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RESEARCH ARTICLE

Optic and echo-acoustic flow interact in bats

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ABSTRACT

Echolocating bats are known to fly and forage in complete darkness, using the echoes of their actively emitted calls to navigate and to detect prey. However, under dim light conditions many bats can also rely on vision. Many flying animals have been shown to navigate by optic flow information and, recently, bats were shown to exploit echo-acoustic flow to navigate through dark habitats. Here, we show for the bat *Phyllostomus discolor* that, in lighted habitats where self-motion-induced optic flow is strong, optic and echo-acoustic flow interact to guide navigation. Echo-acoustic flow showed a surprisingly strong effect compared with optic flow. We thus demonstrate multimodal interaction between two far-ranging spatial senses, vision and echolocation, available in this combination almost exclusively in bats and toothed whales. Our results highlight the importance of merging information from different sensory systems in a sensory-specialist animal to successfully navigate and hunt under difficult conditions.

KEY WORDS: Echolocation, Navigation, Flow field, Flight guidance, Multimodal integration

INTRODUCTION

While navigating and foraging, bats have to adapt their flight manoeuvres to the complex spatial layout of natural habitats. Self-motion-induced echo–acoustic flow is readily available to tackle this challenging task. Echo-acoustic flow is the continuously changing stream of acoustic stimulation derived by echoes reflected from objects in the environment while a bat is flying. Unlike echo delay, echo-acoustic flow provides information not only about object distance but also about the geometric relationship between objects relative to the bat (McKerrow, 2008).

Experimental and theoretical work has provided evidence that bats respond to echo-acoustic flow (Kugler et al., 2016; Lee et al., 1992; Müller and Schnitzler, 1999; Warnecke et al., 2018a, 2016). Although explicit information about the distance of objects is provided by echolocation through the delay between call emission and echo arrival time (Simmons, 1971), echo-acoustic flow is used to adjust the lateral distance along structured surfaces (Kugler et al., 2016; Warnecke et al., 2016). This behaviour is often observed, for example, in bats following the edges of vegetation in commuting flight (Holderied et al., 2006).

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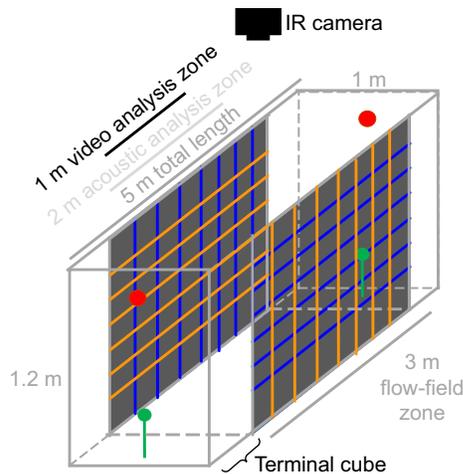H.L., 0000-0002-9835-7715; L.W., 0000-0002-9289-6187; U.F., 0000-0003-2803-3587

However, information gained by echolocation is sparse because of the pulsed pattern of call emission. In addition, the amount of information gained by echolocation can be reduced by echo jamming with other bats, cluttered backgrounds or other factors hampering echo detectability. Merging information from different sensory systems is typically thought to provide a remedy for this problem (Stein and Meredith, 1993). This so-called multimodal integration has been demonstrated for many different animal models and for a number of different senses in behavioural and neurophysiological studies (e.g. Gottfried and Dolan, 2003; Meredith and Stein, 1983, 1986; Verhaal and Luksch, 2016; Winkowski and Knudsen, 2007). Although often disregarded, many bats can rely on vision under dim light conditions (Bell and Fenton, 1986; Bradbury and Nottebohm, 1969; Danilovich et al., 2015; Eklöf, 2003; Horowitz et al., 2004; Orbach and Fenton, 2010; Rother and Schmidt, 1982), and even ultraviolet light sensitivity has been demonstrated (Müller et al., 2009; Winter et al., 2003). Furthermore, the relatively large eyes and use of short, frequency-modulated echolocation calls in many neo-tropical fruit bats seem to closely resemble the features of ancestral bats (Thiagavel et al., 2018). Under light conditions, obstacle avoidance of flying bats was improved (e.g. Orbach and Fenton, 2010; Rother and Schmidt, 1982) and it was shown that both vision and echolocation can integrate with vestibular signals to coordinate motion during complex flight (Horowitz et al., 2004). Perceptual integration of sparse echo information and visual information under dim light might therefore represent a default mode in bats. In particular, the integration of echo-acoustic flow and optic flow (in our case defined as the pattern of apparent motion of surfaces and edges in a visual scene caused by the relative motion between the bat and this scene) might be important.

To test this hypothesis, we designed a flight-tunnel experiment using the neo-tropical fruit-eating bat *Phyllostomus discolor* Wagner 1843. *Phyllostomus discolor* uses short multi-harmonic, broadband calls (40–90 kHz) for echolocation. The bats have frontally oriented, binocular vision (acuity about 1.63 cycles deg^{-1}) and peripheral monocular vision (Hoffmann et al., 2016).

Specifically, we addressed the question of how optic flow interacts with echo-acoustic flow to guide flight of these bats through a structured flight tunnel. Bats were trained to fly through a dimly lit (~10 lx) tunnel with exchangeable black walls that gave rise to either strong or weak optic flow (elicited by vertical or horizontal white stripes; Fig. 1). The flight paths of the bats were monitored with a camera from above. In a second experiment, we investigated how flight guidance is affected when echo-acoustic flow and optic flow are in conflict. For this experiment, the black walls were equipped with ridges that elicit echo-acoustic flow and perpendicularly oriented white stripes that elicit optic flow. Data from an earlier experiment (Kugler et al., 2016) were re-analysed to allow comparison with the echo-acoustic flow-only condition (tunnel walls with weak or strong echo-acoustic flow under complete darkness).

Fig. 1. Schematic diagram of the flight tunnel. The setup consists of a central 3 m-long zone within the flight path, where stimuli were presented, and one terminal cube, separable with curtains, on either side. Audio was recorded with two ultrasonic microphones (red dots), installed at 90 cm height midline position at the tunnel ends. Flight paths were monitored and recorded via an infrared (IR) camera, centred above the flight tunnel. After a tunnel passage, the bats were rewarded in one of the terminal cubes with fruit pulp delivered via a feeder (green dots) with a light barrier. Stimuli producing echo-acoustic flow were sidewalls carrying horizontal or vertical ridges (blue) along the central 3 m of the flight tunnel. Optic flow was produced using white stripes on black side walls in the same arrangement (orange). Conflicting, echo-acoustic versus optic flow stimuli were obtained by presenting ridges and white stripes rotated by 90 deg relative to each other.

MATERIALS AND METHODS

Experimental setup

Previous work (Kugler et al., 2016) examined the effect of echo-acoustic flow on the flight and echolocation behaviour in free-flying bats of the species *P. discolor*. In the present study, we extended the experimental paradigm to quantify the behavioural response to optic flow and the interactions with echo-acoustic flow in the same bat species.

In our previous study (Kugler et al., 2016), bats were exposed to echo-acoustic flow created by structured wooden side walls, while flying from one end of a 5 m long and 1 m wide flight tunnel to the other in darkness. Each side wall could be set to create stronger (vertical orientation of ridges in the side wall) or weaker (horizontal ridge orientation) echo-acoustic flow (see Fig. 1 and photos in Fig. 3). Data from this experiment were reused to compare the bats' behaviour in optic and conflicting flow fields with their behaviour in echo-acoustic flow fields. For a detailed description of the setup, stimuli, data acquisition and analysis procedure in the previous experiment, refer to Kugler et al. (2016).

In the present study, we partly rebuilt the setup used previously (Kugler et al., 2016). The new setup (see Fig. 1) allowed us to create optic flow as well as contradictory echo-acoustic and optic flow. LED strips (24 V white 9.6 W m^{-1} cold-white; Abrams und Mantler GmbH & Co KG, Minden, Germany) were installed bilaterally on the tunnel floor; they were shielded with frosted glass profiles, which dispersed the light (Cover flat, frosted 2 m M-Line, LED-Shop.com, Bad Endbach, Germany), and inserted into acoustic foam in the tunnel floor along the 3 m test section, approximately 20 cm from each side wall. The tunnel floor was covered with white cloth for high contrast in the video. The illumination level was dimmed (LED-Dimmer Controller monochromatic, dimmable LED-Strips 12 V–24 V, revoART ® e.K., Borsdorf, Germany) to 10 lx (measured with a luxmeter at the midline, 1 m into the flight tunnel at the estimated flight height).

Stimuli

In the present study, we used different stimuli to expose the bats to the different flow fields: echo-acoustic flow alone, optic flow alone and echo-acoustic flow versus optic flow. Ridges served as stimuli producing echo-acoustic flow (see Fig. 1), stripes served as stimuli producing optic flow. In the echo-acoustic versus optic flow condition, ridges and stripes were perpendicular to each other, resulting in conflicting information in the auditory and the visual domain. Specifically, we painted the wooden side walls black and applied white, 1.5 cm broad strips (Iso tape, 10 m×15 mm, white, Tesa, Norderstedt, Germany) on the black walls to create optic flow; stripes were repeated at 9 cm intervals (same as the ridging period of the echo-acoustic stimuli), resulting in an inter-stripe distance of 7.5 cm. For presenting only optic flow, the white strips were glued onto smooth, black walls (see Fig. 3, middle row and photo on the left). To create conflicting echo-acoustic and optic flow, the white strips were glued at a period of 9 cm onto black walls with ridges (see Fig. 1 and Fig. 3, bottom row and photo on the left), so that the stripes were orthogonal to the ridges. Ridge/stripe orientation was changed by rotating the sidewall. For optic flow alone, data were recorded in all eight possible experimental conditions (4 arrangements of walls × 2 flight directions): both walls with vertical stripes, both walls with horizontal stripes (symmetric conditions), left or right wall with vertical stripes and right or left wall with horizontal stripes (asymmetric conditions). For conflicting echo-acoustic and optic flow stimuli, only the asymmetric conditions (in both flight directions) were used. Echo-acoustic flow data from bats 3, 4 and 6 were recorded with ridge stimuli at 9 cm separation, and those from bat 7 with ridge stimuli at 4.5 cm separation (Kugler et al., 2016). Please note that flight path deviations from the midline of the flight tunnel evoked by echo-acoustic flow alone were not significantly different for 9 or 4.5 cm ridge separation.

Acoustic measurement of the flight tunnel

To characterize the reflective properties of the tunnel and the different wall patterns (i.e. horizontal or vertical ridges), we used a special ensonification device. This custom-built device consisted of a Knowles FG series microphone (FG-23629-D65) positioned in the left ear at the entrance of the ear canal of a plastic (scale 1:1) replica of a *P. discolor* head (Fig. S1), which was placed directly on top of and in straight alignment with a ScanSpeak D2004/602000 Tweeter. The emission consisted of a single previously recorded *P. discolor* call. The head replica introduced naturalistic head- and pinna-dependent directional monaural acoustic cues (De Mey et al., 2008) to the measurements. The ensonification device was positioned in the flight tunnel at the midline (50 cm from both walls), varying the location along the longitudinal dimension at 0, 3.3 and 6.6 cm from a randomly chosen start position close to the entrance of the tunnel (Fig. S2). In addition, measurements were collected with the device fixed in three orientations (at 0, +30 and −30 deg in the horizontal plane) relative to the midline of the flight tunnel while pointing into the tunnel (see Fig. S2). The received echo signal was digitized at 1×10^6 samples s^{-1} and high-pass filtered (third-order Butterworth filter with a centre frequency of 20 kHz). To arrive at independent samples of the echo strength, we subsampled the envelope of the received echo signal at intervals of 1.5 ms, i.e. the duration of the emission. We considered the three measurements along the longitudinal dimension as independent replications of the same experiment.

Training and data acquisition

Experiments were approved by the Regierung von Oberbayern (55.2-1-54-2532-221-14) and were conducted under the principles

of laboratory animal care and the regulations of the German Law on Animal Protection. Approval to keep and breed the bats was issued by Munich district veterinary office.

Four adult bats (bats 3, 4, 6 and 7 from Kugler et al., 2016; 2 female, 2 male) of the species *P. discolor* were trained in a dimly illuminated flight room to fly back and forth between two feeder platforms. Initially, the bats learned to associate a food reward (a fruit pulp mixture made from banana, melon, oats, honey and minerals) with the feeder platforms, where the pulp was delivered directly from a motor-driven syringe. On this basis, the bats learned to alternate between the two feeder platforms by being rewarded only when flying from one feeder platform to the other. Once the animals were familiar with this simple task, data acquisition commenced in the flight tunnel. Five days of experimental data acquisition were always followed by two resting days. This schedule was maintained during the 20 day data acquisition period. Data acquisition was conducted similar to that in our previous study (Kugler et al., 2016). Each bat was used in one experimental session with a maximum duration of 30 min per experimental day. At the beginning of a session, a single bat was placed into one of the terminal cubes of the setup that could be separated from the rest of the flight tunnel with a curtain. A trial was started when the curtain of the terminal cube was opened. After the bat passed the flight zone and entered the opposite terminal cube, the experimenter closed the curtain behind the bat and stopped data acquisition. The bat was rewarded with fruit pulp automatically after each trial, after interrupting a light barrier on the platform containing the feeder. Audio and video data were recorded as a 5 s ring buffer with sampling rates of 192,000 and 20 Hz, respectively. Data acquisition was carried out using custom-written Matlab® programs (MathWorks, Natick, MA, USA) complemented with SoundmexPro software (HörTech, Oldenburg, Germany) and Matlab® image acquisition and data acquisition toolboxes.

Data analysis

All trials without reversal of the flight direction were included in the data analysis. Reversals of flight direction were observed in less than ~3% of all trials, and could not be correlated to specific experimental conditions. Automated data analysis was carried out using custom-written Matlab® programs: flight paths were reconstructed in the central 1 m of the flight tunnel in 2D by blob analysis; the bats were detected as the dark region within the flight tunnel, and their 2D coordinates were determined as the centroid of the detected blob. On the basis of these 2D coordinates and the video frame rate, the forward flight velocity could be calculated.

Data were analysed for individual bats and flow conditions separately (Kugler et al., 2016): individual analysis was necessary to reveal possible inter-individual differences between the bats.

In total, we applied video analysis on 685 trials (261, 182 and 242 with echo-acoustic flow alone, optic flow alone and echo-acoustic flow versus optic flow, respectively). Acoustic analysis was carried out on a total of 659 trials (235, 182 and 242 with echo-acoustic flow alone, optic flow alone and echo-acoustic flow versus optic flow, respectively); 26 trials (7, 6, 5 and 8 with bat 3, 4, 6 and 7, respectively) acquired with echo-acoustic flow stimuli had to be excluded from audio analysis because of a microphone defect. For tests comparing the bats' performance in different flow-field conditions (echo-acoustic flow alone, optic flow alone and echo-acoustic flow versus optic flow), we included only data from the individuals for which data for all three datasets was acquired.

For analysis of the number of calls emitted by the bats in different flow fields, only audio data acquired in the central 2 m of the flight tunnel were included, because flight path reconstruction and thus determination of a bat's position up to 0.5 m adjacent to the terminal cubes was not always possible. This is because the bat's silhouette sometimes became indistinguishable from the components of the setup in the video.

RESULTS

In the following, we present data for asymmetric conditions only, i.e. where the two walls of the flight tunnel showed different orientations of ridges and/or stripes. Data gained from these conditions are most significant for the specific aims pursued in our present study, the analysis of the behaviour in response to conflicting echo-acoustic and optic flow in comparison to unimodal sensory flow. Data from experiments using symmetric conditions for echo-acoustic flow stimuli have been published previously (Kugler et al., 2016) and are therefore not repeated here. First, we analysed whether the bats altered their echolocation behaviour under the different experimental conditions by counting the calls the bats produced while passing through the central 2 m of the flight tunnel. The different conditions did not affect the bats' echolocation behaviour consistently. Overall, bats produced between 6 and 10 calls per passage of the tunnel (corresponding to a call rate of 13–27 calls s⁻¹ at an average flight velocity of 4.5 m s⁻¹), regardless of whether the tunnel was lit or not (Fig. 2, black and orange bars versus blue bars), or whether there were ridges on the side walls (that produced echo-acoustic flow) or not (Fig. 2, black and blue bars versus orange bars). We additionally analysed the inter-call interval (ICI) of emitted calls during flight in the tunnel. The ICI distribution is shown in Fig. S4. The ICI distribution showed a general pattern for all four bats and in all flow conditions. The distribution had three peaks, located at approximately 30, 60 and 90 ms, with the 25–30 ms peak often being the most prominent. Hence, the bats continued to collect information using echolocation, even when they had access to visual information.

Next, we evaluated whether and how the different sensory flow fields (echo-acoustic flow alone, optic flow alone and echo-acoustic flow versus optic flow) affected the flight behaviour of the bats. Fig. 3 shows an overview of the bats' flight behaviours in different flow fields. To assess changes in the flight path adjustments, we focused on the bats' flight paths within the central part of the flight tunnel. We used data recorded exclusively in the central 1 m of the flight tunnel (the video analysis zone), because the bats required the first metre behind the terminal cubes to induce flight path adjustment. Based on these data, we compared the bats' performance for asymmetric (strong versus weak flow) experimental conditions for the different flow stimuli (echo-acoustic flow alone, optic flow alone and echo-acoustic flow versus optic flow). Data were pooled for the two flight directions. Each plot in Fig. 3 shows the performance of one individual bat with a specific wall arrangement and a specific sensory flow field. Raw data are shown as red dots (spread vertically for clarity) and error bars show median deviations from the midline (with interquartile ranges). In all cases, the bats significantly shifted their flight paths towards the side producing the least amount of sensory flow, both for echo-acoustic flow (as has been shown before; Kugler et al., 2016) and when the side walls elicited only optic flow (two-sided Wilcoxon rank sum test, $P < 0.0001$ for all bats and all sensory flow conditions). During flight conditions when conflicting echo-acoustic flow and optic flow information was present (echo-acoustic flow versus optic flow), all bats shifted their flight paths towards the side producing weaker echo-acoustic but stronger optic flow. Again, a two-sided Wilcoxon rank sum test showed highly significant differences ($P < 0.0001$ for all bats).
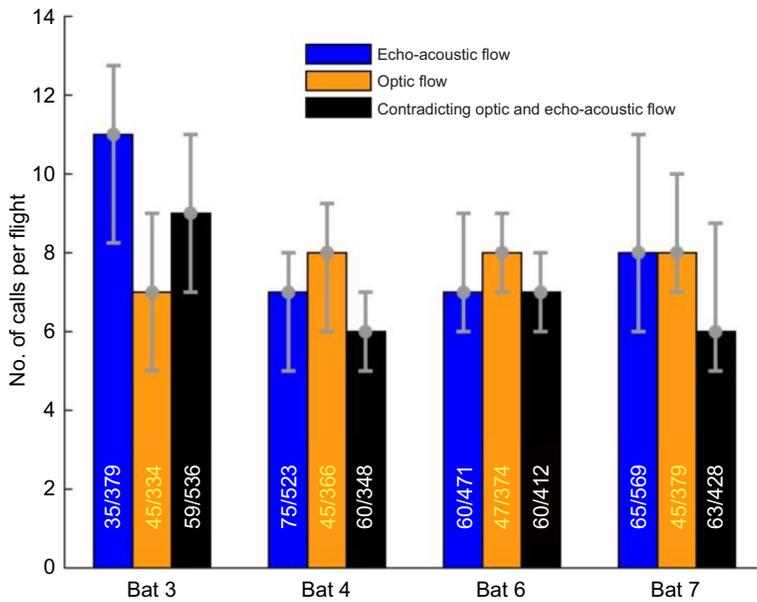
Fig. 2. Echolocation behaviour. Bars show the median number of calls a bat produced per flight in the acoustic analysis zone in the central 2 m of the flight tunnel. Error bars show the corresponding interquartile ranges for each bat and flow-field condition. Numbers within the bars represent the number of trials and calls taken into account for the analysis. Only data from trials in asymmetric conditions (horizontal and vertical grooves and/or stripes of different flight tunnel walls) are shown.

Fig. 4 shows the magnitude of path deviation from the midline for different sensory flow fields. Path deviations were larger in purely echo-acoustic flow fields, whereas for optic flow conditions, paths also deviated in the direction producing weaker sensory flow, but the magnitude of path deviations was smaller than that for the echo-acoustic flow condition. When conflicting echo-acoustic and optic flow information was available, bats deviated towards the side producing weaker echo-acoustic but stronger optic flow, although the overall magnitude of path deviation was smaller than that for the purely echo-acoustic condition.

It has previously been shown (Bhagavatula et al., 2011; Davies and Green, 1990; Lee et al., 1993) that visually guided flyers from various species regulate flight velocity according to the overall optic flow. Therefore, we also examined whether flight velocity differed between flights with weaker (both sidewalls showing horizontal stripes) and stronger optic flow. Bats did not adjust flight velocity to the strength of optic flow (two-sided Wilcoxon rank sum test,

$P=0.6, 0.5, 0.3, 0.6$ for bats 3, 4, 6 and 7, respectively; Fig. 5). This observation is consistent with previous observations that bats do not adjust flight velocity to the strength of echo-acoustic flow (Kugler et al., 2016; Warnecke et al., 2016).

The acoustic measurements of reflective properties of the flight tunnel show that for ensonifications from the midline, echo gain from vertical or horizontal ridges is not much different for reflections over the complete length of the tunnel, as long as the plastic head replica is pointing in the forward direction (Fig. S3). When turning the head, stronger gain differences ($\sim$6–8 dB) are introduced from near-by positions, when the pinna opening points towards walls with vertical ridges.

DISCUSSION
In the present study, we investigated how optic flow interacts with echo-acoustic flow to guide the flight of bats through a structured flight tunnel with conflicting echo-acoustic and visual cues.
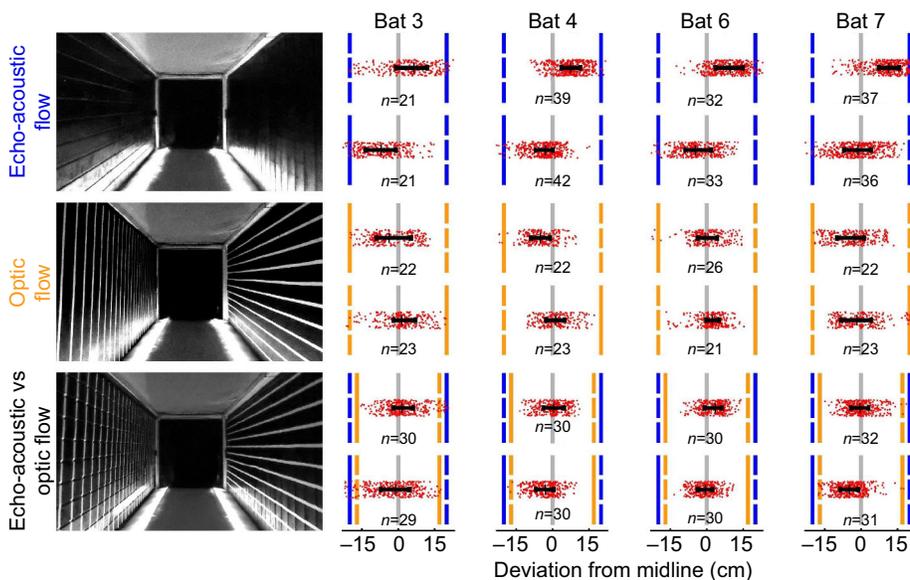

Fig. 3. Flight path adjustments. Left: pictures of the experimental setup in the different flow conditions, photographed from the bat's perspective at the starting position, looking into the tunnel. Right: each column of graphs shows the performance of one bat in the different flow conditions. Each plot depicts a schematic top view onto the flight tunnel. The vertical or horizontal arrangement of ridges (blue; echo-acoustic flow) and/or stripes (orange; optic flow) is depicted as dashed and solid lines, respectively. Red dots show raw data of the bats' position in the video analysis zone in the central 1 m of the flight tunnel, pooled for the two flight directions. Black bars show a bat's median deviation from the midline with interquartile ranges. The last two rows show the bats' performance in conflicting echo-acoustic versus optic flow fields. Here, bats flew closer to the wall producing weaker echo-acoustic but stronger optic flow. *n* represents the number of trials used for the analysis.
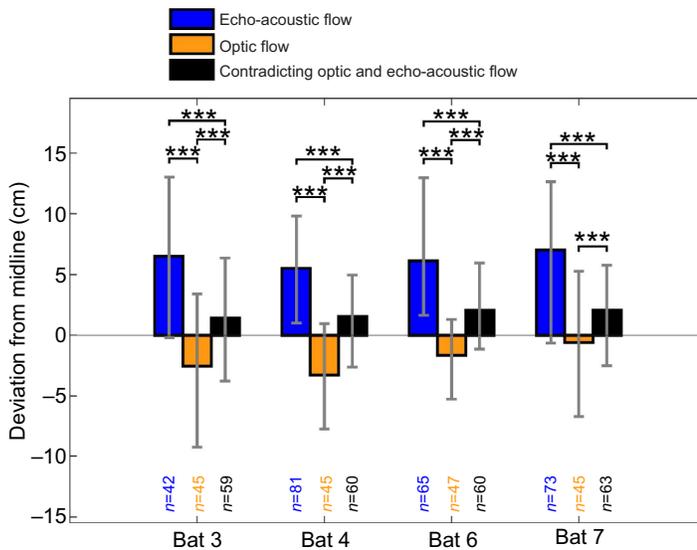
Fig. 4. Summary of flight path adjustments in different sensory flow fields. Bars show the median deviations from midline of a bat in the central 1 m of the flight tunnel in imbalanced echo-acoustic, optic and conflicting echo-acoustic versus optic flow fields. Error bars show the interquartile ranges. (Kruskal–Wallis test, corrected for multiple comparisons, $P<10^{-7}$ for all bats and all comparisons.) The figure shows deviation towards weaker echo-acoustic and/or stronger optic flow as positive values, and deviation towards stronger echo-acoustic and/or weaker optic flow as negative values. n indicates the number of trials that served as the basis for the analysis.

The effectiveness of optic or echo-acoustic flow was measured as flight-path deviations from the midline of the tunnel. The current experiments show that *P. discolor* bats use both vision and echolocation when adjusting their flight paths along structures. The fact that bats showed larger deviations in their flight paths in response to changes in echo-acoustic flow versus optic flow conditions indicates that optic flow information may be less potent in affecting flight paths. Consequently, when echo-acoustic flow and optic flow were in conflict, the echo-acoustic flow information seemed to overrule the optic flow information even in these highly visual bats. However, this only holds true if one looks at the net effect size (Fig. 4): echo-acoustic flow alone evokes a stronger effect than optic flow, and therefore in the contradicting situation the net effect remains dominated by echo-acoustic flow. When looking closer at the differential effect size, it becomes clear that the reduction of the echo-acoustic flow effect is strong compared with the relatively weak effect evoked by optic flow alone. Thus, the weight on optic flow is stronger, and in terms of a weighting of effectiveness, optic flow seemed to overrule echo-acoustic flow.

The current data support the hypothesis that bats integrate echo-acoustic and optic information to navigate in complex environments and corroborate earlier findings on multimodal integration in bats
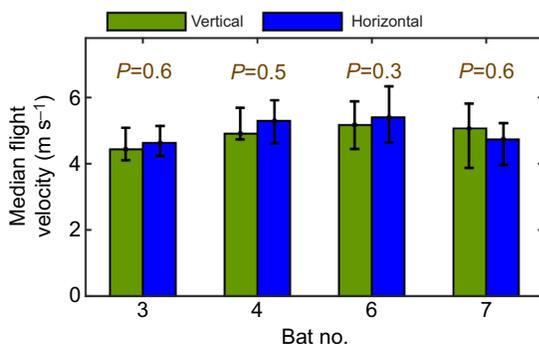
(e.g. Horowitz et al., 2004; Orbach and Fenton, 2010; Rother and Schmidt, 1982). These findings on audio-visual integration are remarkable because bats are typically thought to be sensory specialists that rely predominantly on their elaborated echolocation system. For example, distance information is readily available to bats by analysing the delay between call emission and arrival of the reflected echoes. The bats' central nervous system has evolved special adaptations for the processing of echo-delay information, resulting in a chronotopic map in the auditory cortex (Greiter and Firzlaff, 2017; Hagemann et al., 2010; Suga and O'Neill, 1979). However, our results demonstrate behavioural correlates of audio-visual integration that are comparable to results from 'classical' studies on multimodal integration in animals with less-specialized sensory systems. For example, cats showed a decreased probability of correctly responding to the spatial position of a visual stimulus when a spatially disparate auditory stimulus was presented simultaneously (Stein et al., 1989). The cats often moved to positions half-way between the locations of the two stimuli. In our experiments, the response magnitude to echo-acoustic flow measured in terms of flight-path deviance from midline in a flight tunnel was reduced when conflicting optic flow was presented.

The current data are a good example that in a spatial task the auditory sense can strongly interact with the visual sense. Indeed, space perception is the hallmark of the visual system and when acoustic spatial information is in conflict with visual spatial information, the visual system always overrules the auditory system: classical examples are the ventriloquism effect (e.g. Weerts and Thurlow, 1971) and the localization of objects in elevation. For object localization in elevation, the visual system has even been shown to teach the auditory system in the analysis of elevation-dependent spectral interference patterns generated by the outer ears (Van Wanrooij and Van Opstal, 2005; Zwiers et al., 2003). The fact that this specialized audio-vocal sense can have such a strong effect on vision for a spatial task must be attributed to the development of the highly specialized echo-acoustic system of bats.

The bats in our experiments showed a stronger behavioural response to echo-acoustic flow than to optic flow. It is difficult to compare the relative effectiveness of the two types of sensory flow information, as data on perceptual thresholds for sensory flow are not currently available. However, echoes reflected from the ridges of the tunnel walls and the white stripes used to induce optic flow were

Fig. 5. Flight velocity with strong and weak optic flow. Green bars represent flight velocities with concordant vertical stripes; blue bars represent flight velocities with concordant horizontal stripes. Data are medians and interquartile range of maximum flight velocities measured in each trial. *P*-values show the results of a Wilcoxon rank sum test, testing whether a bat's flight velocity with vertical stripes differs from that with horizontal stripes.

well above the threshold of the respective sensory domains: behavioural studies showed that absolute hearing thresholds in *P. discolor* can be as low as −10 dB SPL in the frequency range of the echolocation calls (Hoffmann et al., 2008). The spatial separation between reflecting ridges in our flight tunnel was 9 cm and thus also far above the threshold for distance resolution by echolocation for *P. discolor* (∼1.3 cm; Goerlitz et al., 2010). Monocular visual fields cover the space up to 155 deg caudally in one hemisphere in *P. discolor* and maximal spatial resolving power was ∼1.63 cycles deg^{-1} (Hoffmann et al., 2016). When the bats were flying in the middle of the tunnel in our experiments (i.e. at 50 cm distance to the wall), structures of ∼0.54 cm should have been resolvable by the visual system (threshold at 1 m distance: ∼1 cm). The white stripes on the flight tunnel walls (width 1.5 cm, separation 9 cm, equivalent to the ridge separation for the echo-acoustic flow stimuli) should therefore be well resolved by the bat. For the bat visual system, Cechetto et al. (2016) report a threshold for green lights (∼540 nm, approximately the peak absorption wavelength of the rod photoreceptors) of ∼0.003 lx. Illumination strength during dusk/dawn is about 1–10 lx, while the flight tunnel was illuminated with about 10 lx. We were therefore confident that our experimental conditions elicited sufficiently strong sensory flow in both the visual and auditory domain.

Why did echo-acoustic flow show such a strong effect on optic flow in conflicting situations in our bats? While optic flow can be easily and completely removed by just switching off any light source, echo-acoustic flow is fully controlled by the bats' emissions. For example, the presence of the feeders will create some echo-acoustic information in the optic flow condition, provoking the bats to produce calls even when the tunnel was illuminated and had smooth walls (Fig. 1). As a result, the behavioural deviation in the flight path in response to optic flow might have been pre-emptively diminished compared with a condition in which the bats could not rely on any echo-acoustic information. It is, however, also conceivable that echo-acoustic flow generally initiates a stronger behavioural response than optic flow in bats as a result of the general dominance of echolocation over vision. A similar phenomenon is observed in humans, who typically rely strongly on vision. The perception of illusory self-motion (vection) can be easily induced visually but it is weaker and less robust when only auditory stimulation is used (Keshavarz et al., 2014). Of course, the dominance of echo-acoustic flow over optic flow might be modulated by the relative strength of the two cues. Under natural conditions (i.e. during dusk/dawn or full moon), light intensities are often lower than those used in our experiments.

In a previous set of experiments (Kugler et al., 2016), we showed that the presence of a finer ridge spacing (4.5 cm) often induced higher call rates (and hence evoked adaptation of call rate to the vertical ridge spacing), although ridge periodicity was generally undersampled by the bats. In our current study, echoes in the flight tunnel changed periodically with a frequency of about 55 Hz (11 ridges m^{-1} and flight velocities of about 5 m s^{-1}). Calls emitted with ICIs of around 25–30 ms result in a call rate of ∼33–40 Hz. The bats thus also seem to under-sample the ridge periodicity in our current experiments, but only slightly. Therefore, two functional interpretations are possible (but remain hypothetical). (1) An adaptation of call rate to match ridge periodicity would be an interesting active behaviour to keep echo-acoustic flow constant (like the stroboscopic fine-adjustment for a turntable). This behaviour would indeed further strengthen the importance of echo-acoustic flow in guidance and navigation for bats. (2) If bats were under-sampling the groove periodicity, this would not mean

that echo-acoustic flow information is not available or used by bats. As shown by Fontaine and Peremans (2011), bats can reconstruct wing beat periodicity despite echo-acoustic under-sampling of the period. Of course, both strategies could be used by different individuals or in different situations.

As expected, measuring the intensity of echoes reflected from vertical or horizontal grooves shows that echo intensity strongly depends on the head position of an animal, i.e. the joined emission and hearing axis. With the head oriented towards the frontal midline (0 deg azimuth), echo intensity is not very different for vertical and horizontal grooves (Fig. S3, middle column). However, when the joined emission and hearing axis is oriented ±30 deg off the midline, echo intensity is stronger for vertical panels (Fig. S3, left and right column). Therefore, one has to consider the possibility that the bats simply avoid stronger echoes and thus deviate from the midline towards the tunnel side producing the weaker reflections. In this case, echo-acoustic flow would not be the main cue underlying the behavioural response. However, using an experimental setup comparable to ours, Warnecke et al. (2018b) showed that the flight path of bats was not influenced by echo intensity by varying echo strength (∼7 dB) while leaving the spacing of the reflective surface unaltered. Our own measurements revealed maximum echo intensity differences of reflection from vertical and horizontal ridges in quite a similar range between roughly 6 and 8 dB. Thus, we are confident that in our experiments reported here and earlier in Kugler et al. (2016) the observed flight path deviations were also mainly caused by echo-acoustic flow and not by echo intensity.

At the neuronal level, sensitivity to echo-acoustic flow has been demonstrated in the auditory cortex of bats (Bartenstein et al., 2014). When stimulated with auditory cues containing echo-acoustic flow information, the representation of nearby targets increased in the cortical map of target range and the neural tuning to echo delays became sharper. For the midbrain superior colliculus (SC), typically considered to be a major hub for multimodal integration, no neurophysiological studies have yet investigated the effect of echo-acoustic flow. However, recent studies in free-flying bats indicated that neural processing of target position in the SC is highly adaptive to actual flight path and echolocation behaviour (Kothari et al., 2018). As a congruent representation of visual and acoustic space was also found in the *P. discolor* SC (Hoffmann et al., 2016), it may represent the major site of integration for echo-acoustic and visual stimuli.

Finally, our results inform evolutionary aspects of echolocation and vision in bats. Thiagavel et al. (2018) used phylogenetic comparative methods to investigate the evolution of echolocation from ancestral bats to the present day. The authors show that bats which use a less-specialized multiharmonic frequency-modulated (FM) echolocation have often retained the relatively large eyes of their ancestors, whereas modern constant-frequency (CF) bats, which are highly specialized echolocators, have decreased eye size. Therefore, phyllostomid bats such as *P. discolor*, which have relatively large eyes and use FM multi-harmonic echolocation, seem to represent a more ancestral state with less sensory specialization. Audio-visual integration might have represented the default situation in those bats. This is supported by findings in phyllostomid bats demonstrating the existence of cones expressing short-wave-sensitive opsin tuned to blue and UV light, and therefore representing the ancestral mammalian retinal pattern (Müller et al., 2009). Corneal electroretinograhic recordings in the same study revealed that short-wavelength sensitivity was best at mesopic light levels, i.e. in dim light found at dusk and dawn or in bright moonlight.

In the light of these evolutionary considerations, the interactions between echo-acoustic and optic flow in *P. discolor* might thus be considered a direct consequence of an ancestral state of bat evolution. It would be interesting to study whether, in very advanced echolocating bat species such as the CF-emitting rhinolophid bats, flight guidance relies even more on echo-acoustic information and optic flow might only affect rhinolophid bats in extreme conditions. At least for the use of echo-acoustic flow cues for flight guidance, experiments in hipposiderid bats hint towards differences between CF and FM bats (Warnecke et al., 2018a). However, as animals are opportunistic in using their sensory organs, even in specialists the benefits of multimodal integration are unlikely to be completely discarded.

Data availability
The datasets supporting this article are available from figshare: https://figshare.com/s/c1155740346f1c5ca8c3

Supplementary information
Supplementary information available online at
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References

Bartenstein, S. K., Gerstenberg, N., Vanderelst, D., Peremans, H. and Firzlaff, U. (2014). Echo-acoustic flow dynamically modifies the cortical map of target range in bats. *Nat. Commun.* **5**, 4668.

Bell, G. P. and Fenton, M. B. (1986). Visual-acuity, sensitivity and binocularity in a gleaning insectivorous bat, macrotus-californicus (Chiroptera, Phyllostomidae). *Anim. Behav.* **34**, 409-414.

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

Bradbury, J. W. and Nottebohm, F. (1969). The use of vision by the little brown bat, Myotis lucifugus, under controlled conditions. *Anim. Behav.* **17**, 480-485.

Cechetto, C., Jakobsen, L., Surlykke, A. Warrant, E. (2016). Visual intensity threshold in bats using psychophysics. International Congress of Neuroethology; 2016 March 30-April 3; Montevideo. Poster Session II, Poster Number: II-131, 360.

Danilovich, S., Krishnan, A., Lee, W. J., Borrisov, I., Eitan, O., Kosa, G., Moss, C. F. and Yovel, Y. (2015). Bats regulate biosonar based on the availability of visual information. *Curr. Biol.* **25**, R1124-R1125.

Davies, M. N. O. and Green, P. R. (1990). Optic flow-field variables trigger landing in Hawk but not in pigeons. *Naturwissenschaften* **77**, 142-144.

De Mey, F., Reijniers, J., Peremans, H., Otani, M. and Firzlaff, U. (2008). Simulated head related transfer function of the phyllostomid bat Phyllostomus discolor. *J. Acoust. Soc. Am.* **124**, 2123-2132.

Eklöf, J. (2003). Vision in echolocating bats. *PhD thesis, Department of Zoology, Göteborg University.*

Fontaine, B. and Peremans, H. (2011). Compressive sensing: a strategy for fluttering target discrimination employed by bats emitting broadband calls. *J. Acoust. Soc. Am.* **129**, 1100-1110.

Goerlitz, H. R., Geberl, C. and Wiegrebe, L. (2010). Sonar detection of jittering real targets in a free-flying bat. *J. Acoust. Soc. Am.* **128**, 1467-1475.

Gottfried, J. A. and Dolan, R. J. (2003). The nose smells what the eye sees: crossmodal visual facilitation of human olfactory perception. *Neuron* **39**, 375-386.

Greiter, W. and Firzlaff, U. (2017). Echo-acoustic flow shapes object representation in spatially complex acoustic scenes. *J. Neurophysiol.* **117**, 2113-2124.

Hagemann, C., Esser, K.-H. and Kössl, M. (2010). Chronotopically organized target-distance map in the auditory cortex of the short-tailed fruit bat. *J. Neurophysiol.* **103**, 322-333.

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

Hoffmann, S., Vega-Zuniga, T., Greiter, W., Krabichler, Q., Bley, A., Matthes, M., Zimmer, C., Firzlaff, U. and Luksch, H. (2016). Congruent representation of visual and acoustic space in the superior colliculus of the echolocating bat Phyllostomus discolor. *Eur. J. Neurosci.* **44**, 2685-2697.

Holderied, M. W., Jones, G. and von Helversen, O. (2006). Flight and echolocation behaviour of whiskered bats commuting along a hedgerow: range-dependent sonar signal design, Doppler tolerance and evidence for 'acoustic focussing'. *J. Exp. Biol.* **209**, 1816-1826.

Horowitz, S. S., Cheney, C. A. and Simmons, J. A. (2004). Interaction of vestibular, echolocation, and visual modalities guiding flight by the big brown bat, Eptesicus fuscus. *J. Vestib Res.* **14**, 17-32.

Keshavarz, B., Hettinger, L. J., Vena, D. and Campos, J. L. (2014). Combined effects of auditory and visual cues on the perception of vection. *Exp. Brain Res.* **232**, 827-836.

Kothari, N. B., Wohlgemuth, M. J. and Moss, C. F. (2018). Dynamic representation of 3D auditory space in the midbrain of the free-flying echolocating bat. *Elife* **7**, e29053.

Kugler, K., Greiter, W., Luksch, H., Firzlaff, U. and Wiegrebe, L. (2016). Echo-acoustic flow affects flight in bats. *J. Exp. Biol.* **219**, 1793-1797.

Lee, D. N., van der Weel, F. R., Hitchcock, T., Matejowsky, E. and Pettigrew, J. D. (1992). Common principle of guidance by echolocation and vision. *J. Comp. Physiol. A* **171**, 563-571.

Lee, D. N., Davies, M. N. O., Green, P. R. and Vanderweel, F. R. R. (1993). Visual control of velocity of approach by pigeons when landing. *J. Exp. Biol.* **180**, 85-104.

McKerrow, P. (2008). Acoustic Flow. 2008 Ieee/Rsj International Conference on Robots and Intelligent Systems, Vols 1-3, Conference Proceedings, 1365-1370.

Meredith, M. A. and Stein, B. E. (1983). Interactions among converging sensory inputs in the superior colliculus. *Science* **221**, 389-391.

Meredith, M. A. and Stein, B. E. (1986). Visual, auditory, and somatosensory convergence on cells in superior colliculus results in multisensory integration. *J. Neurophysiol.* **56**, 640-662.

Müller, R. and Schnitzler, H. U. (1999). Acoustic flow perception in cf-bats: properties of the available cues. *J. Acoust. Soc. Am.* **105**, 2958-2966.

Müller, B., Glösmann, M., Peichl, L., Knop, G. C., Hagemann, C. and Ammermüller, J. (2009). Bat eyes have ultraviolet-sensitive cone photoreceptors. *PLoS ONE* **4**, e6390.

Orbach, D. N. and Fenton, B. (2010). Vision impairs the abilities of bats to avoid colliding with stationary obstacles. *PLoS ONE* **5**, e13912.

Rother, G. and Schmidt, U. (1982). The influence of visual information on echolocation in phyllostomus-discolor (Chiroptera). *Mammalian Biology* (formerly *Zeitschrift für Säugetierkunde*) **47**, 324-334.

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

Stein, B. E. and Meredith, M. A. (1993). *The Merging of the Senses*. Cambridge, Mass.: MIT Press.

Stein, B. E., Meredith, M. A., Huneycutt, W. S. and McDade, L. (1989). Behavioral indices of multisensory integration: orientation to visual cues is affected by auditory stimuli. *J. Cogn. Neurosci.* **1**, 12-24.

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

Thiagavel, J., Cechetto, C., Santana, S. E., Jakobsen, L., Warrant, E. J. and Ratcliffe, J. M. (2018). Auditory opportunity and visual constraint enabled the evolution of echolocation in bats. *Nat. Commun.* **9**, 98.

Van Wanrooij, M. M. and Van Opstal, A. J. (2005). Relearning sound localization with a new ear. *J. Neurosci.* **25**, 5413-5424.

Verhaal, J. and Luksch, H. (2016). Multimodal integration in the chicken. *J. Exp. Biol.* **219**, 90-95.

Warnecke, M., Lee, W.-J., Krishnan, A. and Moss, C. F. (2016). Dynamic echo information guides flight in the big brown bat. *Front. Behav. Neurosci.* **10**, 81.

Warnecke, M., Falk, B. and Moss, C. F. (2018a). Echolocation and flight behavior of the bat Hipposideros armiger terasensis in a structured corridor. *J. Acoust. Soc. Am.* **144**, 806.

Warnecke, M., Macias, S., Falk, B. and Moss, C. F. (2018b). Echo interval and not echo intensity drives bat flight behavior in structured corridors. *J. Exp. Biol.* **221**, jeb.191155.

Weerts, T. C. and Thurlow, W. R. (1971). Effects of eye position and expectation on sound localization. *Percept. Psychophys.* **9**, 35-39.

Winkowski, D. E. and Knudsen, E. I. (2007). Top-down control of multimodal sensitivity in the barn owl optic tectum. *J. Neurosci.* **27**, 13279-13291.

Winter, Y., López, J. and Von Helversen, O. (2003). Ultraviolet vision in a bat. *Nature* **425**, 612-614.

Zwiers, M. P., Van Opstal, A. J. and Paige, G. D. (2003). Plasticity in human sound localization induced by compressed spatial vision. *Nat. Neurosci.* **6**, 175-181.