Object-Oriented Echo Perception and Cortical Representation in Echolocating Bats

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Echolocating bats can identify three-dimensional objects exclusively through the analysis of acoustic echoes of their ultrasonic emissions. However, objects of the same structure can differ in size, and the auditory system must achieve a size-invariant, normalized object representation for reliable object recognition. This study describes both the behavioral classification and the cortical neural representation of echoes of complex virtual objects that vary in object size. In a phantom-target playback experiment, it is shown that the bat Phyllostomus discolor spontaneously classifies most scaled versions of objects according to trained standards. This psychophysical performance is reflected in the electrophysiological responses of a population of cortical units that showed an object-size invariant response (14/109 units, 13%). These units respond preferentially to echoes from objects in which echo duration (encoding object depth) and echo amplitude (encoding object surface area) co-varies in a meaningful manner. These results indicate that at the level of the bat’s auditory cortex, an object-oriented rather than a stimulus-parameter-oriented representation of echoes is achieved.

Introduction

For both the visual and the auditory domain, the formation of perceptual objects from physical stimuli is an essential task. Reliable object recognition is complicated by the variability of naturally occurring objects, e.g., in object size. In the visual system, the effect of object size on object recognition and underlying neural substrates has been investigated in detail (for review see Logothetis and Sheinberg [1]). For example, neurons in the inferior temporal lobe can exhibit object-size invariant responses [2].

In the auditory domain, however, the definition of an object is not straightforward [3]. It is hypothesized that the auditory cortex segregates auditory objects depending on the auditory background, i.e., it adjusts its sensitivity for the boundaries of auditory objects along both the auditory time and frequency axes based on the spectrotemporal fluctuation statistics of the auditory background [4]. In humans, the analysis of auditory objects is thought to be implemented along “where” and “what” pathways in the auditory cortex [5,6], as recently corroborated in a combined functional magnetic resonance imaging (fMRI) and magnetoencephalography (MEG) study [7]. The perception of size information in auditory objects, as part of the “what” analysis, has only recently been addressed. Human psychophysical studies have shown that information about speaker size is well preserved in human speech, that the human auditory system can segregate size information from information about the content, and thus that the auditory system can compensate for the effect of speaker size on perceived speech [8]: The same vowel pronounced by an adult and a child differs dramatically in its spectral content. However, it is readily perceived as the same vowel. Smith et al. [8,9] and Ives et al. [10] showed that recognition of vowels as well as the ability to judge relative size of speakers work well beyond the normally occurring range of speaker size. In an fMRI study, von Kriegstein et al. [11] showed that information about the vocal-tract length of a speaker, as an acoustic marker of body size, may be processed as early as the auditory thalamus and that an interaction between a voice’s fundamental frequency (which can also mediate size information) may occur in non-primary auditory cortex.

For an echolocating bat, the definition of an auditory object is readily obtained: it is the information a bat gains from analyzing the echoes of self-generated sounds reflected by a physical, three-dimensional object in its environment. This clear definition of an echo-acoustic object allows the systematic investigation of the perception [12–14] and neural encoding of auditory object features [15,16] in an animal model. Behaviorally, echolocating bats can identify three-dimensional objects exclusively through the analysis of the echoes of their ultrasonic emissions. It was shown that bats use echolocation for object identification to find fruit [17,18] and flowers [19]. Schmidt et al. [20] showed that the gleaning bat Megaderma lyra uses echolocation to identify prey of...
Author Summary

Bats can orientate and hunt for prey in complete darkness using echolocation. Bats use this extraordinary ability, not only to localize objects in space, but also to identify them. The same object, however, can come in different sizes. Here, we use a combination of psychophysical phantom-target experiments and electrophysiological recordings to investigate how echolocating bats perceive objects of different sizes, and how the echoes reflected from these objects are represented in the bat auditory cortex. We trained the neotropical bat *Phyllostomus discolor* to identify virtual objects, and found that these bats spontaneously associated scaled versions of these objects with the corresponding trained object. Interestingly, we identified neurons in the bat auditory cortex that respond to specific objects irrespective of object size. These findings highlight the sensory capabilities and sophisticated neural processes underlying bat echolocation. This suggests that like the visual system, echolocation meets an important requirement of an effective object-recognition system in that it allows the identification of objects independent of object size.

different size. The bats also discriminated edible prey from dummies of the same size.

In all the above-mentioned cases, object normalization is crucial for compensating for inevitable variations in object size. In fact, it has been shown in behavioral experiments that bats are able to discriminate simple shapes independent of their size [21]. Acoustically, an object is defined by its impulse response (IR). The IR is the sum of the reflections when an object is ensonified with a Dirac impulse, i.e., an impulse of theoretically infinite shortness and amplitude. With increasing object size, there is a proportional change in both object surface area and object depth. Because the strength of the reflection depends on the size of the reflecting surface, the IR becomes louder with increasing object size. In addition, the IR becomes temporally expanded because object depth, and thus the temporal delay between single reflections, increases with increasing object size. This expansion corresponds to a compression of the spectral interference pattern. Taken together, much of the information about the three-dimensional structure of an object is represented in its IR, and much of the variability of the IR relates to the size of the object.

An echolocating bat does not perceive the IR as such, but it perceives the IR convolved with its echolocation call. Thus, the acoustic image of an object is imprinted on the echolocation call to produce an echo. The echo carries the acoustic properties of both the echolocation call and the IR. For a bat, it is essential to extract the IR from the echo [13,22]. Specifically, the loudness of an echo will not only depend on the loudness of the IR (encoding object size), but also on the loudness of the call. Echo loudness also depends on the distance between the bat and the object. Note, however, that the bat has full information about these parameters, because both the call loudness and the object distance are encoded in the auditory system. This information allows for a call loudness-independent and object distance-independent evaluation of the IR.

The aim of this study was to search for a size-invariant echo-acoustic object representation in a combined psychophysical and electrophysiological approach.

In the psychophysical phantom-object experiment, fruit-eating bats (*Phyllostomus discolor*) were trained to discriminate echoes of their sonar emissions. These echoes consisted of the emission convolved with the IRs of two objects. Each IR consisted of 12 randomly spaced reflections of different amplitude (Figure 1A, third row). Once the bats had learned this task, test trials were randomly interspersed, in which a scaled version of one of the standard objects was presented (Figure 1A), and the bats' spontaneous classification of these scaled objects was assessed. The bats' psychophysical performance was compared to the performance of a spectrotemporal
pattern recognizer being fed with auditory representations of the echoes, as they were perceived by the bats.

In the electrophysiological experiment, the ability of neurons in the auditory cortex of *P. discolor* to encode a normalized representation of the two objects that have been characterized psychophysically was tested. The IRs of both standard objects were scaled in terms of the delay and amplitude of the reflections and then convolved with a standard *P. discolor* echolocation call. For each object, the resulting stimuli are represented in a 5×3 matrix (Figure 1D) in which object depth and object surface area co-varied in a meaningful manner only along the diagonal axis, thus representing properly scaled versions of the object. Acoustically, the object–surface area parameter is encoded in the echo level, the object-depth parameter is encoded in the echo duration.

The psychophysical results show that *P. discolor* spontaneously classified most scaled versions of standard objects correctly. A population of cortical units was found that reflected normalized object features in their response rates. This population may serve as a substrate for the perceptual compensation of size-induced object variations.

**Results**

**Psychophysics**

Behavioral results are based on a total of 4,500 trials obtained from three bats. Bat 1 spontaneously classified all four scaled versions for both of the two objects significantly correctly (Figure 2A). Bat 2 did so for all four scaled versions of object 1 and two of the scaled versions of object 2 (Figure 2B). Bat 3 showed a similar trend in evaluating the scaled objects, but failed to achieve significant performance in five of eight cases (Figure 2C). The performance of a spectrotemporal pattern recognizer is shown in Figure 2D. This pattern recognizer worked on a representation of the object echoes as generated by the bat’s auditory periphery (see Materials and Methods, Figure 3). The mean-squared differences between the auditory spectrograms of the two standard objects (Figure 3A and 3B) and those of the scaled objects (examples in Figure 3C–3F) were calculated. Based on the mean-squared differences, the recognizer cannot reliably

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**Figure 2. Psychophysical Classification Performance and Simulation Results**

Spontaneous classification of scaled virtual objects by three bats (A–C) and a simulation (D) based on a spectrotemporal pattern recognizer. Significantly correct classification of scaled objects is marked by a single star (*p* < 0.05) or two stars (*p* < 0.01). The number of test trials for each condition is superimposed on each bar. Although the spectrotemporal pattern recognizer cannot classify the scaled objects correctly, the bats’ performance is in the majority of test conditions significantly better.

**Figure 3. Auditory Spectrograms Generated by Echoes As They Are Perceived by the Bats**

Examples of auditory spectrograms produced by echoes generated with the IRs of both standard and scaled objects. The spectrograms incorporate the limits of spectral and temporal resolution of the auditory periphery of *P. discolor*. These spectrograms serve as inputs to the spectrotemporal pattern recognizer that tries to associate the spectrograms generated with different scaled objects (middle column [C–F]) to those generated with the two standard objects (A and B). Simulation results are shown in Figure 2D.

![Figure 2](https://example.com/fig2.png)

![Figure 3](https://example.com/fig3.png)
classify the peripheral representation of the scaled objects as one of the trained standard objects. Thus, even the bat that showed the weakest normalization behavior (bat 3) performed considerably better than the spectrotemporal pattern recognizer. Note that such a model could successfully predict responses in other echo-acoustic playback experiments [12,13]. These simulation results show that a dedicated neural mechanism beyond the auditory periphery is required to explain the bats’ compensation for size-induced echo variations.

**Electrophysiology**

Recordings were derived from a total of 109 units from four bats (two females and two males, weighing between 33 and 44 g, lightly anaesthetized with a combination of medetomidin, midazolam, and fentanyl ([MMF]; see Materials and Methods). All units were tested with both objects. The stimuli for each object consisted of a standard echolocation call convolved with the 25 object IRs. For quantifying the neural responses, we computed the number of spikes for each of the 25 stimuli, with the spikes counted in a post-stimulus time window that was set separately for each unit according to the limits of statistically significant deviations from spontaneous activity (see Materials and Methods). The 25 responses to each object were arranged in a 5 × 5 matrix and normalized such that the maximum response was set to unity. Then, each response matrix was assigned to one of six categories (“scaled,” “depth,” “surface,” “ambiguous,” “insensitive,” and “irresponsive”; see Materials and Methods). An illustration of the categorization principles with synthetic response matrices is shown in Figure 4. Note that because each unit was tested with both objects, and units could respond differently to the two objects, percentages given below add up to more than 100%.

Two recorded examples of response matrices assigned to the “depth” category are shown in Figure 5A and 5B. In these matrices, responses were strongest either to a particular object depth or to a combination of object depths, but were largely independent of object surface area. Such response matrices were recorded in 14/109 units (13%) for at least one of the two objects.

Two recorded examples of response matrices assigned to the “surface” category are shown in Figure 5C and 5D. In these matrices, responses were strongest either to a particular object surface area or to a combination of surface areas, but were largely independent of object depth. Such response matrices were recorded in 62/109 units (57%) for at least one of the two objects.

Most interestingly, in a third category, the response to stimuli along the diagonal axis, and thus to scaled versions of the stimuli, was strongest (“scaled”; Figure 6E and 6F). Such response matrices could be recorded in 14/109 units (13%) for at least one of the two objects. In these recordings, the response was not simply dependent on echo amplitude or duration, but on a meaningful combination of the two. The sophisticated properties of such a response matrix are
reflected in the fact that the unit responded to this object equally strongly no matter whether it was small (the echo is faint and short) or large (the echo is loud and long). Thus, these units reflected normalized object features in their firing-rate. The effect is especially pronounced in the unit shown in Figure 6E. Except for the position in the upper right corner, stimuli along the diagonal axis of the 5 × 5 stimulus matrix evoked almost equally strong responses that clearly exceed the responses from other positions in the 5 × 5 matrix.

In the other unit shown in Figure 6F, the strong responses are also evoked from positions neighboring the diagonal. However, the stimuli from positions in the lower right and upper left corner of the 5 × 5 matrix evoked only weak responses, so that the diagonal orientation of positions with strong responses still prevails. As can be seen from the raster plots and peri-stimulus time histograms (PSTH) in Figure 6A–6D, the stimuli evoked robust responses in both units.

A clear assignment to a distinct response category along one dimension (“depth,” “surface,” or “scaled”) was not always possible because some response matrices showed significant responses along more than one dimension. These matrices were categorized as “ambiguous” and were recorded in 10/109 units (9%) for at least one of the two objects. However, it is noteworthy that in four of these units, a significant response occurred also along the diagonal dimension either in response to object 1 or 2.

A considerable number of matrices did not show a significantly stronger response along any stimulus dimension and thus were categorized as “insensitive.” These matrices were recorded in 57/109 units (52%) for at least one of the two objects.

Finally, in some units, the response to the convolved echolocation calls was generally weak and did not reach significance (“irresponsive,” 8/109 units, 7%).

The number of units for which at least one of the recorded response matrices met the criteria for a response category are summarized in Table 1.

Each unit was tested with two virtual objects. The combination of response categories in response to the two objects is given for each unit in Table 2. This table shows the number of units that, for the two objects, fall into a specific combination of response categories. In only 53/109 of the recorded units (49%), the response matrices for the two objects were assigned to the same category. This is remarkable because the echoes generated with the IRs of both objects had very similar loudness and spectral content; they differed only in their temporal structure. Most units responded best to a particular object surface area (i.e., stimulus amplitude) or did not show any preference for a certain object dimension at all. A smaller number of units...
responsible strongest to a particular object depth (i.e., stimulus duration) or to scaled versions of an object.

Only one recorded unit was assigned to the “scaled” category in response to both objects. Most of the other units that responded in a “scaled” way to one object were “insensitive” to object depth, surface area, or scale in response to the other object.

Spatial Distribution of Response Categories

Recording sites were located at positions along the rostro-caudal axis over about 4,000 µm and along the dorsoventral axis over about 3,500 µm. This area roughly corresponds to the anatomically evaluated dimensions of the auditory cortex in *P. discolor* (S. Radtke-Schuller, personal communication). The different response categories were almost uniformly distributed over the whole cortical area from which recordings were derived, and no topological order could be detected (Figure 7B and 7C). However, units that responded most strongly to scaled versions of objects were absent in the most anterior region of auditory cortex. There is no detailed information published about different cortical fields in the auditory cortex of *P. discolor*. However, in the auditory cortex of a closely related species (*Carollia perspicillata*), dorsally located, non-tonotopic–organized fields have been distinguished from more ventrally located fields with tonotopic organization, presumably the primary auditory cortex and an anterior auditory field [23]. These data, combined with a preliminary partitioning of the *P. discolor* auditory cortex based on 563 units in our laboratory, suggest that scale-invariant response matrices were recorded in the high-frequency parts of the primary auditory cortex and the adjacent anterior auditory field. Notably, scale-invariant responses were absent in the non-tonotopical dorsal fields.

**Influence of Stimulus Level**

The average presentation level for the recordings was set so that the loudest echoes were 20 to 30 dB above unit threshold. The surface-area axis of the presentation matrix translates to an echo-amplitude axis (larger surfaces produce louder echoes). Along the surface-area axis, echo level varies by 15 dB (see Neurophysiology, Materials and Methods). A change of the response category due to the change of stimulus level for either object 1 or object 2 occurred in 18 out of 21 units tested. The effect of changing the overall presentation level by 10 dB is illustrated in Figure 8. The data show that the categorization of a unit as “scaled” depends on the choice of an adequate range of presentation levels.

**Discussion**

In a combined psychophysical and electrophysiological approach, this study tested the ability of the echolocating bat *P. discolor* to normalize for size-induced variations of virtual echo-acoustic objects. The psychophysical results showed that the bats spontaneously associated most scaled virtual objects with the corresponding standard object. A simulation of the psychophysical paradigm based on spectrotemporal pattern recognition indicates that a complex central auditory circuitry is required to explain the bats’ performance. A neurophysiological correlate of this perceptual accomplishment is found in size-invariant responses to echoes from these virtual objects recorded from units in the auditory cortex. The population of these units comprised 13% of all cortical units investigated.

The psychophysical experiment was implemented as a real-time playback experiment. Consequently, any changes in the bat’s echolocation call loudness, or in the distance between the bat and the virtual object in the setup, are preserved in the echo. If for example, a bat chose to emit a louder call towards a relatively faint IR from a small object, the perceived echo may be louder than the echo of a fainter call towards a louder IR. Thus, as in a natural echo-imaging task, this experimental paradigm requires the bats to evaluate the IR independent of the echo variations caused by call-loudness or

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**Table 1. Categorization of the Recorded Units into Six Response Categories**

| Unit Categorization | Scaled | Depth | Surface | Ambiguous |Insensitive | Irresponsive | Sum |
|---------------------|--------|-------|---------|------------|------------|-------------|-----|
|                     | 14     | 14    | 62      | 10         | 57         | 8           | 109 |

The numbers shown represent the number of units for which at least one of the two recorded response matrices met a specific category. Because the total number of recorded matrices is double the number of recorded units (each unit was tested with two objects), the numbers add up to more than the 109 recorded units.

**Table 2. Observed Combinations of Response Categories for the Two Investigated Objects**

| Categorization of Responses to Object 1 | Categorization of Responses to Object 2 |
|----------------------------------------|----------------------------------------|
| Scaled       | Depth       | Surface     | Ambiguous | Insensitive | Irresponsive | Sum    |
|--------------|-------------|-------------|-----------|-------------|--------------|--------|
| Scaled       | 1           | 1           | 1         | 0           | 4            | 0      | 7      |
| Depth        | 1           | 1           | 3         | 0           | 2            | 1      | 8      |
| Surface      | 1           | 2           | 26        | 2           | 16           | 0      | 47     |
| Ambiguous    | 2           | 0           | 3         | 0           | 2            | 0      | 7      |
| Insensitive  | 3           | 3           | 8         | 1           | 18           | 0      | 33     |
| Irresponsive | 0           | 0           | 0         | 0           | 7            | 0      | 7      |
| Sum          | 8           | 7           | 41        | 3           | 42           | 8      | 109    |

The numbers given correspond to the number of units whose response matrices for the two objects fall into the specific combination of response categories. For example, one unit was found for which the response matrices in response to both objects fall into the “scaled” category (top left). Eighteen units were found that were insensitive to any object feature for both object 1 and 2 (third row from bottom, third column from right). The right-most column and the bottom row show the summed numbers of units in a category for objects 1 and 2, respectively.

*discolor*.
Object-Oriented Echo Processing

The limits of the two scatter plots (B and C) are superimposed on the photograph of the P. discolor brain (A) (box). The scatter plots show the recording sites for units tested with objects 1 (B) and 2 (C). Units selective for scaled versions of an object (shown as filled red circles) were absent in the most anterior region of auditory cortex.

doi:10.1371/journal.pbio.0050100.g007

Comparison with Previous Studies

In nature, a bat can evaluate an object by integrating the echo information of the object from different ensonification angles. In fact, the echo spectra of bat-pollinated flowers changed significantly with the angle of incident sound, whereas echoes from single positions often were quite similar [19]. von Helversen [21] showed correct size-independent discrimination of real targets performed by the echolocating bat Glossophaga soricina. von Helversen proposed that correct object classification is guided by the changes in the spectral pattern of the returning echoes at different ensonification angles. Object recognition could then depend on serial integration of acoustic signals. The ability to integrate over a sequence of signal has been demonstrated in songbirds and mammals (e.g., see [24, 25]). In contrast, the bats in the current study were confronted with the IRs of static, virtual objects.

Figure 8. Influence of Overall Presentation Level on Responses of Cortical Units

(A) The unit shown in the figure responded best to properly scaled versions of object 2 (diagonal axis) only at an adequate range of presentation levels. (B) A 10-dB change in the overall presentation level pushed the stimuli out of the range the unit was sensitive to. The overall presentation level is indicated above each panel. Abscissa: normalized object depth. Ordinate: normalized surface area. maxSp, maximum number of spikes.
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Figure 7. Location of Recording Sites in the Auditory Cortex of P. discolor

The limits of the two scatter plots (B and C) are superimposed on the photograph of the P. discolor brain (A) (box). The scatter plots show the recording sites for units tested with objects 1 (B) and 2 (C). Units selective for scaled versions of an object (shown as filled red circles) were absent in the most anterior region of auditory cortex.
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standard. assign most responses to scaled versions of both objects to the correct
scaled versions of object 2, but also to discriminate the two objects and
cortical unit. The unit was not only able to respond best selectively to
(C and D) show the results of a simulation of both the behavioral
Paradigm
Hypothetically, the bats’ echo-acoustic object-normalization
ability will improve further when they can evaluate sequences
information from different ensonification angles was missing.
Thus, a normalized object representation requires response
invariance for a meaningful co-variation of these two
acoustical parameters.
Thus, the bats received only a one-dimensional echo-acoustic
image of these virtual objects. Nevertheless, the bats were able
to classify scaled versions of these IRs correctly, although the
information from different ensonification angles was missing.
Hypothetically, the bats’ echo-acoustic object-normalization
ability will improve further when they can evaluate sequences
of echoes from different ensonification angles.
Cortical Representation of Objects
The stimuli used in this study differ fundamentally from
those used in many previous investigations in which cortical
sensitivity for a specific stimulus parameter is investigated.
Namely, echoes from objects of different size differ both in
echo intensity (larger objects produce louder echoes) and in
the echo duration (larger objects produce longer echoes). Thus,
a normalized object representation requires response
invariance for a meaningful co-variation of these two
acoustical parameters.
Heil [26] showed that neurons in the cat auditory cortex
were tuned to the slope of the stimulus onset. In principle,
such a mechanism could account for the described scale-
invariant responses. When the size of the presented virtual
objects changes, the change in signal duration affects the
stimulus slope linearly. However, the corresponding change
in surface area affects the stimulus slope quadratically (see
Materials and Methods). Consequently, the slope of the
stimulus onset is not constant over different scales of the
same virtual object. Thus, tuning to onset slope cannot fully
account for the described scale-invariant responses.
Galazyuk and Feng [27] showed that the best duration in
duration-sensitive neurons in the auditory cortex of the little
brown bat decreased with increasing amplitude. In our
experiments, echo duration increased with object depth and
thus co-varied in a systematic manner with echo amplitude
for scaled versions of objects. Thus, stimulus intensity-
dependent duration sensitivity of cortical units might be
the underlying mechanism in units that encoded scaled
versions of complex objects.
An important parameter influencing object normalization
might be the bats own vocalization. It was shown for neurons
in the inferior colliculus of the greater horseshoe bat
(Rhinolophus ferrumequinum) that the bat’s own vocalization altered the response to pure tones and frequency-modulated
stimuli, presumably via direct neuronal influence of vocal
activity onto collicular neurons [28]. This finding makes it
highly likely that the response to scaled versions of acoustic
objects is also influenced by the bat’s own vocalization. As
mentioned above, a mechanism like this would also be very
favorable for bats, because the bats could compensate for
differences in their outgoing echolocation calls that could
otherwise be misattributed to object properties.
Units that responded most strongly to scaled versions of
objects were not found in the most anterior parts of the
auditory cortex, but were mostly located in the high-
frequency parts of primary auditory cortex. This finding is
interesting, because units that were sensitive to echo rough-
ness were mainly located in anterior regions of the auditory
cortex of P. discolor [15]. Because the processing of temporal
envelope features is a prerequisite for encoding of echo
roughness, the stimuli used in the present study provided no
temporal envelope cues that could be used for object
normalization; the spatial distribution of roughness-encoding
units on the one hand and size-invariant units on the other
hand seems to reflect a principle separation of encoding
properties in anterior and posterior parts of the auditory
cortex of P. discolor.
The current study supports the hypothesis that the auditory
system has dedicated mechanisms to deal with the
compensation of size-induced variations of acoustic sources.
The fact that the size-invariant neural responses were
obtained from individuals that had not been exposed to the
stimuli behaviorally, indicates that this mechanism is not
experience dependent, but hard wired.

Materials and Methods
Experimental animals. The experimental animal used in this study
was the New World bat Phyllostomus discolor (family: Phyllostomidae).
The animals originated from a breeding colony in the Department
Biologie II of the Ludwig-Maximilians-University in Munich. P.
discolor emits short (<3 ms) broadband downward-modulated multi-
harmonic echolocation calls in the frequency range between 40 and
90 kHz (see Figure 1E). It feeds mainly on fruit, pollen, and insects
[29], the insects being gleaned mainly from the vegetation.
Psychophysics. The psychophysical experiments were imple-
mented as virtual-object playback experiments. The bats were
required to evaluate echoes of their own echolocation calls. These
echoes were generated by convolving in real time the calls with IRs of
a virtual object. Thus, unlike in classical psychoacoustic experiments,
the bats did not hear a sound unless they emitted echolocation calls.
Five adult P. discolor (four females, one male, body weight 30 to 40
g) took part in the training. The animals were housed in boxes (80 cm
× 40 cm × 50 cm) with free access to water, and separated for sexes. In

Figure 9. Putative Performance of Single Units in the Psychophysical
Paradigm
(A and B) show the rate responses of a unit to all versions of both
standard objects. This unit responded best to scaled versions of object 2.
Responses to object 1 were assigned to the “depth” category.
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discolor emits short (<3 ms) broadband downward-modulated multi-
harmonic echolocation calls in the frequency range between 40 and
90 kHz (see Figure 1E). It feeds mainly on fruit, pollen, and insects
[29], the insects being gleaned mainly from the vegetation.
Psychophysics. The psychophysical experiments were imple-
mented as virtual-object playback experiments. The bats were
required to evaluate echoes of their own echolocation calls. These
echoes were generated by convolving in real time the calls with IRs of
a virtual object. Thus, unlike in classical psychoacoustic experiments,
the bats did not hear a sound unless they emitted echolocation calls.
Five adult P. discolor (four females, one male, body weight 30 to 40
g) took part in the training. The animals were housed in boxes (80 cm
× 40 cm × 50 cm) with free access to water, and separated for sexes. In
these boxes, they were only fed on days without training sessions, i.e., only for 2 d after a 5-d training period. During the training sessions, they were fed with banana pulp for reward. On the days without training, they had access to mealworms (larvae of Tenebrio molitor) ad libitum.

**Immiscible responses.** A hundred IRs with 12 single reflections each of random relative level were generated (750 samples, 1.86 ms). Then, those two IRs with the largest mean-squared difference in the time domain were determined. These two IRs were taken as standard IRs of virtual objects 1 and 2.

Both IRs then were scaled with the following scaling factors (Scf): 0.67, 0.8, 1.25, and 1.5. The time delay of each reflection was scaled with Scf. The amplitude of each reflection was scaled with Scf² because the level of a reflection is proportional to surface area and the latter is proportional to the square of the radius. Consequently, the IR produced with an Scf of 0.67 was 15 dB fainter than the same IR scaled with Scf 1.5. The duration of both objects is shown in Figure 1A. All IRs had frequency-independent, white magnitude spectra. A convolution of these IRs with an echolocation call is equivalent to the multiplication of the spectra of call and IR. Thus, the high similarity of the echo spectra shown in Figure 1C reflects the similarity of the IR spectra. This similarity occurs because the 12 reflections with random delays do not produce a systematic spectral ripple.

**Experimental set up.** The bats were trained in a two-alternative, forced-choice (2-AFC) playback setup as used in former experiments with bats [30]. It consisted of two boxes mounted on the wall of an echo-attenuated chamber at an angle of 45°. A starting perch was located at the top end, and a feeder was mounted at the end of each leg. The angle between the legs was 60°. A ¼-inch microphone (Microtech Gefell MK 301; Gefell, Germany) was located in the middle of the maze to pick up the bats’ sonar emissions. The transfer function of the concentric-microphone was measured at varying sound pressure levels and transferred to a 2048 matrix in which object surface area (vertical dimension) and object depth (horizontal dimension) co-varied in a meaningful manner only along the diagonal axis, thus representing properly the auditory periphery up to the stage of the auditory nerve. First, we convolved each IR of each object with a standard echolocation call. Then, the convolved signal was sent through outer and middle ear filters that mimicked the absolute thresholds of P. discolor as described by Esser and Daucher [51]. To simulate the inner ear characteristics, we applied a gammatone filter bank, consisting of 25 channels with center frequencies equally spaced on a log frequency scale between 20 and 110 kHz. The transfer function of the gammatone filters is designed to mimic the shape of the distortion product, otoacoustic-emission tuning curves of P. discolor as measured by Wittekindt et al. [52]. After half-wave rectification and exponential compression, we applied a phase-locking filter (cutoff frequency: 1 kHz; 12 dB/octave). The resulting signal is then resampled at 20 kHz. Thus, we obtained the auditory spectrograms generated by echoes as they are perceived by the bats in the experimental setup.

The auditory spectrograms generated with the two standard objects IRs, as shown in Figure 1C, were rescaled for four of the eight scaled versions of the standard objects, as shown in Figure 3C–3F.

Next, the mean-squared differences between the auditory spectrograms generated with scaled objects and those generated with standard objects were calculated. Based on these mean-squared differences, the simulation classified the auditory spectrograms of the scaled objects as either similar to the spectrograms of standard object 1 or standard object 2. Thus, the simulation works as a spectro-temporal pattern recognition method. The difference simulation has no concept of scaling, and thus it serves as a null hypothesis for a behavioral test of echo-acoustic object normalization.

**Neurophysiology—surgery.** Four adult bats were used for the neurophysiological experiments. All experiments compiled with the principles of laboratory animal care and were conducted under the regulations set forth in the current German Animal Protection (approval 209.1211-2531–68/03, Reg. Oberbayern). The principle surgical procedure has been described in detail elsewhere [33]. In brief, bats were anesthetized using MMF (medetomidin 0.4 μg, midazolam 4 μg, and fentanyl 0.04 μg per gram body weight). The skin overlying the skull was opened by means of a trephine. The dura was freed from tissue. A small metal tube was fixed to the skull using a microglass composite in order to fixate the animal to a stereotactic device, and the accurate skull position in stereotactic coordinates was determined as described in detail elsewhere [34].

**Neurophysiology—stimulus production and recording of neural responses.** Experiments were conducted in a sound-attenuated chamber. Acoustic stimuli were computer generated (Matlab; Mathworks), digital-analog converted (RX6 [Tucker Davis Technologies, Gainesville, Florida, United States], sampling rate: 200 kHz), attenuated (PA5; Tucker Davis Technologies), and binaurally presented via ultrasonic earphones with a flat frequency response (≤3 dB) between 10 and 100 kHz [35].

Stimuli consisted of a typical echolocation call of P. discolor (Figure 1E) convolved with IRs identical to the IRs used in the psychophysical experiments. The resulting IRs were amplified in the test trials, the bats’ spontaneous classification of the scaled objects as either object 1 or object 2 was assessed. Data acquisition stopped when the slowest animal had performed at least 30 trials for each scaled object. The spontaneous performance for each scaled object was numerically tested for significance using a χ² test based on 10,000 repetitions of the 2-AFC experiment with the given number of trials and a random performance. Significance was set at p < 0.05.

**Simulation of the classification of scaled virtual objects via auditory spectrograms in P. discolor.** A bat does not hear the IR of an object itself, only object-related sounds. In the simulation, two object-related sounds are convolved with each other, and the resulting IRs can be presented to bats. However, these convolved IRs are modified in the auditory periphery of the bats. To simulate the classification of the scaled objects used in this study, we have to take account of these modifications. We simulated the object-oriented echo processing.
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### Data analysis

Spike responses from all 25 stimuli for each object were displayed as raster plots (see Figure 6C and 6D). An analysis window was set automatically by moving a 10-ms window in 1-ms steps over the time course of recorded activity and computing a Wilcoxon signed rank test (p < 0.01). Matlab Statistics Toolbox, Mathworks) over the 25 stimuli and the first 10–50 ms preceding each stimulus (spontaneous activity). The first point at which two successive windows led to significant responses was taken as the start of the analysis window; the last position of two successive significant windows was taken as the end of the analysis window. Spikes were summed over the analysis window; normalized such that the maximum number of spikes was set to unity. The normalized responses were arranged as color-coded plots in a 5 × 5 matrix corresponding to the stimulus matrix (cf. Figure 1D). If no significant response was detected by the analysis window, the neuron was categorized as “irrelevant” (Figure 4J).

In all other cases, a Kolmogorov-Smirnov test (kstest2, Matlab Statistics Toolbox, Mathworks) was used to test whether the five responses, e.g., for a specific object depth, belonged to the same, continuous distribution as all other responses in the response matrix. Exemplary, synthetic response matrices, in which this hypothesis can be rejected with p < 0.05, are illustrated in Figure 4A and 4F. This analysis was performed, not only along the vertical dimension (object depth), but also along the horizontal dimension (object surface area, Figure 4B and 4G) and along the diagonal dimension (object scale, Figure 4C and 4H). The test along the diagonal was performed for the main diagonal and one parallel above and below. This was done in order to avoid misclassifying units due to the comparison of only low numbers of positions in the 5 × 5 matrix (the shortest diagonal line in the matrix would contain only two positions). If the null hypothesis could be rejected for more than one of the three tested dimensions, the response matrix was categorized as “ambiguous.” If the null hypothesis could be rejected for none of the three dimensions, the response matrix was categorized as “insensitive.”

### Acknowledgments

We thank Susanne Radtke-Schuller and Claudia Schulte for their help with the reconstruction of recording sites and histological procedures, and Horst König for technical help. Special thanks go to Susanne Hoffmann for help during the experiments. We would like to thank Benedikt Grothe, Roy Patterson, and Gerhard Neuweller for many fruitful discussions about the current topic. We also thank two anonymous reviewers for very constructive comments on earlier versions of the manuscript.

### Author contributions

UF, MS, JEG, and LW conceived and designed the experiments. UF and MS performed the experiments. UF, MS, and LW analyzed the data. GS contributed reagents/materials/analysis tools. UF, MS, and LW wrote the paper.

### Funding

This work was supported by the Volkswagen Foundation (I/79782).

### Competing interests

The authors have declared that no competing interests exist.

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