

RESEARCH ARTICLE

Echo-acoustic scanning with noseleaf and ears in phyllostomid bats

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ABSTRACT

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

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

INTRODUCTION

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

requires that larger volumes are investigated successively, i.e. that larger volumes are scanned.

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

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

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

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

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

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

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

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

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

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

MATERIALS AND METHODS

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

Experimental setup

The setup consisted of a cart (see Fig. 1A) that was driven along a 6 m long linear rail (ITEM Industrietechnik GmbH, Solingen, Germany). The cart was moved via a geared belt drive and a motor (DC Servomotor Serie 3268 BX4 AES, Dr Fritz Faulhaber GmbH & Co. KG, Schönaich, Germany) that was controlled by a computer. The position of the cart was recorded at a sampling rate of 10 Hz. The bat was positioned in a holder on the cart, mounted on a ramp (25 deg slope), which kept the animal's body in a steady position while its head and ears remained motile. The holder was stuffed with soft foam to avoid injury to the bats, covered with tissue adhesive tape for easy cleaning and lined with exchangeable cloth. It was composed of two half-shells that were sealed by hook-and-loop fasteners to prohibit the animal from escaping. On each side, a pole in the front part of the holder was

placed between the head and the respective wing. Hence, the animal could not escape to the front.

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

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

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

Reflectors

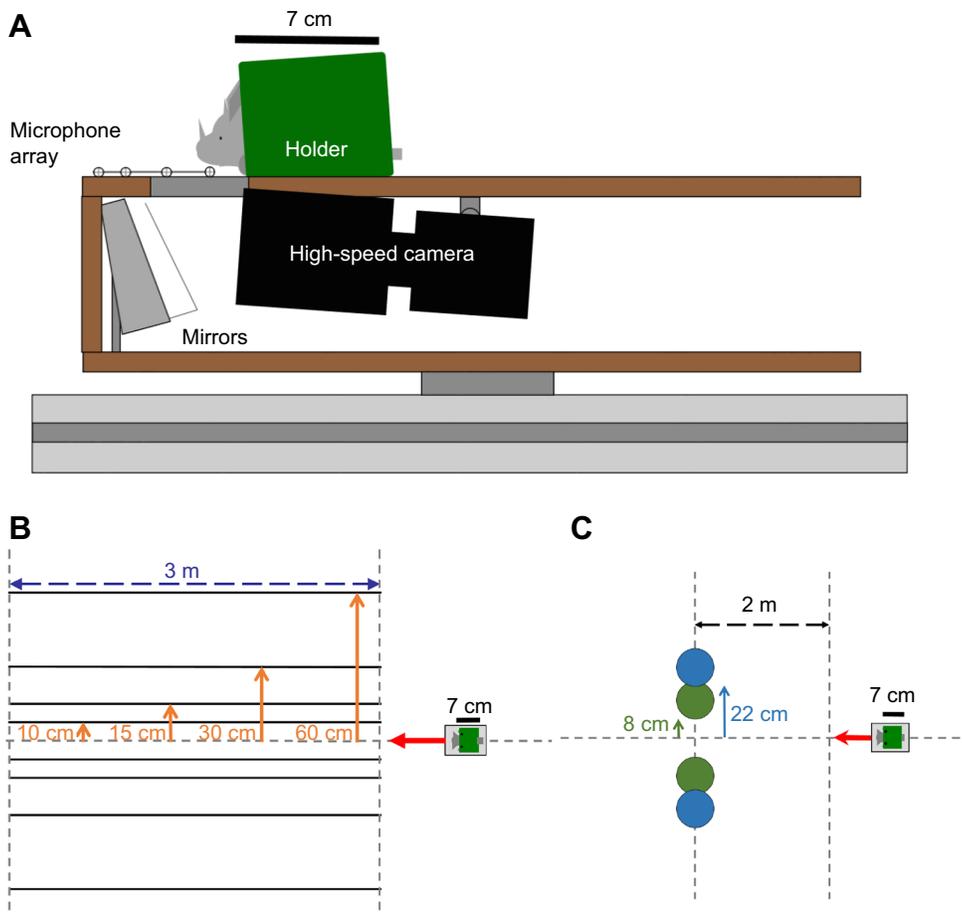
We define reflectors as objects in the surroundings that the bat can detect using echolocation while in the apparatus. We used two types of reflectors: wall and column reflectors. The wall reflectors flanked the test section, constituting elongated structures which produced a relatively constant sensory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

Ridged wall reflectors

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

Column reflectors

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

**Fig. 1. Experimental setup.**

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

Procedure and data acquisition

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

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

Analysis

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

Video analysis

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

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

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

For the call-correlated ear movements, the distance between the markers at half-height of the frontal rim of the ear was calculated

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

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

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

Audio analysis

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

Animals

The experimental animals were 6 adult specimens of the lesser spear-nosed bat, *Phyllostomus discolor* Wagner 1843. Data were recorded for 3 individuals (2 males, 1 female) with the ridged wall

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

RESULTS

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

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

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

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

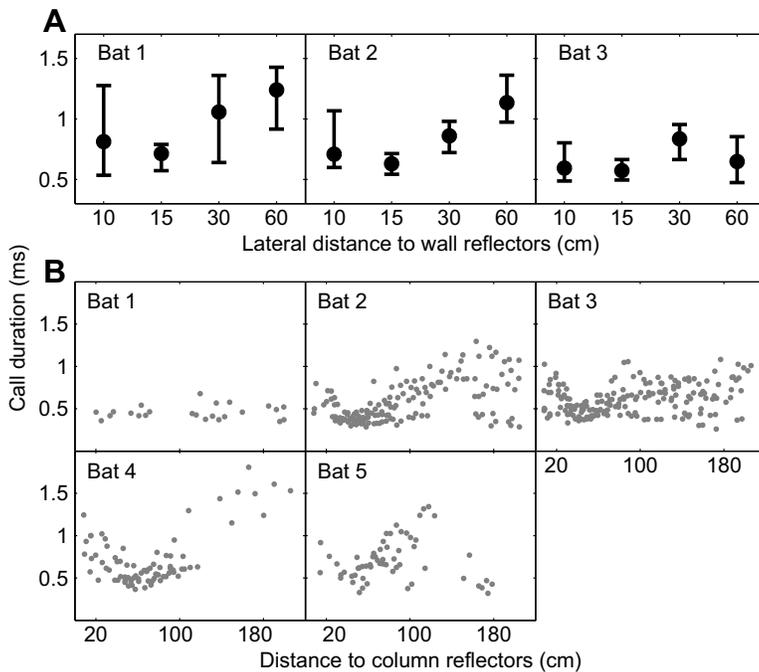


Fig. 2. Analysis of the duration of echolocation calls dependent on reflector distance. (A) For the experiment with wall reflectors, call durations for all calls an animal emitted within vertically orientated walls were pooled for each lateral reflector distance (10, 15, 30 or 60 cm). The graphs show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

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

Call-correlated movements of the bats' ears likewise conformed to a stereotyped pattern: for each call, both ears were raised and set in an upright position pointing both ears towards the front, before the tension was released again. Raising of the ears led to a decrease in the distance between the ear markers (see Fig. 5A). The release of the tension caused a downward movement of the ears to a more lateral and suspended position. This downward movement was often omitted when the call was the first in a group, i.e. when the following call occurred within the next 35 ms. Again, these movements were quantified as distance measures between the two

markers on the bats' ears. An example trace of the distance is shown in Fig. 5C. Fig. 5B shows how the distance between the ears changes as function of time to call emission. The data show that the ears start to rise about 35 ms before call emission and that this ends about 45 ms after emission. Ears are maximally raised about 2–8 ms after call emission (Fig. 5D).

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

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

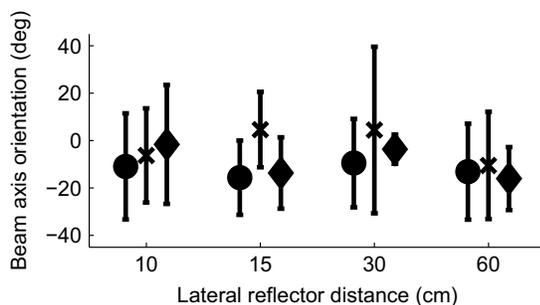


Fig. 3. Beam axis orientation for different lateral reflector distances while bats are moved between the wall reflectors. The beam axis orientation is defined as the direction in which the bats point their sonar beam. Negative values indicate beam orientations towards the left, positive values indicate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

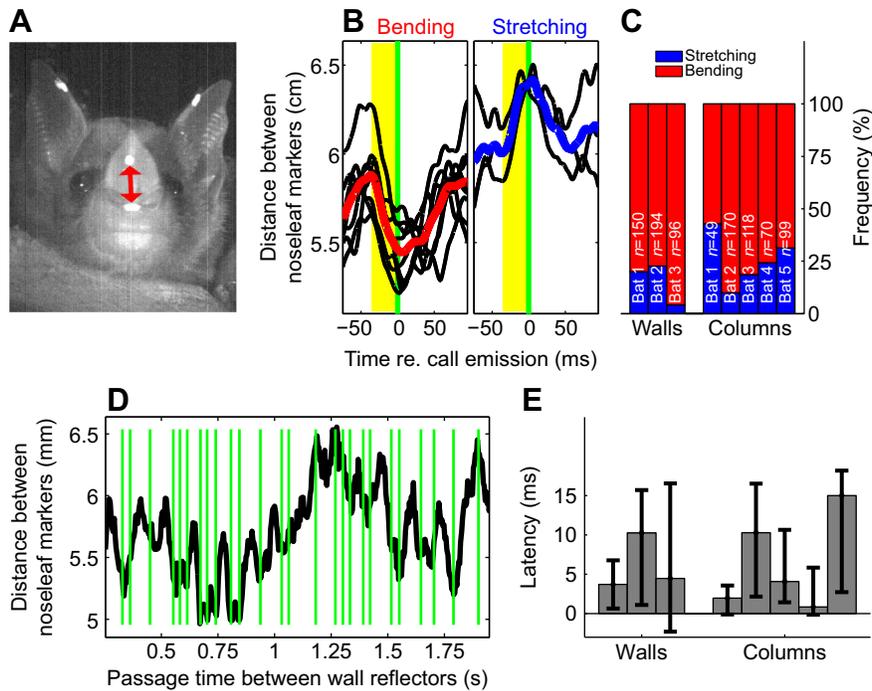


Fig. 4. Call-correlated movements of the noseleaf. (A) A bat performing the experiment. Markers were placed at the base and at half-height of the noseleaf and at half-height of the frontal rim of each ear. A movement of the noseleaf is quantified as a change in distance between the two markers (red arrow)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

their ears more when the reflectors were closer. This effect was significant for bat 2 with the wall reflectors and for bats 2, 3 and 5 with the column reflectors. Overall, these data indicate that the

call-correlated ear movements of the bats are not stereotyped motor programmes but depend on the echo-acoustic layout of the environment.

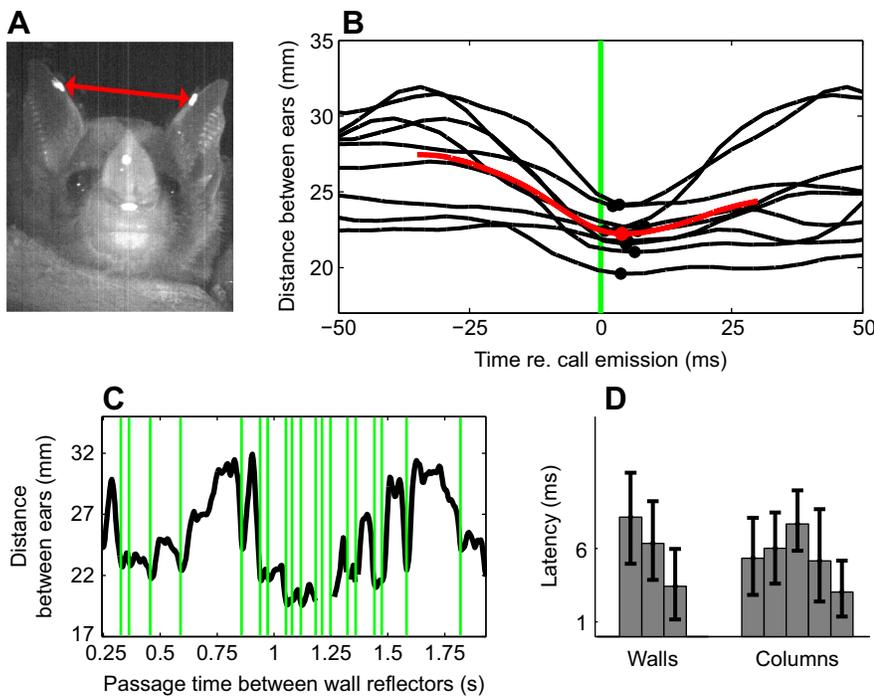


Fig. 5. Call-correlated ear movements. Same format as in Fig. 4. (A) The distance measure taken to quantify ear movements. The distance decreased when the bats raised their ears and increased when the ears moved down and sideways. (B) The distance between the two ear markers in a fixed time window around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

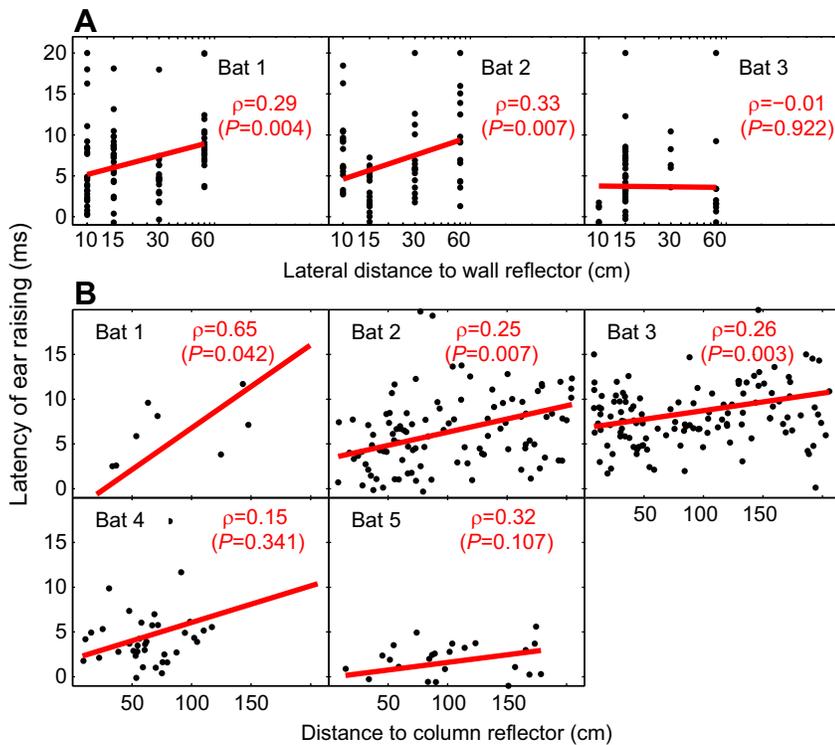


Fig. 6. Correlation between the latency of ear raising and distance to reflectors. (A) Lateral distance to the wall reflectors and the latency of ear raising. Bats 1 and 2 both raised their ears significantly earlier when the walls were closer. (B) Direct distance to the column reflectors and latency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

DISCUSSION

The novel bat cart setup allowed for very accurate 3D reconstruction of facial structures on the move: we were able to monitor noseleaf and ear movements as well as echolocation behaviour while moving the bats along different lateral reflectors. In our experiments, most bats adjusted their echolocation behaviour according to their distance to the reflectors by reducing call duration when closer reflectors caused the risk of

echo overlap. This is in line with previous observations (Aytekin et al., 2010; Griffin, 1958; Moss and Surlykke, 2010; Neuweiler, 1989; Schnitzler and Kalko, 2001). Our results also reveal that the characteristic noseleaf and ear movements that had been reported anecdotally in previous publications are precisely timed with call emission, even though a previous paper claimed otherwise for phyllostomid bats (Pye and Roberts, 1970). However, there remains plasticity in this behaviour, as the bats are able to adjust

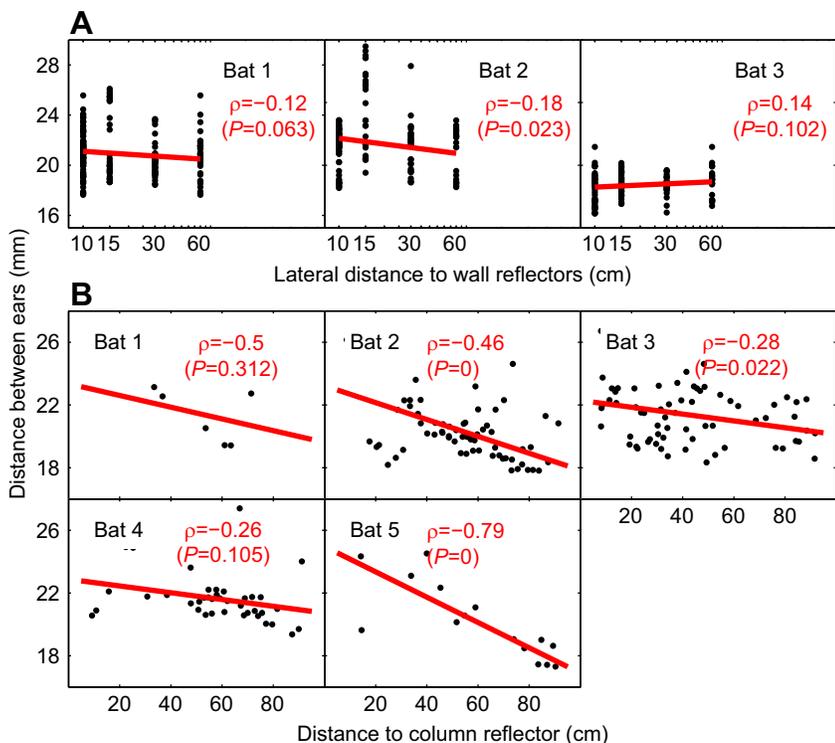


Fig. 7. Correlation between magnitude of ear movement and distance to reflectors. The magnitude of ear movement was expressed as the distance between the ears. (A) Lateral distance of the wall reflectors and the minimal distance that was detected for the respective ear-raising movement. Bats 1 and 2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

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

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

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

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

The way an incoming sound is changed by the ears depends on both sound frequency and the angle of incidence. These

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

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

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

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

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Competing interests

The authors declare no competing or financial interests.

Author contributions

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

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Supplementary information

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