Biosonar navigation above water II: exploiting mirror images

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Biosonar navigation above water II: exploiting mirror images. *J Neurophysiol* 113: 1146–1155, 2015. First published November 19, 2014; doi:10.1152/jn.00264.2014.—As in vision, acoustic signals can be reflected by a smooth surface creating an acoustic mirror image. Water bodies represent the only naturally occurring horizontal and acoustically smooth surfaces. Echolocating bats flying over smooth water bodies encounter echo-acoustic mirror images of objects above the surface. Here, we combined an electrophysiological approach with a behavioral experimental paradigm to investigate whether bats can exploit echo-acoustic mirror images for navigation and how these mirrorlike echo-acoustic cues are encoded in their auditory cortex. In an obstacle-avoidance task where the obstacles could only be detected via their echo-acoustic mirror images, most bats spontaneously exploited these cues for navigation. Sonar ensonifications along the bats' flight path revealed conspicuous changes of the reflection patterns with slightly increased target strengths at relatively long echo delays corresponding to the longer acoustic paths from the mirrored obstacles. Recordings of cortical spatiotemporal response maps (STRMs) describe the tuning of a unit across the dimensions of elevation and time. The majority of cortical single and multiunits showed a special spatiotemporal pattern of excitatory areas in their STRM indicating a preference for echoes with (relative to the setup dimensions) long delays and, interestingly, from low elevations. This neural preference could effectively encode a reflection pattern as it would be perceived by an echolocating bat detecting an object mirrored from below. The current study provides both behavioral and neurophysiological evidence that echo-acoustic mirror images can be exploited by bats for obstacle avoidance. This capability effectively supports echo-acoustic navigation in highly cluttered natural habitats.

echolocation; mirror image; navigation; elevation dependence; delay coding

FUNFAIR EXPERIENCE TELLS US how visual orientation is challenged in a hall of mirrors. Visual research has highlighted both the discriminative power of mirror experiments and the neural processing strategies recruited for the perceptual compensation of the dramatic spatial distortions elicited by mirrors (Logothetis and Sheinberg 1996). In humans, there are two main visual processing streams responsible for creating the cortical representation of a visual object: the ventral what and the dorsal how (Felleman and Van 1991; Goodale and Milner 1992; James et al. 2003; Ungerleider et al. 1983). Parts of the processes subserved by the dorsal stream are sensitive to mirror images, indicating their relevance for navigation (Dilks et al. 2011).

Mirror images can also be encountered in other sensory systems, for example, the active acoustic (sonar) system: bats use sonar to probe their environment by analyzing the echoes of their ultrasonic vocalizations (Griffin 1944; Jones and Holderied 2007). As in the visual system, a key challenge for bats is the segregation of these echoes into auditory objects. Unlike for passive listening, where the definition of an auditory object is difficult (Griffiths and Warren 2004), the definition of an auditory object for an echolocating bat is straightforward: it is the sum of those echo components that are reflected by the object back to the bat and that are within the bat's audible frequency range. This strict correspondence of real objects and auditory objects is an important advantage of bats over other mammals for studies of auditory-object formation and recognition, but how straightforward is this correspondence in an echo-acoustic mirror situation?

Visual mirror images represent a (1st-order) reflection of an (externally illuminated) object from a highly reflective smooth surface. Specifically, irregularities of this surface must be smaller than half of the wavelength of the light. With larger irregularities, the light is scattered and the surface is not a mirror. In echolocation, already the 1st image of an object is a reflection, namely that of the emitted call. Thus an echo-acoustic mirror image is a reflection of (at least) 2nd order, i.e., the emitted echolocation call is reflected 1st by an object and then by a highly reflective surface before it is perceived by the emitter. Again, irregularities of this highly reflective surface must be small relative to the wavelength of the (typically ultrasonic) sonar signal.

A bat navigating above water is faced with this mirror situation because a smooth water surface not only acts as a visual mirror, but also reflects a bat's ultrasonic vocalizations in a mirrorlike fashion (Siemers et al. 2001, 2005). The perceptual and neural basis for detecting and dealing with the dramatic distortions introduced into sonar imaging by acoustic mirrors is entirely unclear.

Siemers et al. (2001, 2005) showed that prey above water will become more conspicuous and thereby more easily detected by the acoustic mirror effect. Zsebok et al. (2013) demonstrated that not only echo-acoustic prey detection, but also discrimination is optimized by a spatiotemporal integration of direct and indirect reflections from a water surface, termed the echo-acoustic ground effect.

Which neural circuits may support spatiotemporal integration required for successful echolocation above water? In the auditory cortex (AC) of the mustached bat, combination-sensitive neurons show integrative responses to combinations of acoustic elements of their echolocation call and echo. These so-called FM-FM neurons typically respond best to distinct time delays between the call and echo component and thus encode target-distance information (O'Neill and Suga 1979). When the object is presented above an echo-acoustic mirror

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surface, the direct echo from the object is followed by a second echo from a lower elevation. The influence of additional vertically displaced echoes on the response of target-distance-encoding neurons is so far unknown. To extract neural response characteristics to elevation-dependent temporal echo patterns, the application of virtual acoustic space stimuli that evolve in time is ideal. This method was applied first by Jenison et al. (2001), and Hoffmann et al. (2013) show that the thereby acquired spatiotemporal response maps (STRMs) are a valid description of spatiotemporal tuning of auditory neurons engaged in sonar analysis.

If an echolocating animal is familiar with the occurrence of echo-acoustic mirror images at the boundary between water and air, it may exploit those mirror images not only for prey detection and discrimination, but also for navigation. This is relevant when a potential obstacle in a bat's flight path is only detectable via its echo-acoustic mirror image: low-hanging branches could force a bat, hunting above water bodies for insects, to maneuver below the branches. Such a situation is illustrated in Fig. 1. In the bats' natural habitat, they are very likely to encounter situations where the read-out and spatially correct interpretation of echo-acoustic mirror images would be beneficial for navigation.

Here, we designed a behavioral paradigm to investigate formally whether bats spontaneously show navigation via echo-acoustic mirror images. When the echo-acoustic mirror images of the hidden obstacle were conspicuous enough, three of five bats indeed exploited these cues for navigation. Biophysical measurements and analysis of the echo-acoustic mirror images along the bats' flight path reveal the critical echo properties that underlie the behavioral performance.

The corresponding electrophysiological experiments investigate how echo-acoustic elevation cues important for navigation in the vertical plane are encoded in the AC of bats. Specifically, we measured vertical STRMs of neurons in the AC of *Phyllostomus discolor*. Interestingly, many neurons were found, which based on the spatiotemporal arrangement of excitatory areas in the vertical plane could effectively encode echoes coming from lower elevations and with longer delays as
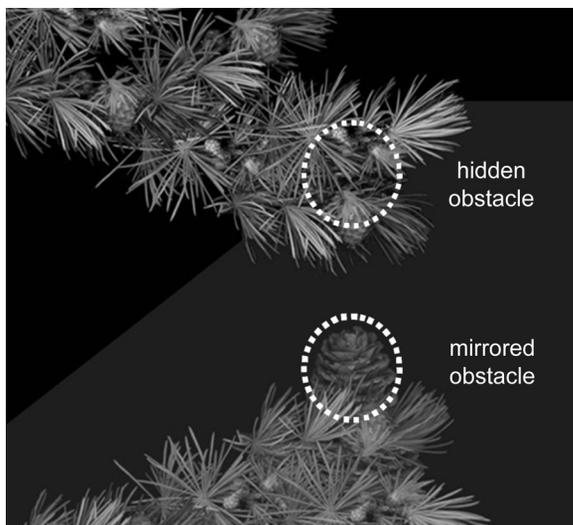

Fig. 1. A low-hanging branch is mirrored by a reflecting surface below. A potential obstacle, a pine cone, behind the branch is only visible on the mirroring surface.

they would bounce back from an obstacle via the water surface. This elevation-delay combination can occur when an object is mirrored on a reflective surface, thereby detected actually from below, but delayed in time. This response specialization may play an important role in the exploitation of echo-acoustic mirror images.

MATERIALS AND METHODS

Rationale

We designed both a behavioral experiment to investigate whether bats can exploit information from echo-acoustic mirror images and corresponding electrophysiological experiments to search for neural correlates of the observed behavior. Specifically, the bats were trained to fly back and forth between two chambers via one of two parallel tunnels, for which entries and exits could be blocked by obstacles. We tested whether the bats spontaneously detected blocked tunnel exits via echo-acoustic mirror images of the obstacles from the smooth floor of the tunnels. Electrophysiologically, STRMs of cortical units were acquired and analyzed with respect to their capability to encode higher-order sonar reflections as a function of the elevation from which these reflections returned to the bat.

Behavioral Experiments

Experimental setup. All experiments were performed in two connected, echo-attenuated chambers (*chamber 1*: 2.1 × 1.8 × 2.1 m, *chamber 2*: 2.1 × 1.2 × 2.1 m). In one corner of each chamber, a landing platform with a feeder attached was monitored with an infrared-light barrier. The chambers were connected to each other via a 2- × 0.9-m opening. Positioned on the floor of the opening was a wooden box (1.3 × 0.9 × 0.3 m) with a middle division along its length and a 0.2-mm smooth polyvinyl chloride (PVC) board on the bottom. The opening above the box could be closed off with a thicker PVC curtain. This construction resulted in two tunnels through which the bats could fly from one chamber to the other. A side and top view of the tunnels is depicted in Fig. 2, *A* and *B*, respectively. The tunnels were monitored with infrared-light barrier curtains (Leuze lumiflex Eco ET 30-225; Leuze Electronic, Owen, Germany), and four ultra-sonic microphones (SPM0204UD5; Knowles Acoustics, West Sussex, United Kingdom), mounted on the four outer tunnel sides, recorded the last 5 s of echo-acoustic activity before a light-barrier curtain was interrupted. Microphone recordings were digitized at 192 kHz using an M-Audio ProFire 610 audio interface and MATLAB-based software. Each tunnel opening could be blocked by an obstacle. Two experiments were conducted differing in obstacle-type: cardboard fans (*obstacle 1*) or artificial branches with leaves (*obstacle 2*). The fans covered the entire tunnel opening, and the branches loosely covered ~3/4 of each opening. With the aid of a servomotor (Reely S689-2BB/MG), the obstacles could be swung to the left or right, thereby blocking or unblocking a tunnel opening. Sketches of the setup with *obstacle 1* are depicted in Fig. 2. A digital video camera (DCR-TRV520; Sony, Minato, Tokyo, Japan) continuously recorded the openings of the tunnels in *chamber 1* during the whole length of each session. A bat detector (Mini-3 Detector; Ultra Sound Advice, London, United Kingdom) set to 45- to 50-kHz heterodyning frequency and connected to the audio input of the video camera allowed a simultaneous recording of the heterodyned echolocation calls. The activity in both chambers was externally monitored by the experimenter via two infrared cameras and infrared lighting mounted above the tunnel openings.

Procedure. The bats were trained to fly back and forth between *chambers 1* and *2* through one of the tunnels to receive a food reward from the landing platforms. In the standard trials, only one tunnel entrance and its corresponding exit were open. The other tunnel was
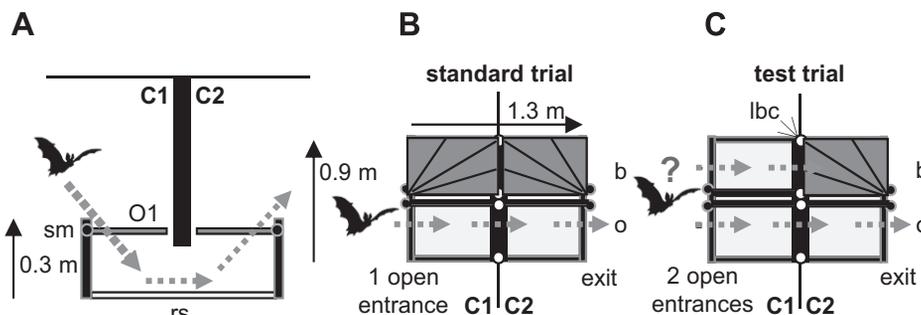
Fig. 2. Schematic of the tunnels. *A* shows the side view of how the bats had to fly from *chamber 1* (C1) to *chamber 2* (C2) through a tunnel with the reflecting surface (rs) and the servomotor (sm) moving the obstacles (depicted here as *obstacle 1*, O1). The height of each tunnel was 0.3 m with a length and width of 1.3 and 0.45 m, respectively. *B* illustrates from a bird's eye view the positions of the obstacles for a standard trial where 1 tunnel entrance and its exit are blocked (b) and the other tunnel entrance and its exit are open (o). A test trial where both tunnel entrances are open but only 1 exit can be viewed in *C*. The fly-throughs were monitored via the light-barrier curtains (lbc).

blocked by an obstacle at both its entrance and exit. A random sequence determined whether the left or right tunnel was opened.

Once the bats had learned this task, test trials were randomly interspersed with a probability of 25%. In the test trials, both tunnel entrances but only one tunnel exit were opened. The other tunnel exit was blocked by an obstacle. It is important to note that a bat could only detect the obstacle blocking a tunnel exit via indirect echoes mirrored by the tunnel floor and not via direct echoes from the obstacle.

A standard or test trial was registered as correct when a bat flew through the tunnel with its exit not blocked by an obstacle. The opening and closing of the tunnel entrances and exits by the obstacles, the light-barrier curtain signals, the microphone recordings, contact with a landing platform, and the resulting feeder reward were all controlled automatically with MATLAB (MATLAB R2007b; Math-Works, Natick, MA) and SoundMexPro (HörTech, Oldenburg, Germany) run on a computer.

Capturing the echo-acoustic properties of an open or closed tunnel exit. A tunnel, with either its exit open or closed off by either *obstacle 1* or *2*, was ensonified, and the reflected echo was recorded. An ultrasonic speaker (R2904/700000; Scan-Speak, Videbæk, Denmark) and a coaxially mounted ultrasonic 1/4-in. microphone (B&K 4135 with 2671 preamplifier and 2610 measuring amplifier; Brüel & Kjær, Nærum, Denmark) were positioned at a height of 45 cm, 30 cm from the PVC curtain at different elevations (-80 to $-30°$ in 10° steps). This position of the microphone-speaker unit corresponded well to that point above the tunnel entrance where the bats appeared to make their decision whether to try to pass the tunnel. The outgoing ensonification signal (a 10-s broad-band noise, high-pass filtered at 12 kHz) was computer-generated (MATLAB 5.3). The noise was sent out by a real-time processor [sampling rate: 260 kHz; RX6; Tucker-Davis Technologies (TDT), Gainesville, FL], amplified (Harman Kardon AVR 445; Harman Deutschland, Heilbronn, Germany), and emitted via the ultrasonic speaker. The amplified signal from the microphone was recorded during the noise presentation by the real-time processor. The computer cross-correlated the outgoing ensonification noise with the incoming echo, thus generating the acoustic impulse response (IR) of the tunnel. The IR describes all acoustic reflections from an object when ensonified by an acoustic impulse; it can be regarded as an echo-acoustic image of the setup layout (Aubauer and Au 1998; Grunwald et al. 2004).

Neurophysiological Experiments

Animal preparation. The principal surgical procedure is identical to the one of Hoffmann et al. (2008) and is described briefly in the accompanying paper ["Biosonar navigation above water I: estimating flight height" (Hoffmann et al. 2014)].

Generation of virtual acoustic space stimuli. Virtual acoustic space stimuli consisted of a typical echolocation call of *P. discolor* that was convolved with a left- and a right-ear pair of head-related IRs (HRIRs) of this bat (Firzlaff and Schuller 2003). The resulting echo includes all relevant spatial cues, including elevation-specific changes in the spectral profile of returning echoes. Echoes were generated as emerging from 23 equally spaced positions between -82.5 and $82.5°$ (step width: 7.5°) in the midsagittal plane.

To measure vertical STRMs, spatiotemporally randomized echo sequences with a duration of 5, 10, 20, or 40 min were constructed from the 23 virtual echoes. This was done by sequentially aligning randomly drawn single echoes with random temporal intervals between 0 and 80, 0 and 200, 0 and 500, and 0 and 1,000 ms, respectively. This resulted in average stimulus densities (SDs) of 575, 230, 92, and 46 echoes per second, respectively. As auditory cortical neurons habituate very fast when stimulated at high SDs, the STRM of a neuron was at least measured with 2 different SDs. The recording with the SD that yielded the strongest response was used for further analysis.

Electrophysiological recordings. Experiments were conducted in a sound-attenuated chamber. All recordings were done under general anesthesia [see Hoffmann et al. (2008) and the accompanying paper, Hoffmann et al. (2014)]. Details of the recording methods are specified in the accompanying paper (Hoffmann et al. 2014). Because it was not always possible to discriminate clearly the activity of a single neuron, the term unit will be generally used in the following to describe the collective activity of one to three neurons recorded at a recording site. For measuring the vertical STRM of a unit, echo sequences (see above) were digital-to-analog (DA)-converted (sampling rate: 192 kHz; RME-Audio Fireface 400; Synthax, Haimhausen, Germany) and presented binaurally to the bats via custom-made earphones (Schuller 1997). The detailed presentation method is described in the accompanying paper (Hoffmann et al. 2014).

Delay tuning. Delay tuning was measured with pairs of virtual stimuli (see above) separated by temporal delays between 1 and 15 ms in steps of 1 ms. The two stimuli were presented from spatial positions in the center of the first and second excitatory area in the STRMs of *class 1* units (see RESULTS). The spatiotemporal presentation order of the stimuli was adjusted according to the spatiotemporal order of excitatory areas in the STRMs. Afterward, the spatiotemporal presentation order of the two stimuli was reversed. In addition, the two stimuli were presented alone.

In these experiments, spikes were amplified, filtered, and analog-to-digital (AD)-converted using TDT equipment (sampling rate: 25 kHz; RA16 and RX5) and recorded with BrainWare (TDT). Stimulus pairs and single stimuli were presented in random order with 20 repetitions at a repetition rate of 1.3–3 Hz.

Please note that, unlike in classic experiments on delay tuning (e.g., O'Neill and Suga 1979), in our paradigm, no intensity difference between the two stimuli was introduced.

Data analysis. All data were analyzed using programs written in MATLAB. To construct the vertical STRM of a unit, a reverse correlation method was used. In accordance with the method described by Jenison et al. (2001), for each spike, the spatiotemporal stimulation pattern within a 50-ms interval preceding the action potential was averaged. To evaluate the level of stimulus-uncorrelated background activity, matrices were constructed from randomly drawn 50-ms segments of the stimulus sequence according to the method used by Lesica and Grothe (2008). For each neuron, the number of randomly drawn segments was equal to the number of spike-triggered segments. Excitatory (positive) and suppressive (negative) deviations from this background activity were considered to be significant based on a pixelwise *t*-test. Formation of excitatory and suppressive areas was based on a clustering (>3) of space-time bins with significant ($P < 0.01$) positive or negative *t* values (±1.9), respectively. For each area, the position of the center of gravity (centroid) and the positions of borders were determined in the spatial and temporal domain.

As the construction of STRMs is based on a spike-triggered average, vertical STRMs were generated only for units from which at least 500 spikes could be evoked during the time of stimulus presentation.

To determine whether a unit was delay-tuned, we tested whether the response elicited by stimulus pairs was significantly stronger (*t*-test, $P < 0.05$) than the sum of the responses elicited by the two stimuli presented alone, a criterion indicating facilitative interactions (e.g., Olsen and Suga 1991).

Animals

P. discolor is a New World subtropical omnivorous bat feeding on fruit, pollen, and insects (Nowak 1994). It belongs to the *Phyllostomidae* family and can be found on the South American continent. It emits short (<3 ms), broad-band, downward-modulated, multiharmonic echolocation calls in the frequency range of 40–90 kHz. All experimental animals originated from a breeding colony in the Department Biology II of the Ludwig-Maximilians University in Munich, Germany, and are kept under seminatural conditions (12:12-h day-night cycle, 65–70% relative humidity, 28°C). Five adult *P. discolor* (2 male and 3 female) took part in the behavioral experiments. They always had free access to water. During the training periods, consisting of 5 consecutive days, the bats were fed with banana pulp from automated feeders in the experimental setup. On days without training, they were fed mealworms and a fruit mix consisting of banana, melon, and mango. The animals that were subjected to the neurophysiological experiments were kept separated from other bats and had free access to food and water. All experiments complied with the principles of laboratory animal care and were conducted under the regulations of the current version of the German Law on Animal Protection (for the neurophysiological experiments: approval 55.2-1-54-2531-128-08, Regierung von Oberbayern).

RESULTS

Behavioral Experiments

Experiment 1: spontaneous detection of obstacle 1. Five *P. discolor* were successfully trained to fly back and forth between two chambers using one of two parallel tunnels. In the first experiment, cardboard fans (*obstacle 1*) were used as obstacles for the tunnel openings. The results are plotted in Fig. 3*A*. The performance in percentage correct for all bats is plotted for the standard trials (left bars) and the test trials (right bars) with the number of trials above each bar
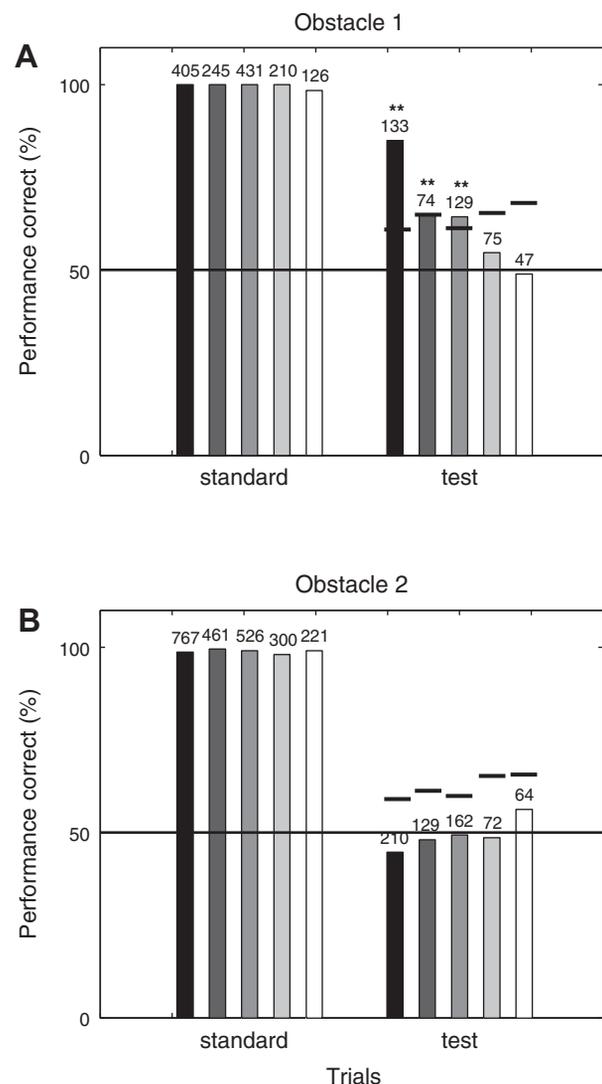

Fig. 3. The results for the behavioral *experiments 1* and *2*. The performance in percentage correct for 5 bats (gray-coded) is plotted for the standard trials (1 open tunnel entrance, left bars) and the test trials (2 open tunnel entrances, right bars) with the number of trials above each bar. The chance level is marked by the black line at 50% (2-alternative forced-choice), and the highly significant thresholds ($P = 0.01$) for each individual are indicated by the short, vertical black lines. Highly significant performances are labeled by 2 asterisks above the bars. The results for *obstacle 1* (fan) and *obstacle 2* (branch) are plotted in *A* and *B*, respectively.

and the thresholds (significantly different from chance level) for each individual indicated by the short, horizontal black lines. Highly significant performances ($P = 0.01$) are labeled by two asterisks above the bars. In the standard trials where only one tunnel entrance and its corresponding exit were open, all bats reliably chose the tunnel with the open entrance to fly to the other chamber, independent of which tunnel was blocked (left bars). In the test trials, which occurred with a probability of 25%, both tunnel entrances but only one exit were open. Two bats (*bats 4* and *5*) performed at chance level, but *bats 1*, *2*, and *3* achieved highly significant ($P = 0.01$) correct performances with 65, 84, and 64%, respectively (right bars). Thus these animals spontaneously detected this hidden obstacle via echolocation and thereby navigated through the correct tunnel.

Experiment 2: spontaneous detection of obstacle 2. In the second behavioral experiment, echo-acoustically less conspicuous obstacles (artificial branches) were used to block the tunnel openings. Results are shown in Fig. 3*B*. As in *experiment 1*, all bats reliably chose the tunnel with the open entrance to fly to the other chamber in the standard trials independent of which tunnel was blocked (left bars). In the test trials, however, none of the bats significantly preferred the tunnel for which the exit was open (right bars). Thus the bats were not able to detect spontaneously the branch obstacle above the tunnel exit through its echo-acoustic mirror image.

To test whether the bats could learn to detect the artificial branches via echo-acoustic mirror imaging, only test trials were presented. A slight performance improvement for some animals was measured, but this improvement was correlated to tunnel-side preferences of the individual animals. Therefore, the bats also cannot learn to use the echo-acoustic mirror image of the branch obstacle for navigation.

Echo-acoustic properties of the open and closed tunnel exit. A tunnel entrance was ensonified from a position of 45 cm above and 30 cm from the division of the two chambers because informal observations indicated that the bats decided around that position onto which entrance to choose (see in DISCUSSION, *Target Strength of Echo-Acoustic Mirror Images*). The measurements of the echo-acoustic properties of a tunnel with either its exit open or closed by either *obstacle 1* or *2* can be seen in Fig. 4. Depicted is the magnitude of the measured IR in decibels as a function of time for elevations from -30 to $-80°$. The light gray area shows the IR magnitude with the tunnel exit open, the black area with the tunnel exit closed by *obstacle 1*, and the dark gray area shows the IR magnitude with the tunnel exit closed by *obstacle 2*. The IR magnitude of *obstacle 2* is up to 10 dB stronger than that of the open tunnel for certain elevations. However, *obstacle 1* generates an up to 15-dB stronger echo for a broader time range and more elevations. These measurements confirm the assumption that target strength of the echo-acoustic mirror image is a decisive factor for a successful navigation through the tunnels.

Sound analysis. The analysis of the call number obtained for both tunnel entrance sides during the test trials revealed a significantly higher number of calls directed toward the tunnel that the animal flew through (Wilcoxon rank sum test, *P* values for all animals <0.0005). However, the individual echolocation behavior did not differ significantly between correct and incorrect test trials (Wilcoxon rank sum test, *P* values for all animals >0.2).

Neurophysiological Experiments

Extracellular recordings were derived from 175 acoustically responsive single and multiunits in the AC of both hemispheres of 4 anesthetized *P. discolor*. Eighty-two percent (143/175) of units showed robust responses to stimulation in virtual acoustic space. The spatiotemporal arrangement of excitatory areas in the vertical STRM allowed classification of these units into 4 different classes. Units of *classes 2-4* are described in the accompanying paper (Hoffmann et al. 2014). Here, we focus on *class 1* units, which amount to 51% (73/143). Figure 5, *A–E*, shows examples of vertical STRMs for *class 1* units. These units show a 1st excitatory area close to 0° elevation, which is followed by 1 or more excitatory areas that were spatially and
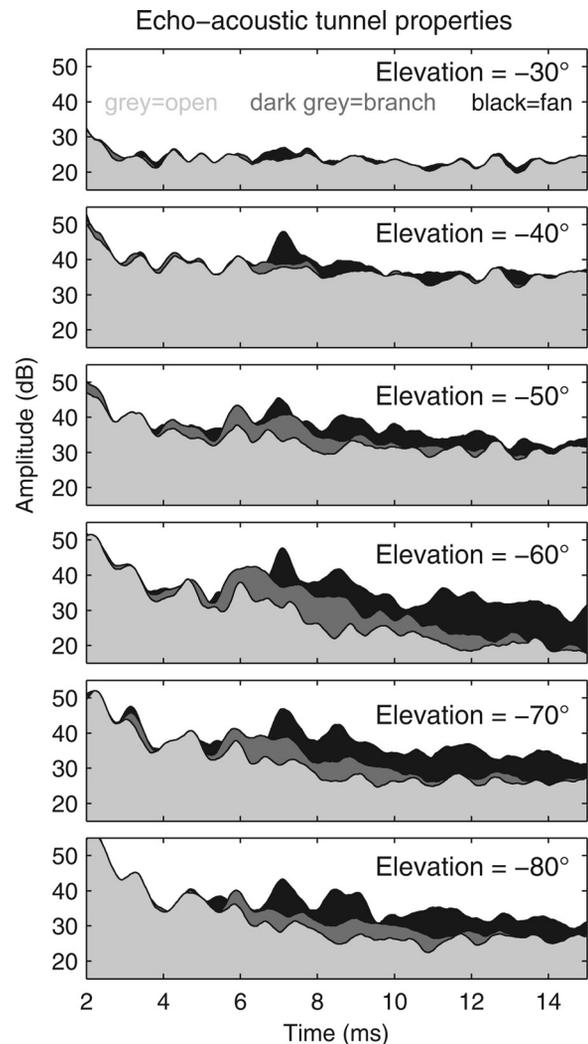

Fig. 4. The echo-acoustic properties of a tunnel with either its exit open or closed. Shown is the magnitude of the measured impulse response in decibels as a function of time in milliseconds for elevations from -80 to $-30°$ in 10° steps. The measurements for an open exit and for an exit closed by *obstacle 1* or by *obstacle 2* are coded by gray, black, and dark gray, respectively.

temporally separated from the 1st. The spectrum of virtual echoes presented close to 0° elevation is very similar to the spectrum of the bat's echolocation call [see Fig. 2, *B* and *F*, in the accompanying paper (Hoffmann et al. 2014)]. The 1st excitatory area in *class 1* units would therefore be particularly sensitive for the bat's emitted echolocation pulse ("pulse-encoding"). This hypothesis is strengthened by the fact that a bat would perceive its emitted echolocation pulse as originating from ~0° in elevation. The spectrum of virtual echoes presented as originating from spatial positions above or below the horizon increasingly differs from the echolocation call with increasing separation in elevation. In addition, the amplitudes of these virtual echoes decrease in contrast to the amplitude of the call with increasing separation in elevation. Therefore, excitatory areas, which are spatially and temporally separated from the first one, may encode echoes, returning to the bat from different elevations ("echo-encoding"). Although the stimulus sequence included only randomly presented sonar signals, one can infer that the two excitatory areas reflect the spatiotemporal arrangement that would occur for the emitted pulse and the
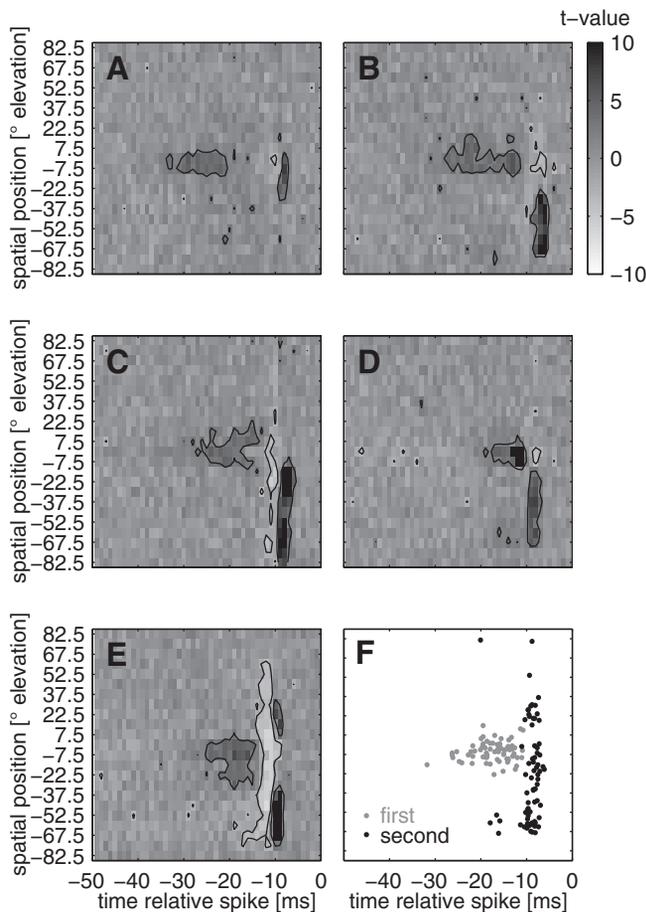
Fig. 5. *A–E*: examples of vertical spatiotemporal response maps (STRMs) for *class 1* units. Excitation in the STRMs is shown in dark colors, and suppression in light colors. Significant pixels ($P < 0.05$, black contour) in the STRMs correspond to a *t* value of ±1.9. *F*: population data for *class 1* units ($n = 73$). Gray dots indicate the position in elevation of the center of the 1st ("pulse-encoding") excitatory area, and black dots indicate the position in elevation of the center of the 2nd ("echo-encoding") excitatory area.

reflected echo in a natural situation. Figure 5*F* shows population data for all *class 1* units, represented by the spatiotemporal position of the centroids of the first excitatory area (pulse) and the second excitatory area (echo) in their STRMs. Whereas the latency of the echo-encoding excitatory area is quite stable, ~9 ms across units, latencies of the pulse-encoding excitatory area span from 11 to 32 ms. The ability of auditory neurons in bats to encode certain temporal separations between emitted echo-location calls and returning echoes has been described as pulse-echo delay tuning (O'Neill and Suga 1979). To test the hypothesis that *class 1* units are also able to encode pulse-echo delays, we stimulated 44 of 73 (60%) *class 1* units with pairs of virtual stimuli separated by temporal delays between 1 and 15 ms (see MATERIALS AND METHODS). Figure 6*B* shows responses to stimulus combinations that were spatiotemporally arranged according to the spatiotemporal order of the pulse- and echo-encoding areas in the STRM [i.e., the stimulus from 0° was presented 1st and was followed by the stimulus from −67.5°; Fig. 6*A*]. Stimulus combinations with delays between 7 and 15 ms elicited a strong late component in the neural response that could be seen in neither the response to stimulus combinations with shorter delays nor in the response to presentations of single stimuli. Figure 6*C* shows responses to

stimulus combinations with a reversed spatiotemporal arrangement (i.e., the stimulus from −67.5° was presented 1st and was followed by the stimulus from 0°). Note that the strong late component in the neural response shown for certain delay steps in Fig. 6*B* cannot be found when the spatiotemporal arrangement of stimuli is opposite to that in the STRM (Fig. 6*C*). Figure 6, *D* and *E*, shows bar plots of the mean spike counts
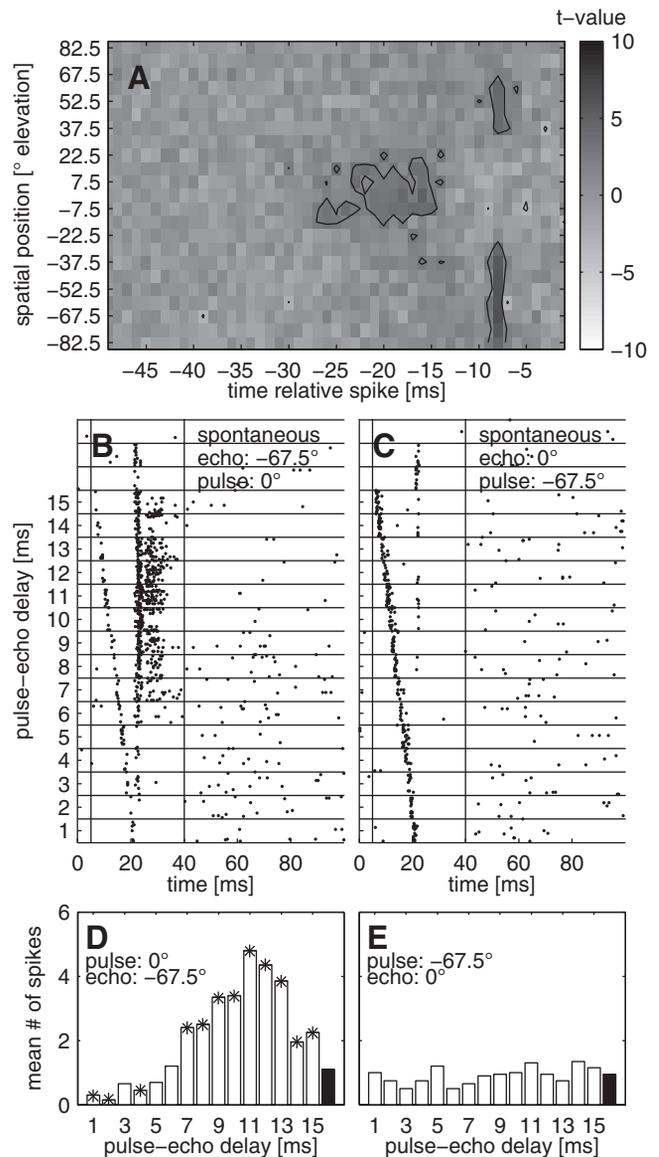

Fig. 6. Example of a *class 1* unit tested for delay tuning. *A* shows the vertical STRM of the unit. Excitation in the STRM is shown in dark colors, and suppression in light colors. Significant pixels ($P < 0.05$, black contour) in the STRM correspond to a *t* value of ±1.9. *B* and *C* show raster plots of responses to single stimuli and stimulus pairs with temporal delays between 1 and 15 ms for the *class 1* unit shown above. Stimuli were presented as originating from spatial positions located in the center of pulse- and echo-encoding areas (pulse: 0°; echo: −67.5°). *B* shows responses to stimulus combinations that were spatiotemporally arranged according to the spatiotemporal order of the pulse- and echo-encoding areas in the STRM, and *C* shows responses to stimulus combinations with a reversed spatiotemporal arrangement. *D* and *E* show bar plots of the mean spike counts elicited by stimulus combinations with temporal delays between 1 and 15 ms (white bars) and the mean spike count of the combined response to single presentations of the 2 stimuli (black bar). Black asterisks indicate significant differences (*t*-test, $P < 0.05$) in spike count between the combined responses and the response to stimulus combinations.

elicited by stimulus combinations with temporal delays between 1 and 15 ms (white bars) and the mean spike count of the combined response to single presentations of the two stimuli (black bar) as shown in Fig. 6, *B* and *C*. Delay steps at which the response was significantly different (*t*-test, $P < 0.05$) from the sum of the responses to presentations of single stimuli are indicated by black asterisks.

In most of the units that were tested for delay tuning (26/44, 59%), response strength was significantly (*t*-test, $P < 0.05$) higher for stimulus combinations with certain delays than the sum of responses for the two stimuli presented alone. This facilitatory effect, however, could only be observed when the spatial arrangement of the two stimuli was similar to the arrangement of pulse- and echo-encoding areas in the STRM. If the spatial order was reversed, no facilitatory effect was observable.

These results support our interpretation of *class 1* STRMs: the first excitatory area encodes the echolocation pulse, and the subsequent excitatory areas encode echoes. Therefore, we can assume that the temporal separation between the centroids of the pulse- and echo-encoding area in the STRM represent the pulse-echo delay a unit is sensitive to. STRM delays (temporal separations between pulse- and echo-encoding area in the STRM) of *class 1* units ranged between 1 and 18 ms (Fig. 7*A*) with a median of 7 ms. This is in line with earlier findings on pulse-echo delays encoded by neurons in the auditory system of bats (Dear et al. 1993; Hagemann et al. 2010, 2011; O'Neill and Suga 1979).

The current STRMs, however, describe the tuning of the units also along the elevation axis. Figure 5*F* shows that whereas the pulse-encoding excitatory area is typically located at elevations $\sim0°$ (median: $-5°$), the echo-encoding area is most often located at a lower elevation (median: $-26°$). Especially in units sensitive to long delays >10 ms, the echo-encoding excitatory area is almost exclusively located below horizon (Fig. 7*B*).

From the spatiotemporal arrangement of pulse- and echo-encoding areas in the STRMs of *class 1* units, we suggest that these units will respond preferentially to echoes from objects located below the horizon and at distances ~120 cm (corresponding to a 7-ms STRM delay).

The preference of *class 1* units for echoes with these properties matches quite well with our measurements of the echo-acoustic properties of our flight tunnels: the IRs of the tunnel (Fig. 4) show that closing the tunnel exit with *obstacle 1* increases IR magnitude for delays above ~6 ms and for echo

elevations below about $-40°$. These echo-acoustic features of our behavioral setup match well with the STRM delay and elevation preferences of *class 1* units as illustrated in Fig. 7. Thus the spatiotemporal tuning of *class 1* units is well-suited to detect the changes in the echo-acoustic properties of the flight tunnels introduced by *obstacle 1*.

DISCUSSION

The current behavioral results show that three of our five bats spontaneously avoided flying through that tunnel for which the exit was blocked by a hidden obstacle. The IR measurements showed that this hidden obstacle increased the loudness of later reflections out of the tunnel mouth. Complementing this behavior, we have described the spatial tuning of delay-tuned cortical units along the elevation axis, and we see that the tuning of these units to echoes from low elevations and, relative to the tunnel dimensions, long echo delays supports the neural encoding of such echo-acoustic mirror images. In what follows, we will discuss this echolocation feat with respect to different sensory aspects.

Mirror Images vs. Reverberation

In room acoustics, an increase of late reflections is interpreted as an increase in reverberation. In fact, reverberation and acoustic mirror images are closely related because reverberation comes about through multiple specular (mirrorlike) reflections of a sound in a confined space. Accordingly, modern approaches to calculate acoustics of virtual spaces use a mirror image approach to quantify both early reflections and (late) reverberation (Blauert 1997). These physical reflection properties, however, must not be confused with their percepts. Specifically, reverberation in human hearing refers to a blurry, ambient sound property imposed on an external sound source. Bats, however, are specialized to navigate based on echoes of self-generated sounds. Our behavioral data and IR measurements show that bats can exploit even late, higher-order specular reflections for navigation. Here, we relate this capability to the percept of acoustic mirror images, but relating it to the analysis of reverberation is, due to the close relationship between the two, equally appropriate.

Stereotype Flight Paths and Tunnel Side Preferences

Höller and Schmidt (1996) showed that bats, while navigating in a known surrounding, will rely mainly on spatial memory, supported by visual and echo-acoustic information. The resulting stereotyped flight paths have also been observed in other studies, which also indicate that bats often at first ignore changes in their surrounding that would be easily detectable via their sonar system (Barchi et al. 2013; Griffin and Grinnell 1958; Holderied et al. 2006; Holland and Waters 2007; Jensen et al. 2005). The stationary layout of the tunnels in the current study supported the bats in developing stereotyped flight paths, which, in the standard trials, only had to be adapted according to which tunnel entrance was open. In the test trials, however, both tunnel entrances were open, and only the echo-acoustic mirror image of the obstacle would indicate which tunnel allowed a passage. Thus the echo-acoustic mirror image had to be conspicuous enough for the bat to divert from its stereotyped flight path and use the other tunnel for the fly-through.
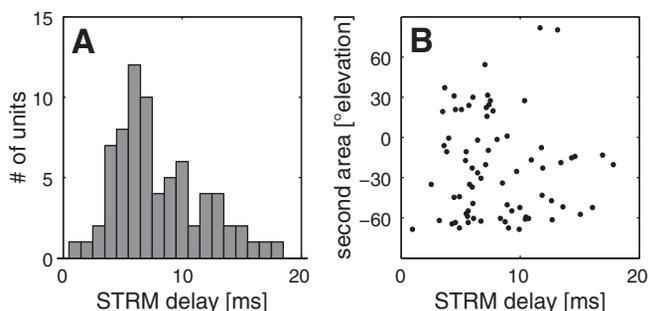

Fig. 7. *A*: distribution of temporal separations (i.e., STRM delays) between pulse- and echo-encoding areas in the STRMs of *class 1* units. *B*: relationship between STRM delays and spatial positions of the centroids of echo-encoding areas in STRMs of *class 1* units.

Target Strength of Echo-Acoustic Mirror Images

When approaching the tunnel entrances of our setup, a bat would be limited from which angle and height combinations it could receive the echo-acoustic mirror image of the obstacle at the tunnel exit. With a too steep downward or too horizontal ensonification angle, the echolocation calls would only be reflected by the floor or tunnel walls but not travel through the tunnel and back again (cf. Fig. 4). These limitations should therefore be reflected in the behavioral approach toward the entrances. Indeed, infrared video recordings of some experimental sessions confirm that, in the test trials, the animals decided for the tunnel when they were ~45 cm above the tunnel floor and ~30 cm in front of the middle wall of the tunnel. The point of decision was evident behaviorally as the bat would suddenly swerve away from one tunnel entrance and choose the other, correct tunnel. This corresponds to an ensonification angle of about −40 to −60°.

When *obstacle 1* was replaced by *obstacle 2*, which had a much lower target strength (cf. Fig. 4), only *bat 4* would rarely display this obstacle avoidance behavior toward the mirrored obstacle. Moreover, none of the bats reliably learned to avoid *obstacle 2* even when they were trained specifically on this task (data not shown). This indicates a detection criterion determined by the target strength of the obstacle.

Target strength discrimination has been measured for various bat species. Heinrich et al. (2011) found a target strength discrimination threshold of 5 dB at a (virtual) object distance of 114 cm for the bat *P. discolor*. Stamper et al. (2008) measured target strength discrimination thresholds of 1–2 dB at a (real) object distance of 25–34 cm for the bat *Eptesicus fuscus*. Studies analyzing prey in clutter detection in flight measured increased threshold distances between prey and clutter for the bats to detect prey successfully (Moss et al. 2006; Moss and Surlykke 2001). Figure 4 shows that the additional (mirror) reflections of *obstacle 1* increases IR magnitude by up to 15 dB at longer echo delays above ~6 ms. This effect is much weaker for *obstacle 2*. Regarding the above data on target strength discrimination, it is plausible to assume that bats in the current study relied on target strength differences introduced by the echo-acoustic mirror image of *obstacle 1* to do the task.

Analysis of "Abstract" Echo-Scapes

An early human study (Priel and de Schonen 1986) showed that for children who are not familiar with the visual mirror concept, their capacity to relate real to reflected space is distinctly impaired. Second, the ability to discriminate mirror images is enhanced by literacy in human viewers (Fernandes and Kolinsky 2013; Kolinsky et al. 2011). Finally, several studies revealed depth misjudgments in humans exploring mirror reflections (Hecht et al. 2005; Higashiyama and Shimono 2012; Seyama and Nagayama 2006). These studies indicate that the interpretation of mirror images is an active cognitive process influenced by previous experience. This is likely to be the case for bats as well. For a bat approaching the tunnel entrance in our study, the echo of the mirrored obstacle arrives with a longer delay than the physical distance between the bat and the obstacle but also with a misleading (too low) elevation. This spatial uncoupling of the obstacle and its echo-acoustic image results in an abstract echo-acoustic situation. Prior knowledge of the reflection characteristics of a

mirroring surface is likely to be required to handle such an abstract echo-acoustic situation. Greif and Siemers (2010) showed that bats perceive echo-acoustically smooth surfaces innately as a water surface and that this percept is mediated via their sonar system. A bat flying above an extended, smooth surface and experiencing the direct echo from below may learn how to deal with echoes arriving from low elevations and unusually long delays and interpret them correctly. This presupposes a capability of forming a spatial three-dimensional representation of their surroundings, which several studies have implied (Geipel et al. 2013; Genzel et al. 2012; Neuweiler and Mohres 1967).

Neural Coding of Abstract Echoes

Echolocating bats use the temporal delay between emitted pulse and returning echo to determine target distance (O'Neill and Suga 1979; Simmons 1971). Delay-tuned neurons, sensitive to certain pulse-echo delays, have been found in the midbrain (Dear and Suga 1995; Portfors and Wenstrup 1999; Valentine and Moss 1997), thalamus (Olsen and Suga 1991; Wenstrup 1999), and AC (Dear et al. 1993; Feng et al. 1978; Hagemann et al. 2010; Suga et al. 1990; Sullivan 1982) of different bat species. Because of the large spatial receptive fields of delay-tuned neurons, they do not seem to be suited to process directional information of echoes in the AC of the mustached bat (Suga et al. 1990). In the midbrain superior colliculus of *E. fuscus*, however, Valentine and Moss (1997) found that responses of delay-tuned neurons depended not only on the temporal delay between pulse and echo, but also on the direction of stimulation. They could show that the best delay of all delay-tuned neurons changed with the azimuthal position of sound presentation. The dependence of delay tuning on the vertical stimulation position was tested only in two neurons, and therefore the authors did not make a clear statement on the general influence of the vertical stimulation position on delay tuning.

Class 1 units found in the present study may serve as delay-sensitive units in the AC of *P. discolor*. This hypothesis is strongly supported by the delay tuning experiments done with pulse-echo stimulation in a subpopulation of *class 1* units. With the STRM recordings, we could clearly show that delay-tuned responses of these units strongly depend on the elevation of the echoes. In the vertical STRMs of *class 1* units, the first (pulse-encoding) excitatory area was always located ~0° in elevation, whereas the vertical positions of the second (echo-encoding) excitatory area showed a broader distribution mainly at lower elevations. Since the main energy of the sonar emissions of *P. discolor* is also directed slightly below horizon (Vanderelst et al. 2010), echoes from objects located at lower elevations would be strongest. Thus both the emission and reception characteristics of the bat sonar system facilitate the encoding of echoes from elevations below the horizon.

Best delays of delay-tuned neurons of every investigated nucleus in the ascending auditory system of different bat species have always been found to range roughly between 1 and 25 ms with delays below 10 ms being overrepresented (O'Neill and Suga 1979; Portfors and Wenstrup 1999; Valentine and Moss 1997; Wenstrup 1999). Temporal separations between the two excitatory areas in the vertical STRMs of *class 1* units in the present study ranged between 1 and 18 ms,

and thus STRM delays fell well into the range of best delays described for delay-tuned neurons in earlier studies. Furthermore, also in our study, delays below 10 ms were overrepresented. On average, the first and second excitatory area was temporally separated by 7 ms, corresponding to a target distance of 1.2 m.

Especially temporal parameters of neural responses can be affected by anesthetics. The conformity of delay range found in our study with those found in other bats, however, makes a strong influence of drugs used for anesthesia on the STRMs unlikely. Regarding overall influence of anesthetics on STRM recordings, please see also the DISCUSSION section of the accompanying paper (Hoffmann et al. 2014).

The current electrophysiological and behavioral experiments were independently designed and performed. As such, it appears as a "lucky coincidence" that the best delay and elevation preference for the cortical units match the echo-acoustic layout of our behavioral experiments. Indeed, we would argue that this match is not lucky, but, as we designed our behavioral task with the dimensions of the tunnel to fit the maneuverability of our bats, we think that the delay and elevation preference of the cortical units is evolutionarily shaped to match also the maneuverability of our bats. Thus it is possible that the cortical representation of echo delay and elevational tuning may be quite different for a high-and-fast flying species. In fact, the distribution of characteristic delays in cortical delay-tuned neurons of different species like *Pteronotus parnellii*, *P. quadridens*, and *Carollia perspicillata* is different (Hechavarria et al. 2013). Best target detection distances therefore seem to be related to differences in foraging behavior in these species.

General Conclusion

Overall, the current psychophysical and electrophysiological data highlight the neuroethology of sonar-guided navigation in a special but ecologically highly relevant context, namely above water. In the accompanying paper (Hoffmann et al. 2014), we could show that adjustment of flight height requires the detailed analysis of elevation-dependent spectral cues. When this analysis was hampered by reversibly manipulating the bats' head-related transfer functions, adjustment of flight height was significantly impaired. Both corresponding biophysical and electrophysiological analyses point to the bats relying on spectral information around 50–60 kHz to solve this task. We hypothesize that behavioral performance is mediated by cortical neurons for which STRMs reveal tuning that is relatively inconspicuous along the time axis but sharply tuned in space to very low elevations. Conspicuous tuning both along the elevation and time axes, on the other hand, is needed to support navigation via the echo-acoustic mirror images by water. Specifically, we found that delay-tuned units in the bat AC preferentially respond to echoes from lower elevations and with delays ~7 ms. Compared with the dimensions of our behavioral setup, such units are well-suited to encode the echo-acoustic mirror image of an obstacle in the bats' desired flight path. These data provide the first evidence that bats can indeed exploit the dramatic distortions in space that are introduced by water bodies for navigation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

D.G. and L.W. conception and design of behavioral research; S.H. and U.F. conception and design of neurophysiological research; S.H. and S.P. collected and analyzed neurophysiological data; D.G. performed behavioral experiments and analyzed behavioral data; D.G., S.H., U.F. and L.W. interpreted results of experiments; D.G. and S.H. prepared figures; D.G., S.H., U.F. and L.W. drafted manuscript and approved its final version.

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