrounding (Nelson & MacIver, 2006). In this thesis, "active" sensing is exclusively used in this sense – and is contrasted to passive listening, which does not involve the generation of echolocation calls. By actively generating its signals, the animal controls its flow of sensory information itself. Visually, this can be compared to being in the dark with a flashing light: every time the light is flashing, the propagating light pulse is reflected from the surrounding objects, enabling us to see a short snapshot of the environment. In electrolocation, weakly electric fish generate an electric field by modified muscle cells (electrocytes), which is altered by objects in the fish's surrounding, and which they perceive by electroreceptors (Rasnow, 1996; Graff *et al.*, 2004; von der Emde & Fetz, 2007). In echolocation, an acoustic signal is reflected by the objects in the animal's environment, thus generating echoes which are reflected back to the animal and perceived and analysed by the auditory system.

Sophisticated echolocation is only known from bats and toothed whales (Odontoceti). Almost all echolocating bats produce calls generated in the larynx, whereas toothed whales and the bat-genus *Rousettus* generate short acoustic clicks to probe their surroundings. Other forms of echolocation used for orientation are known from the oilbird (*Steatornis caripensis*) and the cave swiflets (tribe Collocaliini within the Apodidae). Some studies also suggested the presence of simple forms of echolocation in insectivores and rodents (Gould *et al.*, 1964, review in Thomas & Jalili, 2004), tenrecs (Gould, 1965), and seals (Renouf & Davis, 1982); and it is also employed by humans (Griffin, 1944).

History of echolocation research We know that Lazzaro Spallanzani, the bishop of Padua, conducted experiments in the 1790's with flying bats in darkness. He could show that bats do not need vision for orientation, but he could not imagine which sense they might use instead to find their way. During the next 150 years, many researchers thought about that (reviews in e.g. Griffin & Galambos, 1941; Galambos & Griffin, 1942; Dijkgraaf, 1946), until Hartridge (1920, p. 56) suggested "that bats during flight emit a short wavelength note and that this sound is reflected from objects in the vicinity." The emission of ultrasonic calls was later shown by D. Griffin with the new ultrasonic microphone of G. W. Pierce (Pierce & Griffin, 1938) and also, independently, recognised by Dijkgraaf (1943). Griffin & Galambos (1941) and Dijkgraaf (1946) repeated and extended Spallanzani's experiments and could all show that bats rely on acoustic signals for orientation. Since this fundamental question of how bats perceive their surrounding was settled, many details of their perception and the underlying mechanisms were discovered.

Echolocation behaviour Echolocating bats emit (mostly) ultrasonic calls and evaluate the spectrotemporal properties of the returning echoes to gather information about their surrounding. Conceptually, object perception can be divided into object detection, localisation and classification (Schnitzler & Kalko, 2001), all with different requirements on signal design and auditory processing.

Two general types of echolocation signals are distinguished, frequency-modulated (FM) and constant-frequency (CF) calls, with many subtypes and combinations (Jones & Teeling, 2006). Most bats employ a type of FM call, with varying duration and degree of frequency modulation dependent on their phylogeny, habitat and sensory needs (Neuweiler, 1989; Schnitzler *et al.*, 2003; Jones & Teeling, 2006). Specialised CF bats such as the Rhinolophoidea, Hipposideridae and *Pternonotus parnellii* developed a specialized hearing system with extremely fine frequency analysis enabling them to use their long, constant frequency calls as carrier for fine amplitude and frequency modulations generated by fluttering insects (Schnitzler & Flieger, 1983; Kober & Schnitzler, 1990).

The first step in object perception is detection, meaning a bat has to decide whether a suprathreshold echo to an own echolocation call is present or not. Object detection is best accomplished with loud calls of long duration, that concentrate their energy in a narrow bandwidth, and a long temporal integration time to add up reflected echo energy. Detection distances of large objects like trees, buildings and other possible landmarks were estimated to be up to 90 m, whereas smaller objects like flowers or insects are only detectable in a few meters distance, in particular with high call frequencies (Griffin, 1971; Holderied & von Helversen, 2003; Stilz, 2004). Tens of centimetres to a few meters is also roughly the observed detection distance of bats for small objects (Griffin *et al.*, 1960; Kick, 1982; Kalko & Schnitzler, 1993). Echolocation is thus mainly a short-range sense. Reasons for this are in the first place atmospheric attenuation, especially of high frequencies, but also geometric attenuation, low target strength, signal-to-noise-ratio, and even fog (Griffin, 1971; Pye, 1971; Crocker, 1998; Stilz, 2004).

In object localisation, the distance and direction to an object are determined. Binaural (interaural intensity and time differences; Erulkar, 1972; Grothe, 2000) and monaural cues (spectral interference pattern; Wotton *et al.*, 1996) are used to measure echo direction. The range to an object is encoded in the time delay between call emission and returning echo (Simmons, 1973). If several objects are present, their intertarget distance is another parameter which can be measured in the temporal domain, when objects are far apart and their echoes are therefore temporally resolvable, or in the spectral domain, when objects are close and their echoes fall into the temporal integration time of auditory filters, generating object specific spectral interference patterns (Schmidt, 1988). Shorter, broadband signals are best suited for the localisation of objects. The accuracy of distance measurement is the topic of chapter 3.

Complex objects have object specific spectral reflection patterns (von Helversen *et al.*, 2003) or differ in stochastic reflection parameters (Muller & Kuc, 2000; Stilz, 2004). Object classification involves the analysis of these object-specific temporal and spectral reflection patterns in the echoes (e.g. Schmidt, 1992; Grunwald *et al.*, 2004; von Helversen, 2004), requiring broadband signals and a fine temporal and/or frequency resolution. Chapter 2 compares the influence of spectral distortions by background noise on the processing of spectral information during passive listening and active echolocation.

Each bat species has a distinct repertoire of echolocation calls with features that can be adjusted to its current sensory needs. A well studied example is the change in spectro-temporal call structure when an insectivorous bats flies towards a prey item. The sequence of echolocation calls during the pursuit of an insect is generally divided into three stages: the search, approach and terminal phase. Bats emit calls which are optimised for the detection of small objects during the search phase. After prey detection, the calls of the approach phase become shorter and broader in bandwidth to increase temporal and spatial resolution. Calls are also emitted with shorter intervals to increase the information update rate. The terminal phase starts when the bat closes in on its prey and emits the final buzz I and II, where the call interval and the call itself become even shorter and the call frequency lower (Griffin *et al.*, 1960; Kalko & Schnitzler, 1989; Kalko, 1995). Although this echolocation pattern was described in all hunting bats and is partly stereotyped, the function of the final buzz is unclear and was not yet experimentally investigated.

Echolocation sequences during orientation are similar to search phase calls, which are regarded as the ‘standard’ type of an echolocation call used for general tasks. Only recently some studies investigated echolocation behaviour during natural transfer flights (Verboom *et al.*, 1999; Holderied & von Helversen, 2006; Schaub & Schnitzler, 2007a,b) and during landing control (Melcón *et al.*, 2007). The echolocation pattern while approaching a landing position is similar to prey capture, but sometimes without a distinct final buzz II phase (Melcón *et al.*, 2007).

The question behind these detailed studies is about the perception and internal representation of a world that is not easily accessible to us. How do bats "see" the world with a stroboscopic, directional acoustic signal? What kind of internal representation do they build from the sensory input they receive? Understanding this allows us to understand the sensory world of bats, including signal processing and their evolution into their ecological niches.

Hearing in general and echolocation In the centre of a proximate understanding of echolocation is the hearing system. The bat's auditory specialisations are based on a normal mammalian auditory system (Moss & Sinha, 2003; Covey, 2005). Sound transduction starts at the pinna, imprinting direction-dependent spectral patterns onto the received signals. The middle ear ossicles transmit the sound energy to the inner ear cochlea, where the frequency-place transformation occurs and tonotopic neuronal excitation is generated at the inner hair cells.

The first specialisations for echolocation occur already at the level of the cochlea in CF-bats, having an acoustic fovea. The frequency resolution at the acoustic fovea is heavily increased by mechanical adaptations of the basilar membrane and an over-representation of the individual's echo frequency along the cochlea (Vater *et al.*, 1985; Kössl & Vater, 1990), resulting in extremely sharply tuned auditory neurons. The ascending auditory pathway has hypertrophied auditory nuclei (e.g. Covey & Casseday, 1991), showing the importance of acoustic information in bats. Interestingly, the binaural properties of brainstem nuclei differ between species with different call types. CF-species, for example, have a monaural medial superior olive (MSO), but pure FM-bats have a binaural MSO (Grothe *et al.*, 1992, 2001).

In the cortex of bats, several auditory fields were found that are sensitive to specific echo-acoustic parameters or even parameter-combinations. Often, these fields are arranged in a map-like fashion, comparable to the tonotopic organisation of the auditory system. Suga & O'Neill (1979) were the first to describe a neuronal map which represented the range of objects. Such maps contain neurons that are sensitive to a fixed delay between emitted call and returning echo (Feng *et al.*, 1978; O'Neill & Suga, 1979). The properties of the cortical ranging neurons are already generated at lower levels of the ascending auditory pathway, as delay-tuned neurons are also present in the midbrain and thalamus (Moss & Sinha, 2003). Signal duration is another temporal parameter that is coded in specialised neurons that combine excitatory and inhibitory inputs (Casseday *et al.*, 1994; Fuzessery & Hall, 1999; Faure *et al.*, 2003). Furthermore, many neurons show additional sensitivity to sound direction, level, bandwidth or temporal rate, thereby coding additional stimuli features.

All sensory and motor processes involved in echolocation are modulated and adjusted to the current situation, generating a complex network of interdependent processes. A temporal processing window for echo analysis is opened each time a call is emitted (Roverud & Grinnell, 1985b), and head and pinna movement are influenced (Moss & Sinha, 2003). Neuronal echo analysis on all processing stages, from the cochlear hair cells over the brainstem to the midbrain, is adjusted to the changing spectrotemporal properties of the calls and the returning echoes by descending projections from the cortex (Yan & Suga, 1998; Xiao & Suga, 2002; Moss & Sinha, 2003). For example, the received echo level leads to an adaptation of call level in order to reduce the dynamic range of the received echo levels (Hartley, 1992). CF-bats during flight lower their call frequency so that the Doppler-shifted echoes return at the frequency of their acoustic fovea (Schnitzler, 1973; Lancaster *et al.*, 1992).

Why study bats and echolocation? In my opinion, there are two main and very general reasons for studying bats and echolocation: bats are a fascinating group of mammals, for several reasons, giving us the chance to learn a great deal about organismic biology. To these interesting features of bats belong their unusual life history compared to other small mammals, their diverse adaptive radiation into a multitude of ecological niches, their physiology and energetics, with extremes such as a very high metabolism with fast heart beat and breathing rate on one side and torpor on the other side, their ability to powered flight and echolocation and their variety of social systems. All this together defines the typical lifestyle of a bat, which is unique and special in all its interrelations and mutual interferences.

Second, echolocation is a very special sensory systems, but grounded on the general mammalian hearing system. The ability of bats to solve a huge variety of tasks, either in their natural habitat or in experiments, is astonishing. So even more, as echolocation cannot deliver such a highly resolved picture of the world, as can the binocular, multichromatic, two-dimensional visual system with its high spatial resolution.

Comparative research of echolocation, passive listening and other active sensing systems will increase our understanding of the evolution of echolocation and how the auditory system, the vocal tract and the corresponding neural pathways adapted to accomplish this new feat.

Furthermore, echolocation offers a unique opportunity for researchers in sensory ecology, physiology and information theory alike: the need for sensory input of an echolocating animal can be directly observed, simply by recording its calls. Correlating the calls' temporal and spectral pattern to the animal's current situation, manipulating the tasks it has to solve, measuring the neuronal responses and observing its motor behaviour and the animal's interactions with its environment, its conspecifics and its prey, will help us understanding how sensory systems work in general. Analysing the interplay between information gathering, motor programs and behaviour will show how information about the outer world is translated into behavioural decisions.

With this in mind, there are still a variety of unexplored fields in echolocation research.

While many studies elucidated how bats perceive the features of single objects, we know little about how the echoes of several objects and of consecutive calls are perceptually organized in the internal representation of an acoustic scene. This includes the recognition and grouping of acoustic objects, acoustic stream segregation and the comparison of novel information with stored representations and the combination of auditory with other sensory information (Bregman, 1990; Moss & Surlykke, 2001).

The next step from the controlled laboratory into the field and from simple tasks to naturalistic situations has to be taken. A very fruitful approach, which was already applied in echolocating whales, was adopted in a few bat studies where the echolocation signals in freely flying bats were recorded (Lancaster *et al.*, 1992; Hiryu *et al.*, 2007). In whales, such automatic recording devices gave fascinating combined results on the movement, foraging and echolocation behaviour of single individuals and its group of conspecifics (e.g. Johnson *et al.*, 2004; Tyack *et al.*, 2006). On the neuronal site, Ulanovsky *et al.* (2007) started to record neuronal responses in freely moving – but not yet flying – bats to record neurons while the animal is engaged in its usual behaviour.

The evolution of echolocation and echolocation call design is still not clear (Jones & Teeling, 2006), especially the often conflicting impact of ecology and phylogeny on a given call design. More comparative studies will give further insights into the adaptive value of a certain call design. This needs to be combined with research on niche differentiation and resource partitioning between related bat species, and the interaction and coevolution between bats and their prey.

In general, bats should be regarded and used as a model system for general biological phenomena of broad interest, such as the physiology and ecology of sensory perception and the neuronal basis of behaviour, the evolution of specialized traits, predator-prey-interactions or niche differentiation and resource partitioning between competing species.

The real-time, phantom-target generation technique We know about the behaviour of bats through many field studies, whereas the abilities and limits of echolocation were mainly explored in a variety of psychoacoustical lab studies, testing the bats' performance in precisely controlled acoustic situations. This is done by either presenting real objects as echo reflectors (Simmons, 1971; Habersetzer & Vogler, 1983; Simon *et al.*, 2006), or by playing back in real time the echoes of computer generated virtual echo reflectors. The second method, the real-time, phantom-target generation technique (Simmons, 1973; Schmidt, 1988; Aubauer & Au, 1998), uses the acoustic impulse response of an object to generate the object's echo in response to recorded echolocation calls.

The impulse response (IR) of any system is its output ('response') when excited with an impulse of theoretically infinitely short duration and infinitely high amplitude, containing the same energy at all frequencies (the so-called Dirac-Impulse δ). The acoustic impulse response of an object is thus its echo ('response') when ensonified with an acoustic impulse, i.e., simply its temporal reflection pattern (**Fig. 1.1 A**). The spectral analogue of the impulse response is the transfer function of an object, which describes its spectral reflection pattern. Both can be calculated from each other by means of a Fourier Transformation or an inverse Fourier Transformation. The acoustic impulse response of an object can be seen as the acoustic image of that object, in the same way as the visual image of an object is its visual impulse response (or visual reflection pattern), which was taken with a camera and a flash.

As a one-dimensional, linear, time-invariant and dynamic system is completely described by its impulse response, the impulse response can be used to calculate the response of this system to any other stimulus. This is the principle behind the phantom-echo generation technique employed in chapters 2 and 4 of this thesis. This calculation can be either done in the time domain by convolution of the time signals or in the frequency domain by multiplication of their amplitude spectra and addition of their phase spectra. The convolution of two signals is the integral of the product of both signals, after one was time-reversed and shifted along the time-axis point by point. If one signal is the impulse response of an object, and the other signal the recorded echolocation call of a bat, the echo reflected back by this object can directly be calculated and played back to the bat with a loudspeaker (**Fig. 1.1 C**). In this way, the echo of virtual objects can be easily generated, manipulated and presented to investigate the behaviour of bats, without the need to move around real objects. The generated echo is exactly the same as if the bat had ensonified the real object (**Fig. 1.1 B**).

1.3 Perception and action in the context of sensory physiology and sensory ecology

Sensory physiology aims to understand the neuronal mechanisms of sensory systems ('proximate mechanisms'), whereas sensory ecology aims to understand the evolution of a sensory system and its adaptive value for the ecological niche of an animal ('ultimate mechanisms').

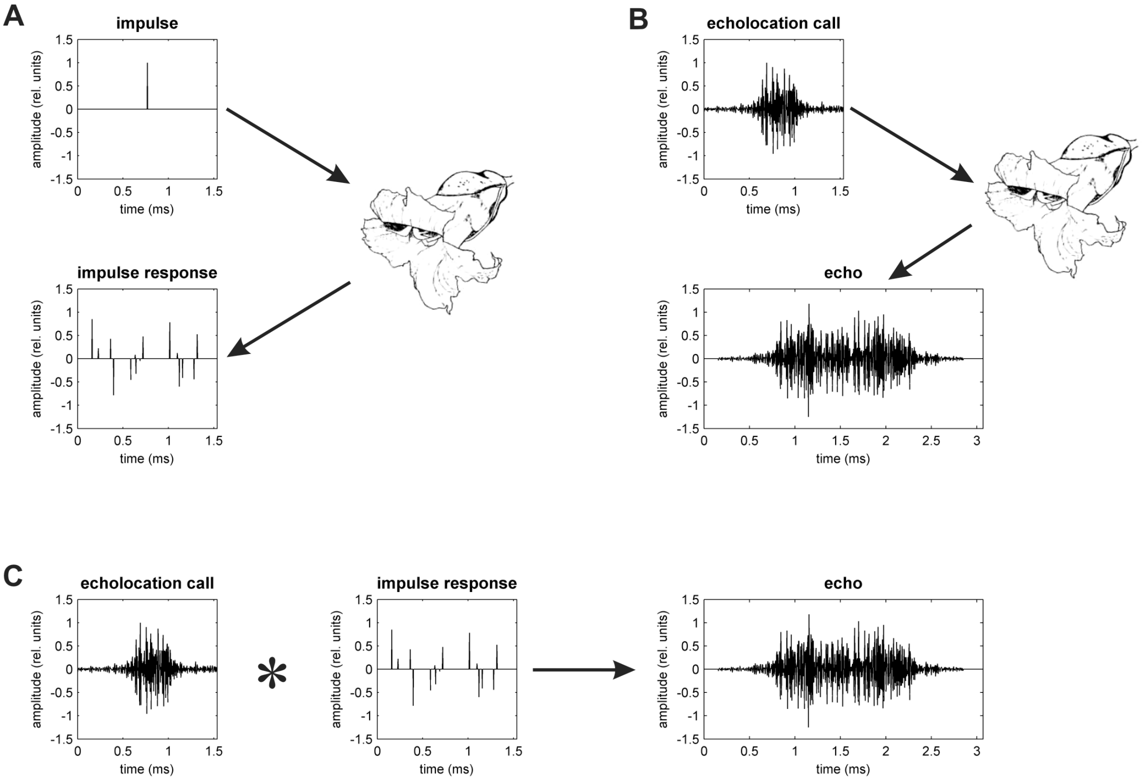


Figure 1.1: The impulse response and the generation of echoes.

A) The impulse response of an object is the echo of the object when ensonified with an impulse. The impulse response contains all object specific acoustic information, e.g. the temporal gaps between the reflections are defined by the distances between the reflecting surfaces, and their amplitudes by the size of the reflectors.

B) When the object is ensonified with a different signal, e.g. an echolocation call, an echo is reflected as a combination of the object- and call-specific acoustic features, i.e., the object's impulse response is imprinted on the echolocation call.

C) The echo which was reflected from the object when it was ensonified with an echolocation call can be generated by convolution of the object-specific impulse response with the echolocation call.

The drawing of the flower was taken from von Helversen *et al.* (2003).

Sound is used in many contexts and is ubiquitously and inevitably coupled to almost all animal behaviour, e.g. during locomotion and communication. Sound generated somewhere in the environment of an animal spreads out, is distorted during transmission and eventually reaches the animal's ear. This animal may be the intended receiver, e.g. the calling pup's mother or the courted partner, but also an unintended receiver, such as a predator. The receiver has to detect, analyse and judge this sound. Then, after decoding the acoustic information and deciding whether this sound is behaviourally relevant or not, the receiver has to start an appropriate reaction, which may be approaching, attacking and catching

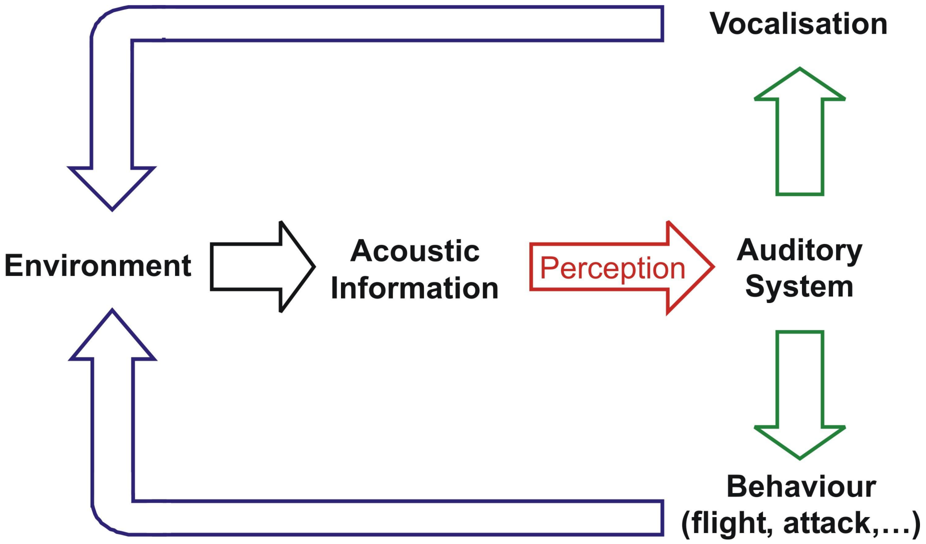


Figure 1.2: Perception-action-loop for bats and their auditory-based interaction with their environment. The environment provides acoustic information (black arrow), which is perceived and processed in the auditory system (red arrow). Based on this and other information from other sensory systems and on the internal state of the animal, it activates its motor system to generate behaviour (green arrows), thus interacting with its environment (blue arrows). The characteristic of echolocation is that by vocalising, the bat has active control over its sensory input. This perception-action-loop can be adapted for all other sensory systems and animals.

a prey, answering to a potential mate, escaping from a predator or threatening towards it, or just paying more attention to the next sound or other sensory information coming from this direction. All these behavioural reactions change the animal's location within its environment and/or its attentional state and thus influences the auditory information it will perceive the next time. Such a perception-action-loop is schematized in the lower half of **Fig. 1.2**.

Active sensory systems, such as echolocation, possess the additional feature that the information receiver actively generated its received sensory input by generating a signal, e.g. an echolocation call, to probe its environment. An echolocation call is reflected from objects in the surrounding and transports information about those reflectors back to the bat. Based on this sensory input, the bat then can flexibly adjust a variety of call parameters to match the current sensory situation – this represents another perception-action-loop, depicted in the upper half of **Fig. 1.2**.

Focus of this thesis This thesis focuses on several parts within this perception-action-loop. All work was experimental, which means that the animal's environment and its acoustic information was altered. The perception of the acoustic information was then investigated by observing the behaviour in two conditioning experiments and in one experiment with unconditioned, wild, freely moving and experimentally naïve bats. Unconditioned behavioural reactions in response to altered acoustic information were analysed for one thing as alterations in the call's frequency structure and for another thing as flight path changes.

- The first project investigated the spectral processing of transient sounds. It was investigated whether bats compensate for the distortion of the spectral envelope of acoustic signals during passive listening, and whether bats process echoes differently from passively presented sounds in the same experimental situation. In addition, the frequency structure of the bat's vocalisation was analysed to see whether bats actively adjust their echolocation calls in response to spectral distortions. This study thus compared passive and active auditory processing and analysed the interaction between echo-perception and vocalisations. The stimuli for this work were presented via the real-time, phantom target generation technique, i.e., the echoes of computer-generated virtual objects were presented via loudspeakers.
- In the second project, the accuracy of the perception of an important echoacoustic parameter was investigated, namely echo-delay. Bats measure the range to objects by the time delay between call emission and the returning echo. The accuracy of delay measurement tells us about the bat's orientation accuracy in the wild, and also about the processing of acoustic information in the brain. In contrast to the first project, real targets instead of virtual targets were used to present the time-varying stimuli. This project also lead to a comparison between passive acoustic information and active echolocation.
- In the third project, we investigated the flight behaviour of free-flying wild bats. This work used again the real-time, phantom target generation technique to present and manipulate several echo-acoustic object parameters. The unconditioned flight paths of naïve bats served as a measure for their perception of echo-acoustic information in order to understand how bats relate given echo-acoustic information to object features and how they use this information to navigate by echolocation.

The following chapter was submitted as manuscript to The Journal of Experimental Biology *under the title "Comparing passive and active hearing: Spectral analysis of transient sounds in bats", authored by Holger R. Goerlitz, Mathias Hübner and Lutz Wiegrebe. A revised version of the originally submitted manuscript is currently under review.*

Lutz Wiegrebe, Mathias Hübner and I conceived the experiment and the analysis. Mathias Hübner wrote the experimental program and collected the data of the part about compensation during passive hearing. Lutz Wiegrebe proof-read the manuscript. I rewrote the experimental program and collected the data of the part about spectral processing during echolocation, analysed all behavioural data and the recorded echolocation calls and wrote the manuscript.

The revised manuscript, which is currently under review, was extended for this thesis in the following way: The methods were extended by the section 2.2.1 and the figure 2.1 to give more information on the experimental animal Phyllostomus discolor *and by the figures 2.2, 2.4 and 2.5 to include more details about the experimental setup. The section 2.3.1 with figure 2.6 was included into the results.*

***Addendum**: The manuscript of this chapter was accepted for publication (28.02.2008) and was published in* J. Exp. Biol.*, 211(12), pp. 1850–1858 (30.05.2008).*

2 Comparing Passive and Active Hearing: Spectral Analysis of Transient Sounds

2.1 Introduction

The colour-constancy of the visual system is a well-known perceptual phenomenon that enables the constant perception of an object's colour despite changes in the spectral composition of the light illuminating the object (Smithson, 2005). Humans perceive the colour of a white object still as white and the colour of a red object still as red, independent of illumination by yellowish candle light or greenish neon light.

The timbral constancy of the auditory system (Risset & Wessel, 1982) is an analogous perceptual phenomenon that allows the perception of a sound signal's spectral shape (i.e., its timbre or acoustic colour) despite spectral changes caused by the environment (Watkins, 1991). The spectral shape of a sound signal is one major component for the identification, grouping and segregation of different auditory signals (Bregman, 1990; Moss & Surlykke, 2001; Griffiths & Warren, 2004). However, the spectral shape of acoustic waves is distorted during propagation in an unpredictable manner (Wiley & Richards, 1978; Lawrence & Simmons, 1982), thus blurring the information content of the sound. The spectrum of a sound signal reaching the ear is hence the product of the spectrum of the sound source and the spectrum of the surrounding.

Two options exist to cope with such acoustic distortions: either the sender adapts its vocalization behaviour, or the receiver compensates for these distortions. Species of almost all vertebrate classes show the first option, i.e., changes in their vocalization behaviour, e.g. frogs (Feng *et al.*, 2006), birds (Lengagne *et al.*, 1999; Slabbekoorn & Peet, 2003; Brumm, 2004; Slabbekoorn & den Boer-Visser, 2006), monkeys (Egnor *et al.*, 2007) and whales (Au *et al.*, 1985; Miller *et al.*, 2000; Foote *et al.*, 2004). The second option, the timbral constancy of perceived sounds, was only investigated in humans. Despite the ubiquitous distortions of the spectral envelope of acoustic signals, humans can easily identify them. The auditory system compensates for the spectral characteristics of the environment, resulting in an undistorted perception of the original spectrum of the signal (Risset & Wessel, 1982;

Watkins, 1991). When the frequency response of the environment is experimentally manipulated, timbral constancy leads to several perceptual phenomena, such as the 'phoneme boundary shift' between intergradient vowels (Watkins, 1991), the 'flat spectrum vowel effect' (Summerfield *et al.*, 1987) and the 'Zwicker tone' (Zwicker, 1964).

Echolocating bats allow comparison between potentially different mechanisms of auditory spectral processing, since they use both passive and active hearing. The spectral information in both contexts is of great importance and needs to be perceived correctly. Passive hearing is used for vocal communication and rustling prey detection (Esser & Schubert, 1998; Boughman & Wilkinson, 1998; Arlettaz *et al.*, 2001) and we would expect bats to compensate for spectral distortions in the same way as humans. Active hearing, or echolocation, is used for spatial orientation and airborne prey detection (Schnitzler *et al.*, 2003) by listening for the echoes of self-generated ultrasonic calls and evaluating their temporal and spectral properties. For example, bats identify and discriminate objects exclusively based on their different spectral reflection patterns (von Helversen & von Helversen, 2003). Bats even separate and generalize the size-invariant spectral reflection patterns of the same object with different sizes from their size-variant spectral reflection patterns (von Helversen, 2004; Simon *et al.*, 2006).

The neuronal processing of auditory information is different in passive hearing and active echolocation and is closely coupled to and directly influenced by the vocalisations during echolocation (Suga & Schlegel, 1972; Schuller, 1979). The behavioural responses to auditory stimuli may thus differ in the same experimental task between passive and active hearing. However, whether this is the case was only investigated in one study on the temporal processing of communication sounds and echoes (Schuchmann, 2006).

In this study, we examined in two experiments how bats classify the spectral shape of transient foreground stimuli in relation to a simultaneous noise background with a constant spectral shape. The two experiments differed only in the type of signal that needed to be classified: (i) in the passive-acoustic experiment, the bats had to classify passively presented filtered impulses, which are temporally uncorrelated to their echolocation calls. (ii) In the active-acoustic experiment, filtered replica of their recorded echolocation calls were played back in real-time through a loudspeaker, thus generating a virtual echoacoustic object. During both experiments, a continuous noise background was presented, whose spectral shape was constant during a given trial and could be altered between trials.

During passive hearing, we expected the bats to use the background as a reference for the spectral analysis of the transient foreground stimuli, as humans do (Watkins, 1991). Thus, if the spectral shape of the background was changed, we hypothesised to see a shift in the classification as a result of the compensation for the changed environmental transfer characteristics. During echolocation, we hypothesised to find no influence of a passively

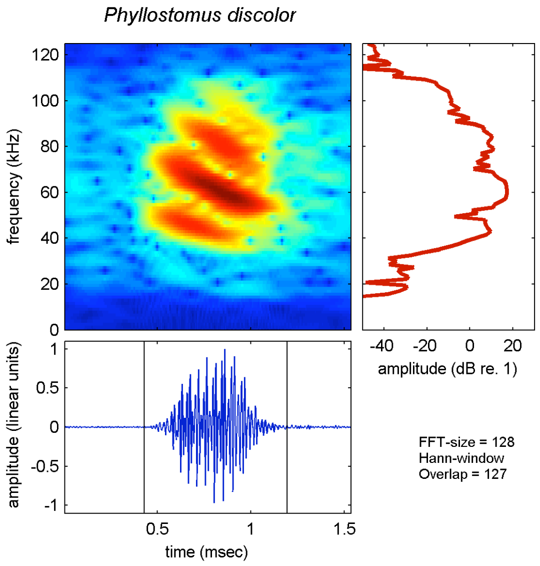


Figure 2.1: Echolocation call of *Phyllostomus discolor*, shown as spectrogram (top), as oscillogram (bottom) and as amplitude spectrum (right). The spectrum was calculated from the marked section in the oscillogram.

presented constant noise on the spectral classification of echoes, if bats processed echoes differently, and independently, from passive acoustic signals.

2.2 Methods

In two separate experiments on passive and active hearing, bats of the species *Phyllostomus discolor* were trained to classify signals with different spectral shape as containing more energy either in the high or low frequencies. After a training period with two signals that were easily distinguishable by their spectral shape, the bats' spontaneous classification of intermediate test signals was assessed depending on the spectral shape of a noise background.

2.2.1 *Phyllostomus discolor*

Phyllostomus discolor is the smallest (30–40 g) of the four species in the neotropic genus *Phyllostomus*, occuring from southern Mexico to northern Argentinia and in Trinidad (Nowak, 1994; Simmons, 2005b). *P. discolor* is an omnivorous species, foraging on fruits, pollen, nectar and insects, which are often caught in flowers and not on the wing (Nowak, 1994). However, Giannini & Kalko (2004, 2005) classified *P. discolor* as phytophageous, as a great amount of its food is plant material.

The echolocation calls are downward frequency modulated in the range from 40 to 90 kHz, with most energy in the second to fourth harmonics (Rother & Schmidt, 1982, see also the results section of this chapter and **Fig. 2.1**).

2.2.2 Animal housing and training procedure

Nine adult, male pale spear-nosed bats, *Phyllostomus discolor* Wagner 1843, were trained in the experiments. They were housed in groups of two to five animals per cage (80 × 60 × 80 cm) on a 12/12 h dark-light cycle, but could fly in a large room for several hours each day. Animals had free access to water. On training days, they received food only as a reward in the experiment. Additional food (fruit, mealworms) was given at the weekends. Bats were usually trained daily for 20–30 min at 5 d per week, followed by a 2 d break.

Bats were trained in a two-alternative, forced-choice paradigm to discriminate a highpass filtered training stimulus (+6 dB/octave) from a lowpass filtered training stimulus (-6 dB/octave) during continuous playback of white noise. Bats indicated their decision by crawling into the left or right arm of a Y-shaped maze (**Fig. 2.2**). Correct decisions were rewarded with banana-pulp; wrong decisions were neither rewarded nor punished. The next trial started when the animal had crawled back to the starting position at the top of the Y-shaped maze. The presented stimulus, and thus the rewarded side, was selected pseudo-randomly (Gellermann, 1933).

Data collection of the test conditions began after the bats classified the two training stimuli correctly with $>75\%$. Presentation of training stimuli was continued, serving as controls for a constant classification performance, while the test stimuli were interspersed between these control trials with a probability of 20–40% (Experiment 1) or 20% (Experiment 2). During a test-trial, one out of ten different test stimuli was selected randomly (see the next sections for description of the stimuli). Test trials were always rewarded, independent of the bat's decision. Only the test trials of days with a control performance $>75\%$ correct were included into the analysis.

We collected data of three bats in Experiment 1 (passive hearing) and of two bats in Experiment 2 (echolocation). Five animals (two in Experiment 1, three in Experiment 2) did not learn to classify the control stimuli and could thus not be used for testing. Most of these individuals were too calm and did not explore the setup. This was e.g. the case for Bat 2, which was successfully trained in Experiment 1, but did not cooperate anymore in Experiment 2. Other bats were too active and crawled quickly through the setup without paying enough attention. For example, one bat in Experiment 2 learned the task, but only showed a stable performance once or twice within several weeks.

2.2.3 Experiment 1: passive hearing

Stimuli A noise background of 45–55 dB SPL re. 20 μPa and 0–100 kHz bandwidth was continuously presented to the bats. The spectral envelope of the noise was either white (i.e. having a flat amplitude spectrum (0 dB/octave), used during training and testing) or

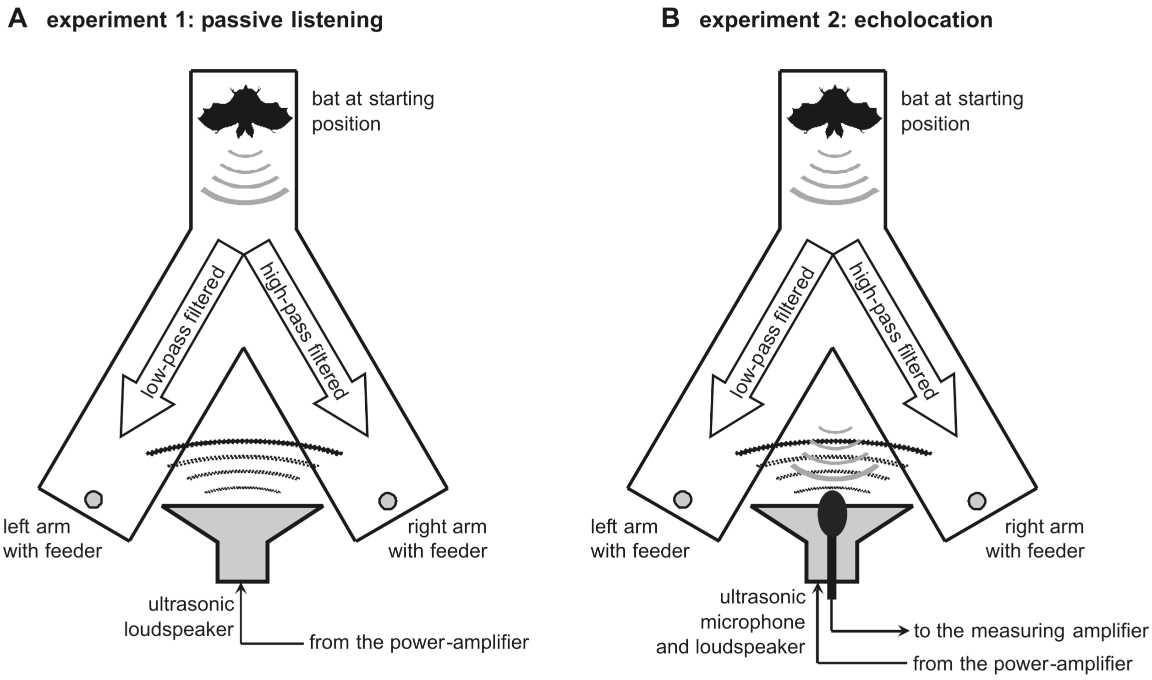


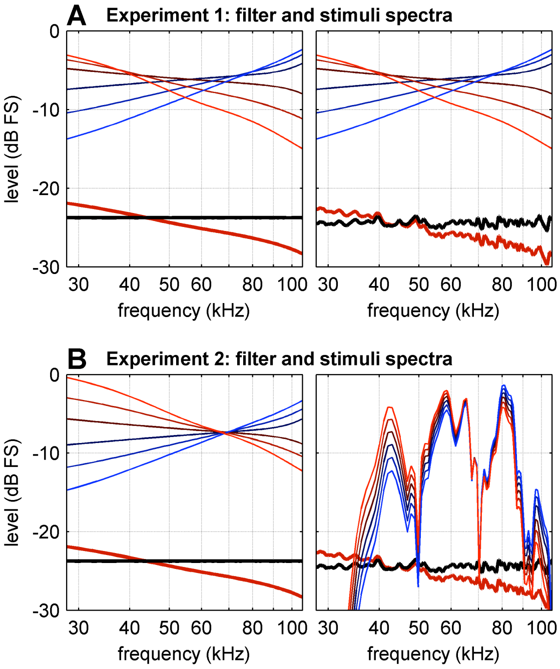
Figure 2.2: Y-shaped training platform. The bats were trained to wait at the starting position at the top of the the platform and to crawl to the end of one of the two arms after the presentation of a foreground signal. When the foreground signal was lowpass-filtered, they had to crawl into the left arm. When it was high-pass-filtered, they had to crawl into the right arm.
A) Training platform in Experiment 1 (passive hearing): A speaker was positioned between the two arms, which presented the noise background and the stimulus foreground.
B) Training platform in Experiment 2 (echolocation): A microphone was placed on top of the speaker. The microphone picked up the echolocation calls, which were filtered and added to the noise background and then presented to the bat via the same loudspeaker as in Experiment 1.

pink (i.e. an amplitude spectrum decreasing by -3 dB/octave, test trials only; **Fig. 2.3**).

Impulses of 7 μs duration with a flat frequency spectrum were used as foreground stimuli. They were presented at a repetition period of 500 ms, uncorrelated to the bats' echolocation calls. Bats were trained to discriminate impulses that were either high- or lowpass filtered, such that the amplitude of their spectrum either increased or decreased with 6 dB/octave (**Fig. 2.3**). After the bats had learned this task, we tested their spontaneous classification of impulses which were filtered by filters having transfer functions of intermediate slopes (±3.6 and ±1.2 dB/oct.; **Fig. 2.3**). The level of the filtered impulses was roved between ±6 dB to prevent the bats from using overall amplitude cues for classification.

Altogether, we had two training- (= control-) conditions, which were the ±6 dB/oct.-stimuli filters superimposed on white noise, and ten test-conditions, which were the four remaining stimuli filters of ±3.6 and ±1.2 dB/oct. superimposed on white noise and all six stimuli filters superimposed on pink noise.

Figure 2.3: Filter transfer function and amplitude spectra of example stimuli.
A) Experiment 1 (passive hearing).
B) Experiment 2 (echolocation).
The graphs on the left show the transfer functions of the filters used to generate the foreground stimuli and the noise background. The graphs on the right show examples of the amplitude spectra of the finally presented stimuli, i.e. after convolution of the filters with a foreground signal (an impulse or a recorded echolocation call) and with white white noise.
Thick solid lines: noise background (filter slope of 0 dB/oct. or -3 dB/oct.).
Thin solid lines: foreground stimuli (filter slopes from -6 dB/oct. to +6 dB/oct.).
The amplitudes of the spectra do not illustrate the amplitudes of the finally presented stimuli, which cannot be easily compared and additionally depended on the applied attenuation and on the call amplitude.



Stimulus generation The impulses (impulse generator HP 8012B, Hewlett-Packard, Palo Alto, CA, USA) and white noise background (noise generator 1405, Brüel & Kjær, Nærum, Denmark) were separately digitized and continuously filtered with 21-point digital filters (DAP-boards 5200a, Microstar Laboratories, Bellevue, WA, USA; 250 kHz sampling rate). All filters were normalized to their RMS to maintain a constant signal level. The analogue outputs of the DAP-boards were lowpass filtered at 100 kHz (FT 6, Tucker-Davis-Technologies, Alachua, FL, USA), attenuated (Crystal 3310, Cirrus Logic, Austin, TX, USA) and then summed together (SM 3, Tucker-Davis-Technologies). The combined stimulus was amplified (Yamaha M 35, Yamaha Corp., Hamamatsu Shizuoka, Japan in Exp. 1 and Rotel RB 960 BX, Rotel, Halle, Germany in Exp. 2) and played back via one ultrasonic loudspeaker (Technics EAS 10 TH 800D, Matsushita Electric Industrial, Osaka, Japan), which was placed in the middle between the two arms of the Y-shaped maze. Stimulus generation and presentation is summarised in **Fig. 2.4**.

Subjects Data of three bats were collected in Experiment 1 with 23-30 trials per subject and test-condition. The number of control trials for white and pink noise background, respectively, were 346/354 (Bat 1), 270/268 (Bat 2) and 273/250 (Bat 3).

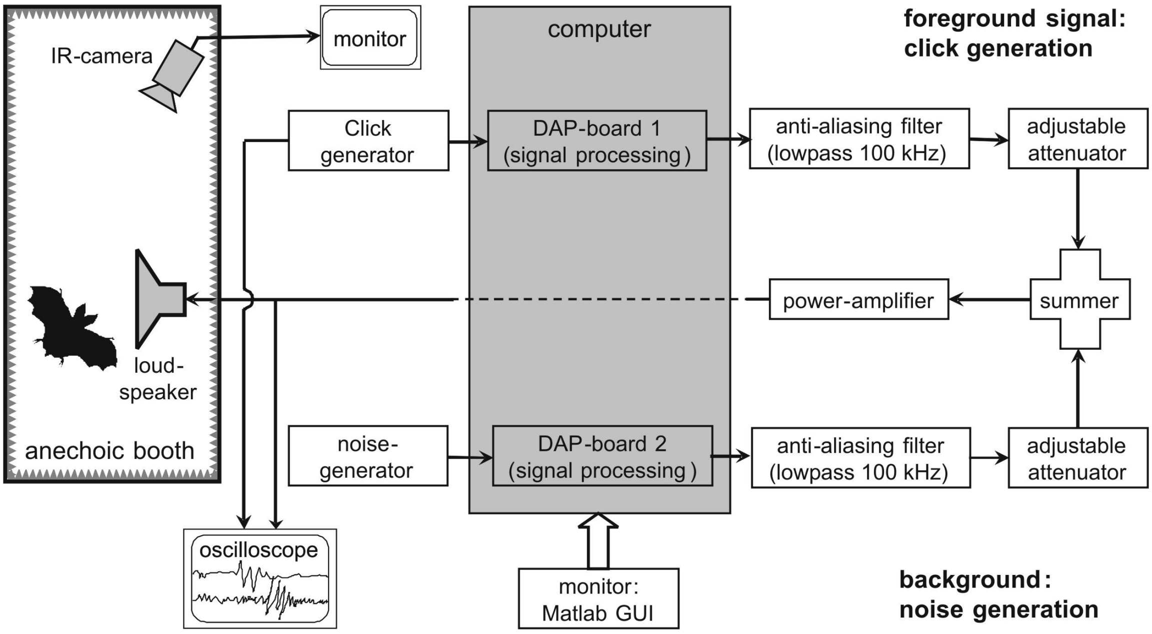


Figure 2.4: Block diagram of the stimulus generation- and presentation-setup in Experiment 1 (passive hearing).

2.2.4 Experiment 2: echolocation

Stimuli Instead of impulses, filtered replicas of the bats' echolocation calls were used as foreground stimuli, thus presenting virtual objects reflecting an echo every time the bat was emitting a call. The filters used for the foreground and background were the same as in Experiment 1 (**Fig. 2.3**). We thus presented again two training-/control-conditions and ten test-conditions.

Stimulus generation Echolocation calls were picked up with a microphone (MTG MV301, protection grid off, Microtech Gefell, Gefell, Germany; pre-amplifier 2671, Brüel & Kjær), which was placed on top of the loudspeaker in the middle between the two arms of the Y-shaped maze. Calls were amplified (measuring amplifier 2610, Brüel & Kjær) and bandpass filtered (model 3550, 4$^{\text{th}}$-order bandpass filter, 30–100 kHz, Krohn-Hite, Brockton, MA, USA), before they were processed by the DAP-board as described for Experiment 1 and then played back via the loudspeaker. In addition to normalizing the stimulus filters to their own RMS, they were normalized to the RMS of a model call of *P. discolor* filtered with the respective filter. Virtual object target strength was about -10 dB, which is around the upper bound of large three-dimensional objects relevant for orientation, such as tree trunks (Stilz, 2004). The electronic delay was 3 ms, thus positioning the virtual object about 50 cm behind the loudspeaker. The noise background was generated and filtered as

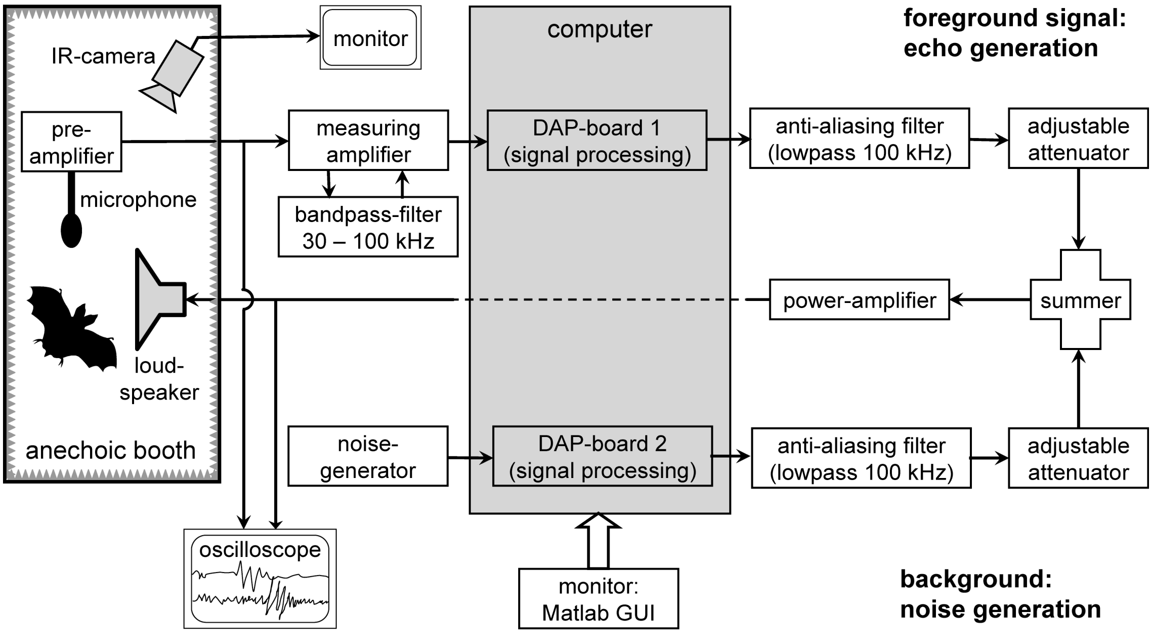


Figure 2.5: Block diagram of the stimulus generation- and presentation-setup in Experiment 2 (echolocation).

in Experiment 1. Stimulus generation and presentation is summarised in **Fig. 2.5**.

Subjects Data of two bats were collected with 42 (Bat 4) or 64 (Bat 5) trials per subject and test-condition. The number of control trials for white- and pink noise background, respectively, were 878/916 (Bat 4) and 1416/1439 (Bat 5).

2.2.5 Echolocation call recording and analysis

We recorded echolocation call sequences of the preceding 4 sec before decision during almost all trials in Experiment 2 (Phase 24, Terratec, Herrenpfad, Germany; 192 kHz sampling rate, 24 bit), altogether 2134 call sequences for Bat 4 and 3457 for Bat 5. We analysed the calls automatically by a custom written routine (Matlab 7.1, The Mathworks, Natick, MA, USA). Calls were detected as regional maxima above a constant threshold in the lowpass filtered (700 Hz) Hilbert-envelope and then extracted from the time signal, containing 5–95% of the total noise-corrected call energy. All calls with a signal-to-noise ratio $<$ 30 dB and a peak-amplitude $<$ -20 dB FS were removed and the remaining calls were checked visually to exclude obvious artefacts, e.g. clicks or external noise. From the five calls with the largest signal-to-noise ratio per sequence (= per trial), we calculated the mean per sequence of six call parameters (best frequency, -20 dB bandwidth and its corresponding lower and upper cut-off frequencies, frequency centroid and fundamental frequency). For further analysis, the sequence means were grouped, either per stimulus or per noise background, to calculate the respective second-order means per stimulus or per noise background.

2.2.6 Analysis and statistics

Perceptual classification boundary A perceptual classification boundary k was calculated for each animal according to the phoneme boundary used by Tyler *et al.* (1982) and Watkins (1991). The perceptual classification boundary is defined as the filter-slope that results in 50% of highpass and lowpass classification by:

$$k = m \cdot P - c \; . \tag{2.1}$$

where P is the mean percentage of highpass classifications of all six signals (0–100%). k is defined as 0 for $P = 50\%$, which is half of all possible highpass classifications, and k is defined as +6 when the steepest highpass filter (+6 dB/oct.) receives only 50% of highpass classification, and the others none ($P = 8.33\%$). This yields values of $m = -0.144$ and $c = -7.2$.

Roving level simulation We analysed the possible influence of the roving level on echo classification in Experiment 2 by applying a roving level of -6 to +6 dB to the five selected calls per sequence and calculating their frequency centroid on a logarithmic frequency axis. The frequency centroid divides the amplitude spectrum into two parts with equal energy and can therefore be used as a measure for the frequency distribution.

Statistics As we were limited to a small number of animals, we used each animal as its own control for the behavioural data. Behavioural classifications of the same foreground stimuli with different noise backgrounds were compared using Fisher's-Exact-test. Behavioural mean data were tested using Student's paired one-sided t-tests, testing the null-hypothesis against the one-sided alternative hypothesis that the percentage of highpass classification increases with pink noise background. Student's two-sided t-tests were used to compare means of the call parameters across backgrounds (Matlab 7.1). Significance levels are marked with $*$ if $p < 0.05$, with $**$ if $p < 0.01$, and with $***$ if $p < 0.001$.

2.3 Results

2.3.1 Training performance

Training and data collection took about 22 months with Bats 4 and 5. The daily performance over these period is presented in **Fig. 2.6** for both bats. Training until the bats

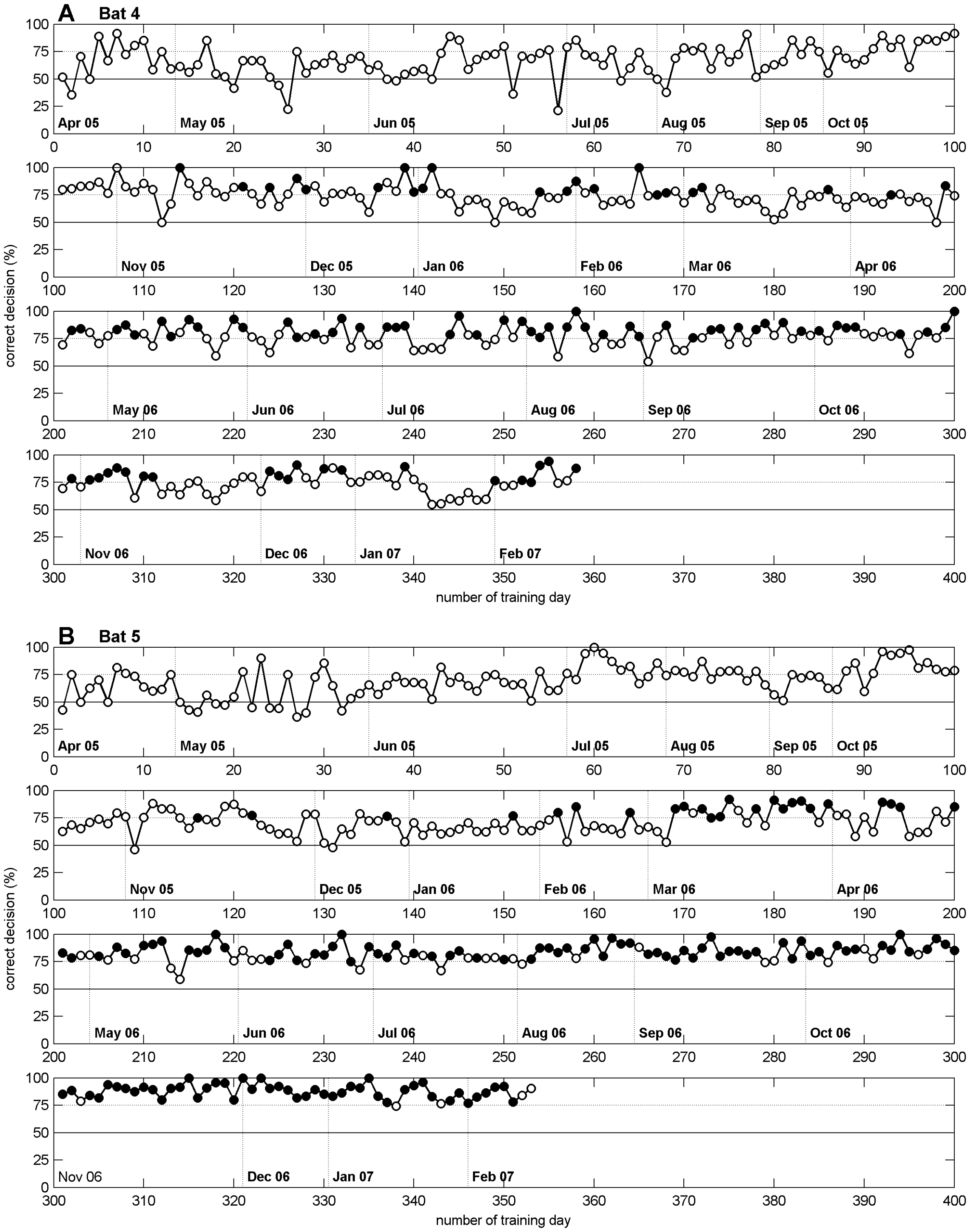


Figure 2.6: Learning curves of Bat 4 (**A**) and Bat 5 (**B**) in Experiment 2 (echolocation), showing the performance in the control-trials (±6 dB/oct., white noise). Chance level and 75%-threshold are marked with horizontal lines. Open symbols: training days without data collection, closed symbols: days with data collection.

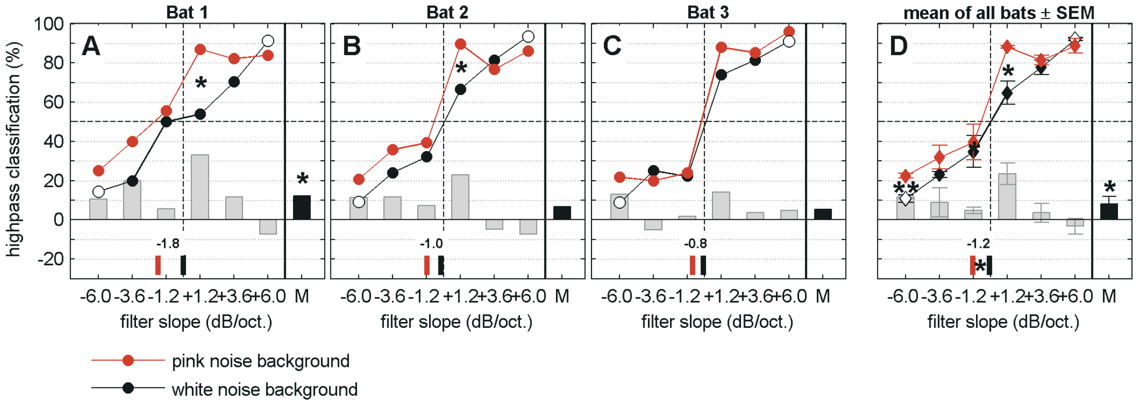


Figure 2.7: Experiment 1 (passive hearing): Classification of filtered impulses. Classification of filtered impulses by three bats (**A**–**C**) and their mean ± SEM (**D**).
The performance is plotted as percentage of highpass classification *versus* the filter slope. Trials with presentation of white noise background are shown with black symbols, and trials with pink noise background are shown with red symbols. The two training-/control-conditions (±6 dB/oct. and white noise) are indicated with open symbols.
The grey bars show the difference in highpass classification between pink and white noise background, their mean difference across all six filter slopes (marked 'M') is shown as black bar.
The perceptual classification boundaries per background are indicated by vertical lines in the lower part of each panel (black: white noise background, red: pink noise background). The shift in the perceptual classification boundary from white to pink noise background is given above the vertical lines in units of dB/oct.
Only the mean perceptual classification boundary could be tested for significance.

reached a stable performance took between seven months and one year. After the bats had learned the classification, their performance was mostly stable above 75%, especially in Bat 5.

2.3.2 Experiment 1: perceptual compensation during passive hearing

Three bats were successfully trained to discriminate highpass filtered impulses (+6 dB/oct.) from lowpass filtered impulses (-6 dB/oct.), which were superimposed on a white noise background (control, χ^2-test, $p < 0.0001$). The open symbols in **Fig. 2.7** show that in these trained conditions, all three bats performed highly reliably in that highpass filtered impulses were classified as highpass in about 90% of the trials and lowpass filtered impulses were classified as highpass in only about 10% of the trials. With a white noise background, the bats' spontaneous classification of the test stimuli with intermediate filter slopes depended in general monotonically on the filter slope (solid black symbols in **Fig. 2.7**). The perceptual classification boundary (vertical black lines in the lower part of the panels, **Fig. 2.7**) is on average at -0.1 dB/oct., and thus very similar to the physical filter boundary of 0 dB/oct.

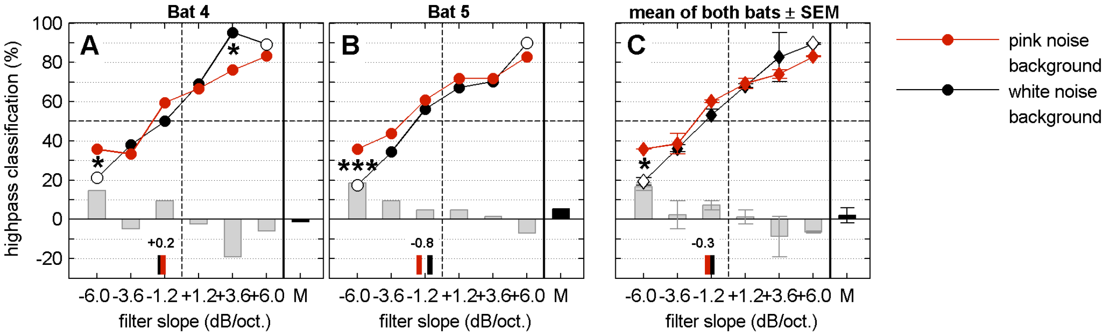


Figure 2.8: Experiment 2 (echolocation): Classification of filtered calls. Classification of filtered echolocation calls by two bats (**A**–**B**) and their mean ± SEM (**C**) during presentation of white noise (black symbols) and pink noise background (red symbols). Symbol notation is the same as in Fig. 2.7.

With a pink noise background, however, the bats classified the same stimuli more often as highpass filtered (red symbols in **Fig. 2.7**). Consequently, the perceptual classification boundary shifted towards negative slopes (vertical red lines in the lower part of the panels, **Fig. 2.7**). For the individual bats, this downward shift of the perceptual classification boundary amounted to 1.8, 1.0, and 0.8 db/oct., with a significant mean downward shift of 1.2 dB/oct. (Student's one-sided paired t-test, $t(2) = 5.52$, $p = 0.0156$). Accordingly, the mean difference in highpass classification between pink and white noise background (black bar in **Fig. 2.7 D**) is significantly larger than zero (Student's one-sided t-test, $t(5) = 2.21$, $p = 0.0391$). In summary, all three bats compensated for the spectral shape of the noise background.

Note that the possible shift of the perceptual classification boundary is limited by ceiling effects. These are apparent in the averaged psychometric functions (**Fig. 2.7 D**) where the degree of highpass classification of signals with a slope of +3.6 or +6 dB/oct. did not increase further when the background was changed from white to pink.

2.3.3 Experiment 2: spectral processing during echolocation

In this experiment, the bats were trained to classify the spectral transfer function of a filter, and not the presented sounds themselves. As the filter was excited by the bats' echolocation calls, the perceptual task was to evaluate spectral changes of the perceived echoes, relative to the emitted calls.

Two bats were successfully trained to discriminate echoes generated with a highpass filter (+6 dB/oct.) from echoes generated with a lowpass filter (-6 dB/oct.), which were superimposed on white noise background (control, χ^2-test, $p < 0.0001$, open symbols in **Fig. 2.8**). As we showed for the passive-acoustic classification of impulses, the spontaneous

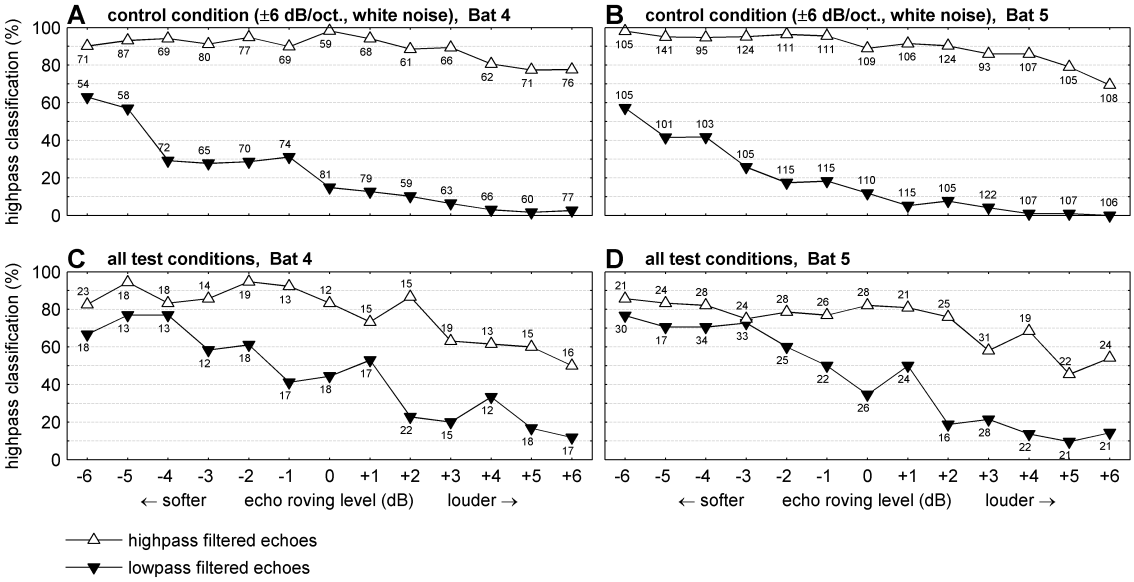


Figure 2.9: Experiment 2 (echolocation): Influence of the roving level on signal classification.
Classification of the same filtered echoes by the same bats as in Fig. 2.8, but here plotted as a function of echo level.
A, B) Control signals (±6 dB/oct., white noise background).
C, D) Test signals (white noise: ±1.2 and 3.6 dB/oct., pink noise: ±1.2, 3.6 and 6.0 dB/oct.).
Numbers above data points give the number of trials.

classification of filters with intermediate spectral slopes and a white noise background depended monotonically on the filter slope (solid black symbols in **Fig. 2.8**). However, in contrast to the passive-acoustic experiment, the classification of the same test filters was not affected by the change from a white to a pink-noise background (red symbols in **Fig. 2.8**). The shift of the perceptual classification boundary was positive in Bat 4 and negative in Bat 5, with a non-significant mean shift of -0.3 dB/oct. Accordingly, the mean difference in highpass classification between pink and white noise background equals almost zero (black bar, **Fig. 2.8 C**).

2.3.4 Effect of the roving level

In both experiments, the foreground level was roved to prevent the bats from using overall amplitude cues for classification. In addition to the filter slope, which was the reinforced cue, this roving level also influenced the classification during Experiment 2 (echolocation), but not during Experiment 1 (passive hearing). **Fig. 2.9 A, B** illustrates this effect for the control data of Experiment 2, which are plotted as a function of echo level. This shows that highpass controls were mostly classified as highpass ($>90\%$), with a small decrease in performance for louder echoes. In contrast, lowpass controls were classified correctly at high levels, but incorrectly at low levels with errors of up to 60%. A similar, more pronounced pattern is visible for the test echoes (**Fig. 2.9 C, D**).

2.3.5 Echolocation calls

P. discolor emits multiharmonic, downward frequency-modulated calls. Calls were very short, having 90% of their energy within less than 0.7 ms (median 0.4 ms, **Fig. 2.10 A**). The mean fundamental frequencies were 18.4 and 19.5 kHz for Bats 4 and 5, respectively (**Fig. 2.10 H**). The -20 dB bandwidth was around 55 kHz with cut-off-frequencies at 36–38 kHz and at 91–93 kHz (**Fig. 2.10 C–E**), which was about the same for both bats. Inter-individual differences were only found in the energy distribution across frequencies: the frequency centroid of Bat 4 is about 4–5 kHz lower than in Bat 5 (**Fig. 2.10 F**), and its best frequency is about 15 kHz lower than in Bat 5 (**Fig. 2.10 G**). Thus, calls of Bat 4 had most energy in their third harmonic (58–60 kHz), whereas calls of Bat 5 had most energy in their fourth harmonic (75–76 kHz). Hence, both bats performed the spectral classification task with partially different spectral call structures.

The noise background and the different filter slopes only partially influenced the call structure. Bat 4 showed no systematic background-correlated differences in its call parameters. Bat 5 slightly increased the high-frequency content of its calls during the presentation of pink noise background: the -20 dB bandwidth and the frequency centroid were about 1 kHz higher (**Fig. 2.10 D, F**, $t(3099) = -4.04$, $p < 0.0001$), which was caused by an increase in the upper cut-off frequency (**Fig. 2.10 E**, $t(3099) = -4.04$, $p < 0.0001$). It is possible that the bat accomplished this frequency increase by reducing its fundamental frequency by 0.1 kHz (**Fig. 2.10 H**, $t(3099) = 3.49$, $p = 0.0005$), thus also reducing its fifth harmonic by 0.5 kHz. In consequence, the fifth harmonic moves further down into the pass band of the bat's vocal tract, and thus shifts the frequency centroid upwards.

2.4 Discussion

In both experiments, bats were successfully trained to classify the spectra of the control foreground signals which differed in filter slope by 12 dB/oct. (**Fig. 2.3**). Despite the differing frequency structure of impulses and echolocation calls, both source stimuli were sufficiently broadband for a stable classification of these spectral changes. As the stimulus level was roved, the bats were prevented from using potential overall amplitude cues caused by the filtering. The bats thus learned to evaluate the filter-generated spectral changes.

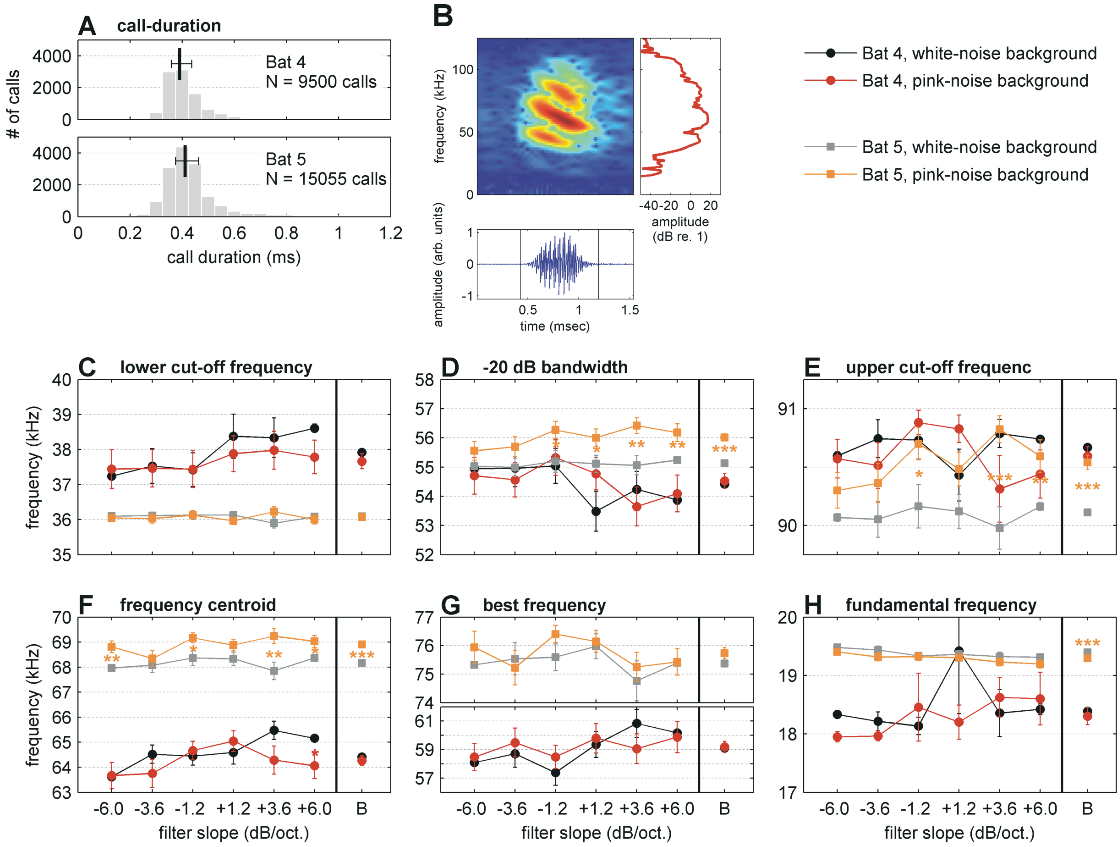


Figure 2.10: Call parameters of *Phyllostomus discolor* in Experiment 2 (echolocation). **A)** Histogram of the durations of the analysed calls. Median and quartiles are indicated by the vertical lines.

B) One example call of Bat 4 plotted as spectrogram (top), as oscillogram (bottom) and as amplitude spectrum (right). The amplitude spectrum was calculated from the marked section in the oscillogram.

C-H) Spectral parameters. For analysis, we used the five calls with the highest signal-to-noise-ratio per sequence and calculated the mean per sequence of every parameter. The means per sequence were then grouped, either per stimulus (i.e., the combinations of filter slope and noise backgrounds; larger part of the panels, between -6.0 to +6.0 dB/oct., N = 33–59 sequences per test stimulus, 766–1252 sequences per control stimulus) or per noise background (smaller right part of the panels, marked ‘B’. N = 214–2694 sequences per background), and their second order mean ± SEM was plotted.

Note the different scales of the y-axes. Grey symbols: white noise background, coloured symbols: pink noise background.

2.4.1 Experiment 1: perceptual compensation during passive hearing

With pink noise background, the bats classified the same passively heard impulses more often as highpass filtered than during the presentation of a white noise background. This shows that bats employ a compensatory mechanism during passive hearing similar to the one described in humans (e.g. Summerfield *et al.*, 1987; Watkins, 1991). The perceptual compensation in humans is presumably based on the different rate of spectrotemporal variation between two different filters (Furui, 1986; Repp, 1987; van Dijkhuizen *et al.*, 1987; Haggard *et al.*, 1987): while the spectrum of transmission channels is fairly constant over time, the spectrum of natural sound sources varies rapidly. By taking the constant fraction of the overall perceived spectrum as a reference, the auditory system compensates for it and evaluates only relative changes to it. This results in a perceptual whitening of the environmental spectrum and in the perception of the undistorted spectral shape of the transmitted sound signal.

We found the same compensatory mechanism during passive hearing in bats. When the noise background was filtered with a constant lowpass filter, the bats compensated for this filter and perceived the same transient foreground stimuli as containing more high frequencies. Such a compensation allows animals to perceive the frequency content of acoustic signals as it had been at the sound source, i.e., to perceive the spectral characteristics of the sound source despite changes in the absolute frequency content of the acoustic signal that is impinging on the ear.

This timbral constancy of the auditory system is a high-level perceptual process (Watkins, 1991), as is the colour constancy of the visual system (Smithson, 2005); the colour constancy being mediated by cells in area V1 and V4 of the visual cortex (Zeki, 1983; Wachtler *et al.*, 2003). As such, they are likely to be under cognitive control and may be employed only if necessary. This was investigated for the auditory system in Experiment 2.

2.4.2 Experiment 2: spectral processing during echolocation

In contrast to passive hearing, the classification of echoes during active hearing was independent of the passively presented noise background. This shows (i) that the spectral processing of echoes differs from the spectral processing of transient passive acoustic stimuli, even in the identical experimental situation, and (ii) that the spectral processing of echoes is independent of simultaneous, passively presented acoustic signals.

In contrast to the auditory processing of passive sounds, the neuronal processing of echoes is coupled to and influenced by the vocalisation pattern (Suga & Schlegel, 1972; Schuller, 1979), for example by opening temporal processing windows (Roverud & Grinnell, 1985b). Based on neurophysiological data, it was suggested for the gleaning bat *Antrozous* *pallidus* that it processes passively heard prey sounds and actively acquired echoes in two different, parallel auditory pathways (Fuzessery, 1994; Razak & Fuzessery, 2002), which converge later in the auditory cortex (Razak *et al.*, 1999). Behaviourally, it was suggested that the auditory mechanisms for the temporal analysis of spatial echo information are under cognitive control and that they may be employed during passive hearing, but not during echolocation (Schuchmann *et al.*, 2006; Schuchmann, 2006). The current study provides behavioural evidence that also spectral processing differs between passive and active hearing. The reference for the spectral evaluation of echoes is thus not the constant spectral characteristics of external passive acoustic signals, but an internal reference, which can either be an efference copy of the motor signals that generated the last call, or an auditory reference generated from hearing the outgoing call.

The evaluation of spectral echo characteristics independent of passive acoustic signals may not be confounded with masking effects by background noise, which will still occur if the background level was high enough.

2.4.3 Effect of the roving level

In Experiment 2, echo classification was influenced by echo level; softer echoes were more often classified as highpass filtered than louder echoes. Three explanations are conceivable for this influence: first, the bats might have used residual overall loudness cues to discriminate the filter slopes of -6 dB/oct. and +6 dB/oct., thus rendering the applied roving level ineffective. To test this hypothesis, we filtered the recorded calls with the experimentally used filters and calculated their RMS-level. The level of lowpass filtered calls was 3–4 dB lower than the level of highpass filtered calls (**Fig. 2.11 B**). This finding is in contrast to the experimentally observed level effect (**Fig. 2.9**) which showed that softer echoes were more likely to be classified as highpass. Therefore, we reject this hypothesis.

Second, the spectral shape of the echo above hearing threshold might vary with echo level. To test this hypothesis, we applied a roving level of -6 to +6 dB to every recorded call and calculated the frequency centroid of the roved echo above hearing threshold on a logarithmic frequency axis as a measure for the perceived spectral shape. The hearing threshold of *P. discolor* was taken from Hoffmann *et al.* (2008), and for frequencies above 80 kHz from Esser & Daucher (1996, magnitudes reduced by 28.5 dB to match the audiogram of Hoffmann *et al.*). The frequency centroid increased by about 2 kHz for softer echoes (**Fig. 2.11 C**). In comparison, the filters used for echo generation changed the frequency centroid by about 5 kHz (**Fig. 2.11 A**), which is about twice as much as caused by the roving level. Frequency cues caused by the roving level should therefore be overruled by the direct spectral changes and are not sufficient to explain the influence of the roving level on their own.

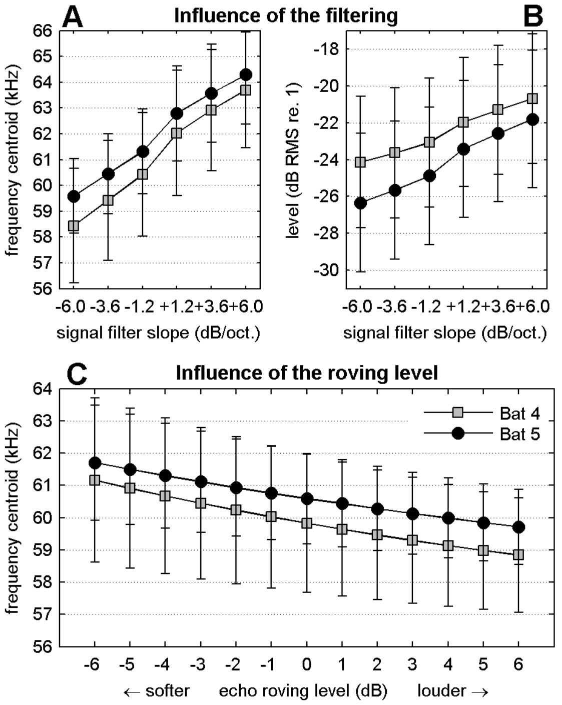


Figure 2.11: Calculated influence of the stimulus filters and the roving level on the frequency centroid and the level of the recorded calls (mean ± std. of 9500 calls for Bat 4 and 15055 calls for Bat 5).

A) The stimulus filters, going from lowpass (-6 dB/oct.) to highpass (+6 dB/oct.), increased the frequency centroid of the recorded calls by about 5 kHz.

B) The stimulus filters, going from lowpass (-6 dB/oct.) to highpass (+6 dB/oct.), increased the RMS-level of the recorded calls by 2-3 dB SPL.

C) Increasing roving levels, i.e., higher overall levels, reduced the frequency centroid of the recorded calls by about 2 kHz.

Third, timbre, pitch and loudness are auditory perceptual dimensions that mutually interact in a complex non-linear fashion. For example the reaction time for stimulus classification in one of these dimensions depends in non-reciprocal ways on the other dimensions (Melara & Marks, 1990), with high pitch and high timbre both corresponding to loud signals, but high timbre corresponding to low pitch. In echolocation, small targets give more high frequent echo content than larger ones. Therefore, the bats may tend to classify low level targets together with high-pass filtered ones.

In summary, the joined effects of the bats perceptual associations between different stimulus qualities and the spectral composition of the echolocation calls combined with the bats' absolute hearing threshold can at least qualitatively explain the level dependence of the behavioural performance in Experiment 2. As the shape of the psychometric functions did not change if only a part of the trials was analysed, e.g. all trials with a roving level >0 dB, the effect of the roving level on classification does not impact the findings of the spectral classification.

2.4.4 Echolocation calls

In addition to perceptual compensatory mechanisms, which are used during passive hearing, bats could compensate for spectral distortions during echolocation by adapting the call spectrum to the spectral shape of passive acoustic signals. Bats constantly adjust their signals to meet changing perceptual requirements. For example, call bandwidth is changed during prey interception (Schnitzler *et al.*, 2003), presumably to increase object resolution (Siemers & Schnitzler, 2004; Boonman & Ostwald, 2007), and depending on the surroundings (Kalko & Schnitzler, 1993) and the calls of conspecifics (Gillam *et al.*, 2007). If bats compensated for the lowpass characteristic of the passively presented noise background, they would need to increase call energy in the lower frequencies. However, every consistent effect we found was into the opposite direction, and thus not supporting the compensation hypothesis. This is further evidence that echo spectral processing is not influenced by passive acoustic stimuli. Passive and active hearing thus represent two different and separated modes of operation of the auditory system, where the spectral shape of passive sounds does not influence the evaluation of signals during active hearing.

2.4.5 Conclusion

The presented data show that the bat *Phyllostomus discolor* adapted the processing of passive acoustic signals to compensate for spectral distortions. Communication signals, or other passively heard sounds, can thereby be corrected for spectral changes generated by the environmental transfer function. Such changes are discernible from the constant spectrotemporal part of the same or other ongoing signals, such as ambient sounds, transmitted through the same transmission channel. In contrast, when the bats had to classify self-generated echoes instead of passively presented transient signals in the exactly same experimental paradigm, their classification was independent of the passively heard noise background. The spectral analysis of transient stimuli during echolocation, i.e., spectral echo analysis, thus recruits different auditory processing mechanisms than the spectral evaluation of passively heard sounds. The data provide psychophysical evidence for the hypothesis that bats switch to an independent mode of auditory processing during echolocation which does not necessarily obey to the general rules of auditory spectral analysis. Thus, during echolocation, spectral echo analysis is not interfered by ambient sounds.

Acknowledgments

This work was supported by a stipend of the DFG graduate program 1091 (‘Orientation and Motion in Space’) to HRG.

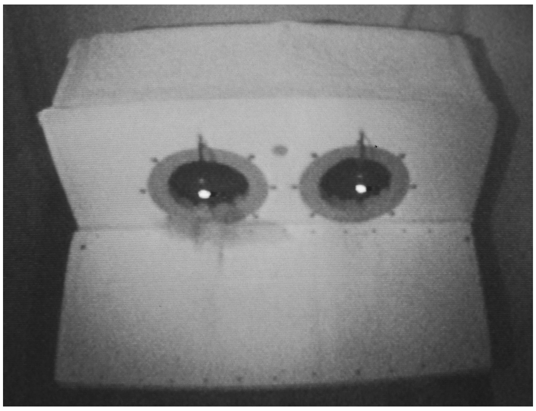
The experiments in this study are non-invasive and do therefore not require an experimentation approval. Permission to keep and breed *Phyllostomus discolor* in fulfillment of § 11, Abs. 1 TierSchG has been given to the Dept. Biologie II der Ludwig-Maximilians-Universität München by the Landratsamt München, dated April 20, 2005.

Appendix: Transponder

One subcutan transponder (Trovan, Douglas, Isle of Man, UK) per bat was injected below the skin of the neck and back, for most of the bats after the end of the experiment, with the following numbers (internal lab name given first, which is based on the shape of the scissored hair.):

1laengs:	000 0648CB5	(bat 4 of this study)
GrossT:	000 6995ABC	(bat 5 of this study)
1quer:	000 62F00CE	
2Punkt:	000 62F00CE	
Stan:	000 62F0E91	

Lutz Wiegrebe and I conceived and planned the experiment. I developed, built and programmed the experimental setup, ran the experiment and analysed the data. A manuscript of this chapter, possibly together with data of Kristian Beedholm (University of Southern Denmark, Odense) on his attempt to train Eptesicus fuscus *in a similar experiment (see the discussion for details), will be submitted to PLoS ONE.*



3 Detection of Jittering Real Targets by Passive Listening and Echolocation

3.1 Introduction

Bats use echo-delay to measure the distance to objects, called ranging. This was first conjectured by Hartridge (1945) and later shown by Simmons (1973), who used real targets with varying distances on the one hand and loudspeaker playbacks of echoes with varying temporal delays on the other to demonstrate their equivalence. A delay of 1 ms between the emission of the call and the returning echo corresponds to an object distance of about 17 cm.

The earliest psychoacoustical studies to determine the accuracy of distance measurement in echolocation used two real targets with different distances to the bat. The bats were trained to fly or crawl to the closer target. These studies gave distance accuracy thresholds for FM-bats of about 6–17 mm respectively 36–100 μs (see **Tab. 3.1**). Objections to these studies were raised because the head movements which the bats needed to face first one and then the other object potentially changed the bats' distance to the object and thereby deteriorated the threshold measurements (Simmons, 1973; Simmons & Grinnell, 1988; Simmons *et al.*, 1995). In addition, the accuracy of the internal representation of the measured distance could deteriorate over time. James Simmons and colleagues introduced therefore the virtual jitter paradigm (Simmons, 1979; Simmons *et al.*, 1990a, 2003), which was replicated several times (Menne *et al.*, 1989; Moss & Schnitzler, 1989). The published delay-accuracy constantly decreased from initially 1 μs to later 10 ns (Simmons, 1979; Simmons *et al.*, 1990a, 2003, see **Tab. 3.2**). The discussion about the validity of those data, possible methodological artefacts and its implications for signal processing in the bats' brain did not stop since then (e.g. Schnitzler & Henson, 1980; Altes, 1981; Menne & Hackbarth, 1986; Pollak, 1993; Simmons, 1993; Beedholm & Mohl, 1998; Beedholm, 2005; Schörnich & Wiegrebe, 2008).

Table 3.1: The time and distance thresholds measured in **distance discrimination experiments** by several authors, ordered by threshold.

thrs. μs	**thrs.** mm	**condition**	**authors**
36	6	*Eptesicus fuscus*, virtual targets, one-channel 2-AFC	Miller (1991)[1]
36	6	*E. fuscus*, virtual targets, one-channel 2-AFC	Masters & Raver (1996)[1]
55	9	*E. fuscus*, virtual targets, one-channel 2-AFC	Surlykke (1992)[1]
59	10	*E. fuscus*, virtual targets, two-channel 2-AFC	Simmons (1973)
70	12	*Phyllostomus hastatus*, real targets, 2-AFC	Simmons (1973)[2]
70	12	*E. fuscus*, virtual targets, two-channel 2-AFC	Masters & Jacobs (1989)[1]
76	13	*E. fuscus*, real targets, 2-AFC	Simmons (1973)[2]
76	13	*Noctilio albiventris* real targets, 2-AFC	Roverud & Grinnell (1985a)
80	14	*E. fuscus*, virtual targets, two-channel 2-AFC	Denzinger & Schnitzler (1994)
88	15	*Pipistrellus pipistrellus* real targets, 2-AFC	Surlykke & Miller (1985)
94	16	*Pteronotus suapurensis*, real targets, 2-AFC	Simmons (1973)
100	17	*E. fuscus*, virtual targets, two-channel 2-AFC	Denzinger & Schnitzler (1998)
176	30	*Rhinolophus ferrumequinum*, real targets, 2-AFC	Simmons (1973)

[1] These studies used an open-loop target presentation method, i.e., a stored model echo and not a real copy of the actual recorded echolocation call was played back.

[2] The same data are also published in Simmons (1971) and Simmons & Vernon (1971).

3.1.1 Experiments on temporal accuracy

Tab. 3.1 gives an overview of the results of several studies on distance discrimination. For distance discrimination, two (real or virtual) objects are presented at different distances, either simultaneously or consecutively, and the bats were trained to detect the closer or more distant one. In summary, for all species and methods, the threshold is roughly around half a centimetre to two centimetres, corresponding to a temporal accuracy between 30–100 μs. Only the threshold of *R. ferrumequinum* is a little larger due to the constant frequency calls of this species, which are less suited for ranging tasks.

Table 3.2: The time and distance thresholds measured in **jitter detection experiments** by several authors, ordered by threshold.

thrs. μs	**thrs.** μm	**condition**	**authors**
0.01	1.7	*E. fuscus*, two-channel 2-AFT, target positions: t and $t \pm \Delta t/2$	Simmons *et al.* (1990a)
0.01	1.7	*E. fuscus*, two-channel 2-AFC target positions: t and $t \pm \Delta t/2$	Simmons *et al.* (2003)
0.01	1.7	*E. fuscus*, two-channel 2-AFC target positions: t and $t \pm \Delta t/2$	Simmons *et al.* (2004)
< 0.4	< 70	*E. fuscus*, one-channel 2-AFC, target positions: t and $t - \Delta t$	Moss & Schnitzler (1989)
< 0.4	< 70	*E. fuscus*, two-channel 2-AFC, various presentation protocols	Menne *et al.* (1989)
1[1]	170	*E. fuscus*, two-channel 2-AFC, target positions: t and $t \pm \Delta t/2$	Simmons (1979)
225	38000	*E. fuscus*, one-channel 2-AFC target positions: t and $t \pm \Delta t/2$	Masters *et al.* (1997)[2]
intertarget spacing jitter detection[3]			
0.9	150	*E. fuscus*, one-channel 2-AFC, three targets, no overall range shifts	Masters *et al.* (1997)[2]
< 10	< 1700	*E. fuscus*, one-channel 2-AFC, two targets, no overall range shifts	Masters *et al.* (1997)[2]
10–70	1700–11900	*E. fuscus*, one-channel 2-AFC, two targets, plus overall range shifts	Masters *et al.* (1997)[2]

[1] Simmons (1979) is referring to this threshold as 0.5 μs. However, 0.5 μs is the value of $\Delta t/2$, so the change in delay between to consecutive echoes is Δt.

[2] This study used an open-loop target presentation method, i.e., a stored model echo and not a real copy of the actual recorded echolocation call was played back.

[3] In the intertarget spacing jitter detection task, two echoes with a certain temporal delay (intertarget spacing) were presented instead of only one echo. The bats had to decide whether the intertarget spacing was constant or jittering. In one experiment, the overall delay (or range) of the two-echo-complex was additionally shifted over several presentations.

To estimate the true accuracy of the internal distance representation of bats, Simmons (1973) and Simmons *et al.* (1975) tried to correct for the head movements of the bats while they were echolocating the two different targets in the experiments. Their estimated thresholds are between 3–8 mm (17–46 μs) for FM-bats, which is about one half to one third of the original thresholds. Yet, to determine internal time or distance accuracy even more precisely and to minimize head movement errors from the very beginning, Simmons (1979) introduced the virtual jitter experiments. Instead of presenting targets at two different distances with two different echo delays t_1 and t_2, he generated one virtual target at a fixed distance, i.e. with a constant echo delay t, and another target with an echo delay that was alternating ('jittering') from call to call. Thus, every second call, the presented echo delay was $t + \Delta t$, and every other call, it was $t - \Delta t$. By reducing the size of Δt, thresholds for the detection of call-to-call echo-delay changes were measured.

Already the first jitter detection experiment found a smaller threshold of 1 μs than the previous distance discrimination experiments (Simmons, 1979). Follow-up studies by the same and another laboratory found even smaller thresholds of first less than 0.4 μs and then down to only 0.01 μs (= 10 ns; **Tab. 3.2**). The proposed distance accuracy of those studies goes down to less than 2 μm. Only Masters *et al.* (1997) found a much larger threshold, when they were comparing their intertarget spacing jitter detection experiments (for explanation see Tab. 3.2) to the classical time jitter detection experiments. However, it is not quite clear from their paper, whether an overall range shift was added to the single jittering target (which would make detection harder), as stated in the abstract ("...detect jitter added to a single moving target...", p. 279), or not, as described in the methods ("...a single target *at 80 cm*. On half of the trials the target was jittered by adding or subtracting a standard value from the offset delay, and on half the delay was constant at 3780 μs.", p. 283), although the second possibility of a constant range is much more likely from the argument of the whole paper.

Disregarding the results of Masters *et al.* (1997), which they did not further explain, it is evident that the jitter detection thresholds are at least two orders of magnitude smaller than the range discrimination thresholds. This seemed to confirm the original arguments about movement errors which led to the introduction of the jitter experiments.

3.1.2 Objections to the jitter experiments

The general criticism of the jitter experiments questioned the biological need for this accuracy and asked for the neuronal implementation (Pollak, 1993; Beedholm & Mohl, 1998). Despite these criticism, the measured data needed to be explained, and many authors suggested additional cues correlated to the jittering signal (Pollak, 1993), for example caused by impedance mismatch of the delay lines (Beedholm & Mohl, 1998).

Beedholm (2005) simulated a jitter detection task and showed that thresholds of some tens of nanoseconds are indeed physically realisable under optimal conditions, but that thresholds increase as soon as realistic transfer functions and the naturalistic variability in call structure are taken into account. Simulating the setup used by Simmons *et al.* (1990a), he calculated a maximally detectable jitter threshold of 355 ns.

The virtual jitter results, especially the 10 ns data, were interpreted as so-called hyperacuity (Altes, 1989; Simmons *et al.*, 1990a, 2003), which was contrasted to the ordinary acuity used during 'normal' behavioural tasks and which was claimed of having been estimated to be on the order of 1 μs (Simmons *et al.*, 2003). The hyperacuity was interpreted as the intrinsic temporal accuracy of auditory signal representation, which is blurred during behavioural task due to self-motion of the bats (Simmons, 1973; Simmons & Grinnell, 1988; Simmons *et al.*, 1995). A need for the hyperacuity is hard to comprehend if it cannot be applied during normal behavioural tasks. The unified hypothesis of target representation therefore assumed that objects are exclusively represented along the temporal range axis after they were transformed from the spectral domain into the temporal domain (e.g. Simmons & Chen, 1989; Simmons *et al.*, 1990b; Saillant *et al.*, 1993). According to this hypothesis, the temporal hyperacuity is used to represent object distance with high accuracy and to resolve closely spaced objects on a temporal axis. However, a temporal accuracy of 10 ns corresponds to a distance accuracy of 1.7 μm, which is once more much more accurate than it seems to be necessary for the resolution of closely spaced objects. In conclusion, if no use for a temporal hyperacuity can be envisaged, it seems to be very unlikely that it evolved as a by-product of another task, especially as such a task could also not be envisaged.

3.1.3 Aim of this study

The aim of this study was twofold. First, I wanted to test the jitter detection performance in an experimental situation completely different from all other studies, using real-targets which will not be subject to the potential artefacts discussed for the virtual jitter experiments (though to others, see below).

Second, all jitter detection experiments so far were laboratory studies, where the bats unnaturally sat on a platform and had to crawl to the jittering target. The aim of this study was therefore not to measure once more a potentially arbitrary jitter detection threshold, but to measure the distance accuracy of a nectarivorous bat (where studies are especially rare) under semi-natural conditions, i.e. while the bat was flying and echolocating on real targets. This mimics under laboratory conditions the naturalistic foraging situation as close as possible, where the experimental animal, *Glossophaga soricina*, has to locate flowers and to position itself within the flower openings to lick the nectar.

G. soricina is well suited to measure minimal jitter detection thresholds under semi-natural conditions, as it is able to hover in front of objects, thus minimizing potential errors caused by self-motion.

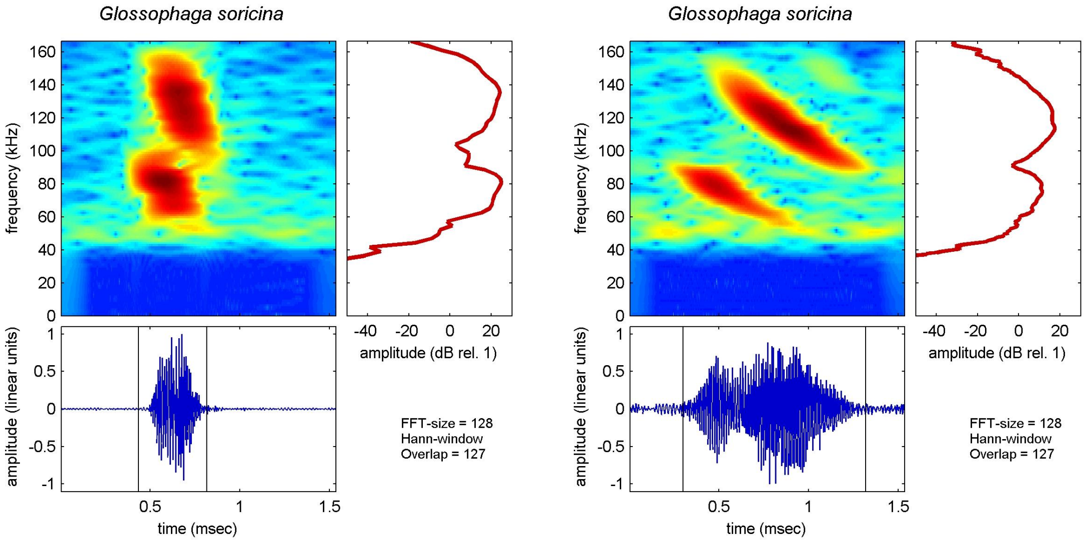


Figure 3.1: Two examples of **echolocation calls of *Glossophaga soricina***, shown as spectrogram (top), as oscillogram (bottom) and as amplitude-spectrum (right). The spectrum was calculated from the marked section in the oscillogram.
The call duration varies between 0.4 to 1 ms. The second harmonic is the most prominent one with its peak around 120-140 kHz. The calls were recorded with a sampling frequency of 333 kHz and later highpass filtered at 50 kHz.

3.2 Methods

3.2.1 *Glossophaga soricina*

Glossophaga soricina Pallas, 1766 is a neotropic bat species living in the area from northern Mexico to Paraguay and northern Argentina, and on Jamaica and the Bahamas (Nowak, 1994). It is regarded as nectarivorous, and with its ability for hovering flight, its long snout and its papillae-bearing tongue it is well adapted for the exploitation of flower calices. Its metabolism is able to use the nectar carbohydrates quickly and efficiently to fuel the high metabolic needs of its flight muscles (Voigt & Speakman, 2007; Welch *et al.*, 2008). However, besides nectar, *G. soricina* also feeds on fruit, pollen, flower parts and small insects (Nowak, 1994). Accordingly, all individuals in my experiments readily took dead blowflies (*Calliphora vicina*).

Glossophaga soricina emits, as is typical for members of the family Phyllostomidae, short and faint multiharmonic, downward-frequency modulated (FM) echolocation calls (**Fig. 3.1**). The calls of *G. soricina* are of very high frequency, with most of the energy above 100 kHz.

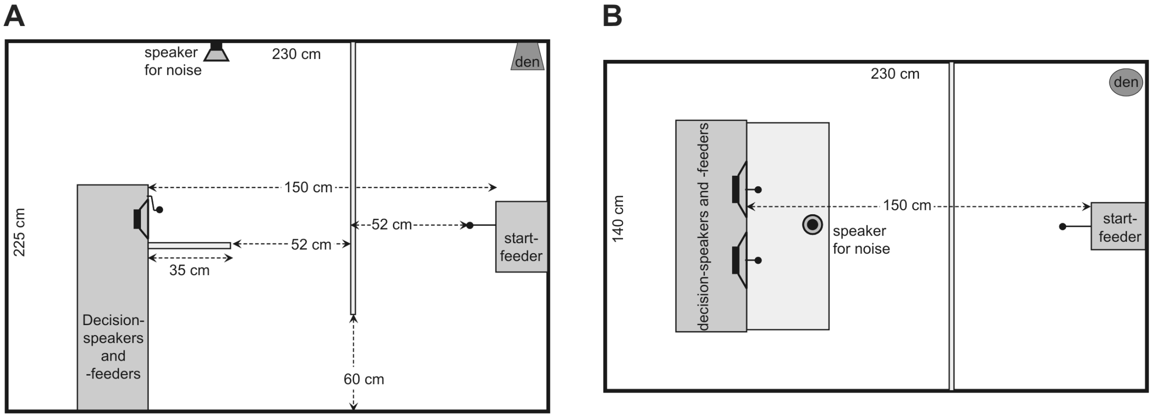


Figure 3.2: Experimental chamber of the real-target jitter experiment.
A) Side view. The bats were trained to start a trial with a visit to the start feeder and then to fly towards the feeder above the vibrating speaker membrane. The chamber was divided with a curtain. A shield below the two bass speakers ensured that the bats approached from directly ahead. An ultrasonic speaker at the ceiling played band-pass filtered noise to mask passive acoustic cues. A den was mounted in one corner of the chamber.
B) Top view. Each bass speaker was associated with a computer-controlled feeder.

3.2.2 Housing

One individual *Glossophaga soricina* at a time was housed in a climate-controlled chamber (230 × 140 × 225 cm) at 26°C and 60% humidity with a 12/12 h dark-light cycle for 2-3 months. The chamber contained a feeder on one side (the start feeder), and the experimental setup with two bass-loudspeakers and two associated feeders on the opposite side (**Fig. 3.2**). Experiments were started after we had received the permission to keep *G. soricina* and run from September 2007 to March 2008. Data from three individuals were collected.

Daily experiments started with the beginning of the dark phase and lasted for 12 h until the beginning of the light phase. The bats were mainly fed with a 18% (w/w) sugar solution of a mixture of sucrose, fructose and glucose (1 : 1.5 : 1.5) in water, mimicking in part the natural concentration of nectar (Baker *et al.*, 1998). The sugar solution was delivered by the three feeders during the experiment. The bats got some additional food about 3-5 h before the start of the experiment: 1.5 ml of a solution of honey (16% w/w) and NektarPlus (10% w/w; Günter Enderle Necton, Pforzheim, Germany) in water, ~0.1 g of pollen, and one blowfly (*Calliphora vicina*). Water was always available *ad libitum.*

3.2.3 Experimental setup

The setup consisted of a vertical wooden board which contained two openings of 16 cm diameter with a centre-to-centre distance of 30 cm. Behind each opening, a bass-loudspeaker

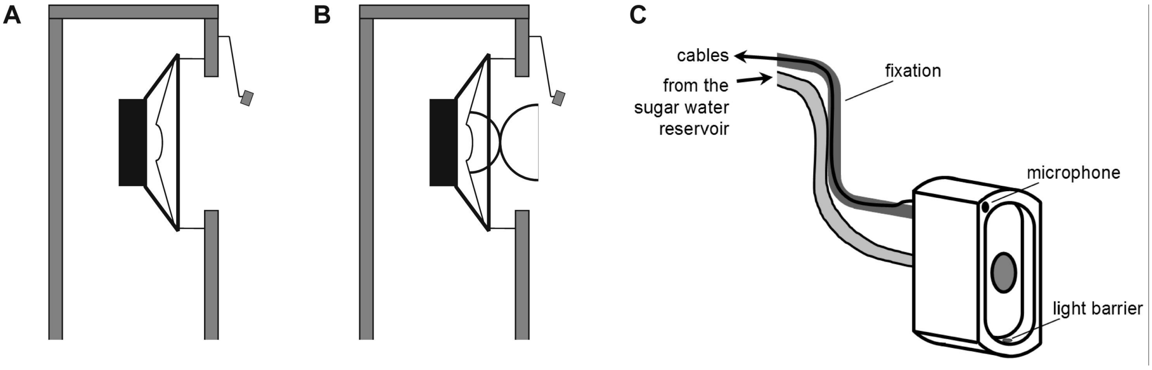


Figure 3.3: Sketch of the setup and the feeders.
A) Standard configuration: Both loudspeakers were fixed inside the setup, behind an opening in the setup wall of 16 cm diameter. The membrane of the speaker was 5.5-8 cm behind the opening. The feeder head was positioned about 4 cm before and about 3 cm below the rim of the opening.
B) Hollow hemisphere configuration: A hollow hemisphere with a diameter of 10 cm was mounted with its opening facing to the bat on a second, inverted hollow hemisphere (8 cm diameter), which was glued on the membrane. A hollow hemisphere is a good echo-reflector, which reflects sounds back into the same direction where it originated from. In addition, the hollow hemisphere was closer to the bat, which will presumably make jitter detection easier.
C) Feeder head: The feeder head was fixed on a threaded rod (dark grey) coming out of the setup wall. The feeder head contained a hole in the middle (dark grey) for the delivery of the sugar water reward, which was supplied by a tubing (light grey). Small holes in the bottom and on the top contained the light barrier.

(Peerless XLS 10, Tymphany, San Rafael, CA, USA) was mounted so that its membrane had a distance of 5.5-8 cm to the front of the wooden board (**Fig. 3.3 A**). The rear part of the setup was enclosed by a wooden case so that only the frontal openings with the speakers were accessible by the bats. A feeder which delivered the reward was fixed in the frontal setup wall above each loudspeaker. The head of each feeder was about 4 cm before and 3 cm below the rim of the opening.

The feeders were build as gravity feeders. To the bats, only the head of each feeder was accessible (**Fig. 3.3 C**), which were made from PVC with a oval-shaped opening in the front for the bat's snout. A Tygon silicone tube (0.8 or 1.6 mm inner diameter; VWR, Darmstadt, Germany) with a LuerLock tube connector (Rotilabo, Carl Roth, Karlsruhe, Germany) was inserted from the back into the feeder head to deliver the sugar water. Inside the setup, the tube passed through a magnetic valve (W295A112, Asco valve, Inc., Florham Park, NJ, USA) and was connected to a syringe as sugar water reservoir, containing 20 ml of sugar water at a height of 8 cm. Each feeder head was equipped with an infrared light barrier (infrared-emitting diode IRL80A, Siemens, Berlin & München, Germany; infrared-sensitive transistor IS471F, Sharp, Osaka, Japan) to register the bat's visits, which was connected to the serial port of a personal computer running Matlab 6.5 (The Mathworks, Natick, MA, USA). The magnetic valves were activated via relays, which were controlled by the serial port of the same computer. Each time the bat received a reward, the magnetic valve was opened to deliver a fixed amount of sugar water, which was 14–17 μl at the two decisions feeders and 5 μl at the start feeder. The two decision feeders were additionally equipped with a small microphone (FG-23329-P07, Knowles Electronics, Itasca, IL, USA) which allowed the recording of echolocation calls.

3.2.4 Description of the stimuli

The stimuli presented to the bats were sinusoidal displacements of the speaker membrane with 10, 25 or 50 Hz (standard configuration, **Fig. 3.3 A**). Glosso3 was additionally trained in the hollow hemisphere configuration (**Fig. 3.3 B**), where a hollow hemisphere was glued to the membrane as a better echo reflector to facilitate the detection of the vibrating membrane. Training signals had peak-to-peak-displacements of 20 or 24 mm at 10 Hz, 13.5 mm at 25 Hz and 10 mm at 50 Hz. Only one of the two membranes was vibrating at a time, and the bats were trained to detect the vibrating membrane and to fly towards the feeder above that membrane.

The speakers were driven by a stereo-signal of the duration of one cycle of the vibration frequency, with one of the two channels containing a sinusoidal voltage change. This signal was played continuously from a ringbuffer by the Soundmex 1.1 software (HörTech, Oldenburg, Germany) through the computer's soundcard (Sound Blaster Audio PCI, Creative Technology Ltd., Singapore, Singapore) and was amplified (RB 971, Rotel, Halle, Germany) and fed into the loudspeakers.

The time-variant acoustic impulse response of the vibrating speaker membrane as measured by ensonification with white noise (0-130 kHz) and cross-correlation of the outgoing with the reflected noise is shown in **Fig. 3.4 A**. The time-variant spectrum of the acoustic impulse response is presented in **Fig. 3.4 B**, showing that no spectral artefacts were generated by the vibrating membrane.

Calibration The displacement of the speaker membranes was initially calibrated with an accelerometer (type 4374, and charge amplifier type 2635; Brüel & Kjær, Nærum, Denmark; signal digitized with Firewire Audiophile, M-Audio, Avid Technology, Tewksbury, MA, USA, sampling rate 32000 Hz) and later regularly checked with a laser displacement sensor (optoNCDT 1700 ILD 1700-100, Micro-Epsilon, Ortenburg, Germany, sampling rate 2500 Hz) and was linear and constant in the relevant range.

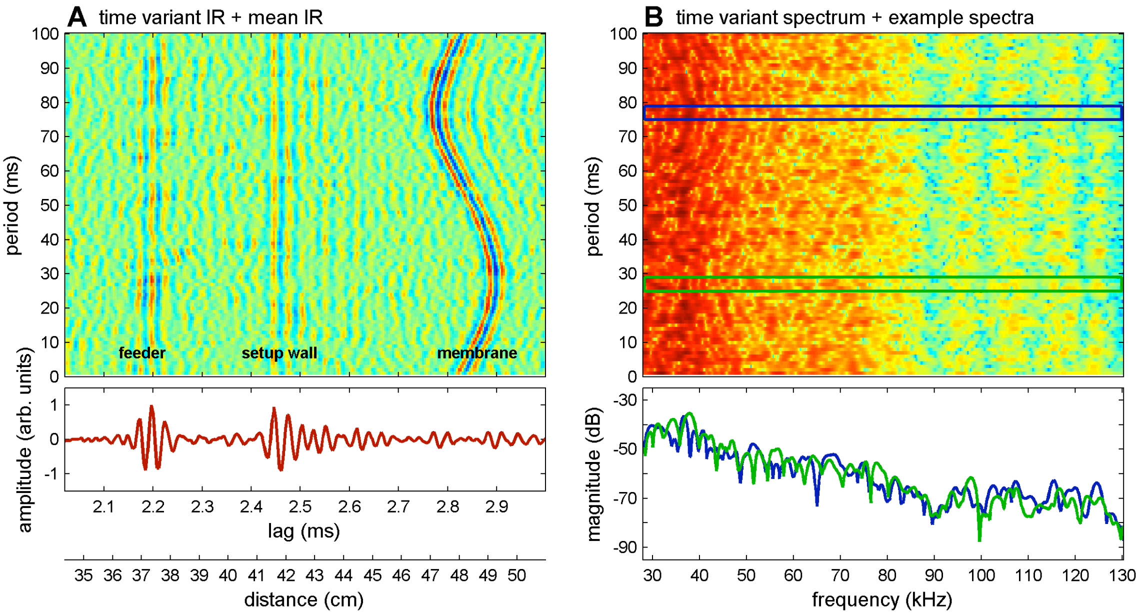


Figure 3.4: Time variant impulse response of the vibrating speaker membrane and its spectrum at a vibration frequency of 10 Hz with a pp-displacement of 22 mm (127 μs time jitter). The vibrating membrane was ensonified with white noise and the reflected noise was recorded (sampling rate 260 kHz). A running cross-correlation between outgoing and reflected noise was calculated to extract the impulse response in steps of 1 ms of one vibration cycle.
A) Time-variant impulse response. The upper graph shows the colour-coded impulse response plotted along one cycle (0-100 ms) of the displacement. The lower graph shows the mean across all impulse responses. The reflections of the feeder, the wall and the membrane are clearly visible.
B) Time-variant spectrum of the impulse response. The upper graph shows the colour-coded spectrum of the impulse response plotted along one cycle (0-100 ms) of the displacement. The lower graphs show the spectrum at the two most extreme displacements, which are marked by the green and blue boxes in the upper graph.

3.2.5 Experimental paradigm

The experiment was implemented as a two-alternative, forced-choice (2-AFC) paradigm and was run automatically by a custom-written program for Matlab 6.5. The program continuously monitored the light barriers of all three feeders. When the bat was detected at one of the feeders, the appropriate action was taken and the bat's decision was stored.

Training The training consisted of two steps. In the first step, each subject had to learn to alternate between the start and the decision feeders. Therefore, the first visit at one of the two decision feeders was rewarded after the bat had visited the start feeder, and *vice versa*. This training took between two to five days.

After the subject had learned the alternation procedure, it was trained in the second step to detect the vibrating membrane at 10 Hz vibration frequency and 20 or 24 mm peak-to-peak-displacement, corresponding to a maximal echo-delay difference, or time jitter, of 115 or 139 μs (calculated with a sound velocity of 346.5 m/s, which is appropriate for the temperature and humidity in the setup). The bat first had to visit the start feeder to start a new trial. When it was registered at the start feeder, one of the two bass-speakers started to vibrate and the bat received a reward at the start feeder. Further visits to the start feeder remained unrewarded until the bat made a decision at one of the two decision feeders or a time-out occurred. If the bat selected the non-vibrating speaker membrane, no reward was delivered and the start feeder remained inactive for 30 or 60 s as punishment. If the bat did not decide for one of the two speakers within 1 min, the trial was aborted and the bat punished with an inactivation of the start feeder for 15 s.

The vibrating membrane, and thus the rewarded side, was selected pseudo-randomly (Gellermann, 1933), with no more than three consecutive rewards at the same side.

Tests The tests were run for several nights after the bat had learned to detect the vibrating membrane with >80% correct. During tests, the displacement of the membrane was reduced in predefined steps. The sequence started with 20 repetitions of a large displacement, and then contained ten repetitions of smaller displacements, usually in steps of 2 mm (10 Hz) or 1 mm (25 and 50 Hz). This sequence was repeated several times per night. The performance of all nights with a given vibration frequency was pooled to obtain the psychometric jitter detection function.

Control experiments Control experiments were run during which the echoacoustic access to the vibrating membranes was prevented by occluding the speakers behind plastic disks (**Fig. 3.5**). The disks either closed the opening in front of the speakers completely (**Fig. 3.5 B**), or only partially (**Fig. 3.5 B, C**), so that air and passive acoustic cues could easily pass by.

The control experiments showed that the bats were able to perceive the position of the vibrating membrane based on passive acoustic cues only, generated by the vibrating membrane. To prevent the use of passive acoustic information, band-pass filtered white noise (noise generator, model 1405, Brüel & Kjær; model 442, 4$^{\text{th}}$-order filter, Wavetek Rockland, Inc., San Diego, CA, USA) was played back by an ultrasonic loudspeaker (Technics EAS 10 TH 800D, Matsushita Electric Industrial, Osaka, Japan), mounted on the ceiling of the experimental chamber above the setup (**Fig. 3.2**). The noise was bandpass filtered between 5 or 10 and 45, 50, 65, 80 or 100 kHz (stated at the results). Its RMS-level was adjusted to 72–75 dB SPL re. 20 μPa at the position of the decision feeders.

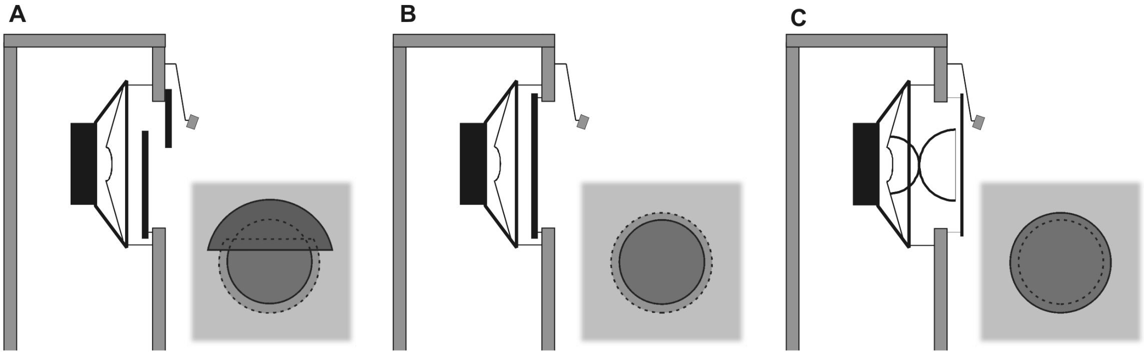


Figure 3.5: Sketch of the setup used to control for the perception of passive acoustic cues and air draught. The opening in front of the speakers was closed with different sets of disks to prevent the use of echolocation cues. The inset to the right of each sketch shows a frontal view of the occluded speaker.
A) Part-disk control: Two disks, which were cut at their top or bottom, allowed passive acoustic signals and air to pass, but prevented that the bat could echolocate the vibrating membrane.
B) Full-disk control: One disk completely closed the opening in the setup wall, preventing air draught and echo-acoustic access to the vibrating membrane. Passive acoustic cues could pass on the side of the disk.
C) Distant-disk control: One disk was fixed in a distance of 3.5 cm in front of the setup (e.g. in the hollow hemisphere configuration) and blocked the echolocation access to the vibrating membrane. Passive acoustic cues could pass sideways behind the disk.
Note that in addition, masking noise could be presented from an ultrasonic loudspeaker as an additional control for passive acoustic cues.

3.2.6 Call recordings and simulation of the perceived temporal jitter

A bat does not perceive the distance to an object continuously, but only every time it emits a call and gets back an echo. In the experiment, the bats thus sampled their distance to the membrane at their call rate. The perceived change in distance to the membrane, respectively echo delay, is therefore not the same as the peak-to-peak-displacement of the membrane, but depends on the bat's call rate and its perceptual integration of several echo delays.

In the virtual jitter experiments, the echoes were always played back with one out of two possible delays and the time jitter had therefore one fixed value. In contrast, in this experiment, we do not know which jitter was perceived by the bat. We only know the maximally possible time jitter, which is the time that the call needs to travel between the minimal and maximal displacement of the membrane. However, the bat would only perceive this maximally possible jitter when its pulse interval is exactly half the period of the vibration frequency and when it would exactly sample at the minimal and maximal displacements of the vibrating membrane. With every other PI, its perceived temporal

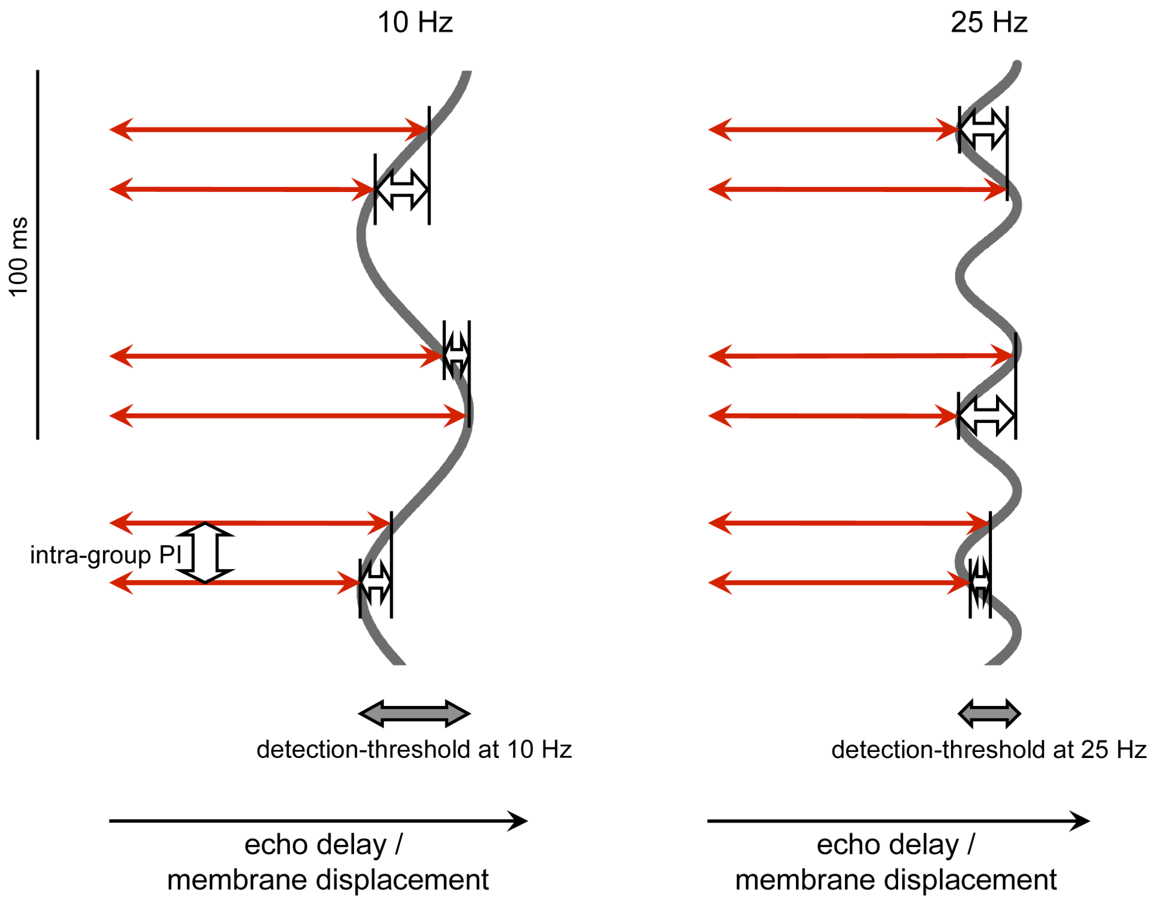


Figure 3.6: The perceived jitter (white-filled arrows) depends on the vibration frequency and the pulse interval (PI). If the pulse interval between consecutive calls (red arrows) is not exactly the duration of the half period of the vibration frequency, the perceived jitter will always be smaller than the peak-to-peak jitter of the membrane. At 10 Hz (left), the detection threshold in terms of peak-to-peak-displacement of the vibrating membrane is larger than at 25 Hz, because the membrane is vibrating more slowly and the change in echo delay (jitter) is hence smaller between two consecutive calls.

jitter will be smaller (**Fig. 3.6**).

We estimated the perceived temporal jitter with a simulation, based on the assumption that the bats always compared the echo delay of two consecutive calls, and based on the average measured pulse interval (PI) between consecutive calls within a group of calls. Bats regularly emit calls clustered in groups shortly before prey interception (Moss *et al.*, 2006) or during landing control (Melcón *et al.*, 2007), and it is assumed that call groups help bats to sharpen their perceptual representation of spatial relations. We therefore used the PI of consecutive calls within a group (the intra-group PI) to simulate the perceived temporal jitter. This also most closely mimics the situation in the virtual jitter experiments, where the bats always had to compare two consecutive echo delays.

To measure pulse intervals, calls were recorded during several nights for every bat (D940 Ultrasound Detector, F2000 Control/Filter Unit and BatSound Pro 3.1 recording software, all by Pettersson Elektronik AB, Uppsala, Sweden; PCM-DAS16/330 PCMCIA-card, Computer Boards, Inc., Middleboro, MA, USA; 333 kHz sampling rate). The PI is the interval from the centre of one call to the centre of the next call and was extracted from the highpass filtered spectrograms (30 kHz 8^{th}-order butterworth highpass-filter).

The perceived jitter was simulated at the detection threshold of each bat, based on 10,000 simulated trials. In each trial, ten call groups of two calls each with the average intra-group PI of 16 ms were randomly positioned on the sinusoidal displacement and the difference between their two echo delays (= temporal jitter) was calculated. From these ten independent jitter estimates per trial, we calculated the mean and the maximum perceived jitter per trial, which are the mean value of the ten jitter estimates per trial and the largest value of the ten jitter estimates per trial.

Increasing the number of simulated call groups per trial (e.g. 20, 50 or 100 trials) does not influence the value of the perceived jitter, but only its distribution, which gets narrower for more simulated call groups per trial.

3.2.7 Statistical methods

After the training and during the controls, the performance per night was tested using a cross tabulation χ^2-test, testing the null-hypothesis that the measured distribution was not different from chance distribution versus the two-sided alternative hypothesis that they were different.

Psychometric functions were fitted to the jitter detection performance data with a sigmoid function defined by the general formula

$$y = \frac{b}{1 + e^{-\lambda * x - a}} + c \; . \tag{3.1}$$

The parameter c, which defines the lower asymptote of the function, was fixed to 50%. The three other parameters were fitted by minimizing the squared error between the fit and the measured data (Matlab 7.1, The Mathworks, Natick, MA, USA). The inflection point of the fitted function was taken as the most probable estimate of the jitter detection threshold (Schmidt, 1995).

Significance levels are marked with $*$ if $p < 0.05$, with $**$ if $p < 0.01$, and with $***$ if $p < 0.001$ and with *n.s.*, if the null-hypothesis could not be rejected.

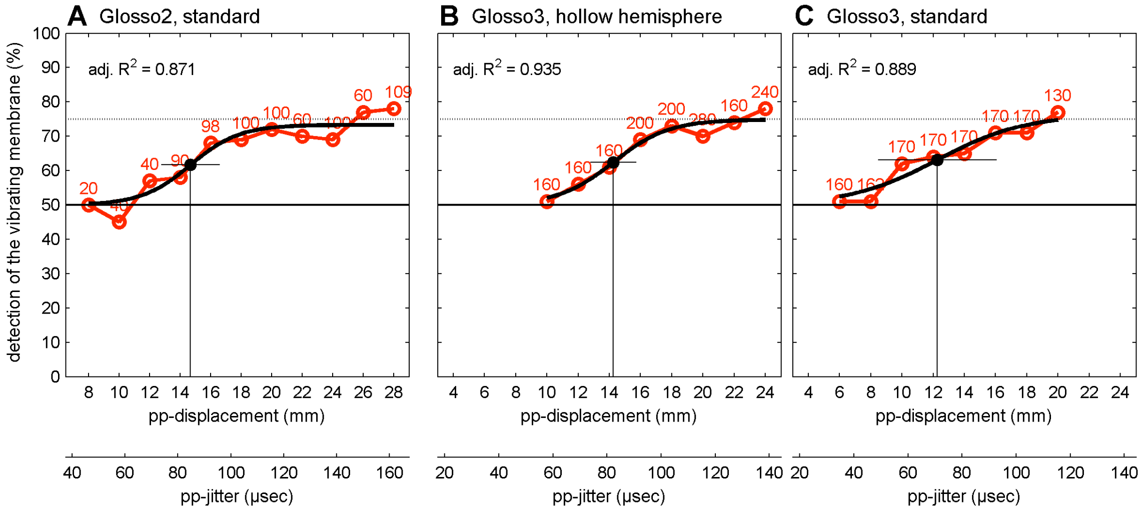


Figure 3.7: Temporal jitter detection: Temporal jitter detection performance (red) and the fitted psychometric functions (black) for a vibrating speaker membrane with 10 Hz vibration frequency for Glosso2 and Glosso3, using echo-acoustic cues. Note that the data were collected in the standard configuration with Glosso2 and in the hollow hemisphere and standard configuration with Glosso3.
Small numbers above the data give the number of trials per condition. The threshold is marked with a vertical line, and its 95% confidence interval by a horizontal line.

3.3 Results

3.3.1 Echo-acoustic detection of temporal jitter in Glosso2 and Glosso3

The two bats Glosso2 and Glosso3 were successfully trained to detect the vibrating membrane with echo-acoustic cues; Glosso2 in the standard configuration and Glosso3 first in the hollow hemisphere and than in the standard configuration. Their jitter detection performance and the fitted psychometric functions are shown in **Fig. 3.7**. All three psychometric functions have a shallow slope, and the bats did not reach a performance better than 75-80% even for large displacements. The thresholds of the functions are similar and lie at 14.7 ± 1.9, 14.3 ± 1.5 and 12.3 ± 3.8 mm peak-to-peak displacement ± 95% confidence interval, respectively. The thresholds correspond to echo-delay-differences of 85 ± 11, 83 ± 9 and 71 ± 22 μs, respectively. The mean of all three thresholds is 13.8 mm (80 μs).

3.3.2 Detection of jitter in Glosso1

Glosso1 was the first bat that was trained in this experiment. After an initial training of twelve days in the final configuration of the setup, Glosso1 reached a performance of over 80% correct detection of the vibrating membrane at 10 Hz and 21 mm pp-displacement. Jitter detection performance and the fitted psychometric jitter functions for 10 and 25 Hz

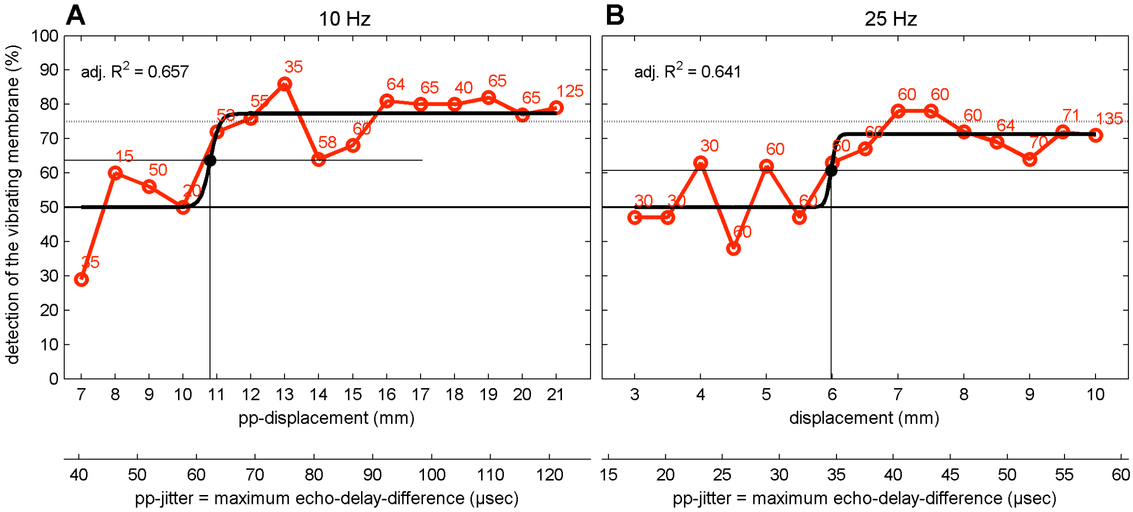


Figure 3.8: Jitter detection for Glosso1: Jitter detection performance (red) and the fitted psychometric functions (black) for a vibrating speaker membrane with 10 and 25 Hz vibration frequency for Glosso1. Note that no controls for the use of passive-acoustic information were performed for Glosso1.
Small numbers above the data give the number of trials per condition. The threshold is marked with a vertical line, and its 95% confidence interval by a horizontal line.

are shown in **Fig. 3.8**. The bat transfered the learned detection of the 10 Hz vibration frequency immediately to the 25 Hz vibration frequency without further retraining. Note that no controls for the use of passive acoustic information were conducted for Glosso1. It can therefore not be ensured that these psychometric functions are based on the use of echo-acoustic information, in particular as Glosso2 and Glosso3 did also use passive acoustic cues as their first means to detect the vibrating membrane (see the next section).

The performance at both frequencies was less smooth than the performance of Glosso2 and Glosso3, indicating the potential use of a cue which was not linearly correlated to the displacement, possibly passive acoustic cues.

For the sake of comparison with Glosso1 and Glosso2, jitter detection thresholds were calculated although the the use of temporal jitter is not sure. The threshold for the 10 Hz vibration frequency is at 10.8 mm pp-displacement of the membrane, corresponding to 62 μs temporal jitter. This threshold was about 3.5 mm (20 μs) smaller than the threshold of Glosso2 and Glosso3. For 25 Hz vibration frequency, the threshold is 6.0 mm pp-displacement (35 μs temporal jitter), i.e. only 56% of the displacement at 10 Hz vibration frequency. However, both threshold estimates have very large 95% confidence intervals, which sometimes exceeded the range of the measured displacements, but the adjusted R^2-values of the fits were still good (0.657 and 0.641).

3.3.3 The use of passive acoustic cues

Glosso2 Glosso2 was initially trained in the same way as Glosso1 before, i.e. without the presentation of background noise. First psychometric functions were obtained for 10, 25 and 50 Hz (**Fig. 3.9 A–C**), having thresholds of 11.3, 8.5 and 5.2 mm pp-displacement, respectively, corresponding to 65, 49 and 30 μs temporal jitter. This was about 3 mm (17 μs) smaller than during temporal jitter detection (**Fig. 3.7 A**). The adjusted R^2-values of the fitted psychometric functions are high and the 95% confidence intervals of the thresholds are about the same as during echo-acoustic jitter detection (**Fig. 3.7 A**).

However, subsequent controls revealed that Glosso2 did not need echo-acoustic information. The bat was still able to detect the vibrating membrane at the training displacement when the echo-acoustic access to the membranes was occluded with disks mounted in front of them (**Fig. 3.9 D, E**; χ^2-test, $p(1) < 0.05$). However, the performance dropped to chance level in a next control with continuous playback of white background noise (0-100 kHz, 75 dB SPL, **Fig. 3.9 F**; χ^2-test, $\chi^2 = 0.015$, $p(1) = 0.9025$). These controls showed that Glosso2 could use passive acoustic information to detect the vibrating membrane, which could be masked by noise playback. This control also excluded the possibility that the bat had used air draught generated by the moving membrane to detect it.

Sound recordings revealed that the right vibrating membrane indeed produced clicks containing frequencies of up to 60 kHz (**Fig. 3.10 A, B**). Depending on the integration time, the RMS-level of the click was up to 5 dB above the background noise floor.

Glosso2 was subsequently retrained to detect the jittering membrane using echo-acoustic information only during continuous playback of white noise, which was bandpass filtered between 10 and 45 kHz. Only 10 Hz was further used for training and data acquisition, as the membranes produced even more ultrasound when vibrating at higher frequencies, which could not be masked. After three weeks of retraining, Glosso2 had learned to detect the vibrating membrane with 75% correct, which is significantly above chance level (χ^2-test, $p(1) < 0.05$). This performance was based on echo-acoustic information as was shown by the part-disk control (**Fig. 3.9 G**, χ^2-test, $\chi^2 = 0.813$, $p(1) = 0.3672$). After this retraining was achieved, the temporal jitter detection function, which was already shown in **Fig. 3.7 A**, was measured during continuous playback of noise (10–45 kHz). Subsequent continued training did not improve the performance any further.

Glosso3 Glosso3 was initially trained in the same way as Glosso2 was trained at the end, i.e. during continuous presentation of bandpass filtered noise (10–50 kHz for Glosso3). After Glosso3 had learned to detect the vibrating membrane (**Fig. 3.11 A, left bar**, χ^2-test, $p(1) < 0.05$), several controls were conducted (**Fig. 3.11 A, right bars**). These controls

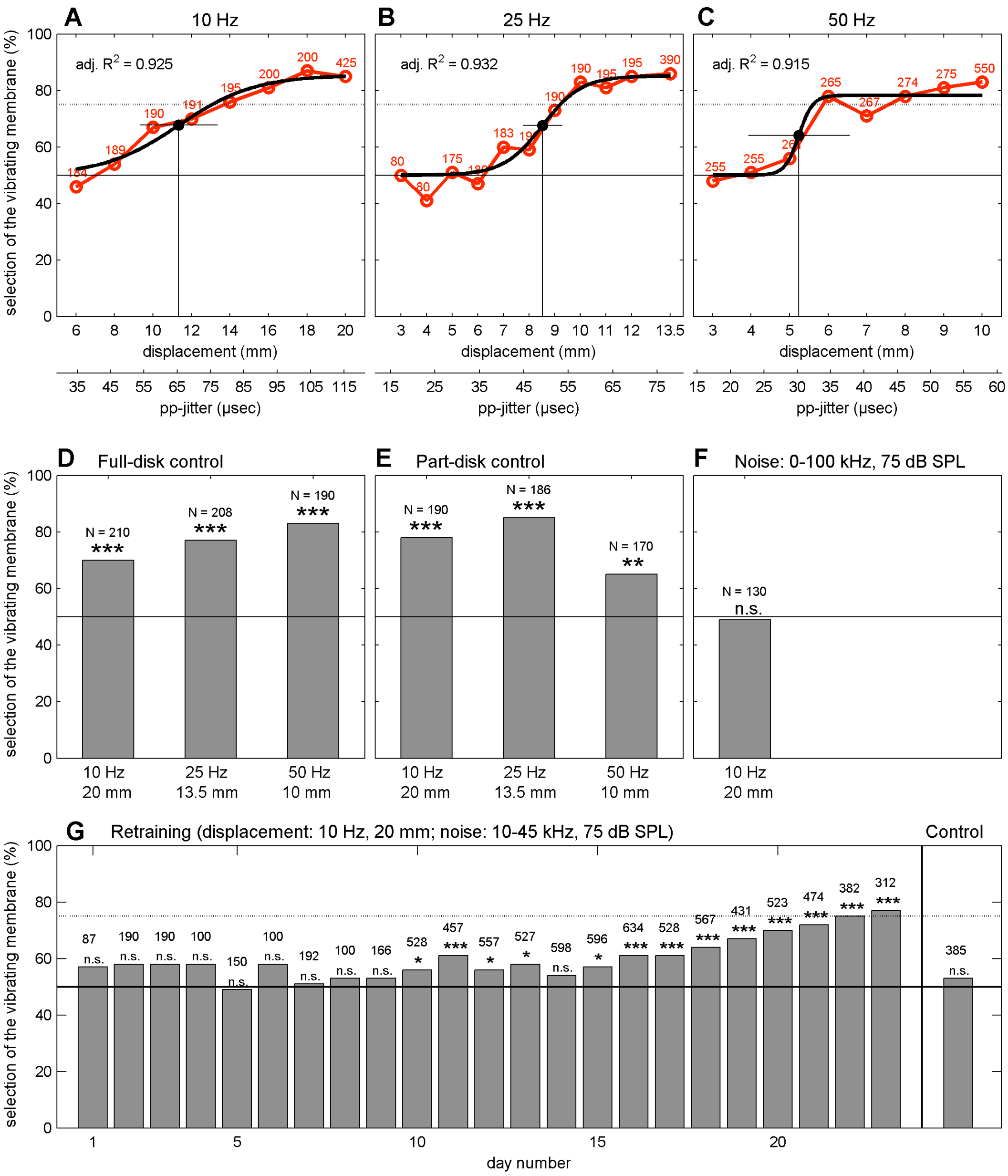


Figure 3.9: Initial test, control and retraining performance of Glosso2.

A–C) Jitter detection performance (red) and the fitted psychometric functions (black) for 10, 25 and 50 Hz vibration frequency, without noise playback. Threshold are marked with a vertical line, and their 95% confidence interval by a horizontal line.

D–F) Subsequent controls after the measurement of the jitter detection thresholds. The bat could still detect the vibrating membranes after they were either occluded with one disk (full-disk control, **D**) or with two parted disks (part-disk control, **E**). The presentation of white noise reduced the performance to chance level (**F**).

G) After three weeks of retraining, the bat had learned to detect the vibrating membrane. During the part-disk control (right bar), the performance decreased to chance level.

Small numbers above the data give the number of trials per condition.

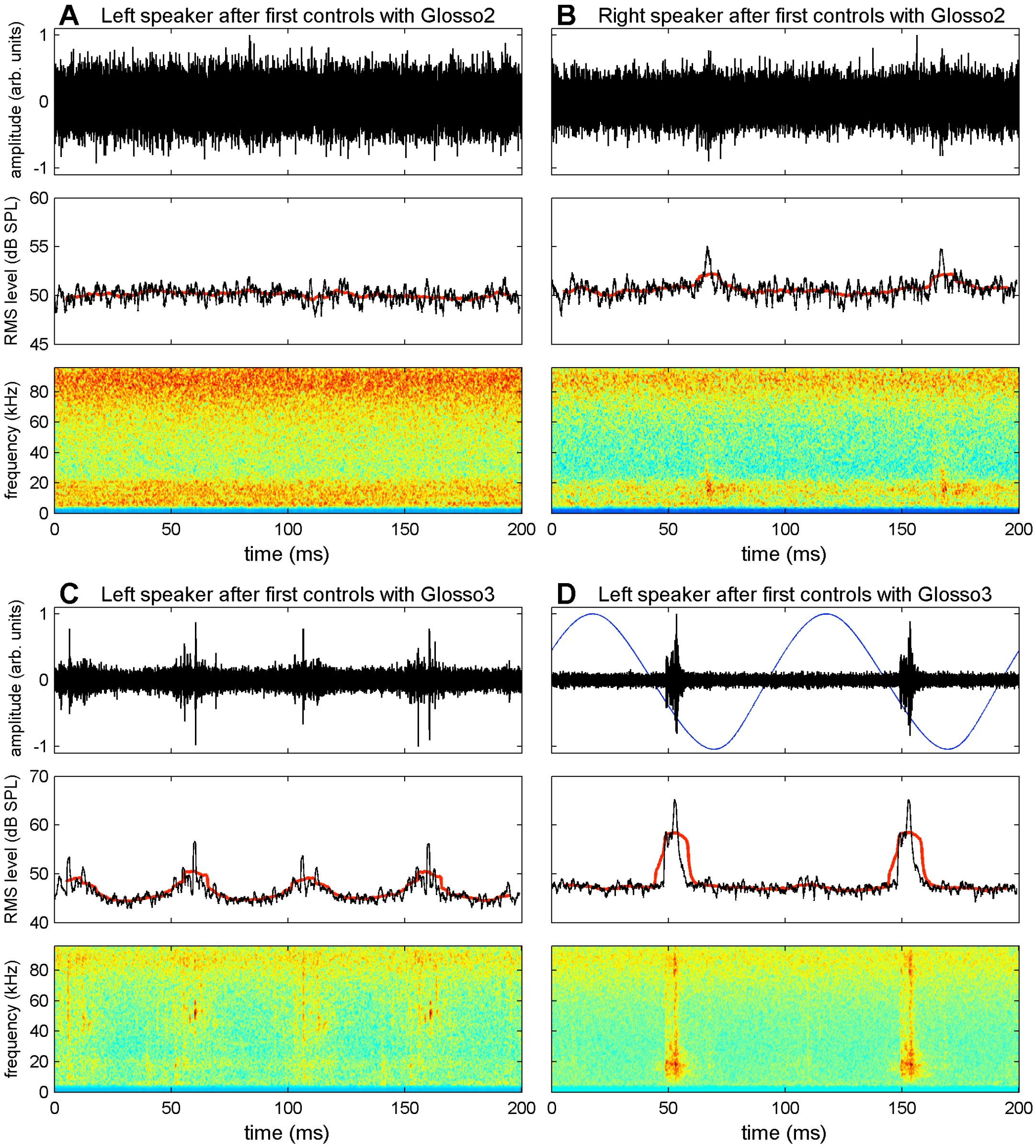


Figure 3.10: Sound recordings of the vibrating membranes, highpass filtered at 5 kHz and shown as time signal, running RMS-level (black: 1 ms integration window, red: 10 ms integration window) and spectrogram (512 point FFT, 97.5% overlap; the increase in energy above 80 kHz is caused by the microphone characteristics).

A, B) Recordings of the vibrating membranes (10 Hz, 20 mm pp-displacement) after the first controls with Glosso2. The right vibrating membrane produced one faint click per cycle, which could be masked by noise playback.

C, D) Recordings of the vibrating membranes (10 Hz, 20 mm pp-displacement) after the first controls with Glosso3. Both vibrating membranes produced more intense clicks as before, which could not be masked anymore. Both speakers were therefore replaced. The blue line in **D** gives the measured displacement of the speaker membrane, showing the phase-locking of the click to the cycle of the displacement.

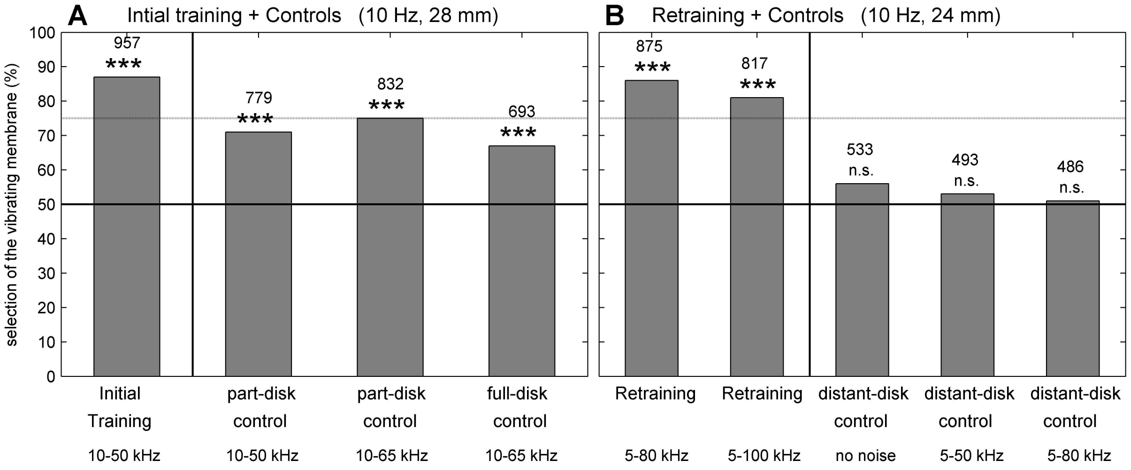


Figure 3.11: Training and control performance of Glosso3.
A) Jitter detection performance of single nights in the initial training and subsequent controls. Passive acoustic cues were sufficient to detect the vibrating membrane and could not be masked with noise between 10–50 and 10–65 kHz.
B) Jitter detection performance of single nights in the retraining and subsequent controls. After the retraining with new loudspeakers and band pass filtered noise, the performance in the control conditions with occluded membranes decreased to chance level.

revealed that the bat was still able to detect the vibrating membrane, also with noise of broader bandwidth (10–65 kHz; χ^2-test, $p(1) < 0.05$).

Acoustic measurements of the vibrating membranes with the microphone directly above the loudspeakers showed that both loudspeakers were now producing clicks once or twice per vibration cycle containing frequencies up to 100 kHz and beyond and having RMS-levels of 10–15 dB above the noise floor (**3.10 C, D**). The clicks were fainter and contained less ultrasound when the microphone was placed in front of the loudspeakers at the position of the feeders. Yet, the clicks could not be masked by noise playback. Therefore, both loudspeakers were replaced and Glosso2 was successfully retrained within two weeks during continuous noise presentation in the hollow hemisphere configuration (**Fig. 3.11 B, left bars**; χ^2-test, $p(1) < 0.05$). The performance dropped to chance level in the distant-disk control, showing that the retraining on echo-acoustic cues was successful (**Fig. 3.11 B, right bars**; χ^2-test, $\chi^2 = 3.28 - 0.066$, $p(1) = 0.0703 - 0.7972$). The temporal jitter detection functions, which were already presented in **Fig. 3.7 B** were then measured during continuous playback of noise (5–80 kHz and 5–100 kHz).

3.3.4 Simulation of the perceived temporal jitter

All bats echolocated during the experiment. When they approached the feeder, they increased their call rate and changed the temporal call patterning, indicating that they

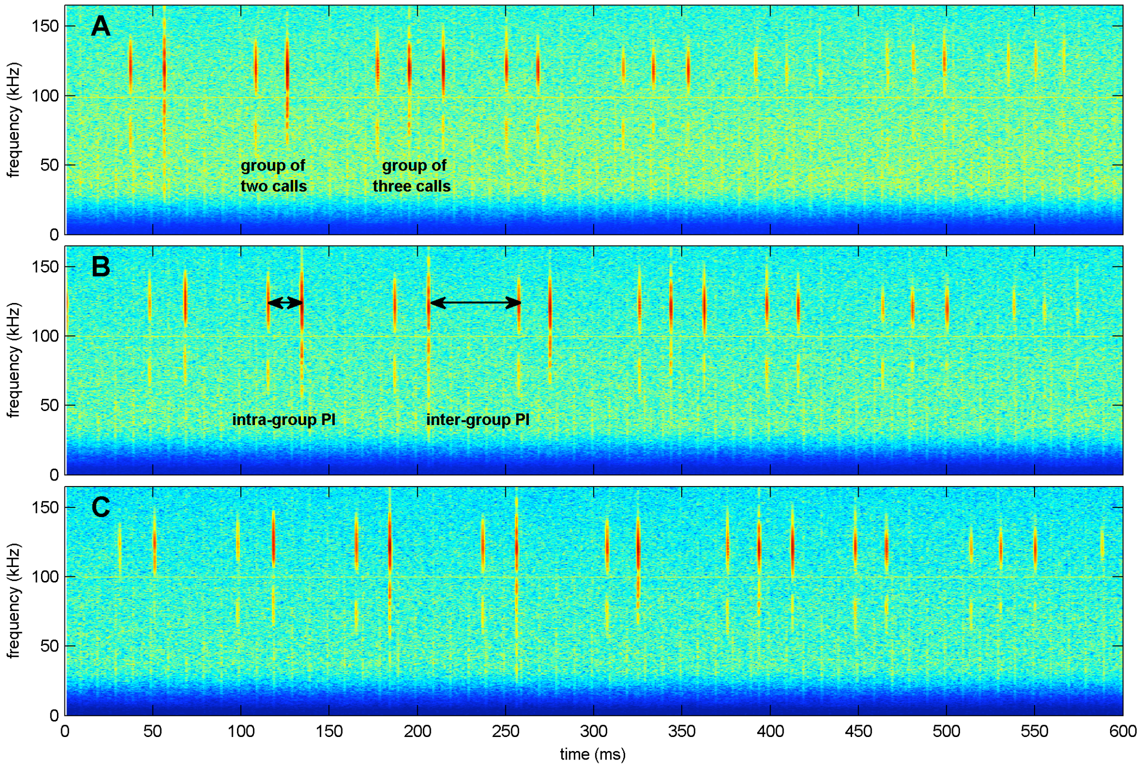


Figure 3.12: Call sequences of Glosso1 (**A**), Glosso2 (**B**) and Glosso3 (**C**) shortly before visiting the rewarded feeder. The end of the shown call sequences is about 400–500 ms before the sound of the opening feeder was registered on the recordings.

relayed on echo-acoustic information at least to control their approach to the feeder. Call sequences contained calls which were emitted as single calls or in groups of calls. Call groups contained mostly two or three, sometimes also four calls. Example call sequences of all three bats shortly before visiting the rewarded feeder are shown in **Fig. 3.12**. Groups of two and three calls and the analysed pulse intervals (PIs) are indicated.

The pulse intervals of calls within a group (intra-group PI) and the pulse intervals between two consecutive groups (inter-group PI) are given in **Tab. 3.3**, separately per bat and group type and averaged over all intra-group PIs. On average, the intra-group PI was 16 ms with a standard deviation of 3 ms. This PI was used to simulate the mean and the largest perceived temporal jitter per trial.

The distribution of the mean and largest perceived temporal jitter per trial at the detection threshold of each bat is shown in **Fig. 3.13**. The perceived jitter is always smaller than the maximally possible one, because the PI is shorter than half the period of the vibration frequency. For Glosso2, the mean perceived jitter at threshold was around 25 μs, corresponding to a distance accuracy of 4.3 mm (**Fig. 3.13 A**). The mean perceived jitter

Table 3.3: Pulse intervals of call sequences. The pulse intervals are given as means ± standard deviation of several pulse intervals per bat, and over all bats.

	intra-group PI of groups with			
	2 calls	**3 calls**	**4 calls**	**inter-group PI**
	ms	ms	ms	ms
Glosso1	19 ± 2, N = 24	16 ± 2, N = 46	(not observed)	44 ± 7, N = 70
Glosso2	19 ± 3, N = 14	16 ± 2, N = 61	14 ± 2, N = 34	45 ± 11, N = 51
Glosso3	19 ± 4, N = 28	17 ± 3, N = 52	14 ± 2, N = 6	43 ± 8, N = 62
all bats	19 ± 3, N = 65	16 ± 3, N = 160	14 ± 2, N = 40	44 ± 9, N = 160
		16 ± 3, N = 265		

at threshold for Glosso3 was between 20 and 25 μs (3.5–4.3 mm), depending on whether the hollow hemisphere or the standard configuration was used. The largest perceived jitter for both bats is 35–40 μs, corresponding to a distance accuracy of 6.1–6.9 mm.

The mean perceived jitter at 10 Hz of Glosso1 (if its performance was based on echo-acoustic information) is at 20 μs (3.5 mm), which is also the lower limit of Glosso3. At 25 Hz, the mean perceived threshold for Glosso1 is also around 20 μs. The largest perceived jitter at both frequencies is also similar at 30–33 μs, i.e. at a distance accuracy of 5.2–5.7 mm. Thus, the almost twofold difference in peak-to-peak-displacement thresholds between 10 Hz and 25 Hz vibration frequency disappears when the perceived jitter thresholds are considered. The pp-displacement threshold at 25 Hz is smaller than at 10 Hz as the membrane moves faster and covers a larger distance within one PI for 25 Hz than for 10 Hz. However, the actually perceived jitter at threshold is the same for both frequencies.

3.4 Discussion

This experiment has two general findings. First, at least two bats relied on passive acoustic cues as their first and predominant means to detect the vibrating speaker. Second, the bats required a long retraining to detect the vibrating membrane after passive acoustic cues had been excluded, only one bat reached a performance as good as with passive listening and the echo-acoustic jitter detection thresholds are three orders of magnitude larger than in the virtual jitter detection experiments.

It is most important to bear in mind that all experimental animals learned the procedural task of alternating between the start feeder at one side and the decision feeders at the other

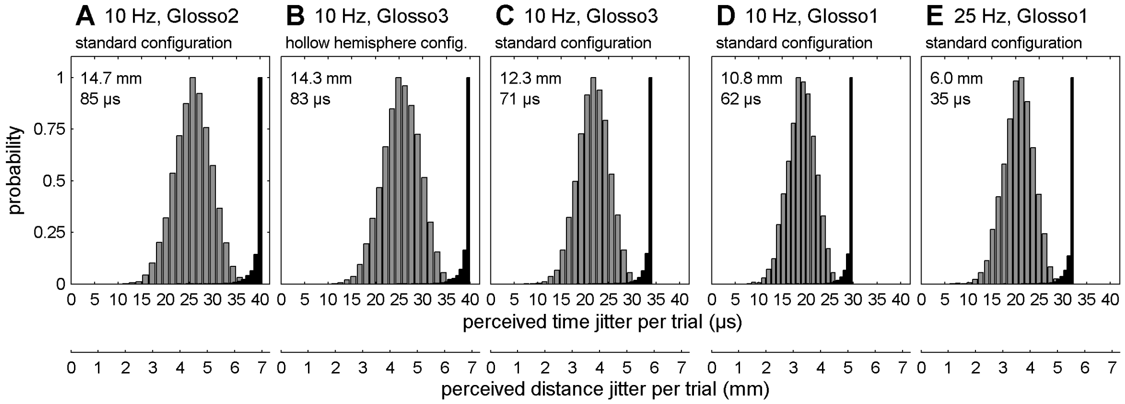


Figure 3.13: Simulated perceived jitter at threshold. The perceived jitter was simulated based on the average intra-group pulse interval of 16 ms and the assumption that the bats compared the delay of two consecutive echoes of one call group to measure the time jitter. The grey bars give the distribution of the mean perceived jitter per trial, when ten call groups per trial were simulated. The black bars give the distribution of the largest perceived jitter per trial.

The individual detection threshold of each bat in terms of the peak-to-peak-displacement of the vibrating membrane and the corresponding maximum temporal jitter is given in each panel. Remember that the use of echo-acoustic information cannot be ensured for Glosso1 (**D, E**) and that its pp-displacement thresholds have very large 95% confidence intervals.

side of the experimental chamber, that all bats echolocated during the experiment, and that at least two, if not all, bats could be trained to use passive-acoustic cues to detect the vibrating speaker. Obviously, it was not the lack of echo-acoustic information nor the failure to train the bats on the procedural task which prevented them initially to detect the vibrating speaker based on the jittering echo delay. In fact, it seems that the passive-acoustic clicks of the vibrating membrane were more salient for the bats than the generated echo delay. These results question the 10 ns threshold of virtual jitter detection tasks.

3.4.1 Potential cues in the current jitter detection experiment

The worst case in every psychophysical study is another physical cue that changes in accordance with the nominal manipulated cue. Therefore, other potential cues will be analysed first before the obtained temporal jitter detection thresholds and the unexpected use of passive acoustic information will be discussed, to make sure that, besides passive acoustic cues, no other non-intended cues were present.

Doppler-shift One potential cue are Doppler-shifts of the reflected echoes caused by the movement of the membrane. The maximum velocity of the membrane was 0.75 m/s, generating Doppler-shifts of less than 0.5% of the incident frequency. At treshold, the

Doppler-shift was around 0.25–0.27% for both vibration frequencies. Just noticable frequency differences are much larger in mammals in general and also in bats (except for CF-bats, see Schnitzler & Flieger, 1983), which will render these Doppler-shifts indetectable. Just noticable frequency differences where found to be 11% (Genzel & Wiegrebe, 2008) or 6–13% in *Megaderma lyra* (Schmidt, 1992) and 7–21% (mean: 16%) in *Glossophaga soricina*, the species in this study (Simon *et al.*, 2006). Direct measurements of just noticable Doppler shifts with *Eptesicus fuscus* found thresholds between 2 and 4% (Wadsworth & Moss, 2000), and thresholds of 1.0–1.4% even for the CF-FM-bat *Noctilio leporinus* (Wenstrup & Suthers, 1984). These thresholds are all larger than the Doppler-shifts in this study.

Spectral ripples Another possibility are spectral patterns of temporally varying two-wavefront echoes, generated by the reflection of the call at two (or even more) surfaces of the setup. If one of the surfaces is moving, the reflected echo-complex will have varying spectral patterns. This idea is extensively discussed in Sum & Menne (1988) and Grossetête & Moss (1998) for the detection of a fluttering target by *Pipistrellus stenopterus* and *Eptesicus fuscus*, simulated by a rotating propeller (the moving target) in a box (the stationary target). As typical FM-bats with low duty cycles (only short periods of time are filled with signal), both species could neither evaluate Doppler-shifts nor detect echo-acoustic glints to measure the rate of the propeller, and therefore spectral ripples seemed to be the perceptual cue in these experiments.

Three lines of evidence argue against the use of spectral ripples in the current jitter detection task:

The speaker membrane in our setup was 55–80 mm behind the front wall of the setup. Generated spectral ripple will be between 3.1 kHz (55 mm) to 2.1 kHz (80 mm), which is a very fine spacing. These constant spectral ripples are present at both membranes, but will begin to vary at the moving membrane. The width of the spectral ripples will maximally change between 1.8 and 4.2 kHz at the maximum displacement (±14 mm), and between 1.9 and 3.5 kHz at threshold. Assuming a mean frequency resolution of 16% in *Glossophaga soricina*, the largest ripple of 4.2 kHz can only be perceived with a call frequency of 26 kHz or less. Even the best frequency discrimination performance of 7% found by Simon *et al.* (2006) for *G. soricina* would still require a call frequency of maximally 60 kHz to detect these ripples, but this is the very lower limit of the calls of *G. soricina* (see **Fig. 3.1**).

The next two arguments question the generation of spectral ripples at all, caused by the directionality of the echolocation system and the short temporal integration of the auditory filters. For spectral ripples to occur, two wavefronts need to be reflected from the setup and integrated at the auditory filters.

The amount of energy reflected from the setup surrounding the membranes, in relation to the energy reflected from the membranes directly, depends on the direction and beam width of the echolocation call and the directional characteristics of the ears. The opening for the loudspeaker in the setup's front had a diameter of 16 cm, and was in addition surrounded by a 4 cm wide rim of sound-absorbing foam (altogether an area of 24 cm diameter, which did not reflect back sound energy from the setup's front). Caused by the high directionality of ultrasound, the beam of *Glossophaga soricina* will be narrow. In *Carollia perspicillata* (Hartley & Suthers, 1987) and *Eptesicus fuscus* (Hartley & Suthers, 1989), the sonar beam width at half amplitude is ±25° at 90 kHz. For *Pteronotus parnellii rubiginosus*, the beam width is ±15–20° at 80 kHz (Hartley & Suthers, 1990). In combination with the directional reception characteristics of the pinna and the higher call frequencies of *G. soricina*, its effective beam width will presumably be ±15° or less. If the bat is closer than 45 cm to the setup (which it was during several observations when it changed the approached feeder) and aims its sonar beam at the centre of the membrane (which we, however, do not know), it will not get back an echo from the surrounding front wall. Altogether, we cannot exclude the ensonification of several reflectors, thereby generating several echo-wavefronts, but especially at close distances this is unlikely.

Two or more echo-wavefronts need to be integrated by the peripheral auditory filters to generate spectral interference patterns. The integration time for two transients sounds is only 100–200 μs in *Megaderma lyra* (Weißenbacher *et al.*, 2002) and is presumably limited by the time constant of auditory-filter ringing (Krumbholz & Wiegrebe, 1998; Weißenbacher *et al.*, 2002). The distance between two reflectors of the real-target jitter setup was at least 41 mm (= 55–14 mm), generating two echo-wavefronts with a temporal separation of at least 237 μs. If we assume a similar temporal integration in *Glossophaga soricina*, or even shorter due to the higher frequencies (Wiegrebe, 2008), then spectral interference patterns will not be generated.

Altogether, the perception of spectral patterns generated by two-front targets is unlikely, at least for the standard configuration. The same argumentation is more difficult for the hollow-hemisphere configuration, where a hollow hemisphere of 10 cm diameter was fixed on the vibrating membrane and the distances between the reflecting surfaces are in general closer. However, as the psychometric functions and the thresholds obtained in both configurations did not differ, it is likely that the same echo-acoustic cues were used.

Measurement of spectral artefacts In addition to the above mentioned considerations, the time-variant impulse response of the sinusoidally jittering real targets was measured (**Fig. 3.4**). No spectral changes correlated to the displacement of the membrane could be detected, thereby supporting these considerations.

Temporal jitter and passive acoustic cues Time jitter is therefore the only remaining echo-acoustic cue available in the current experiment, besides the passive-acoustic clicks which were already described and which could be masked by noise playback.

3.4.2 Discussion of the measured thresholds

The published thresholds for the perception of several temporal characteristics of echoes vary by four orders of magnitude, depending on the task and the study – from the 10 ns result for temporal jitter detection (Simmons *et al.*, 1990a, 2003) to the 300 μs of duration discrimination (Schörnich & Wiegrebe, 2008).

In this experiment, the perceived time jitter at detection threshold was 20–25 μs for the mean value per trial, and 30–40 μs for the largest value per trial, corresponding to a ranging accuracy of 4 mm (mean) and 7 mm (largest value). It is difficult to decide whether the mean or the maximal perceived jitter per trial is the better estimate of the perceptual representation of the delay. In both cases, the thresholds are three orders of magnitude larger than the lowest virtual jitter threshold of 10 ns. They are are at the lower end of, and even slightly smaller than, the thresholds of all distance discrimination studies (see **Tab. 3.1**). The jitter method thus slightly reduced the obtained threshold compared to pure distance discrimination tasks.

The thresholds are in a range which seems to be biologically plausible. The most demanding task that requires the highest spatial accuracy for a flower-visiting bat seems to be the orientation in relation to flower openings (**Fig. 3.14**). The width and depth of typical bell-shaped bat-pollinated flowers is around 2 cm and 4 cm, respectively (von Helversen *et al.*, 2003). Such distances can be easily measured with the here determined distance accuracy, and no need for a higher accuracy would be necessary. In addition, tactile information will in addition help to stop the bats' approach after contacting the flower.

In addition, the bat achieved this performance while being in flight, as will be the case while approaching a flower. Behavioural observations during the experiments revealed that the bats only rarely hovered in front of the membranes, but either approached the correct feeder directly or receded and changed to the other one. This indicates that the bats detected the vibrating membrane while being in flight within a few tens to hundreds of milliseconds. The reason to introduce the jitter paradigm was to minimize errors in distance measurements caused by head movements between trials (Simmons, 1973; Simmons & Grinnell, 1988). However, the bats in the current experiment reached jitter detection thresholds which are comparable or even smaller than during distance discrimination tasks, although they were quickly flying instead of sitting stationary on a platform. Body and

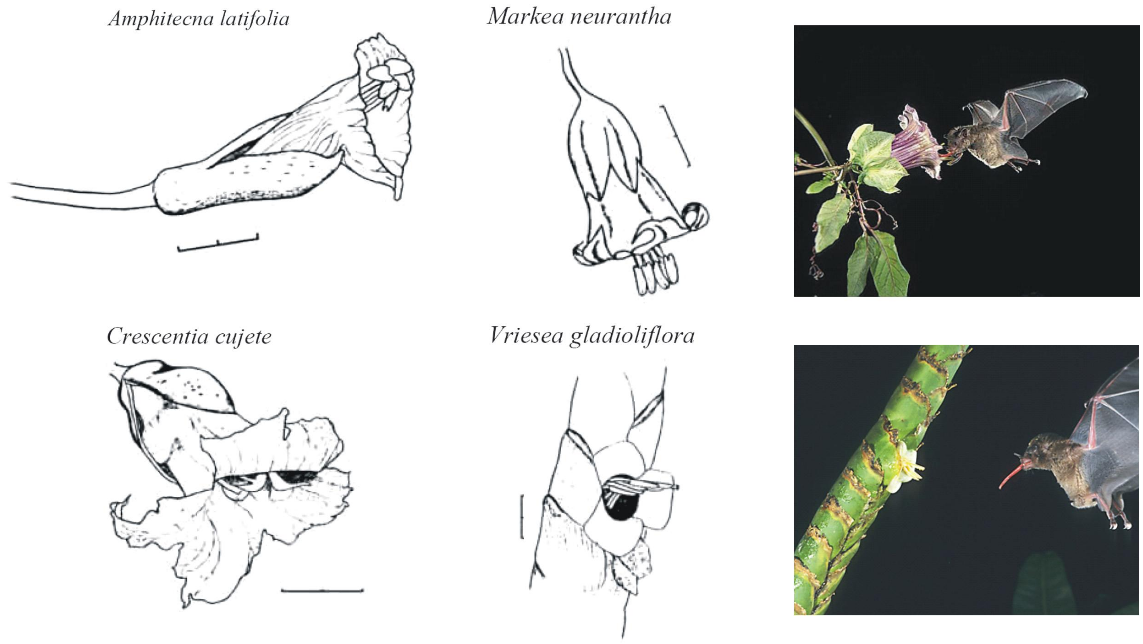


Figure 3.14: Bat pollinated flowers. The drawings show four typical bat-pollinated flowers with bell-shaped openings. The scale bars are 2 cm. The drawings were taken from von Helversen *et al.* (2003). The two pictures show *Glossophaga soricina* approaching open flowers. Photographs by Dietmar Nill (www.dietmar-nill.de).

head movements seem therefore not to play the limiting role in the detection performance, but rather the accuracy of the internal representation of timing.

A comment is needed on the experimental animal: It is often questioned whether the temporal accuracy of nectarivorous and frugivorous bats is as good as the accuracy of insectivorous bats, such as *Eptesicus fuscus*, which was used in most psychoacoustical studies. However, most insectivorous bats (except for rare exceptions like *Craseonycteris thonglongyai*; Surlykke *et al.*, 1993) catch their prey with a pouch formed by their wing or tail membrane (e.g. Griffin *et al.*, 1960; Kalko, 1995), which does not require a very high spatial accuracy. Likewise, Webster (1963) and Trappe (1982) estimated the localization accuracy of bats catching flying insects to be around 1 cm^3 in volume or 2–3 cm in diameter, respectively (cited in Schnitzler *et al.*, 1985). Finally, the threshold of *Phyllostomus hastatus* did not differ from *Eptesicus fuscus* in a real-target distance discrimination task (Simmons, 1973, see also **Tab. 3.1**). As not only insectivorous bats use short and steeply frequency-modulated calls, which are necessary for precise timing (Schnitzler & Kalko, 2001; Siemers & Schnitzler, 2004; Boonman & Ostwald, 2007), but also nectarivorous and frugivorous bats, and all of them have to navigate in dense cluttered spaces, there is no basis for assuming differences in the spatial or temporal accuracy between these species.

In line with that, it is noteworthy that Kristian Beedholm tried to train the insectivorous bat *Eptesicus fuscus* in a real-target jitter detection task, but failed (K. Beedholm, University of Southern Denmark, Odense, *pers. comm.*; see also the first sentence in the methods of Beedholm, 2005). Even this species, which was used in most psychoacoustical studies on bat echolocation, particularly in the virtual jitter detection tasks, failed to detect a sinusoidal real-target jitter. This once more makes appreciable differences in the temporal accuracy between insectivorous and nectarivorous bats questionable, and shows additionally the difficulty of the real target jitter detection task – which I believe is transferable to the virtual jitter detection task.

Finally, in comparison with other known limits of temporal processing, such as the just noticeable interaural time difference in humans (10 μs; Klumpp & Eady, 1956; Zwislocki & Feldman, 1956) and barn owls (reviewed by Knudsen, 1980) and the temporal analysis in weakly electric fish (1 μs; Rose & Heiligenberg, 1985; Heiligenberg, 1991), the threshold of 20–40 μs found here seems reasonable. The putative temporal hyperacuity of 10 ns as revealed by the virtual jitter experiments (Simmons *et al.*, 1990a, 2003) is two to three orders of magnitude below all these thresholds and is not supported by the current data.

3.4.3 The use of echolocation versus passive listening

Both bats tested for the use of passive acoustic cues (Glosso2 and Glosso3) relied on them after the first weeks of training. This does not coercively exclude the simultaneous use of echo-acoustic information. However, the retraining on echo-acoustic information took around two to three weeks; and Glosso2 did not reach a performance as good as in the first training. This strongly indicates that the first psychometric functions measured with Glosso2 (**Fig. 3.9 A-C**) are not based on echo-acoustic information (although their thresholds for 10 and 25 Hz are in the same range as the echo-acoustic based thresholds). In addition, *Eptesicus fuscus* relied also on passive acoustic cues in the aforementioned training attempt by Kristian Beedholm (*pers. comm.*).

The cue employed by Glosso1 has to remain unclear. As the passive acoustic signals grew louder over time, it is possible that its psychometric functions are based on echo-acoustic information. On the other hand, the uneven shape of the psychometric functions indicates the use of a cue which was not consistently related to the displacement of the membrane, e.g. an acoustic click that was louder at certain displacements.

Passive acoustic cues are used in several vertebrate taxa for the detection and localisation of prey, so in owls (Payne, 1971), bats (Bell, 1985; Anderson & Racey, 1993; Faure & Barclay, 1992; Fuzessery *et al.*, 1993; Arlettaz *et al.*, 2001) and primates (Charles-Dominique, 1977; Goerlitz & Siemers, 2007; Siemers *et al.*, 2007). In bats, passive listening is generally employed by so-called gleaning bats that forage close to background vegetation, where background echoes mask prey echoes (Neuweiler, 1990; Schnitzler & Kalko, 2001; Siemers & Swift, 2005). However, *Glossophaga soricina* is not a specialised gleaning bat (Nowak, 1994), but all the same relied on passive acoustic cues to solve an experimental task which should be easily solvable with echolocation if *G. soricina* was able to detect submicrosecond changes in echo delay.

This shows that the sensory access of *G. soricina* to its environment is not restricted to echolocation alone, and presumably will integrate also other senses, such as vision (Bell, 1985; Eklöf & Jones, 2003), olfaction (Korine & Kalko, 2005), touch (Zook, 2005; see also Miller, 2005) or magnetoperception (Holland *et al.*, 2006), which will presumably be combined with its spatial memory (Thiele & Winter, 2005; Toelch *et al.*, 2008). The use of passive listening will complement the orientation based on echolocation, vision and spatial memory and may help in the detection of predators and rustling insects sitting in flowers.

3.4.4 Conclusion

The temporal accuracy in a jitter detection task was measured in a freely moving bat, i.e. under semi-natural conditions. The threshold is in a biological plausible range of a few millimetres, which is sufficient for the bat to position itself in relation to a flower. The measured threshold is three orders of magnitude above the threshold found in some virtual jitter detection experiments (Simmons *et al.*, 1990a, 2003). Interestingly, the bats had difficulties in using temporal jitter and initially based their decisions on passive acoustic clicks generated by the vibrating membrane.

This leads to two conclusions: first, during semi-natural foraging coupled to a jitter detection task, *Glossophaga soricina* did not measure temporal jitter readily and not at all with submicrosecond accuracy (and maybe does not have a hyper-accurate delay representation at all); and second, *G. soricina* relies more easily on other sensory information, such as passive acoustic cues, as previously was expected.

Acknowledgments

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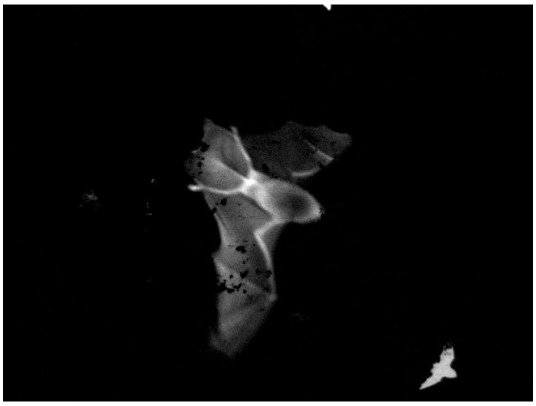
No thresholds without calibration – I thank Phil Brownell for providing the accelerometer and as well Gerd Schuller for the laser sensor.

Conny Geberl temporarily looked after one animal and the experiment during a practical course in our lab and during my field trip to Trinidad. Thank you very much.

This work was supported by financial contributions and a scholarship of the DFG graduate program 1091 'Orientation and Motion in Space' to HRG .

The experiments in this study are non-invasive and are therefore no animal experiments and do not require an experimentation approval. Permission to keep *Glossophaga soricina* in fulfilment of § 11, Abs. 1 TierSchG has been given to the Dept. Biologie II der Ludwig-Maximilians-Universität München by the Landratsamt München, dated October 09, 2007.

The following chapter presents a joint project together with Daria Genzel, LMU München. We both equally conceived and prepared the experiments, together with Lutz Wiegrebe. Daria Genzel and I conducted the experiments, whereas I analysed the data.



4 Orientation Response of Wild Free-Flying Bats to Real and Virtual Objects

4.1 Introduction

Echolocation is used by bats for spatial orientation and navigation and for food acquisition. Whereas most research on echolocation behaviour and signal structure focused on the context of foraging (object detection, discrimination, classification and localisation), the requirements for navigation are only minimally understood (Schnitzler *et al.*, 2003; Holland, 2007). While active, bats are highly mobile and spend most of their time on the wing (Krull *et al.*, 1991). They move during their daily travels from their roosting places to their foraging grounds, while foraging and during social interactions (e.g. swarming; Rivers *et al.*, 2006) and in many, mainly paleotropic species, during their seasonal migrations from their summer roosts to their hibernation places (Petit & Mayer, 2000; Neubaum *et al.*, 2006; Rodrigues & Palmeirim, 2008).

During all types of movement, bats have to orient in their environment and to navigate from place to place. Trullier *et al.* (1997) defined navigation as the ability of animals to find, learn and return to specific places. Schnitzler *et al.* (2003) distinguished three levels of navigation in echolocating bats according to the orientation situation and the necessary echolocation task. Small-scale navigation comprises all tasks where the target of interest is in the perceptual range of the echolocation system, e.g. food items, landing places, obstacles and background objects. In middle-scale navigation, the target of interest is beyond the perceptual range of the echolocation system, but still within the animal's home range, thus also within the spatial memory of the bat. A typical example are commuting flights from the roost to the foraging area, where well-established spatial landmarks and routes along background structures are used for orientation. Large-scale navigation encompasses long-distance travels, such as migration and homing, where echolocation is of little use, but vision (Williams *et al.*, 1966a,b) and celestial cues (Buchler & Childs, 1982), magnetoperception (Holland *et al.*, 2006, 2008) and distant prey sounds (Buchler & Childs, 1981) are employed. Long scale navigation will not be regarded further here.

A typical middle scale navigational task are commuting flights. Many bats fly along stereotyped routes from their roosts to their foraging areas (Bateman & Vaughan, 1974; Krull *et al.*, 1991; Rieger & Alder, 1993; Britton *et al.*, 1997; Robinson & Stebbings, 1997), often along landscape structures, such as alleys, hedgerows or buildings (Krull *et al.*, 1991; Britton *et al.*, 1997; Verboom *et al.*, 1999; Holderied *et al.*, 2006; Schaub, 2007; Schaub & Schnitzler, 2007a,b). During small and middle scale navigation, bats orient in part echo-acoustically, for example by using acoustic landmarks (Verboom *et al.*, 1999; Jensen *et al.*, 2005). However, it should not be forgotten that bats have also good vision, including for ultraviolet wavelengths (Winter *et al.*, 2003), on which they rely on during flight (Rother & Schmidt, 1982), for landing control (Joermann *et al.*, 1988) and prey detection (Bell, 1985; Eklöf & Jones, 2003; Rydell & Eklöf, 2003). Furthermore, spatial memory is of great importance during orientation (Höller, 1995; Thiele & Winter, 2005; Winter & Stich, 2005; Stich & Winter, 2006).

Despite using the same familiar flight paths, bats continue to echolocate while commuting in order to detect and avoid unsuspected obstacles in their flight path. Schaub (2007) measured for the first time changes in flight and echolocation behaviour when bats are unexpectedly confronted with a novel object in their flight path. She described the flight and echolocation behaviour of several bat species while commuting in a gap or edge situation, i.e., on flyways with a vertical background structure (trees, forest, building) on one or both sides of the flyway. Bats normally kept a species-specific horizontal distance of about 1.9–3.7 m to the background and flew within a flyway of 0.7–2.6 m width. She then placed a novel, vertical echo reflector at different distances to the background close to or into the bats' flyways and recorded the orienting reaction in echolocation behaviour and flyway changes in response to this real object. Again, the changes in flight behaviour were species specific and included evasive manoeuvres around the object, mostly on the side remote to the background, but also between background and novel object. Additionally, changes in flyway height and width were observed.

While the study of Schaub (2007) described for the first time systematically the flight changes of bats involved in a spatial novel object avoidance task, she could not quantify the bats' evasive manoeuvres in response to a variety of real objects and to specific acoustic parameters. The question of how acoustic information relates to the auditory percept and to the internal representation of the outer world is still not fully understood. We thus tried to create an experimental situation where we could manipulate the available acoustic object features, present this object to wild, naïve bats and record their flight behaviour as a measure for their precept of this object's size. This would allow us to relate the acoustic features, such as signal level, duration, delay or structure, to the changes in the flight path, which could be used to infer the perceived size of this object.

The challenge of object recognition in echolocation The spatial features of an object are encoded in its impulse response, and are imprinted on the reflected echo after ensonification of the object with an acoustic signal. Based on the perceived echoes, bats extract the object specific features and build an internal three-dimensional representation of their surrounding. For example, echolocating bats measure the range to an object via the time delay between their call and the returning echo (Simmons, 1973). Echolocating dolphins discriminate virtual objects just as well as learned real objects (Aubauer *et al.*, 2000) and can recognize visually learned objects when only echoacoustic cues are presented, and vice versa (Pack & Herman, 1995; Harley *et al.*, 1996; Herman *et al.*, 1998). However, only the object's distance and its depth, i.e., its extent along the longitudinal or range axis, can be encoded unambiguously in the acoustic reflection pattern. Object distance translates into the time delay between emission and returning echo, and object depth into echo duration (given that the emission could reach all reflectors of the object from the front to the rear, e.g. as can be the case for a tree).

However, the two other object dimensions width and height, which are perpendicular to the range-axes, are not directly accessible via echolocation. As acoustic information is inevitably one-dimensional, all information aside from the range axis has to be computed or estimated from other auditory information connected to this information. The shape of an object, i.e., its height and width, cannot be perceived directly and needs to be acquired trough sequential echo analysis from different angles (Nachtigall *et al.*, 1980; Helweg *et al.*, 1996; Genzel, 2007; Genzel & Wiegrebe, 2008). Other object features can be perceived or estimated from different features of the acoustic signal. The surface structure is coded in the spectral interference pattern ("acoustic colour"). The overall reflecting size of a surface (not its shape) can be estimated based on the amplitude of its impulse response. Directional information is calculated from interaural comparison of the intensity and arrival time of acoustic signals at the two ears and from direction dependent spectral interference patterns generated by the outer ear. Altogether, direct and indirect and even mutual dependent auditory information about the spatial characteristics of three-dimensional objects are available, but need a large amount of extensive neuronal processing to extract them.

For comparison, the visual system has direct access to the shape and position of objects from the two-dimensional retinal image. Like in the auditory system, the perceived colour depends on the spectral reflection pattern of the object. In contrast, the depth structure of a visual scene has to be computed by comparing the two-dimensional image between the two eyes, relying on experience and assumptions about the outer world.

Despite the complicated calculations necessary during auditory object recognition, bats can recognize three-dimensional objects (Kalko & Condon, 1998; Thies *et al.*, 1998; Korine & Kalko, 2005) and build object-specific and even size-invariant internal representations

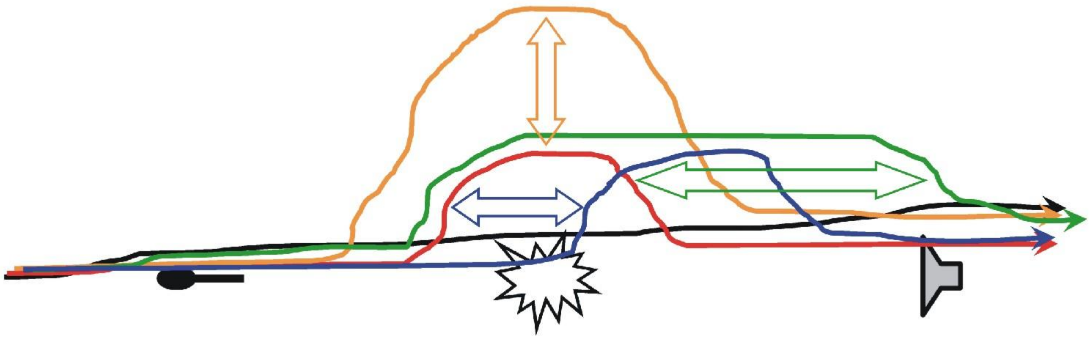


Figure 4.1: Simplified hypothetical flight paths around the virtual object. The flight paths without virtual object presentation should be straight, with minor influences of the microphone and loudspeaker (black). When a simple virtual echo reflector (star) is presented, the flight paths should be deflected sideways (red). Increasing the virtual object's surface area (echo level) should lead to a larger sideways flight path correction (orange). Increasing object length (echo duration) should lead to a longer deflection in the flyway (green). When the position of the object is shifted backwards (echo delay is increased), the flight path change should occur later (blue). The arrows give the change in flight path in relation to the flight path with a simple echo reflector (red).

(von Helversen & von Helversen, 2003; von Helversen, 2004). However, the internal representation of object features and the connection from a given physical stimulus parameter to its psychological percept still needs clarification.

Aim of this study The real-time, phantom-target generation technique (Simmons, 1973; Schmidt, 1988; Aubauer & Au, 1998) is used in our and other labs, mainly in a multitude of psychoacoustical studies. Here, we aimed to transfer this method into the field to naïve, untrained bats, which are only familiar with real objects. Our second goal is to establish a relationship between the flight behaviour of naïve bats in response to controlled manipulation of several echoacoustic parameters. The extent of the bats evasive flight paths around the virtual object should reflect the perceived object size. We hypothesized (**Fig. 4.1**) that (1) flight paths should deflect sideways upon presentation of a virtual object. (2) The lateral extent of the flight path deflection depends on object width and height, which is determined by echo level. (3) The longitudinal extend of the flight path deflection depends on the object length, i.e. echo duration. (4) The beginning of the flight path deflection depends on the position of the object, determined by echo delay.

We chose the Tamana Cave in Trinidad as study site, which is inhabited by thousands of bats that leave the cave in the evening. The great advantage of this site is the high density of animals leaving the cave. It enabled us to record behavioural data of hundreds or thousands of individuals with minimal, potentially adverse, impact on the behaviour of every single individual. The study was designed to avoid the time-consuming training of bats required for many laboratory studies, and to observe an innate behavioural response to an experimentally manipulated situation. Thereby, this study functioned as a critical test for the phantom-target generation method. As the bats were not accustomed to the presented impulse responses, their behaviour will directly show how they perceive the presented virtual objects in relation to real objects.

4.2 Methods

4.2.1 Study site and animals

We performed playback experiments and flight path video recordings in front of the Tamana Cave on the island of Trinidad in December 2007. The Tamana Cave is located on the Tamana mountain in the Middle Ranges, a mountain ridge running in East-Western direction through the middle of Trinidad (**Fig. 4.2**). The Tamana Cave is a cave system with several openings. For our experiments, we chose a large opening with about 3 m in diameter, where most of the bats fly out every night, with a clearing in front of the opening. Bats of up to twelve species (Kenny, 2008; Riskin, 2008; Firzlaff U, Wiegrebe L & Zahn A, *pers. comm.*) emerge from the cave and fly into the surrounding forest. While they fly along the clearing and before they spread out into the forest, their flight paths are approximately straight and in a constant height of 0.5 – 2.3 m above the ground.

Due to the large amount of individuals passing the camera simultaneously, a three-dimensional flight path reconstruction with two or more cameras was not possible: caused by the differing viewing directions of the potential cameras, the same bat would look different on the different video frames. It would thus not be possible to reconstruct individual bats when many bats are visible on the same video frame, neither for a human observer nor for an automatic algorithm. In addition, due to the sheer amount of passing animals, a manual reconstruction would not be feasible. An automatic reconstruction would fail due to the differing illumination (especially in the darker parts of the video frames), the sparse sampling and the concealing of bats by other bats. Therefore, we decided to record with only one camera which was aligned perpendicular to the flight paths, which was possible in front of the Tamana Cave as the bats flew in a more or less constant height on more or less straight paths.

4.2.2 Playback and video-recording setup

We used the real-time, phantom-target generation technique for the presentation of virtual targets, as routinely employed in our lab (e.g. chapter 2, **Fig. 4.3**). We recorded echolocation calls of the emerging bats with an ultrasound-sensitive microphone (CO 100K, Sanken

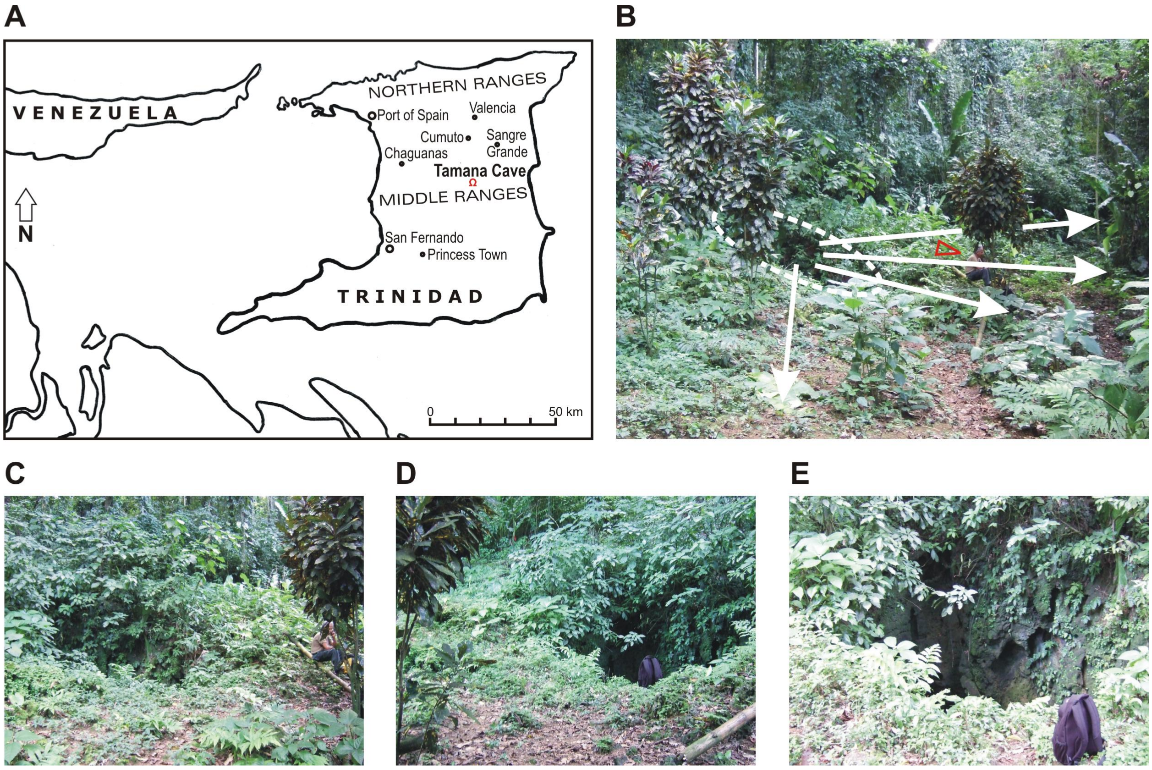


Figure 4.2: The Tamana Cave in Trinidad.

A) Map of Trinidad with the location of the Tamana Cave (red) in the Middle Ranges.

B) Picture of the clearing around the Tamana Cave. The dashed ellipse marks the cave opening; typical flight paths are indicated with arrows. The first bats emerging mainly flew along the rearmost flight path, whereas the bats emerging later increasingly took the frontal flight paths. For size information, please note the person sitting behind the small tree to the right of the opening (red arrowhead).

C) Closeup of the clearing in front of the cave opening.

D) The cave opening seen from the other direction. The cave is located on an ascending hillside, which is steepest behind the opening. The bats are all leaving the cave downhill.

E) Closeup of the cave opening. Note the ascending rocks in the rear part of the opening.

Microphone Co., Ltd., Tokyo, Japan) and presented the echoes of computer-generated virtual objects via an ultrasonic loudspeaker (RT-3Pro, Expolinear, Berlin, Germany) in real time. A video camera (A602f, 1/2 inch chip, Basler, Ahrensburg, Germany), equipped with a 3.5 mm-lens (SV-03514, NET-GmbH, Finning, Germany; 1.4/3.5 mm, supervised area 3.12 × 5.02 m in 2 m distance) and two infrared lightings (IR-294, Infrared Illuminator Microlight Co. Ltd., Moscow, Russia) were positioned midway between microphone and loudspeaker, either on the forest floor and pointing vertically upwards, or mounted on a arch in 2.3 m height and pointing vertically downwards. The speaker was placed on a tripod with one vertical stand in the flight path of the bats; the microphone was either

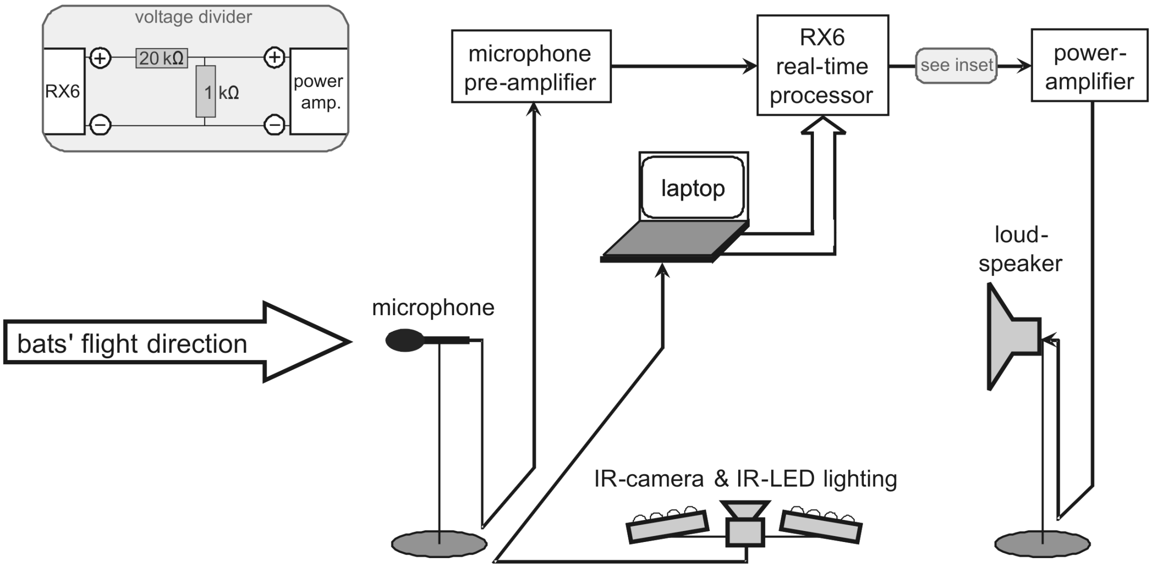


Figure 4.3: Block diagram of the experimental setup. The camera and IR-lighting were positioned mostly on the forest floor below the bats' flight path. The microphone and loudspeaker were mounted on vertical stands in the bats' flight path. The rest of the equipment for signal manipulation and experimental control was positioned outside of the observed area. The cables for power supply and for the camera's trigger signal are not drawn. The voltage divider reduced the voltage by 26 dB, thereby allowing the usage of the full dynamic range of the RX6 real-time processor.

placed on a similar tripod or on the floor close to the cave opening. The electronic delay of the setup was 550 μs, corresponding to a virtual object distance of 9.5 cm. The virtual target thus appeared to be positioned 9.5 cm behind the middle between microphone and loudspeaker.

The remaining units of the technical equipment were positioned further away from the recording site in order to avoid interference with the bats under observation. The calls recorded by the microphone were amplified (Quadmic, RME, Haimhausen, Germany) and digitized by a real-time processor (RX6, sampling rate 220 kHz, Tucker-Davis Technologies, Gainesville, FI, USA). Recorded calls were then high-pass filtered (20 kHz, 2$^{\text{nd}}$ order butterworth filter) and convolved with one out of a set of impulse responses (see section 4.2.3) in order to generate the echo. The echoes were amplified (TDA-7560, STMicroelectronics, Genf, Switzerland) and played back via the loudspeaker. The whole experiment was controlled with a custom written program in Matlab 5.3 (The Mathworks Inc., Natwick, MA, USA) and RPvds (Tucker-Davis Technologies). Video recording was triggered for one second at a frame rate of 30 pictures per second by the real-time processor each time a supra-threshold acoustic signal was detected. Videos were recorded in Streampix 3.17 (Norpix, Montreal, Canada).

4.2.3 Test and control stimuli

Flight path video recording was performed at different positions in front of the Tamana Cave during six evenings. Possible changes in the flight paths caused by the presence of the camera and IR-lighting could not be controlled for, but are highly unlikely, as the flight paths were at least 0.5 m above the ground, and such also above the camera. We controlled for any influence of the remaining experimental setup by the following control experiments. Flight paths were recorded without microphone and loudspeaker present to document the undisturbed flight paths (***camera-only control***). Microphone and speaker were then introduced into the flight paths, but no playbacks presented, to control for flight path alterations caused by the sheer presence of the equipment (***mic-speaker control***). In the next step, broadband noise (20 – 100 kHz, 85 dB SPL re. 20 μPa at 1 m in front of the loudspeaker) was presented to control for flight path alterations caused by non-specific acoustic signals (***noise control***). Control video-recordings were taken every evening at every recording position.

We presented a real and several virtual objects in the bats' flight paths to investigate the evasive flight behaviour in response to the introduced objects. The real object was a vertical shield (70 – 80 cm wide, about 200 cm high), which was positioned in the flight paths at several distances behind the camera.

Virtual echoes were generated by convolution of the recorded acoustic signal with one out of nine impulse responses (IR). IRs differed by the following parameters: level, delay, duration and roughness, corresponding to the object features size, distance, depth and depth structure. The range of presented parameters is given in **Tab. 4.1**. All IRs had a flat amplitude spectrum and were normalized to their RMS-value. The level and delay of the IRs were altered by digitally attenuating and delaying the playback on the real-time processor. The duration and roughness of the test IRs were defined beforehand (during their generation). One test IR was a single reflector which generated an echo that is an exact copy of the recorded signal (***click IR***). The other eight IRs had longer durations between 59 – 7382 μs in about doublings (13 – 1648 samples) and where generated from so-called sparse noise (***sparse noise IRs***; Hübner & Wiegrebe, 2003; Grunwald *et al.*, 2004; Firzlaff *et al.*, 2006). Sparse noise is generated from Gaussian noise by introducing temporal gaps of uniformly random duration with a predefined average temporal gap width. By increasing the average gap width, the resulting sparse noise has less reflectors (with higher amplitudes, if the RMS-value is kept constant), resulting in a stronger envelope fluctuation of the noise. The degree of envelope fluctuation of a signal can be expressed by its 4^{th} moment, which is a measure for the smoothness or roughness of the signal's temporal structure. The 4^{th} moment of a discrete time signal is defined as:

Table 4.1: The acoustic parameters of the impulse responses (IR) used for echo generation and their corresponding (virtual) object features.

IR parameter	presented range	object feature	presented range
level	0 – -24 dB	size	1 – 1/16 relative surface area
delay	0 – 10 ms	distance	0 – 170 cm distance
duration	click (4.5 μs) and 59 – 7383 μs	depth	one-front reflector and 1 – 125 cm object depth
IR roughness	0.5, 1.5 and 2.5 log10M4	depth structure	(very) smooth meadow conifer tree very broad-leafed tree

$$M_4 = \frac{\frac{1}{N}\sum\limits_{n=a}^{b} {y_n}^4}{\left(\frac{1}{N}\sum\limits_{n=a}^{b} {y_n}^2\right)^2} \tag{4.1}$$

with y = amplitude of each sample
and n = sample number, running from a to b
and N = number of all samples.

In the following, the 4th moment is given as its decadic logarithm, indicated by log10M4 behind its value.

Average echo level at 0 dB attenuation was between 80 and 100 dB SPL re. 20 μPa at 1 m in front of the loudspeaker, depending on the used IR. This relates to target strengths of about -20 to -40 dB at 1 m distance. The echoes generated with the click IR had an average level of 87 dB SPL (target strength -33 dB). The general trend for the sparse noise IRs was the longer the IR duration, the larger the target strength and the louder the resulting echo.

Altogether, we thus had the possibility to present virtual objects of varying size, depth and roughness (depth structure) at different distances behind the centre between microphone and loudspeaker (**Tab. 4.1**).

4.2.4 Experimental procedure and stimulus presentation

The experiment was set up before nightfall. Bats began to emerge between 17:05 – 17:25 h, depending on the daily weather conditions and those of the previous days. The experimenters were positioned offside and controlled the signal presentation from there.

On the first day, we recorded the undisturbed flight paths (***camera-only control***) at several positions and time points around the cave opening. Based on these recordings, we developed an ad-hoc field analysis method to evaluate the flight paths off-line after recording to adapt our experimental methods, the placement of microphone and loudspeaker and the recording positions.

On the following days, we first recorded flight paths in response to the real object. This experiment allowed us to quantify evasive flight path changes to real objects and compare them to the recorded responses to playbacks of virtual objects. In addition, we were thus able to test our video equipment under field conditions and to develop a method for flight path analysis, using recordings which definitely showed a change in flight paths.

For most of the time we presented different virtual objects, including the click IR, several sparse noise IRs, and noise (***noise control***). We did not test the full range of acoustic parameters we were able to vary, as the behavioural reactions of the bats were less obvious as expected, but concentrated on obtaining recordings of behavioural responses to a subset of IRs by testing several microphone, loudspeaker and camera positions. We mainly presented the click IR and a few sparse noise IRs with short, medium and long duration at the highest level. We additionally tested different roughnesses, different delays and different levels a few times.

The presented impulse responses were selected by the experimenters in order to test the whole range of parameters and to get the best-visible responses of the bats. As no bat was recorded more than once per day, a pseudo-random stimulus presentation was not necessary. Per impulse response, video recordings of 0.5 – 1 min duration were taken, often several times per evening.

4.2.5 Video analysis and flight path extraction

Recorded videos were exported from Streampix and saved frame per frame as single pictures. In most cases, not the originally recorded raw-frames where analysed, but the difference-frames, which were calculated in Matlab 7.1 as the difference between two consecutive frames (difference-frame$_\mathrm{t}$ = frame$_\mathrm{t+1}$ − frame$_\mathrm{t}$). The subtraction of consecutive frames enhanced the difference between pictures, i.e., the moving bats, whereas the constant background became invisible.

All further analysis was only possible on a pixel-basis. As we had recorded with only one camera, we could not reconstruct the bats' position and flight paths in meters, but only their projection onto the video-frame. The analysed images had a resolution of 656 pixels along the flight direction (longitudinal, or x-axis) and 491 pixels vertical to the flight direction (transverse, or y-axis).

Per recording we analysed about 300 – 600 frames in order to extract mostly about 50 – 100 flight paths per recording (after interpolation, see below). Flight path extraction was manually done in ImageJ 1.39q (Wayne Rasband, National Institute of Health, Bethesda, Maryland, USA; http://rsb.info.nih.gov/ij/) with the plugin MTrackJ (Erik Meijering, Biomedical Imaging Group, Erasmus MC – University Medical Center Rotterdam, The Netherlands; http://www.imagescience.org/meijering/software/mtrackj). The position of every passing bat was manually marked in every frame where this individual was visible, resulting in the x-y-position (in terms of pixel-numbers) of the passing bat over time, i.e., its flight path. Among the extracted flight paths, we only used those for later analysis which met the following requirements. Flight paths had to cover a minimum flight distance, starting before x-pixel 150 and ending after x-pixel 400 (23% – 61% of the frame-length). The first recorded position had to be within the central half along the y-axis, i.e., between y-pixels 123 and 368. Finally, at least four positions of the bat (on four video-frames) had to be recorded. These restrictions excluded low-flying bats, which were recorded only two or three times on the video and ensured that the flight paths covered the relevant range where a reaction of the bats was expected. Each flight path was then interpolated with a cubic spline function (Matlab 7.1) at up to twelve positions (at the x-pixels 50 to 600 in steps of 50 pixels, when they were inside the length of the recorded flight path).

4.2.6 Evaluation of flyways and statistics

Flight paths crossed the observed area from left to right, with real objects or the microphone, the loudspeaker and virtual objects positioned on the midline of the picture (around y-position 245.5). If the bats reacted to the presented objects and flew around it, we expected less bats to be present in the central part of the video frames and more in their lateral parts. In order to test this, we analysed the recorded flight paths by describing the mean flyway of the recorded bats. A flyway is a bundle of individual flight paths (Bateman & Vaughan, 1974) and is described by its centre position and its width, defined by the mean ± the standard deviation of the bundle of individual flight paths.

In different approaches, we calculated two different sets of flyways and analysed their position, their width and their direction (Matlab 7.1). The first approach tested whether the mean flyway of all recorded flight paths (the ***central flyway***) became wider during object presentation, indicating that the bats flew in a larger distance to the midline. We therefore calculated the central flyway from all flight paths for each condition and compared its width along the flight direction (at x-pixels 50 to 600, step width 50 pixels) with a F-test, Bonferroni-corrected for multiple comparisons to the same control. We tested the null-hypothesis that the central flyways have the same width against the one-sided alternative hypothesis that the central flyways are wider during object presentation compared to control-conditions. In addition, the flyway centre position was compared using two-sided Bonferroni-corrected Student's t-tests, testing the null-hypothesis of equal flyway centre position versus the two-sided alternative hypothesis that the flyway centre positions were different between control- and test-conditions.

If the bats avoided the central region of the observed area, where the objects were presented, we would expect to see two flyways on the recorded videos, one passing the central region on each side of the object (the ***lateral flyways***). The second approach tested whether the position of the lateral flyways were shifted outwards during object presentation compared to control conditions. In order to calculate the lateral flyways around the real or virtual objects, the individual flight paths were classified into paths going around the object on either side of the midline. The classification was based on the mean-value of each flight paths' y-position at its last three x-positions. If this mean y-position was above the midline (> 245.5), the flight path was assigned to the upper half of the video-frame. If it was below the midline (< 245.5), the flight path was assigned to the lower half of the video-frame. We compared flyway-centres along the flight direction with Bonferroni-corrected Student's t-tests, testing the null-hypothesis of the same position against the one-sided alternative hypothesis that flyway-centres are shifted outwards during object presentation compared to control-conditions. Lateral flyway width was tested as before, testing the null hypothesis of the same flyway width versus the one-sided alternative hypothesis that the lateral flyway width was smaller during object presentation compared to control-conditions.

In addition, the overall direction of flyways was sometimes described by calculating a linear regression.

4.3 Results

4.3.1 Behavioural observations

The bats normally started to emerge from the cave at dusk between 17:15 to 17:25 h, while it was still light enough for visual orientation. After a few days of rain, the flyout already began at 17:05 h. After about 45 – 60 min, the stream of emerging bats became thinner, but bats kept emerging for at least another 30 min. The first bats emerging took a route slightly uphill, which was not well suited for our recordings. However, while more and more bats emerged from the cave, they also flew straight out of the cave and more into downhill direction, forming flight routes in a fan-like manner across the clearing, with the fan's centre at the cave opening.

The flight heights were about 0.5–2.3 m above the ground, depending on the location in front of the cave. At none of the location the flight heights were distributed less than about 1.2 m. The flight paths were in general distributed in a fan-like manner around the cave opening, but this effect was less pronounced in the smaller field of view of the camera, where the flight paths were parallel enough for the experimental requirements. Despite this general pattern, the paths taken by individual bats were not always a straight line, but were deflected to the left and right or altered in height, presumably to avoid contact with closely flying other bats.

We were able to record the flight paths of passing bats without and with the presentation of a real object in their flight path, and during the playback of virtual echo-acoustic objects. Examples of flight path recordings will be presented together with interpolated flight paths and flyways in the following sections.

4.3.2 Control recordings of undisturbed flight paths

Fig. 4.4 shows the video recordings of several bats without any object present, except for the video camera and infrared lightings on the ground (***Camera-only control***). The examples are shown as maximum-intensity projections of multiple video frames, either of the originally recorded raw-frames, or of the calculated difference-frames. The flight paths are straight, crossing the field of view of the camera from left to right. Based on the straight flight paths, it seems that the bats were not affected or disturbed by the video camera and the infrared lightings. Obviously, we do not have video recordings without the video equipment placed on the ground for comparison. The different heights of individual flight paths are visible when comparing the size of the bat silhouettes. In **Figs. 4.4 C, D**, a low-flying bat is crossing the camera, behind which a high-flying bat is visible with a very small silhouette. In **Figs. 4.4 E, F**, high-flying bats are present, too, whereas the bats in **Figs. 4.4 A, B** all fly in a medium height. The different heights of the flight paths make an automatic analysis of the videos difficult, as the low-flying bats blocked the camera's view to the high-flying bats and will conceal the other bats in the maximum-intensity projections.

Fig. 4.5 shows the extracted and interpolated flight paths of the camera-only control, and the central and lateral flyways to illustrate the method of flight path extraction and flyway calculation. Flight paths run for the most part straight across the observed area. The cushion-shaped distortions of the flight paths, especially visible in the lower half of the picture, are caused by the wide-angle objective and not by an actual bend of the flight paths. No obvious effect of the camera and lighting on the ground below the bats is recognisable.

The slope of a linear regression to the central flyway is $m =$ -0.094, which is significantly smaller than zero ($p < 0.0001, t(10) =$ -7.89). The midline of the camera's field of view was

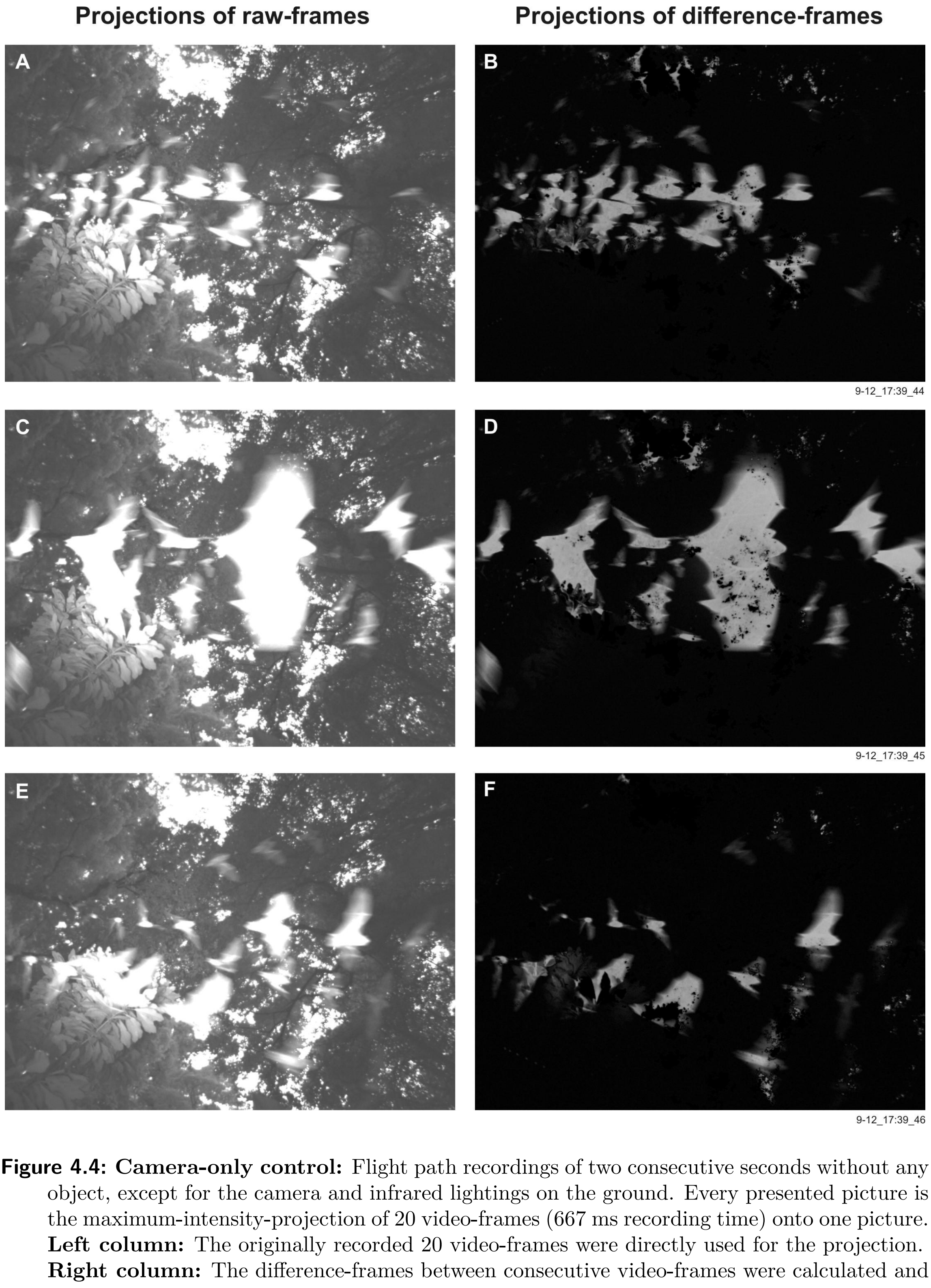


Figure 4.4: Camera-only control: Flight path recordings of two consecutive seconds without any object, except for the camera and infrared lightings on the ground. Every presented picture is the maximum-intensity-projection of 20 video-frames (667 ms recording time) onto one picture. **Left column:** The originally recorded 20 video-frames were directly used for the projection. **Right column:** The difference-frames between consecutive video-frames were calculated and then projected onto one picture.

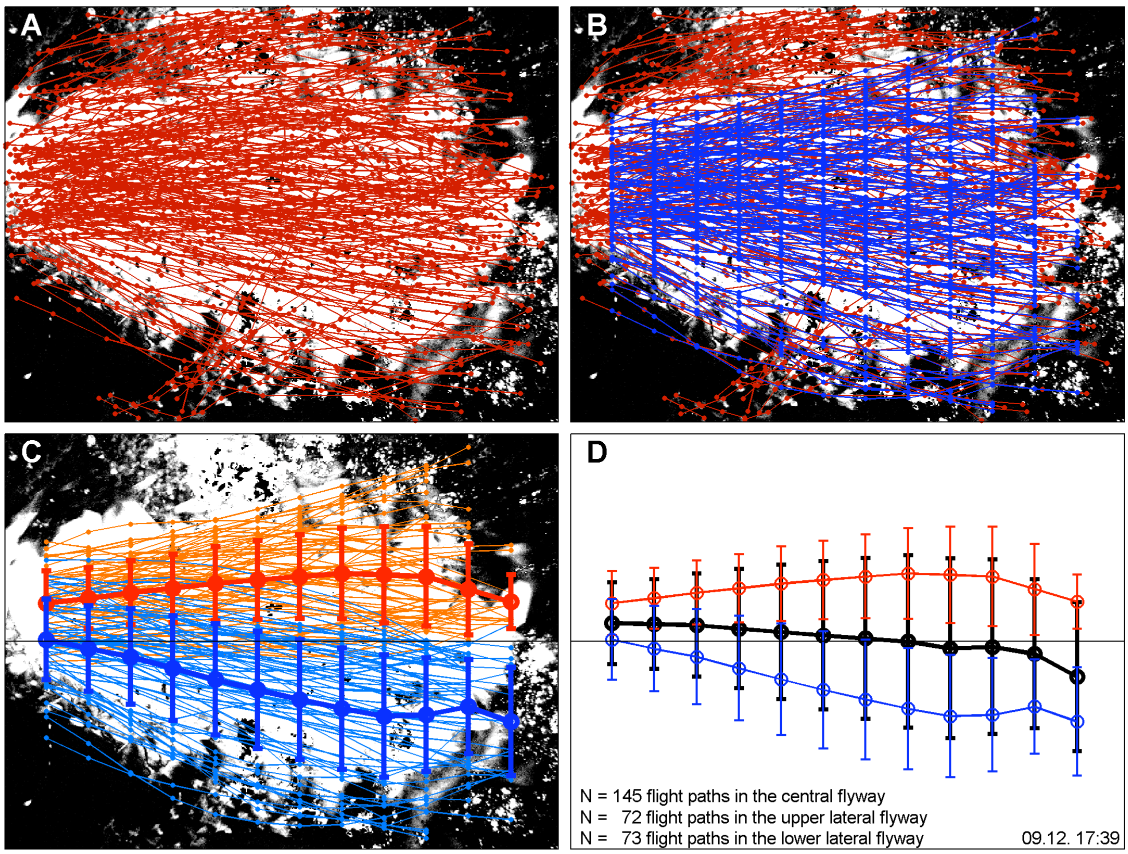


Figure 4.5: Camera-only control: Flight paths in the camera-only control, illustrating the extraction of flight paths and flyways.

A) Maximum-intensity projection of a 20 sec recording with all flight paths that were extracted from the recording (red).

B) Maximum-intensity projection of a 20 sec recording with all extracted flight paths (red) and all interpolated flight paths (blue). The interpolated flight paths fit to the recorded ones. Flight paths were not interpolated where they were either too short or did not run through the relevant range of the frame, which were mainly the paths at the corners and edges of the video frame.

C) Maximum-intensity projection of a 20 sec recording with all interpolated flight paths, classified and colour-coded into paths of the lower (blue) and upper half (red) of the video frame, and their respective lateral flyways ± standard deviation (flyway width).

D) The finally extracted and analysed central flyway, which is based on all individual flight paths (black) and the lateral flyways, which are based on the individual flight paths classified into paths of the lower (blue) and upper half (red) of the video frame, each time ± standard deviation (flyway width).

thus slightly tilted laterally relative to the central flyway direction by 9 pixels per 100 pixels in flight direction. The slope of the linear regression of the upper lateral flyway is not significantly different from zero ($m = 0.026, p = 0.1991, t(10) = 1.38$). The linear regression of the lower lateral flyway has a significant negative slope ($m =$ -0.18$, p < 0.0001, t(10) = -11.44$). The visible spread of the individual flight paths and the significant spread of the flyways illustrates the fan-like pattern of the flight paths in front of the Tamana Cave.

4.3.3 Orientation response to real objects

In **Fig. 4.6**, some examples of video recordings of several bats in response to a vertical shield in the flight path are presented, both as maximum-intensity-projections of the originally recorded raw-frames and of the difference-frames. The flight paths are not straight, but bended laterally around the shield. The three presented examples were recorded at different time points over the course of one evening. At the beginning of the evening (17:30 h), the forest canopy is still clearly visible against the sky, making it difficult to recognise the passing bats. However, the projection of the difference frames shows the bats clearly. At 18:05 h, the bats are visible on the projection of the raw-frames; together with the shield and a brightly lit twig. In contrast, the projection of the difference-frames only shows the passing bats.

Fig. 4.7 shows the interpolated flight paths and the lateral flyways during presence of a real object, presented at different distances behind the camera. Bats clearly reacted to the real object with evasive manoeuvres to the sides of the object. Compared to the camera-only control in **Fig. 4.5**, the flight paths and lateral flyways do not run straight across the video frame, but are bended sideways.

In **Fig. 4.8**, the position and width of the central and lateral flyways during presence of the real object are contrasted to the camera-only control. The solid black lines show the flyways in the camera-only control. The coloured lines show the flyways during presence of the real objects at 40, 50, 80 and 120 cm distance behind the camera. The central flyways are in general not affected and run straight across the video frame (**Fig. 4.8 A**). Albeit some positions of the central flyways during object presentation changed compared to the camera-only control (Student's two-sided t-test, $p < 0.05$), this effect is to be attributed to the uneven distribution of the individual flight paths in the upper and lower half of the video frame (**see Fig. 4.7**), and not to an actual change in overall flight direction. In contrast to the central flyways, the centre positions of the lateral flyways were shifted outwards (**Fig. 4.8 B**), at many positions significantly (Student's one-sided t-test, $p < 0.05$). This outward shift of the centre position of the lateral flyways is also reflected in the width of the central flyways. With a real object, the central flyways became much wider, from

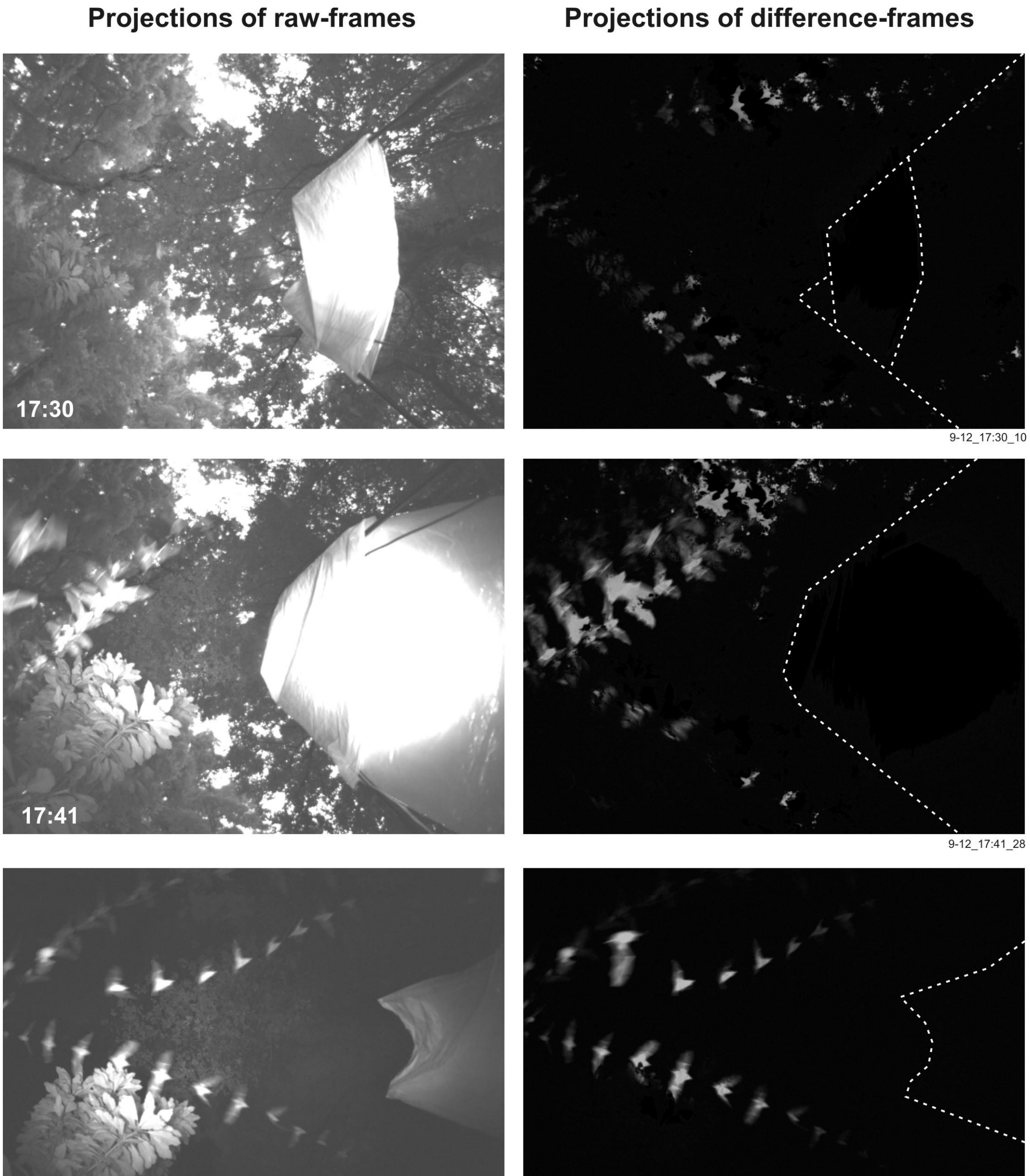


Figure 4.6: Real object: Flight path recordings in response to a real object in the flight path, video-taped at different time points over the course of one evening. Every presented picture is the maximum-intensity-projection of 20 video-frames (667 ms recording time) onto one picture. **Left column:** The originally recorded 20 video-frames were directly used for the projection. **Right column:** The difference-frames between consecutive video-frames were calculated and then projected onto one picture. The dashed line marks the outline of the shield.
Note the change in light intensity over the evening. Although the bats are hardly visible in the raw-frames at 17:30 h (left), the difference-frames highlight them clearly (right).

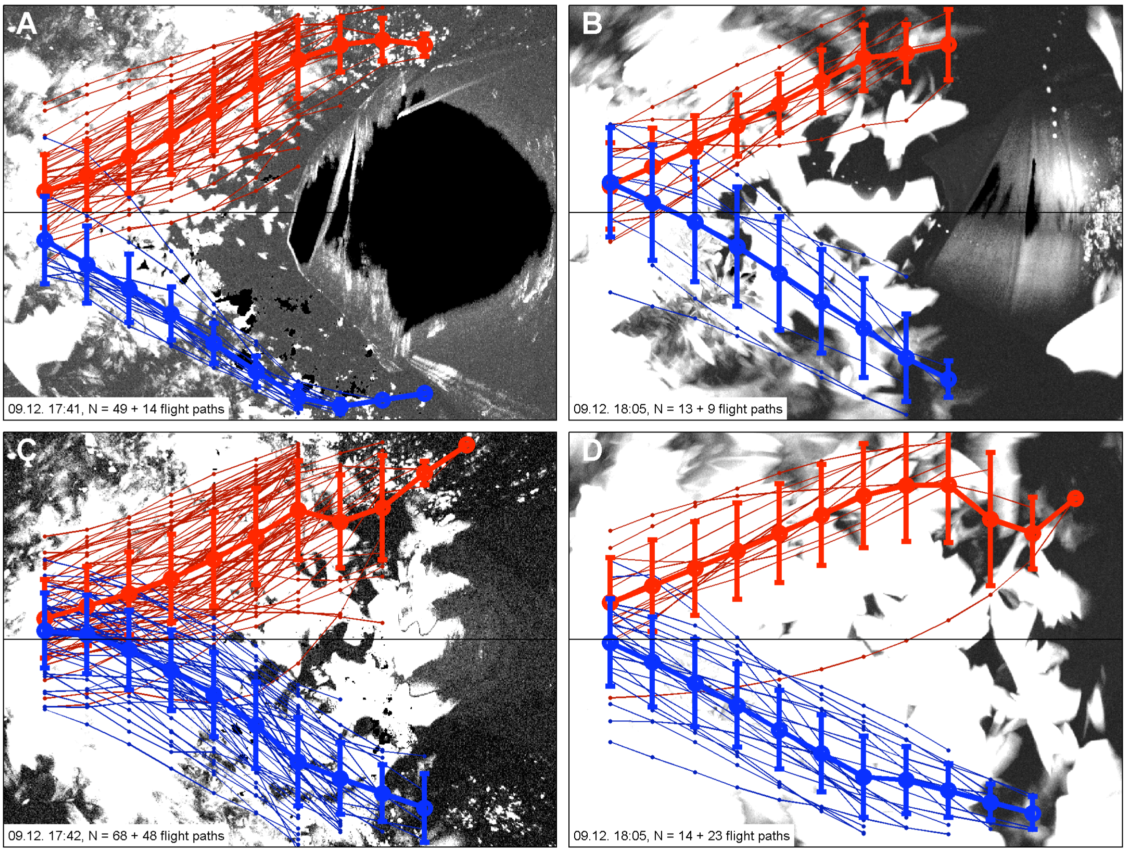


Figure 4.7: Real object: Maximum-intensity projections of 20 sec (**A, C**) or 30 sec (**B, D**) recordings during presence of a real object and the interpolated flight paths and their lateral flyways. The restriction for the length of the flight paths was lowered to the range from x-pixel 150 to 300 as the bats left the observed area sideways very early in response to the real object. **A – D)** The object is presented 40, 50, 80 and 120 cm behind the camera, respectively.

50 – 100 pixels width in the control condition to up to 225 pixels with the real object (**Fig. 4.8 C**), which is also significant at many positions (one-sided F-test, $p < 0.05$). For the real object presented at 40 and 120 cm behind the camera, also a decrease in the width of the lateral flyways was found (one-sided F-test, $p < 0.05$, **Fig. 4.8 D**). However, this may in part also be caused by the lower number of bats recorded under test-conditions compared to the camera-only control and to bats leaving the observed area, and should thus be treated with care.

The first significant reactions of the passing bats to the real object, i.e., a change in flyway width or position, is shown in **Fig. 4.9** in dependence of the object's distance to the camera. A clear systematic change in the first reaction when the object was presented in different distances is not visible. The width of the central flyway changed later when the

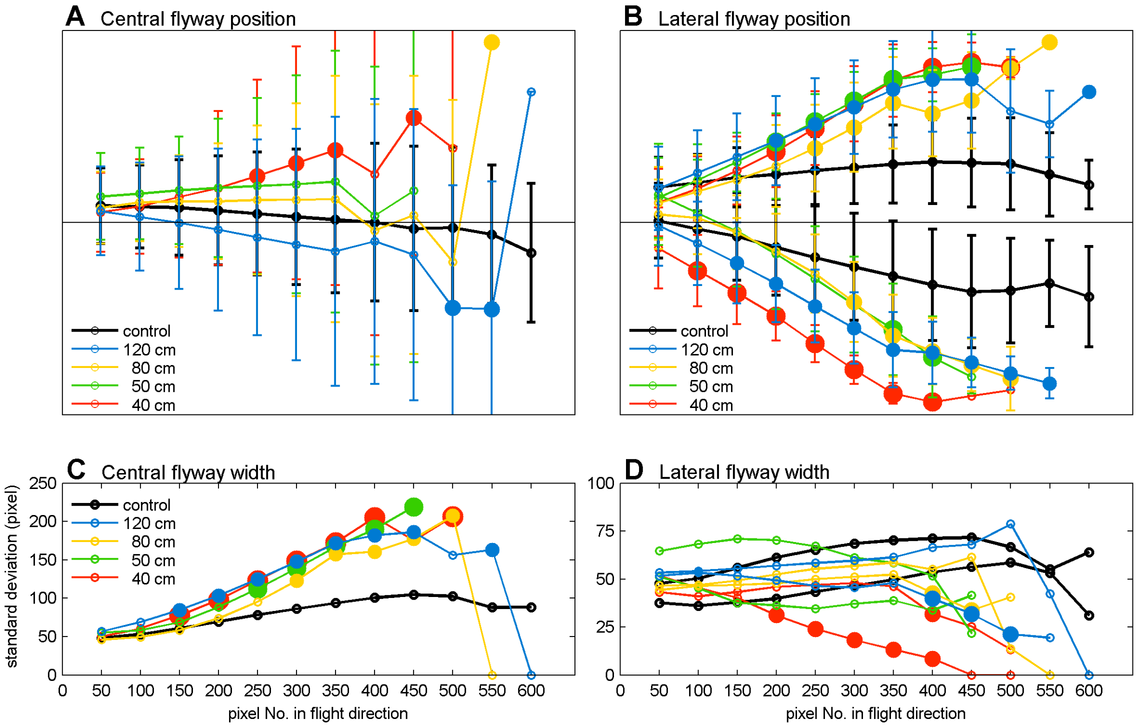


Figure 4.8: Real object: Central and lateral flyways during presence of a real object in 40, 50, 80 and 120 cm distance behind the camera. The restriction for the length of the flight paths was lowered to the range from x-pixel 150 to 300 as the bats left the observed area sideways very early in response to the real object.
A – B) Position of the central (**A**) and lateral (**B**) flyways. Larger, filled symbols mark the positions where the test-flyway centres are significantly different (**A**) or significantly shifted outwards (**B**) compared to the camera-only control.
C – D) Width of the central (**C**) and lateral (**D**) flyways. Larger, filled symbols mark the positions where the test-flyways are significantly wider (**C**) or narrower (**D**) than during the camera-only control.

object was farther away for object distances of 40, 50 and 80 cm, but not for 120 cm. The positions of all lateral flyways on both sides around the object were shifted outwards, mostly around x-pixels 150 – 250, but no clear change in the reaction distance is recognizable. A significant decrease in the lateral flyway width mostly occurred at the end of the observed flyway and is likely to be an artefact. Altogether, the central flyway width and the lateral flyway position seem to be good indicators to detect a change in the flight path distribution in response to an object. Both parameters will be used in the next section to analyse the behavioural reaction of the passing bats to the presentation of echoes of virtual objects.

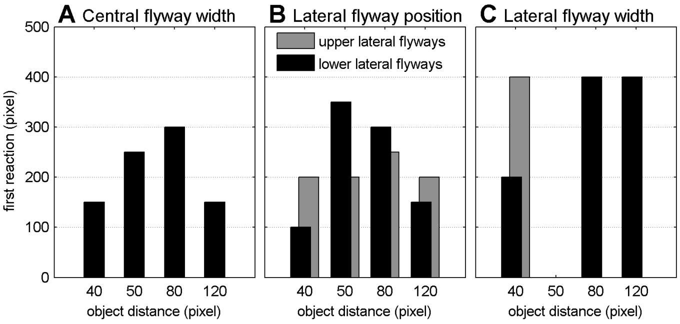


Figure 4.9: Real object: Reaction distances during presence of a real object. The reaction distance is the position of the first significant change in the test flyway compared to the mic-speaker control, either in central flyway width (**A**), lateral flyway position (**B**) or lateral flyway width (**C**).

4.3.4 Orientation response to virtual objects

As a first overview, interpolated flight paths of several control and test recordings of three days are plotted in **Figs. 4.10**, **4.11** & **4.12**. These figures show, without any further analysis, the distribution of the flight paths. In all conditions, many flight paths are more or less straight, but not completely parallel. Rather, the bats fanned out over the observed area. Some flight paths are curved, more often downwards than upwards. No obvious difference in the distribution of the flight paths between the mic-speaker control and the test conditions is visible, but this will be analysed further in the next paragraphs.

The corresponding flyways in response to the virtual objects are shown and analysed in **Figs. 4.13**, **4.14** & **4.15**. The three figures present the flyways of three different recording days. In the upper halves of the figures, the position of the central and lateral flyways (mean of all flight paths) are shown including their width (standard deviation of all flight paths). In the lower halves, only the widths of the central and lateral flyways are shown to compare them. A first look at the flyways already shows that all flyways run along similar positions (panels A & B) and that they have similar widths (panels C & D). This is in contrast to our expectations that the width of the central flyway will increase and the positions of the lateral flyways will be shifted sideways during virtual object presentation. In the following, the flyways will be described in detail and the few significant differences will be mentioned.

The solid black lines show the flyways in the mic-speaker control, i.e. with the microphone and the loudspeaker present in the flight path, but without playback. The mic-speaker control will first be compared to the camera-only control, which is shown with dashed black lines. Significant changes (Student's two-sided t-test, $p < 0.05$) of the central flyway in the mic-speaker control compared to the camera-only control occurred at the beginning (09.12., **Fig. 4.13 A**) and at the end of one flyway (15.12., **Figs. 4.15 A**). The width of some central flyways in the mic-speaker control became significantly larger (F-test, $p < 0.05$) compared to the camera-only control in the recordings of the 15.12. (**Figs. 4.15 C**).

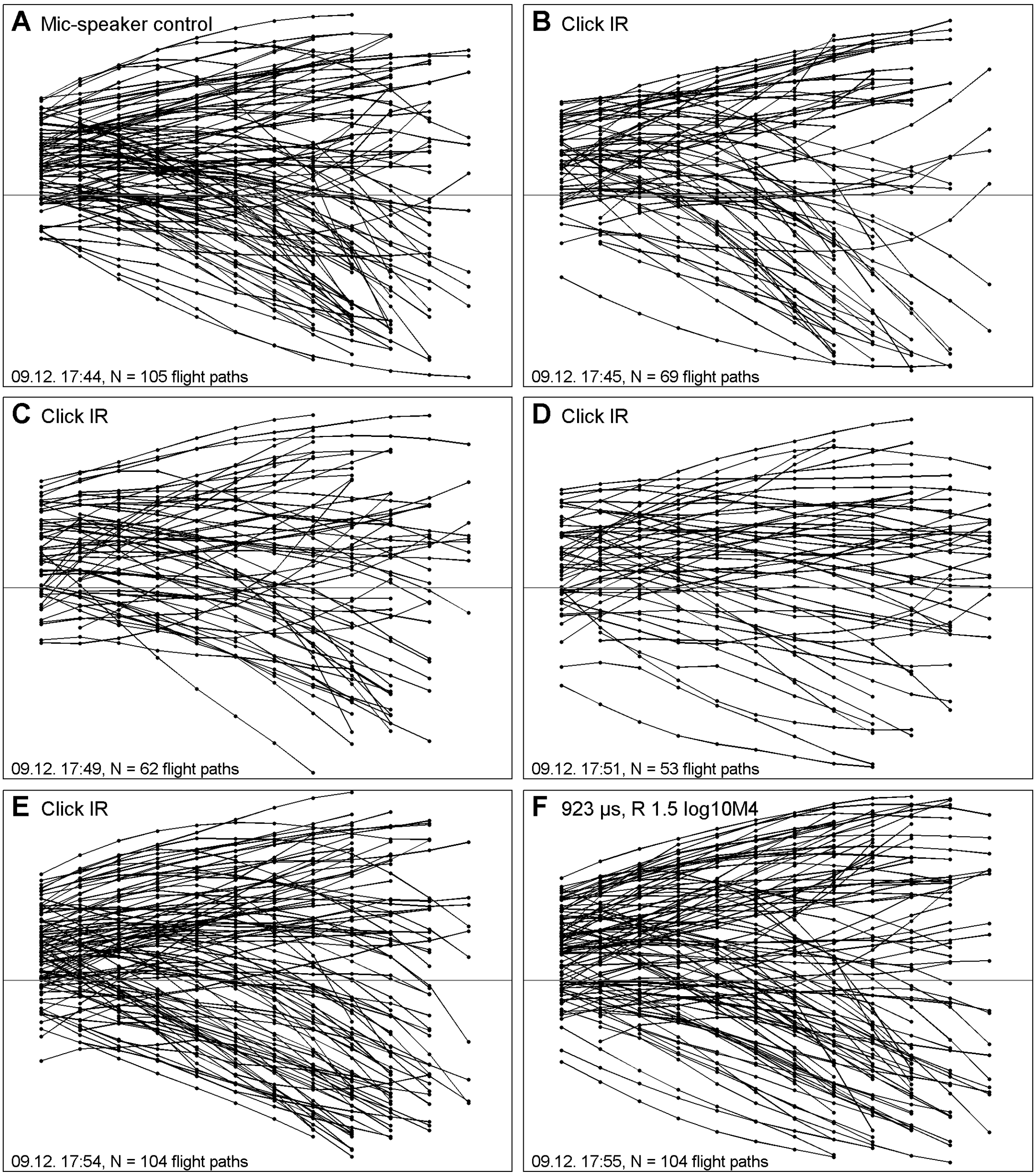


Figure 4.10: Virtual objects: Interpolated flight paths at one recording position during one evening (09.12.07) in the mic-speaker control (**A**) and in response to echoes generated with a click IR (**B**–**E**) and a sparse noise IR (**F**). Loudspeaker at different distances between 1 – 2 m behind the camera.

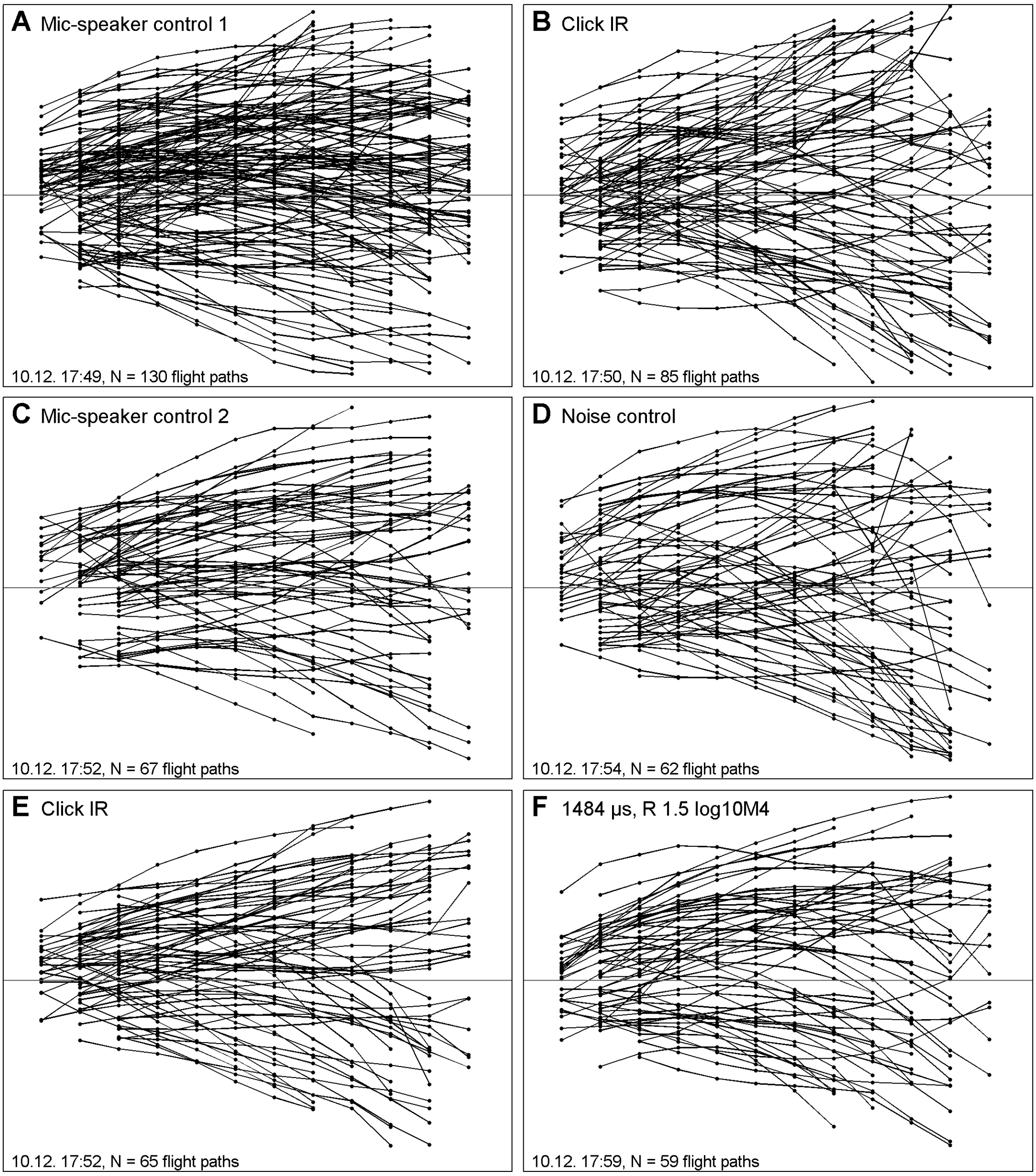


Figure 4.11: Virtual objects: Interpolated flight paths at another recording position during a second evening (10.12.07) in the mic-speaker controls (**A, C**), in the noise control (**D**) and during presentation of echoes generated with a click IR (**B, C**) and a sparse noise IR (**F**). Loudspeaker 1.4 m (**A, B**) or 1.0 m (**C-F**) behind the camera.

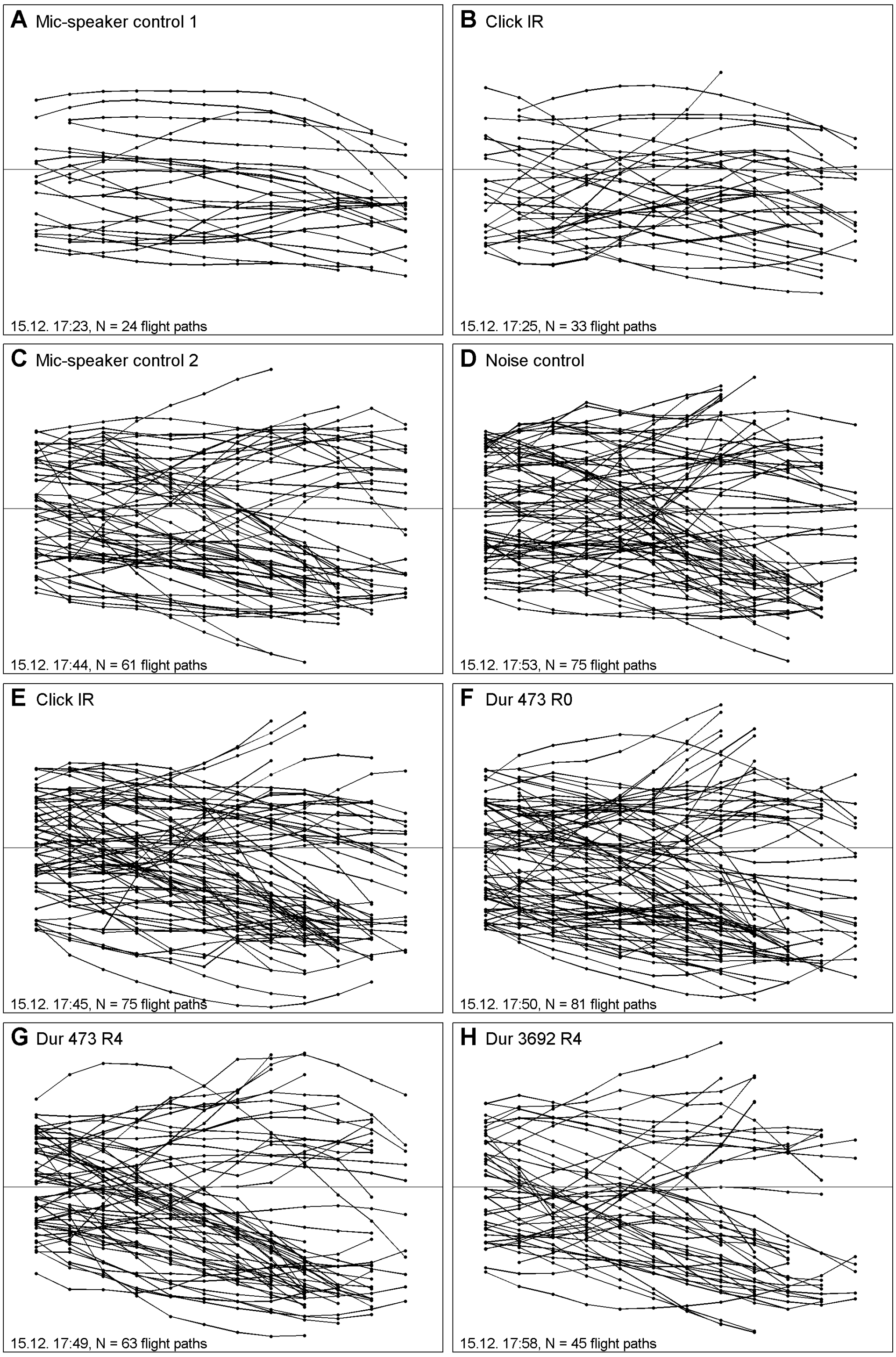


Figure 4.12: Virtual objects: Interpolated flight paths with the camera facing downwards during a third evening (15.12.07) in the mic-speaker controls (**A, C**), in the noise control (**D**) and during presentation of echoes generated with a click IR (**B, E**) and sparse noise IRs (**F-H**). Loudspeaker 1.5 m (**A, B**) or 1.0 m (**C-H**) behind the camera.

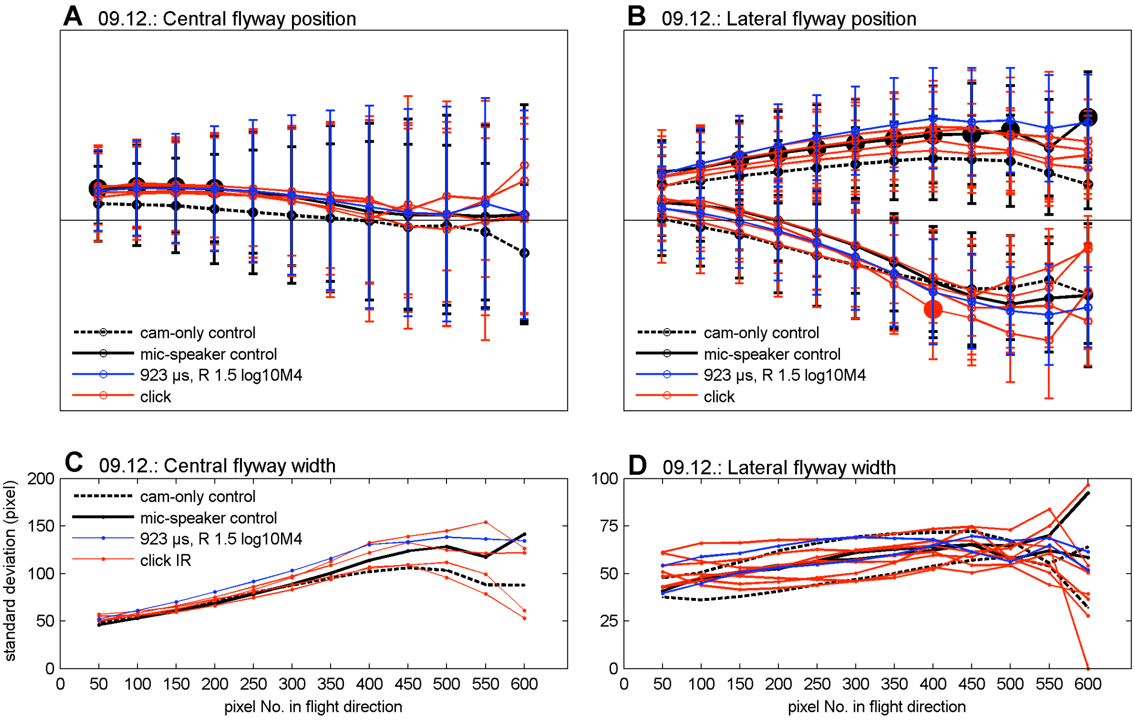


Figure 4.13: Flyways in response to virtual objects, 09.12.: The position (**A, B**) and width (**C, D**) of the central (**A, C**) and lateral (**B, D**) flyways in response to virtual objects at one position during one evening (09.12.07). Flyways were recorded during presentation of echoes generated with a click IR (red) and sparse noise IRs (blue) and are compared to the mic-speaker control (solid black) and the camera-only control (dashed black). Significant differences are marked with large filled symbols.

The lateral flyways of the mic-speaker controls mainly differed in their position compared to the flyways of the camera-only controls. Significant outward shifts (Student's one-sided t-test, $p < 0.05$) were observed along almost the whole flyway at one day (09.12., **Fig. 4.13 B**) and at the end of the flyway of another day (15.12., **Fig. 4.15 B**).

Hence, the bats already slightly avoided the microphone and the loudspeaker. Most of the significant changes occurred in the second half of the flyways where the loudspeaker was positioned. However, additional flight path changes should still be visible if present.

The flyways in response to the virtual objects are shown in red (click IR), blue (sparse noise IRs) and grey (noise control). When any kind of playback was presented, the flyways did almost never change compared to the mic-speaker control. This was true for the click IR, both sparse noise IRs and the noise control. One central flyway increased in width at one x-position (10.12., **Fig. 4.14 C**). The position of two lateral flyways was significantly shifted outwards at to x-positions (09.12., **Fig. 4.13 B** and 10.12., **Fig. 4.14 B**), and one lateral flyway decreased in width at one x-position (15.12., **Fig. 4.15 D**). Altogether, these

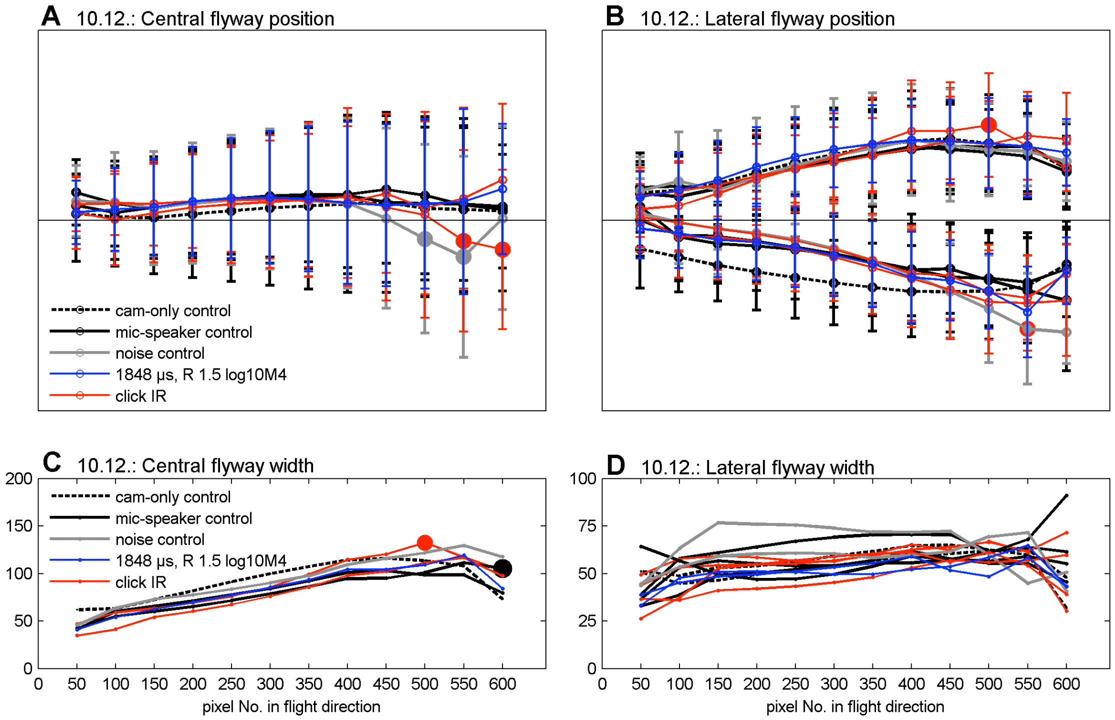


Figure 4.14: Flyways in response to virtual objects, 10.12.: The position (**A, B**) and width (**C, D**) of the central (**A, C**) and lateral (**B, D**) flyways in response to virtual objects at another position during a second evening (10.12.07). Flyways were recorded during presentation of echoes generated with a click IR (red) and sparse noise IRs (blue) and are compared to the mic-speaker control (solid black), the noise control (grey) and the camera-only control (dashed black). Significant differences are marked with large filled symbols.

few changes cannot be taken as indication that the bats changed their flyway in response to the presented playbacks.

4.4 Discussion

Real objects and computer-generated virtual objects were presented to bats emerging from their day roost. This configuration allowed us to establish our method and then to compare the flight behaviour in response to real objects with the flight behaviour during virtual object presentation, which provides access to the selective manipulation of single acoustic parameters.

4.4.1 Real objects

This part validated our protocol as a method to investigate bat flight behaviour and evasive flight path changes. Both evaluation methods, based on the central and lateral flyways,

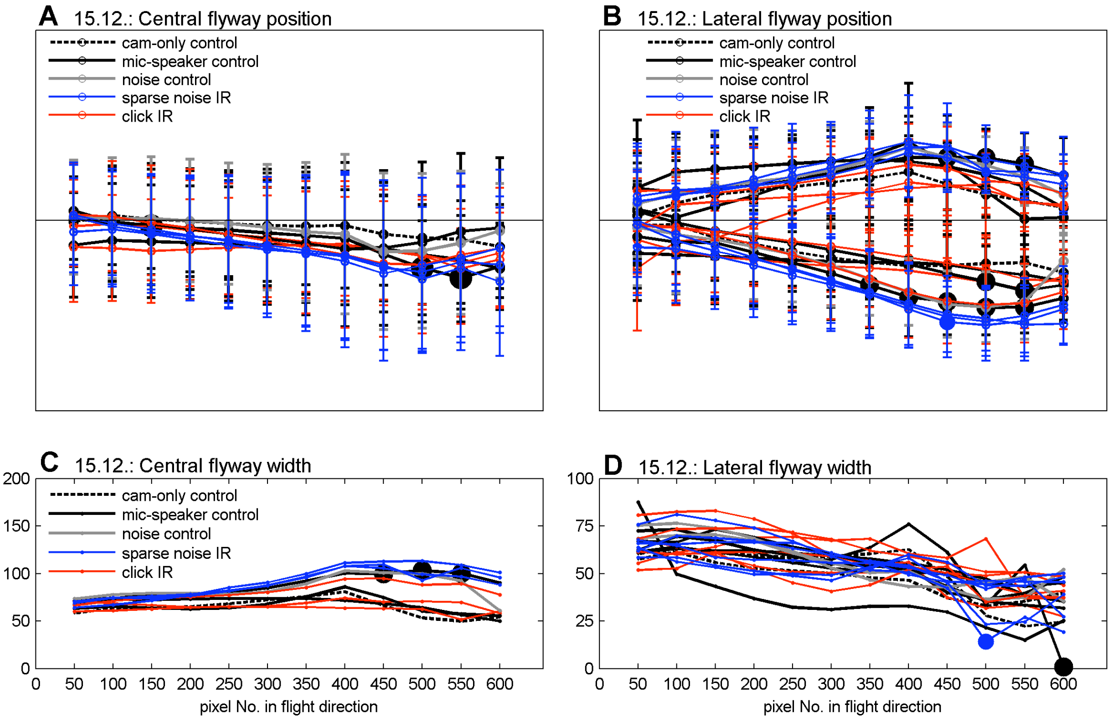


Figure 4.15: Flyways in response to virtual objects, 15.12.: The position (**A, B**) and width (**C, D**) of the central (**A, C**) and lateral (**B, D**) flyways in response to virtual objects with the camera facing downwards during a third evening (15.12.07). Flyways were recorded during presentation of echoes generated with a click IR (red) and sparse noise IRs (blue) and are compared to the mic-speaker control (solid black), the noise control (grey) and the camera-only control (dashed black). Significant differences are marked with large filled symbols.

enabled the detection and description of evasive flight manoeuvres around the real object. The best suited parameters were the central flyway width and the lateral flyway position. The description of the lateral flyways is based on the classification of the flight paths into two different groups. This classification is slightly artificial, especially when the flight paths show a less clear reaction than during real object presentation. But together with the central flyway width, these two parameters seem to allow a realistic description.

The bats avoided the real object and flew around it. In contrast to Schaub (2007), we used a very large object to ensure that we would be able to see flight path changes. We did not try to quantify evasive manoeuvres in response to a variety of shapes, sizes and distances. However, for follow-up studies measuring reactions to playbacks of virtual targets, this would be a prerequisite in order to calibrate the virtual targets.

4.4.2 Virtual objects

We did not find any change in the flyways during the playback of virtual objects, compared to the mic-speaker control, where the silent speaker was positioned in the flyway. The bats slightly avoided the loudspeaker in the mic-speaker control, but flew still close enough to make additional flight path changes visible, if they were present. We thus have to conclude that the presented virtual objects were not perceived as being realistic by the bats. Several explanations are possible for this finding, from potential flaws in the experimental design to perceptual considerations.

The target strengths of the virtual objects were realistic when compared to measurements by Stilz (2004) of German leafed trees and forest edges, which ranged from -27 dB to -36 dB at 1 m distance. Solid structures, such as concrete walls or tree trunks, had measured target strengths of -8 dB to -23 dB. The corresponding echo level of the virtual targets of 80–100 dB SPL re. 20 μPa is way above the hearing threshold of bats (e.g. Long & Schnitzler, 1975; Simmons *et al.*, 1992; Esser & Daucher, 1996; Koay *et al.*, 2002; Heffner *et al.*, 2003; Koay *et al.*, 2003; Bohn *et al.*, 2004; Hoffmann *et al.*, 2008) and higher than the echo level of prey regularly taken by bats (Waters *et al.*, 1995; Rydell *et al.*, 1999). Signal level in itself should thus not have been an issue, and it is not likely that the bats did not perceive the echo at all. More probably, the bats did not perceive the virtual object as an obstacle in their flight path or did not perceive the echo as an realistic object echo at all.

We assume that the missing spatial extent of the virtual object was of major importance. In all studies so far, virtual objects were presented from one loudspeaker, such inevitably failing to present echoes from several directions which are normally present in real objects due to the spatial distribution and spatial extent of the reflectors. Only the object's extent along the range axis is coded in the temporal properties of an echo, whereas its shape is calculated from the reflections reaching the bat from different directions, and from sequential echoes from different aspect angles. Under our experimental conditions with only one loudspeaker the bats will have easily recognised that the sound is only coming from one point in space, and not from a large three-dimensional reflector. In contrast to that, a bat ensonifying a wall or a tree will receive a click-like echo from the wall and a sparse noise-like echo from the tree, but not only from one direction, but from every direction around it which did receive call energy. The high directionality of ultrasound will prevent the bat from receiving echoes from everywhere around it, but the spatial extent will be much larger than during virtual object presentation by one loudspeaker. It is obvious that bats in the laboratory can be trained to use specific temporal cues for object analysis (Grunwald *et al.*, 2004; Firzlaff *et al.*, 2006), but this will not create the same perception as if the real objects were presented. Only an array of associated microphones and loudspeakers with an appropriate set of impulse responses would be able to realistically generate the echo of a three-dimensional object. Laboratory studies on the perception of the spatial extent of echo-acoustic objects are currently under preparation (L. Wiegrebe, *pers. comm.*).

Another potential information source about the object was vision. While the first bats were emerging from the cave, it was still dim. If the presented playback had sounded realistic to the bats, they still would have seen that no real object was present, such generating conflicting cues. But as we did not find a difference in the flight paths between the early and late emerging bats, vision does not seem to influence the perception of the virtual echoes.

Had the bats perceived the virtual object as a realistic small obstacle in their flight path, there are still a variety of possibilities to explain their missing reaction. In a situation like the one in front of the Tamana Cave with thousands of bats emerging from their roost and flying out on familiar routes into a familiar area, bats will presumably rely on spatial memory and employ short range navigation to avoid collision with their neighbours. This has three potential consequences of how bats react to objects in their environment in this situation. (1) The bats at Tamana Cave will be used to nearby objects, e.g. the cave walls while circling in the cave opening before leaving it, the vegetation they pass or most important other nearby flying bats while crossing the clearing. We could constantly observe sudden changes in the flight paths, presumably caused by the presence of other bats. Similarly, bats flew very close to the little tree on the clearing and regularly touched a large banana leaf. If the bats did not perceive the virtual object as a realistic three-dimensional object with a large spatial extent, but only as a small point-like echo reflector, they did potentially not care about such a small reflector a few to tens of centimetres to their side. (2) It is plausible that the bats only evaluated echoes with a very short time delay, i.e. that they focused their attention only on objects directly in front of them. If they did, they would not have reacted to the virtual object while still far away from the centre between microphone and loudspeaker, where the object appeared. On the other hand, when getting closer to the virtual object position, they might have just passed the microphone and thus would not receive an echo from the virtual object anymore. (3) It is furthermore also conceivable that the bats relied in part on a very simple guidance rule of just following the bat in front of them. This in some way simply shifts the question of how the bats are orienting in this situation to a preceding bat. On the other hand, it seems possible that the bats oriented by combining spatial memory, guidance based on preceding bats, and to a smaller degree their own echolocation. For example, Davis & Barbour (1965) showed for *Myotis sodalis* that it relied less on echolocation when visual cues were present during orientation in a novel room. The same may be true for the use of spatial memory versus echolocation in a familiar area. Such a dependence of the use of echolocation versus spatial memory was suggested for *Megaderma lyra* when hunting in unfamiliar space by Ratcliffe *et al.* (2005), and for *Rousettus aegyptiacus* when flying towards the learned position of a perch (Holland *et al.*, 2005). Stamps (1995) even proposed that animals simply apply a fixed site-specific serial motor program in familiar areas. Such a behavioural rule would be easily applicable for the straight flight paths from the cave into the woods.

While flying in a dense flock of bats, every bat will continuously hear the echoes to its own calls, but also the calls of all the surrounding bats and the echoes to their calls. For an organism relying less on auditory information than bats, e.g. us, it is hard to comprehend how bats evaluate the echoes of their own calls, and even more so how they extract the relevant information from such a cluttered and acoustically chaotic situation, and how they then build a mental representation of their environment. The possibility and the extent to which bats use the echoes to calls of conspecifics, and potentially also those of other species, is still not resolved. This so-called eavesdropping (e.g. Xitco & Roitblat, 1996; Gillam *et al.*, 2007) would enable the bats to realise that there is no object, because the calls of those bats that fly ahead and have already passed the microphone will not be echoed back.

In contrast to the work of Schaub (2007), the emerging bats at Tamana Cave did not follow a vertical background structure, to which they echoacoustically kept contact, but flew across an open clearing, potentially only controlling their distance to the ground by echolocation. The behavioural rule at the Tamana Cave can thus not be based on guidance, like ‘from this landmark on follow that structure to the next familiar landmark’ (Denzinger & Schnitzler, 2004). It is more likely that a behavioural rule is connected to the moment when the bats are leaving the cave, which defines for example the direction the bat will take until it reaches the next known place (defined by the surrounding landmarks) or has travelled a certain distance. During the travel time to the next landmark or until the defined time has passed, only little attention may be paid to novel objects, especially as the bats did not employ a contour following mechanism, but crossed an open clearing.

Altogether, the bats might have relied only to a lesser extent on echolocation, as they were at a familiar site in a familiar behavioural situation. Within the restricted general attendance to echolocation, they might only pay attention to objects in close range or to realistic obstacles with a large spatial extent. For all those reasons, our virtual object might still have been to small or inconspicuous for the passing bats.

4.4.3 Methodological discussion

The greatest advantage of our experimental design turned out to be also slightly disadvantageous. The simultaneous video recording of hundreds of bats allowed the sampling of many individuals, which is very rare in behavioural biology, especially in field studies.

The drawback of this situation was that a three-dimensional reconstruction of individual flight paths with several cameras was impossible due to the sheer amount of animals. We thus worked only with one camera, giving us only a two-dimensional projection of the three-dimensional flight paths. The situation at the Tamana Cave seemed to be ideal for this limitation of our video-system, as the bats fly out in a fan-like manner without much variation in height. Yet, the variation in flight height was larger than expected, posing a problem from the very beginning for two reasons. First, some bats already flew above or below the virtual object and had thus no reason to avoid it, if they had recognized its missing spatial extent. Secondly, some bats evaded the loudspeaker and probably also the virtual object by flying above or below it instead of sideways around it. Changes in flight height are not resolvable in our video analysis (expect by changes in the size of the animals, which are not reconstructible in the maximum intensity projections or on the derived x-y-position-plots). Bats flying above or below the loudspeaker will fill the central area of the video frames and thus mask the potential evasive manoeuvres to the sides by other bats.

4.4.4 Conclusion and outlook

Conclusively, this work has to be regarded as a pilot study. We were not able to show a change in flight behaviour upon the playback of echoes of virtual targets. As discussed above, many potential reasons for this, but also caveats of our study, are conceivable.

Potential future work on this topic should include the presentation of a greater variety of real objects. We only aimed to use the real object as a test for our methodology, but not as a quantification for the flight paths during virtual object presentation. The presentation of several real objects with varying sizes would have allowed us to describe the flight paths in dependence of real object size, thus serving as a calibration for the reaction during virtual object presentation. In a longer follow-up study, this should certainly be done. The presentation of several real objects would also yield the potential resolution of the analysis method. However, real objects are difficult to transport, especially at locations like the Tamana Cave, and are difficult to switch between presentations.

In the next step, virtual objects should be presented, and their perceived size then be quantified based on the real object responses. To measure extensive responses to virtual objects, the presentation method has to be improved. It will presumably be most important to generate a realistic spatial extent of the virtual object, for example with several loudspeakers (which will, however, still not generate a realistic spatial extent if only one microphone and real-time signal processor is used). On the other hand, a huge loudspeaker array being capable of generating a realistic object will simultaneously be a larger real echo reflector.

It would be in addition very interesting to test the response to virtual objects at different sites with commuting bats employing different navigational tasks, for example also bats during commuting flights along vertical background structures as investigated by Schaub (2007) and Schaub & Schnitzler (2007b). Different sites with commuting bats will show if the bats at Tamana Cave did not react to the echoes due to a very restricted attention window. Maybe it would furthermore be advantageous to perform these experiments with bats on stereotyped foraging trips, e.g. *Myotis daubentonii* foraging along a river.

Acknowledgments

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5 General discussion

Three projects were conducted in this thesis, which are connected by the two main features of bats, namely sophisticated echolocation and powered flight. Mainly aspects of echolocation were addressed, but also the navigation based on echo-acoustic information. The applied methods included real targets and the generation and playback of virtual targets to investigate echo-processing and behaviour both in trained, captive bats and in wild, free-flying bats.

This discussion is oriented along these lines: After a comparison of methods, the auditory processing of echoes will be discussed, a topic which was strongly influenced by the debate on the jitter experiments, and then briefly regarded in a general context of sensory ecology.

5.1 Comparison of methods

Both methods, the real-time phantom-target generation and the use of real objects, have their advantages and disadvantages.

Virtual objects Virtual objects are presented to the echolocating animal via loudspeakers. As described in chapter 4, one loudspeaker is only able to present virtual objects along one spatial axis, the range axis. The spatial extent of the presented virtual object perpendicular to this axis is zero. This was no problem when certain echo-acoustic parameters were evaluated in psychoacoustical experiments without trying to simulate realistic objects, e.g. in the studies described in chapter 2 and by Schörnich & Wiegrebe (2008) on the processing of spectral and phase information, respectively. Similarly, the classification of echo roughness, a stochastic parameter which differs between tree types, showed that bats can discriminate this specific parameter and thus could classify real trees based on it (Grunwald *et al.*, 2004; Firzlaff *et al.*, 2006). However, these studies cannot tell us yet if the bats perceived, and how they would perceive, a realistic tree.

Besides having a larger spatial extent and therefore reflecting echoes from several directions, real objects give raise to slightly different information at the two ears, generating binaural echo disparities (Holderied & von Helversen, 2006). Binaural echo disparities were mostly considered negligible due to the small distance between the ears, but were only shortly recognized as being of considerable value for object evaluation (Holderied & von Helversen, 2006). Like the spatial extent, binaural disparities cannot be generated by playbacks originating from only one loudspeaker. However, such cues between two receptors are of great importance in vision ('binocular disparities') for the three-dimensional reconstruction of the environment based on the two-dimensional retinal images (reviewed in DeAngelis, 2000) and also in olfaction to localize an odour by 'stereo-smelling' (Rajan *et al.*, 2006; Porter *et al.*, 2007). Binaural disparities are also constantly evaluated by the auditory system for the localization of sound sources (Rayleigh, 1907); and as bats do not exhibit spatial echo suppression (Schuchmann *et al.*, 2006), they could be used for a detailed reconstruction of three-dimensional shapes. Further research into this direction is required to understand the echo-acoustic perception and auditory representation of the world.

Another parameter that is normally missing during phantom target presentations are dynamic changes in the impulse response of the virtual object. In contrast, in a realistic situation the impulse response of the ensonified object is constantly changing when the bat is changing its aspect angle. To simulate such dynamic changes, specialised experimental setups are necessary, as so far only employed by Genzel (2007). As we all know from our own experience, the combination of several single auditory events leads to the perception of musical melody, enables complex languages and allows the grouping and segregation of different auditory streams (Bregman, 1990). Echo analysis over time will similarly create an image much more complex and detailed as can be generated by the evaluation of single echoes only; therefore also research has to apply time-variant stimuli and investigate echo-acoustic processing under conditions as natural as possible.

The flight paths of naïve bats in response to playbacks of virtual objects in a naturalistic situation (chapter 4) suggested that the bats indeed did not perceive the playbacks as being reflections of realistic objects. The real-time phantom-target technique can be thought of being similar to a video screen, the first presenting acoustic images (the impulse response) and the latter visual images. But in the same way as we do not perceive the video screen image as a realistic copy of the world, the bats seem not to perceive the virtual objects as being realistic. In vision, the illusion generated by a video screen can be improved with separate pictures for both eyes, but this method is yet missing in behavioural echolocation research, but can so far be applied in passive hearing using earphones and the individual's head-related transfer function (e.g. Keller *et al.*, 1998), and should be applicable to anaesthetised bats as well.

On the other hand, the great advantage of phantom targets is the complete control over the impulse responses of the virtual object, allowing the generation of every imaginable impulse response. As long as no undetected distortions in the signal processing chain occur, the presented acoustic signals exactly possess the generated characteristics. This allows to present stimuli precisely tailored to investigate one specific acoustic parameter or combinations of parameters.

Real targets Real targets constitute a real echo-acoustic reflector, presenting all the information to the bat that this reflector can possibly generate, including reflections originating from different spatial positions which generates binaural echo disparities and larger spatial extents, and dynamic changes when ensonified from different aspect angles. Real objects can also be manufactured in order to reduce the variability of the available information, e.g. hollow hemispheres with different shapes (Simon *et al.*, 2006). However, already the information available from hollow hemispheres differing only in size is quite diverse and complex. Though the impulse response of real objects can be measured to calculate their reflection pattern and to analyse the presented information, this is very complicated and still not as explicit as directly generating the impulse response. Often, as in the here described experiment with jittering real targets (chapter 3), non-intended information is present, which needs laborious counter-measures.

5.2 Sensory physiology: Spectral and temporal processing

In this thesis, behavioural aspects of spectral and temporal processing were investigated, namely the evaluation of the frequency content of sound signals and the accuracy of echo timing. Such basic sound properties are evaluated to build an internal representation of the external world. How this representation may be organized will be briefly discussed for the representation of echoes.

Acoustic signals can be defined by their time-signal, i.e., their change in sound pressure over time, which is received at the tympanic membrane. Acoustic signals can also be described in the spectral domain by their magnitude and phase spectrum, i.e. the magnitude and phase of each frequency in the signal (Crocker, 1998). All sounds inevitably undergo such a spectral analysis at the level of the cochlea in bats, like in all other mammals. The original acoustic signal is deconstructed into its frequency components and transformed into several parallel temporal excitation patterns across the frequency channels of the ascending auditory pathway (e.g. Moore, 2004).

Auditory information in mammals is thus inevitably two-dimensional, containing time and frequency information (generated from the one-dimensional time-signal impinging on the tympanic membrane). The information that is contained in one of these dimension, i.e. either the temporal excitation pattern within one frequency channel (or summarised across all), or the excitation pattern across frequencies, but without relevant temporal resolution, is always coarser than the combined information, as both undergo transformations that can only be restored (in part) when the other information is added. An example are spectral interference patterns that are generated by two echoes that reach the ear with very short temporal separation, for example as reflected by structured targets. These two signals cannot be resolved in the temporal domain, but in the spectral domain based on their interference pattern (Schmidt, 1988). On the other hand, signals that are separated by an interval longer than the integration time of the auditory filters can only be resolved along the temporal axis. Therefore, both parts of information need to be combined into a unified auditory representation of the acoustic signal, which can then be analysed further in terms of auditory object analysis, for example to differentiate between different speakers or instruments and to combine consecutive sounds to a unified percept of speech, melody or echoes of the same object (Bregman, 1990; Moss & Surlykke, 2001).

Why, at all, is acoustic information separated, if it needs to be restored later? Time and frequency representation of auditory signals can be seen as flipsides of the same coin, and as both representations are limited by physiological mechanisms, either in temporal or spectral accuracy and resolution, one representation alone is not sufficient to represent the full wealth of acoustic signals. Of particular importance are the time constants of the auditory filters, which cannot be sharply tuned to a specific frequency and feature a short temporal integration simultaneously (Hartmann, 1998; Weißenbacher *et al.*, 2002; Moore, 2004; Wittekindt *et al.*, 2005), but also the time constants of neuronal processing.

5.2.1 Auditory receiver models in echolocation

The jitter detection experiments strongly stimulated the discussion on the neuronal representation of acoustic signals, in particular by their claim for coherent auditory processing, which was postulated by Simmons (1979). Schnitzler & Henson (1980) showed that under laboratory conditions only a ranging accuracy of about 10 ns would point to a fully coherent receiver, which has access to the complete phase of a signal. Such a value was later indeed published by Simmons *et al.* (1990a), and since then much of the discussion is about the credibility of these data.

The first papers on distance discrimination in echolocating bats by J. Simmons suggested a semi-coherent receiver that evaluates the envelope of the cross-correlation function between the emitted call and the received echo (Simmons, 1971, 1973). Such a receiver measures the echo delay at the peak of the envelope with the precision of the envelope's width. Further results on jitter discrimination performance, which showed a finer temporal accuracy, were interpreted in favour of a coherent receiver, having full access to the phase of the echo and the fine-structure of the cross-correlation function (Simmons, 1979; Simmons *et al.*, 1990a). Access to the phase would be necessary to calculate the fine-structure of the cross-correlation function and to measure echo delay with a precision corresponding to the width of the peak of the cross-correlation function and not its envelope. The neuronal equivalent of cross-correlation can be implemented as coincidence detection of delayed signals, as proposed by Jeffress (1948) for sound localization and implemented in birds for the coding of interaural time differences (e.g. Carr & Konishi, 1990), but probably not in mammals (Grothe, 2003). Delay-sensitive neurons were indeed found in bats (e.g. Feng *et al.*, 1978; O'Neill & Suga, 1979), but their accuracy is too low to code for the fine-structure of the cross-correlation function. However, Sanderson *et al.* (2003) simulated jitter detection performance with a filter bank model, showing delay accuracies in the range of the experimental results of Simmons *et al.* (1990a), but without requiring the peripheral auditory system to encode the phase of ultrasonic signal. Yet, they had to increase the phase-locking cut-off frequency of the auditory filters to 8 kHz, a value which was not found in mammals so far, but is physiologically possible in birds (Köppl, 1997).

Cross-correlation functions are functions along the temporal axis and thus postulate a temporally coded representation of object properties (Simmons & Stein, 1980). In contrast, Schmidt (1988) showed that the perception of phantom targets with structured surfaces is best explained on a spectral basis and suggested the generation of spectral images in the auditory system. The spectrogram correlation and transformation (SCAT) model incorporated temporal and spectral processing to simulate physiological sound analysis, but postulated a subsequent transformation of the spectral information back into the time domain to reconstruct the target's shape exclusively along the range axis (Saillant *et al.*, 1993, see also Simmons *et al.*, 1990b). Also the models by Peremans *et al.* (1998), Neretti *et al.* (2003), Matsuo & Yano (2004) and Matsuo *et al.* (2004) focused on the analysis of temporal aspects, mainly the extraction of single reflections, in response to the SCAT-model.

A current paper by Wiegrebe (2008) combines a physiologically plausible simulation of the auditory periphery with an autocorrelation within each frequency channel to generate an auditory spectrogram, i.e. the putative representation of echoes in the auditory system along both the time and frequency axis. Based on physiological peripheral data, this model can explain a variety of psychoacoustical studies, but not a delay accuracy of 10 ns.

Depending on the behavioural performances to be explained and the expected auditory representation to be modelled, the models so far differ strongly in their implementation; and the nature of the internal representation of echo-acoustic signals is hence still under discussion. More studies on the behaviour and physiology of echolocation will reveal new details, which can then in turn be incorporated into new models in order to generate new evidence and hypothesis on echo-acoustic processing.

5.3 The context of sensory ecology

Sensory systems serve the animal's need for information about its environment (external senses) and its body (internal senses) and are adapted to its ecological situation (Dusenbery, 1992). In bats, this is obvious from their general and manifold adaptations for echolocation (Neuweiler, 1990; Jones & Teeling, 2006), but also from many smaller details such as enlarged ears in passive listening species (Neuweiler, 2000), cochlear and neuronal adaptations in CF-bats (Neuweiler *et al.*, 1980; Vater *et al.*, 1985; Kössl & Vater, 1990), call patterns to optimize prey detection close to background echoes (Siemers & Schnitzler, 2004) or the reduced size of the eyes (Neuweiler, 2000). In similar environmental conditions, for example in deep oceans or muddy waters, comparable sensory systems evolved, namely echolocation in toothed whales (for reviews see Thomas *et al.*, 2004) or electrolocation in weakly electric fish (reviewed in von der Emde & Fetz, 2007).

In chapter 2, it was shown that the spectral processing of echoes differs from the processing of passively heard sounds. This represents another example of how echo-acoustic processing, which was evolutionary derived from passive listening, adapted to the new requirements of precise spectral perception during echolocation.

An animal interacts with its environment, and reacts to perceived stimuli. Perceived information, not only obtained by echolocation, but from all sensory system, is incorporated into a representation of space (Trullier *et al.*, 1997; Suzuki & Clayton, 2000; Ulanovsky *et al.*, 2007) in order to generate adaptive motor programs to guide the animal's behaviour, for example flight and vocalisation (Ghose & Moss, 2006; Ghose *et al.*, 2006; Moss *et al.*, 2006).

5.4 The end...

...of this thesis is certainly not the end of research into audition, bats and echolocation. A few aspects could be studied here, and many remain, hopefully giving fascinating new insights into neurobiology, behaviour and evolution.

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12/04 – 02/05 – **Research assistant**, Dept. Animal Physiology, Tübingen, Germany

30.11.2004 – **State examination** for lectureship at grammar schools in Biology and Chemistry
16.04.2004 – **Diploma** in Biology
07/03 – 04/04 – Diploma thesis, Dept. Animal Physiology, University of Tübingen, Germany, with Dr. B. Siemers and Prof. H.-U. Schnitzler on "Sensory ecology of arthropod rustling sounds and acoustic classification of prey by Grey Mouse Lemurs"
04 + 09–12/03 – **Field research** in Mandena and Kirindy, Madagascar
10/98 – 11/04 – **Studies in Biology and Chemistry**, Eberhard Karls Universität Tübingen, Germany

09/1997 – Four weeks of travelling in Scotland, England, France, Spain and Portugal

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08/2005 – Summer School “Perception & Action”, Neurobiological graduate programs Bochum, München, Tübingen and Marburg/Gießen
05/2004 – Summer School in Ecology and Biodiversity, European Center of Excellence, Mammal Resarch Institue BIOTER, Bialowieca, Poland

Teaching and work experiences

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2007 – Advanced course “Multimodal Orientation and Learning in Rodents”
2005 – Basic course “Hearing Physiology and Psychoacoustics”
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2002 and 2003 – Extra tuition in animal physiology for an exchange student from Tufts University, USA
1990 – 1992 – Extra tuition and help with homework for foreign primary school children

Work experiences

2003 - 2005 – Freelancer for natural sound recordings, conservation authority *BNL Tübingen*, radio *SWR Tübingen*, museum *Stadtmuseum Tübingen* and five schools of Tübingen
08–09/2001 – Student research assistant, Dept. of Animal Physiological Ecology, University Tübingen

List of publications

Peer-reviewed research papers:

- **Goerlitz HR, Greif S & Siemers BM (2008)**: Cues for acoustic detection of prey: insect rustling sounds and the influence of walking substrate. *J Exp Biol* 211(17): 2799–2806.
- **Goerlitz HR, Hübner, M & Wiegrebe, L (2008)**: Comparing passive and active hearing: Spectral analysis of transient sounds in bats. *J Exp Biol*, 211(12): 1850–1858.
- **Siemers BM, Goerlitz HR, Robsomanitrandrasana E, Piep M, Ramanamanjato J-B, Rakotondravony D, Ramilijaona O & Ganzhorn JU (2007)**: Sensory basis of food detection in wild *Microcebus murinus*. *Int J Primatol*, 28(2): 291–304.
- **Goerlitz HR & Siemers BM (2007)**: Sensory ecology of prey rustling sounds: aoustical features and their classification by wild Grey Mouse Lemurs. *Func Ecol*, 21(1): 143–153.
- **Schill RO, Görlitz HR & Köhler H-R (2003)**: Laboratory simulation of a mining accident: acute toxicity, hsc/hsp70 response, and recovery from stress in *Gammarus fossarum* (Crustacea, Amphipoda) exposed to a pulse of cadmium. *Biometals*, 16(3): 391–401.

Abstracts of posters and talks:

- **Goerlitz HR & Wiegrebe L (2007)**: Ranging accuracy for sinusoidally jittering real targets in a free-flying FM bat. Talk at the 14. International Bat Research Conference. Mérida, Mexico, 19.–23.08.2007.
- **Goerlitz HR & Wiegrebe L (2007)**: Sonar hyperacuity revisited: echo-acoustic evaluation of a jittering suface in the bat *Glossophaga soricina*. Poster for the 7. Göttingen Meeting of the German Neuroscience Society. Göttingen, Germany, 29.03.–01.04.2007.
- **Görlitz HR, Hübner M & Wiegrebe L (2006)**: Perceptual compensation of spectral distortions induced by masking noise in the echolocating bat *Phyllostomus discolor*. Poster for the 5. Forum of European Neuroscience. Vienna, Austria, 08.–12.08.2006.
- **Görlitz HR, Hübner M & Wiegrebe L (2006)**: Perceptual compensation of spectral-envelope distortion in the echolocating bat *Phyllostomus discolor*. Poster, Baltimore, 05.–09.02.2006. In: Santi PA (Ed.): 29. Midwinter Meeting of the Association for Research in Otolaryngology. *Assoc Res Otolaryngol Abs*, 2006: 323.

- **Siemers BM & Görlitz HR (2005)**: Diversity of prey perception in nocturnal primates: lessons from bats' sensory ecologies. Talk, Göttingen 13.–16.12.2005. In: Kappeler PM, Groeneveld LF & Schwibbe M (Eds.): Program of the 5. Göttinger Freilandtage. *Primate Report* 72-1: 42.
- **Görlitz HR & Siemers BM (2004)**: Prey detection and sensory ecology in free-living grey mouse lemurs (*Microcebus murinus*). Poster, Torino, 22.–28.08.2004. In: Giacoma C, Formenti D & Gamba M (Eds.): 20. Congress of the International Primatological Society. *Folia Primatol*, 75(S1): 380–381.

Diploma (Master) thesis:

- **Görlitz HR (2004)**: Sinnesökologie, Nahrungssuche und akustische Beutewahrnehmung bei Grauen Mausmakis (*Microcebus murinus* J. F. Miller 1777) in kurzzeitiger Volierenhaltung. Diplomarbeit der Eberhard-Karls-Universität Tübingen.

Other contributions:

- **Görlitz H (2007)**: Report about the Graduate Student Meeting of the DZG study group "Behavioural Biology". DZG-Rundbrief April 2007, 10-11 (Deutsche Zoologische Gesellschaft) and Etho News **57**, May 2007 (Ethologische Gesellschaft).
- **Görlitz H (2004)**: Sensory ecology, foraging and acoustical prey selection in Grey Mouse Lemurs (*Microcebus murinus* J. F. Miller 1777) in short-term captivity. Summary of the diploma thesis. *Lemur News*, 9: 42.

Ehrenwörtliche Erklärung

Hiermit versichere ich ehrenwörtlich,
dass diese Arbeit von mir selbständig und nur unter Verwendung der angegebenen
Hilfsmittel angefertigt wurde.

München, 27.03.2008

(Holger R. Goerlitz)