

## RESEARCH ARTICLE

# Echolocation behaviour of the big brown bat (*Eptesicus fuscus*) in an obstacle avoidance task of increasing difficulty

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**ABSTRACT**

Four big brown bats (*Eptesicus fuscus*) were challenged in an obstacle avoidance experiment to localize vertically stretched wires requiring progressively greater accuracy by diminishing the wire-to-wire distance from 50 to 10 cm. The performance of the bats decreased with decreasing gap size. The avoidance task became very difficult below a wire separation of 30 cm, which corresponds to the average wingspan of *E. fuscus*. Two of the bats were able to pass without collisions down to a gap size of 10 cm in some of the flights. The other two bats only managed to master gap sizes down to 20 and 30 cm, respectively. They also performed distinctly worse at all other gap sizes. With increasing difficulty of the task, the bats changed their flight and echolocation behaviour. Especially at gap sizes of 30 cm and below, flight paths increased in height and flight speed was reduced. In addition, the bats emitted approach signals that were arranged in groups. At all gap sizes, the largest numbers of pulses per group were observed in the last group before passing the obstacle. The more difficult the obstacle avoidance task, the more pulses there were in the groups and the shorter the within-group pulse intervals. In comparable situations, the better-performing bats always emitted groups with more pulses than the less well-performing individuals. We hypothesize that the accuracy of target localization increases with the number of pulses per group and that each group is processed as a package.

**KEY WORDS:** *Eptesicus fuscus*, Obstacle avoidance, Echolocation, Flight behaviour, Localization accuracy

**INTRODUCTION**

Foraging bats have to perform many echolocation tasks in parallel: spatial orientation, biotope recognition and food finding (Denzinger and Schnitzler, 2013). When orienting in space along background contours, bats continuously determine their position in relation to the environment, a necessity for route following and obstacle avoidance. With decreasing distance to the background, the risk of collision with obstacles increases and the task of avoiding obstacles becomes more difficult. Thus, the localization of potential obstacles should be more accurate the closer the bat flies to the background.

The flight, echolocation behaviour and performance of bats avoiding obstacles have been studied in many species and with different methods. Hahn (Hahn, 1908) and Griffin and Galambos (Griffin and Galambos, 1941) were the first who introduced wires as obstacles, a method adopted frequently since then. The aim of most studies with wire obstacles has been to determine the detection threshold by measuring the minimal threshold diameter at which wires

could still be recognized and avoided. These studies revealed species-specific minimal detection thresholds, for example, 0.12 mm for *Myotis lucifugus* (Grinnell and Griffin, 1958), 0.08 and 0.05 mm for the horseshoe bats *Rhinolophus ferrumequinum* and *Rhinolophus euryale*, respectively (Schnitzler, 1968), 0.065 mm for *Asellia tridens* (Gustafson and Schnitzler, 1979) and 0.8–0.6 mm for *Megaderma lyra* (Möhres and Neuweiler, 1966). Furthermore, it was shown that the reaction distance increased with wire diameter (Grinnell and Griffin, 1958; Gustafson and Schnitzler, 1979; Schnitzler, 1968; Sokolov, 1972; reviewed in Schnitzler and Henson, 1980).

Another approach to measuring the obstacle avoidance performance is to keep the wire diameter constant but to reduce the distance between the vertically or horizontally stretched wires. A constant wire diameter guarantees that the sensory detection task is similar throughout the experiments. The smaller gap sizes, however, make the motor task of passing through the wire array without collision more difficult. The small gap also forces the bats to determine the position of the wires with higher precision to avoid collisions. Such an obstacle avoidance experiment has only been performed with horseshoe bats by Schnitzler (Schnitzler, 1968) who found that the bats exhibited high avoidance scores down to gap sizes of approximately half of the bat's wingspan. The good obstacle avoidance performance of these horseshoe bats proved that they were able to localize the wires with high precision.

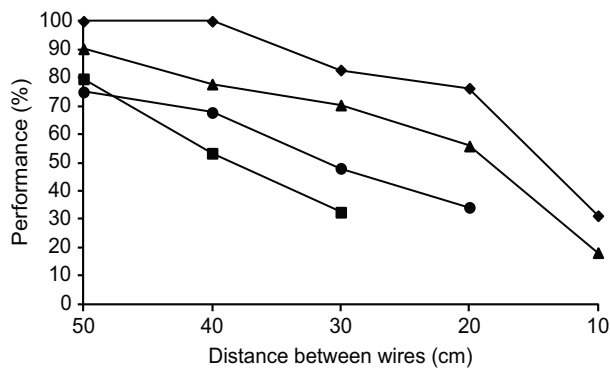
In other studies that address somewhat different questions, bats have been trained to solve increasingly difficult tasks that also required an increase in localization accuracy. Moss et al. (Moss et al., 2006) recorded the echolocation behaviour of big brown bats (*Eptesicus fuscus*) as they captured tethered insects positioned at different distances from background vegetation. When approaching the prey, bats produced groups of pulses with rather stable within-group pulse intervals. The number of groups increased when the distance between prey and clutter was reduced. Moss and Surlykke (Moss and Surlykke, 2001) had described similar groups of sounds with relatively stable intervals in bats approaching prey and termed them 'sonar strobe groups'. Moss and colleagues (Moss and Surlykke, 2001; Moss et al., 2006) suggest that the use of such sound groups may enhance the spatial representation of the environment. Another study (Petrites et al., 2009) investigated how the pulse pattern of *E. fuscus* was influenced by different clutter conditions when flying through corridors of different widths created by rows of vertically hanging chains. When the corridor was narrower, the motor task was more challenging and required higher localization accuracy. Increasing the difficulty by making the corridors narrower produced more groups with two and three pulses and the bats flew more slowly.

From our own preliminary experiments and from the data of Moss et al. (Moss et al., 2006) and Petrites et al. (Petrites et al., 2009), we concluded that grouping of signals may be important for the localization of targets with high accuracy. To understand how the echolocation and flight behaviour changes when bats are forced to

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**Fig. 1.** Performance of four *Eptesicus fuscus* when passing an obstacle of vertically stretched wires with a diameter of 1.5 mm. Performance was measured as the percentage of collision-free flights at different distances between wires (symbol coded: diamonds, EF1; triangles, EF2; circles, EF3; squares, EF4).

increase their localization accuracy, we conducted an obstacle avoidance experiment with *E. fuscus* in which we increased the difficulty of the motor task by systematically reducing the distance between the wires in an array. The increasing difficulty of the motor task implies an increasing difficulty of the sensory task to measure the position of the wires with high accuracy. Our hypothesis was that the flight and echolocation behaviour will change with increasing difficulty of the task, thus revealing the adaptive strategies for achieving the necessary increase in localization accuracy.

## RESULTS

### Obstacle avoidance performance

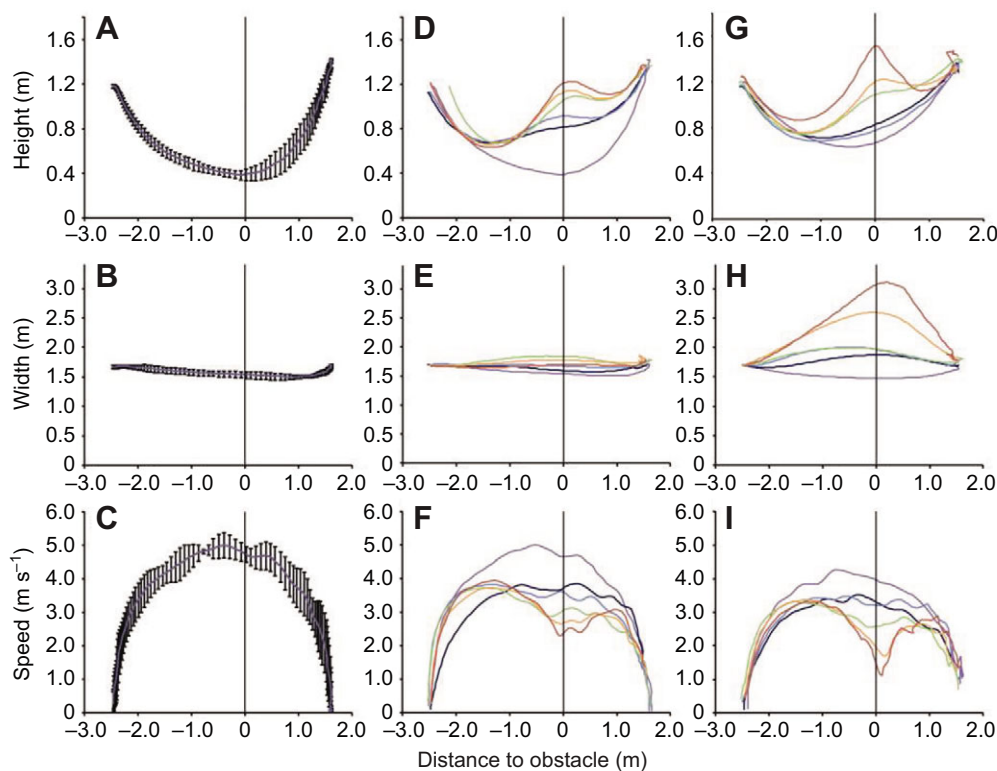
The percentage of collision-free flights through the obstacles depended significantly on the distance between the wires (Pearson  $r_{16}=0.7435$ ,  $P=0.0006$ ). In all bats, the performance dropped with diminishing wire-to-wire distance (Fig. 1), but the four bats showed

distinct differences in their performance: in all conditions, bat EF1 and EF2 performed better than EF3 and EF4. At gap sizes of 50 and 40 cm, EF1 scored 100% collision-free flights and EF2 90% and 80%, respectively, whereas EF4 reached 80% and EF3 only 75% in the 50 cm condition. Individual differences in flight performance were also evident at the other wire separations. The two better-performing bats had at least 70% collision-free flights down to a gap of 30 cm and also had a few flights without contact in the 10 cm condition. The other two bats (EF3, EF4), however, were not able to pass the wires at gap sizes of 10 cm, and even EF4 did not perform successfully at 20 cm wire separation (Fig. 1).

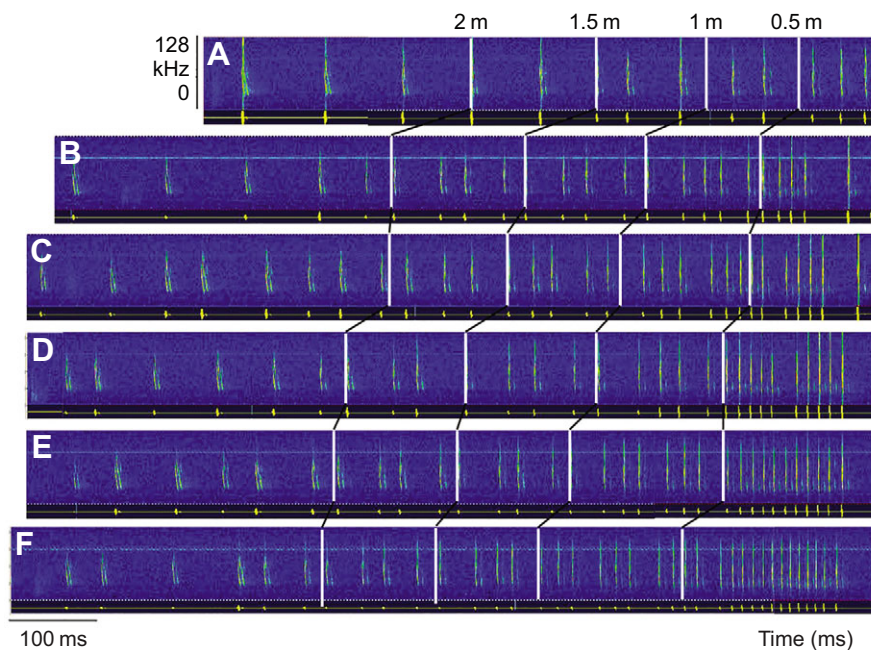
### Flight behaviour

The flight behaviour of the bats was highly stereotyped (Fig. 2). In control flight without obstacles, the bats' flight height decreased after the start and increased again during the approach to the landing grid, resulting in a U-shaped trajectory. The average flight paths differed somewhat between the individuals. In control flights, the flight paths in the section from the start to the frame without the wire array had a maximal vertical extent between 5 cm (EF1, see Fig. 2A) and 18 cm (EF4) and a maximal horizontal width between 6 cm (EF1, see Fig. 2B) and 31 cm (EF3). Except for one bat (EF3), flight paths were smaller than the average wing span, which is approximately 30 cm in *E. fuscus*. In control flights the bats reached maximum average flight speeds between  $3.4 \text{ m s}^{-1}$  (EF4) and  $5.0 \text{ m s}^{-1}$  (EF1) shortly before passing the frame (Fig. 2C).

In the presence of obstacles, the bats changed their flight behaviour. At a distance of 2.0 to 1.5 m to the obstacle, all bats deviated from their control flight path by flying upwards (Fig. 2D,G). Two of the four bats additionally flew to the side (Fig. 2H). The reaction in flight behaviour depended on the distance between wires. At 40 and 50 cm the deviations from the control flights were small and rather similar. They became more prominent at 30 cm and were strongest at 10 cm.



**Fig. 2.** Flight behaviour of two representative bats in control flights and under obstacle conditions. Control flights are shown of EF1 (A–C); flights when passing an obstacle of vertically stretched wires with different wire spacings are shown from EF1 (D–F) and EF2 (G–I). Depicted are the lateral view (A,D,G) and top view (B,E,H) of the flight paths, and flight speed (C,F,I). The vertical solid line at 0 m marks the position of the obstacle frame. The wire spacings are colour coded: purple, control flight; dark blue, 50 cm; light blue, 40 cm; green, 30 cm; orange, 20 cm; red, 10 cm. A–C contain control flight data from bat EF1 (mean  $\pm$  s.d.,  $N=6$ ), D–F contain data from bat EF1 ( $N=55$ ) and G–I contain data from bat EF2 ( $N=50$ ).



**Fig. 3. Typical echolocation sequences of one bat in a representative control flight and under obstacle conditions.** Control flight (A) as well as obstacle conditions with wire spacings of 50 cm (B), 40 cm (C), 30 cm (D), 20 cm (E) and 10 cm (F) belong to the same bat (EF1). Echolocation calls are shown as sonograms from the bat's start until the bat passed the obstacle. Metric numbers and corresponding white solid lines refer to the bat's horizontal distance to the obstacle.

All bats reduced their flight speed with decreasing wire separation (Fig. 2F,I). In the 50 and 40 cm conditions, flight speed was similar. Close to the obstacle it was  $\sim 1 \text{ m s}^{-1}$  below that of the control flight. At 30 cm the flight speed reduction became more prominent. The lowest flight speed was reached in the 10 cm condition. The reduction in speed started between 0.81 m (EF3) and 1.55 m (EF1) from the obstacle and was nearly independent of the distance between the wires.

### Echolocation behaviour

Under control conditions without wire obstacles in the frame the bats emitted either single calls or groups of two calls in the first part of the flight and groups of two calls when approaching the empty obstacle frame (Fig. 3A). Groups are defined as sequences of two or more calls with distinctly shorter within-group pulse intervals compared with the longer inter-group pulse intervals between the beginning of the first call of a group and the beginning of the last call of the preceding group. Groups of three calls occurred very

rarely, just before the bats passed through the frame. Under control conditions the echolocation behaviour of all four bats was rather similar.

When confronted with obstacles, the bats changed their echolocation behaviour in proportion to the difficulty of the obstacle avoidance task. At all gap sizes the bats showed typical approach behaviour and increased the number of calls per group with decreasing distance to the obstacle. With decreasing distance between wires they emitted more groups and increased the number of calls within groups (Fig. 3B–F). The increase in number of calls per group was especially obvious in the terminal group (Spearman  $r_{15} = -0.6524$ ,  $P < 0.0001$ ; Fig. 3, Table 1). The two better-performing bats increased the average number of calls in the terminal group from 4.3 and 4.1 at gap sizes of 50 cm to 11.3 and 14.8 calls at the 10 cm separation. In contrast, the two less-well-performing bats increased the number of calls in the terminal group only slightly from an average of 3.0 calls at a gap size of 50 cm to 5.0 calls at the 20 cm (EF3) and 3.6 calls at the 30 cm wire separations (EF4). At

**Table 1. Average echolocation parameters of the terminal group of all big brown bats (*Eptesicus fuscus*) under all obstacle conditions**

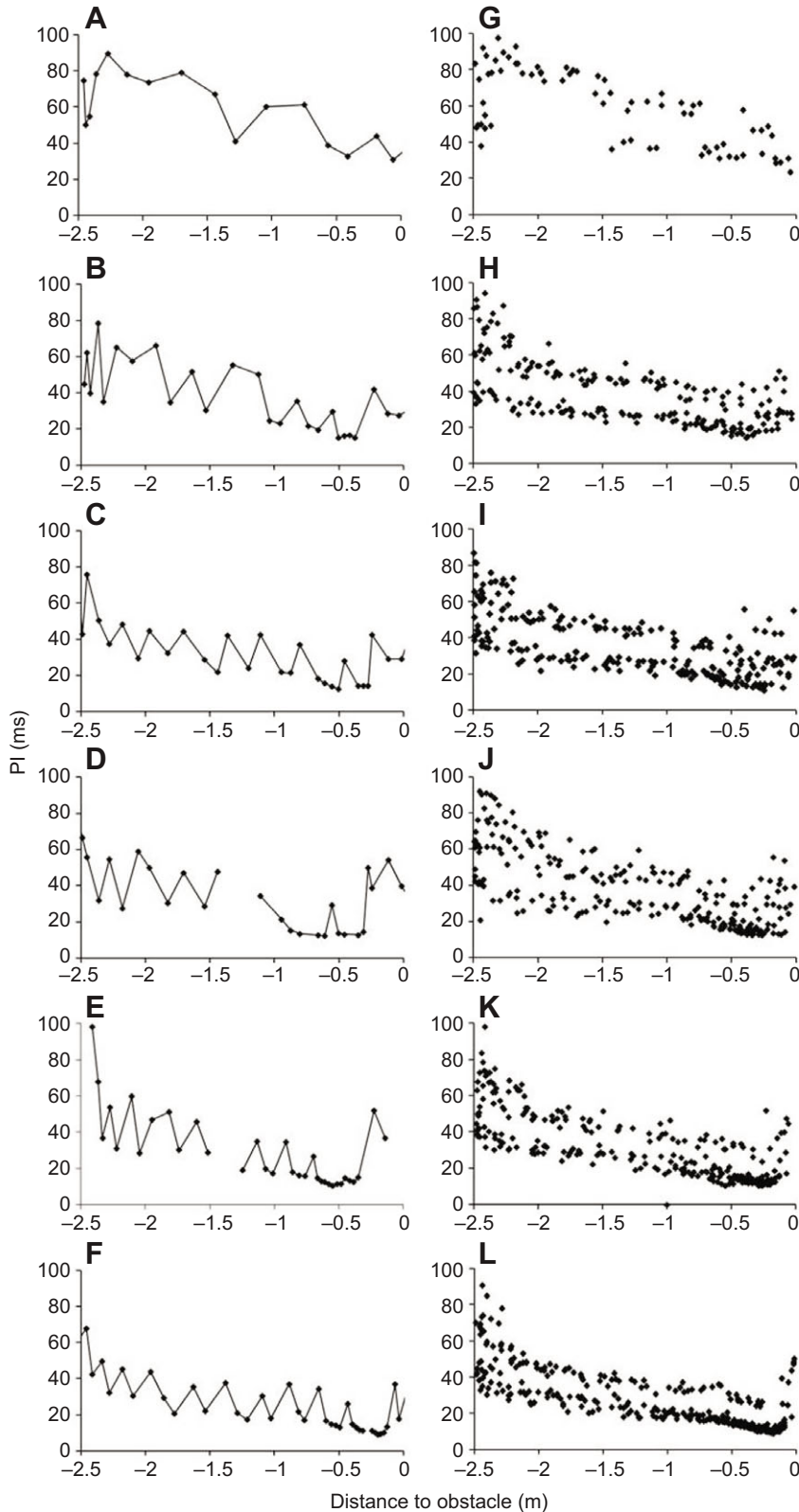
| Obstacle condition                      | EF1                   | EF2                   | EF3                   | EF4                   |
|---|-----------------------|-----------------------|-----------------------|-----------------------|
| Sound duration (ms)                     |                       |                       |                       |                       |
| 50 cm                                   | 2.6±0.1 (2.5; 2.8)    | 2.5±0.1 (2.4; 2.6)    | 2.5±0.1 (2.4; 2.6)    | 3.0±0.2 (2.7; 3.4)    |
| 40 cm                                   | 2.5±0.2 (2.2; 2.9)    | 2.5±0.2 (2.0; 2.7)    | 2.4±0.2 (2.2; 2.5)    | 2.9±0.2 (2.6; 3.1)    |
| 30 cm                                   | 2.4±0.1 (2.3; 2.6)    | 2.3±0.1 (2.0; 2.5)    | 2.3±0.1 (2.2; 2.5)    | 2.5±0.1 (2.4; 2.7)    |
| 20 cm                                   | 2.2±0.1 (2.0; 2.4)    | 2.3±0.0 (2.2; 2.3)    | 2.2±0.1 (2.1; 2.4)    |                       |
| 10 cm                                   | 2.1±0.1 (2.0; 2.2)    | 2.1±0.0 (2.1; 2.1)    |                       |                       |
| Pulse interval (ms)                     |                       |                       |                       |                       |
| 50 cm                                   | 17.6±1.5 (15.8; 19.4) | 20.1±3.7 (16.4; 25.9) | 17.3±1.6 (16.2; 19.2) | 18.5±2.2 (15.5; 21.6) |
| 40 cm                                   | 17.8±4.2 (11.9; 25.4) | 15.6±2.5 (11.5; 19.9) | 21.2±4.8 (17.5; 29.5) | 18.5±1.8 (16.4; 21.2) |
| 30 cm                                   | 15.8±1.9 (13.6; 18.7) | 14.0±1.7 (11.1; 16.8) | 16.5±1.9 (13.5; 20.5) | 16.8±3.6 (14.6; 25.5) |
| 20 cm                                   | 13.2±0.8 (12.1; 14.7) | 12.2±0.5 (11.7; 13.1) | 14.7±1.6 (12.8; 17.0) |                       |
| 10 cm                                   | 11.1±0.6 (10.2; 12.2) | 12.1±0.8 (11.5; 13.2) |                       |                       |
| Number of signals of the terminal group |                       |                       |                       |                       |
| 50 cm                                   | 4.3±0.5 (4; 5)        | 4.1±0.3 (4; 5)        | 3±0 (3; 3)            | 3±0 (3; 3)            |
| 40 cm                                   | 5.2±2.4 (4; 11)       | 5.7±2.8 (4; 11)       | 3±0 (3; 3)            | 3.1±0.3 (3; 4)        |
| 30 cm                                   | 5±0.5 (4; 6)          | 8.7±2.5 (5; 11)       | 3.8±0.4 (3; 4)        | 3.6±0.5 (3; 4)        |
| 20 cm                                   | 8.5±2.1 (6; 12)       | 12.5±3.1 (8; 16)      | 5±1.5 (4; 9)          |                       |
| 10 cm                                   | 11.3±2.1 (9; 14)      | 14.8±4.8 (8; 19)      |                       |                       |

Shown are mean ± s.d. and minimal and maximal (min; max) values.

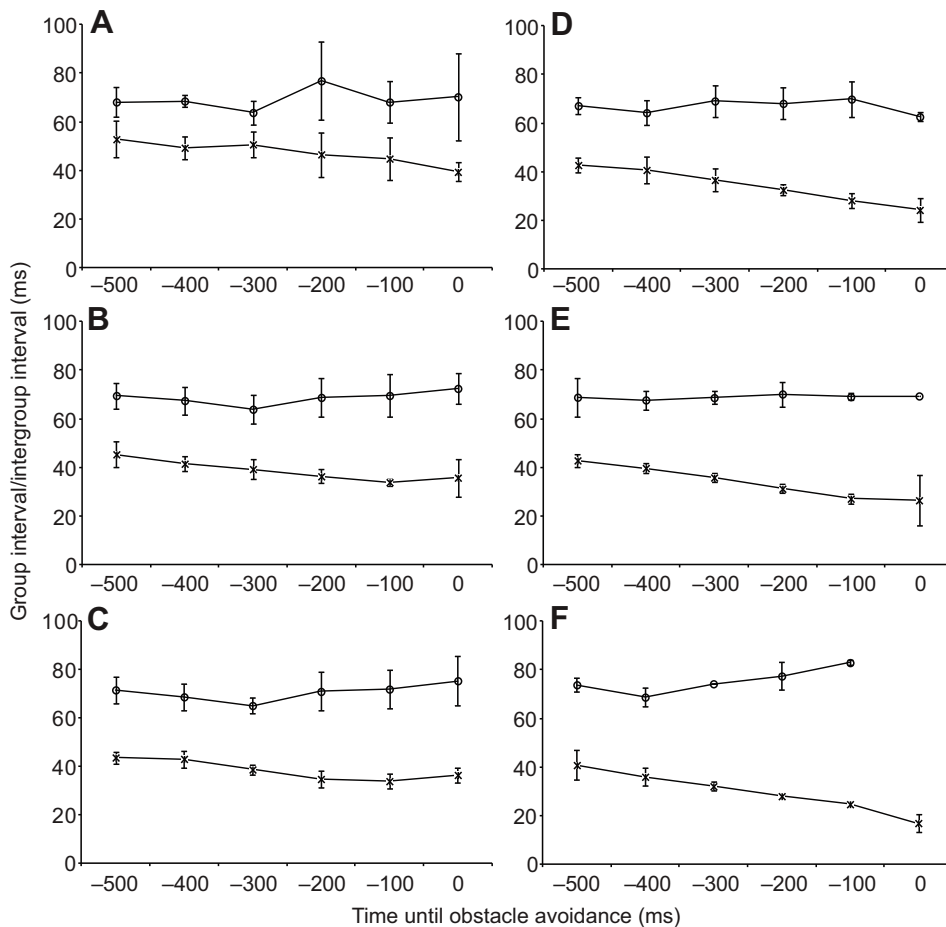
the same gap size the better performing bats always emitted groups with more signals (Table 1). The number of calls per group thus correlated positively with the performance of the bats.

All bats reduced the mean duration (ANOVA;  $F_{4,114}=7.4962$ ,  $P<0.0001$ ) and the mean within-group pulse interval (ANOVA;  $F_{4,114}=3.4956$ ,  $P=0.0099$ ) of the calls in the terminal group when facing decreasing gap size (Table 1). The better-performing bats

decreased the mean duration of the calls in the terminal group from 2.5 and 2.6 ms in the 50 cm spacing to 2.1 ms in the 10 cm spacing, and the mean pulse interval in the terminal group from 17.6 and 20.1 ms in the 50 cm spacing to 11.1 and 12.1 ms in the 10 cm spacing. The short signals and the mean pulse intervals indicate the emission of a buzz-I-like terminal group (Melcón et al., 2007; Schnitzler and Kalko, 2001). However, under none of the conditions



**Fig. 4. Pulse intervals (PIs) of bat EF1 during control and obstacle conditions.** Left: PIs of a typical single flight. Right: PIs of 10 flights. (A,G) Control conditions with empty obstacle frame. Obstacle conditions: 50 cm (B,H), 40 cm (C,I), 30 cm (D,J), 20 cm (E,K) and 10 cm (F,L).



**Fig. 5.** Mean group interval (circles) and mean inter-group pulse interval (crosses) during the last 500 ms of approach to the obstacle frame in control flight and under obstacle conditions. Control condition (A) and wire spacings of 50 cm (B), 40 cm (C), 30 cm (D), 20 cm (E) and 10 cm (F) are depicted. Means  $\pm$  s.d. were determined for time bins of 100 ms (control to 30 cm,  $n=29$ ; 20 cm,  $n=15$ ; 10 cm,  $n=9$ ).

did any of the *E. fuscus* reach constant pulse intervals of 6 ms, which would indicate a ‘buzz II’ (Kalko, 1995).

The terminal group almost always ended before the bats passed the wires (see Fig. 3B–F). Sometimes the bats emitted a single call or a group of two calls immediately before they passed the obstacle. These signals had slightly longer durations (up to 3 ms) and longer within- and inter-group intervals than the signals in the preceding terminal group (Fig. 4). They were most likely directed towards the landing grid behind the obstacle. A similar pattern was reported by Jensen et al. (Jensen et al., 2005) and Surlykke et al. (Surlykke et al., 2009).

The arrangement of signals in groups is indicated by a typical change between shorter within-group pulse intervals and longer inter-group pulse intervals (Fig. 4). In all bats, the within-group pulse interval of successive groups decreased continuously with decreasing distance to the obstacle (Fig. 4). The pulse interval within a single group was not stable but often decreased.

The inter-group interval was also not stable; we found a significant difference in the inter-group interval in the last 500 ms before the passage of the obstacle frame (Kruskal–Wallis;  $\chi^2_4=26.1550$ ,  $P<0.0001$ ). In control flights, the inter-group interval was  $\sim 50$  ms, which dropped down to 40 ms close to the obstacle (Fig. 5A). In trials with obstacles present, the inter-group interval decreased from approximately 40 ms when the bat was 500 ms away from passing through the 10 cm gap to only 18 ms just before passing through the array (Fig. 5F).

In contrast, in control flights as well as under most obstacle conditions, the group interval (which is the interval between the beginning of the first call of a group and the beginning of the first call of the following group, and most likely reflects the wing beat

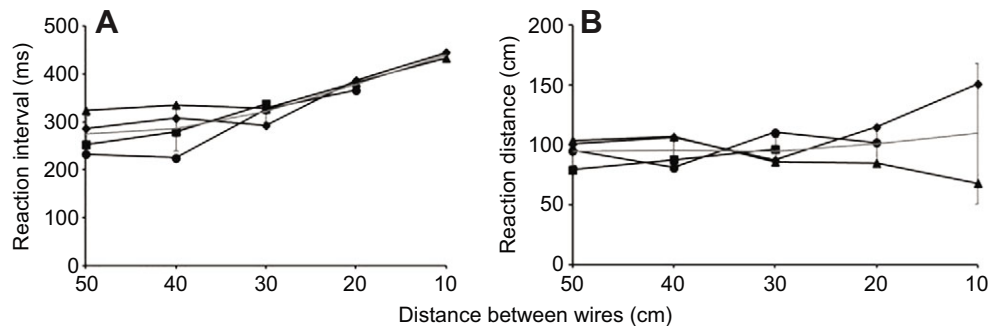
cycle) hardly changed when approaching the obstacle. In the last 500 ms before passing the obstacle frame, it was around 70 ms (Fig. 5). A slightly higher group interval was found only under the 10 cm condition (ANOVA;  $F_{4,79}=2.6270$ ,  $P=0.0406$ ; Fig. 5F), which differed significantly only from the 30 cm condition (Tukey’s,  $P<0.05$ ; Fig. 5D).

The reaction interval, defined as the time span from the first group with three calls to the passage of the obstacle, increased with decreasing wire separation (ANOVA;  $F_{4,158}=20.3211$ ,  $P<0.0001$ ; Fig. 6A). With the 50 cm spacing, bats reacted a mean  $274\pm 40$  ms (mean  $\pm$  s.d.) before reaching the obstacle. With decreasing distance between the wires, the reaction interval increased up to  $440\pm 8.56$  ms for the 10 cm spacing. However, the distance at which bats started to react did not vary significantly between conditions (Kruskal–Wallis;  $\chi^2_4=8.5576$ ,  $P=0.0732$ ) and was a mean of  $99.3\pm 6.3$  cm from the obstacle (Fig. 6B).

Pulse density, the number of calls emitted per meter, differed between tested conditions. In control flights and obstacle flights with wire distances of 50 cm and 40 cm, pulse density was approximately the same during the approach to the obstacle, while the increase in pulse density was especially obvious at gap sizes of 30 cm and below, being highest in the 10 cm wire spacing, which was mastered only by the two better-performing bats (Fig. 7).

## DISCUSSION

Bats avoiding an array of vertically stretched wires face parallel sensory and motor challenges. The motor task of passing between the wires without collision increases in difficulty with a reduction in distance between wires. Reducing the gap size also challenges the



**Fig. 6. Reaction interval and reaction distance of the four bats in flights through the wire obstacle at different wire spacings.** The reaction interval (A) was measured in milliseconds and the reaction distance (B) in centimetres from the beginning of the reaction to the passing through the obstacle. Bats are symbol coded: diamonds, EF1; triangles, EF2; circles, EF3; squares, EF4. The grey solid line shows mean  $\pm$  s.d. values of all bats ( $N=159$ ).

sensory system because the accuracy of localization of the wires must be better at smaller gap sizes. Here we describe an obstacle avoidance experiment in which we forced the bats to increase their localization accuracy by reducing the distance between the wires. Using a constant wire diameter of 1.5 mm, which is far above detection threshold, guaranteed that the difficulty of the sensory task was only determined by the localization accuracy needed to pass the wires. With this approach, we tested the hypothesis that the flight and echolocation behaviour will change when higher localization accuracy is needed.

#### Obstacle avoidance performance

In all bats, the performance dropped with a reduction in the spacing between wires. The task was especially difficult at a gap size of 10 cm, where only two of the four bats were able to avoid the obstacles in any of 10 test flights. These better-performing bats also reached higher avoidance scores at all other gap sizes. This raises the question whether these differences reflect different echolocation abilities of the bats.

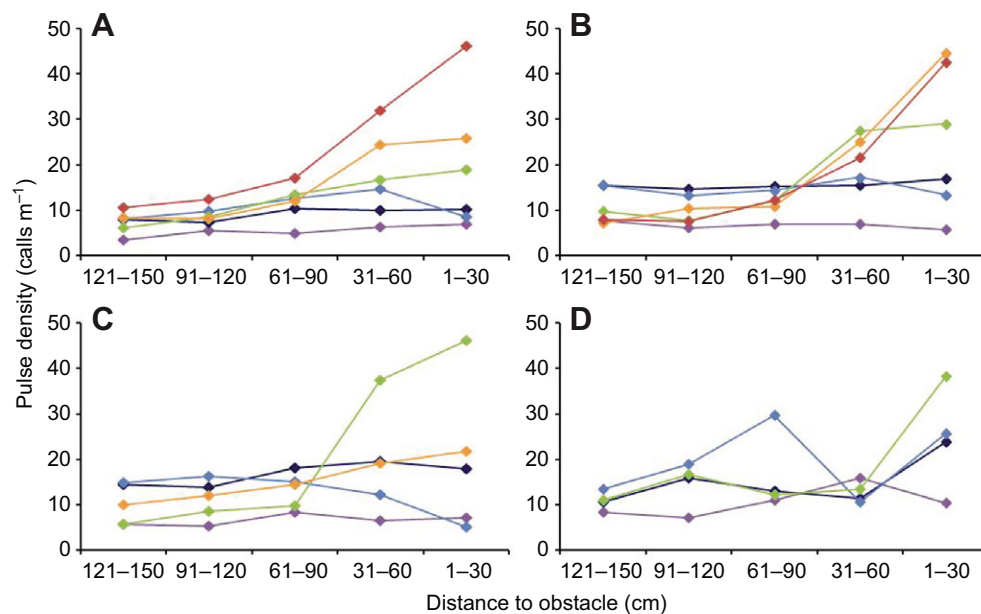
#### Task-dependent changes in flight and echolocation behaviour

The flight behaviour was basically similar in all bats. They increased flight height and flew to the side before they passed the obstacle,

thereby reducing flight speed. This reaction was strongest with the 10 cm spacing, which was only mastered by EF1 and EF2. These changes in flight behaviour resulted in a longer time between the first acoustic response to the obstacle array and passing through the array (the reaction interval), giving the bats more time to collect the information necessary to avoid the obstacles. Independent of gap size, all bats started to react in their echolocation behaviour approximately 1.5 to 1.0 m from the obstacle. This indicates that the echo information which initiated the approach behaviour was much the same under all conditions.

The most obvious change in echolocation behaviour in response to the increasing difficulty was a change in the arrangement of pulses in groups. At all wire spacings, the bats showed characteristic approach behaviour and increased the number of calls per group with decreasing distance to the obstacle. With decreasing gap size, the number of groups was increased as the bats reduced flight speed. The number of calls within a group was also increased and the within pulse intervals became shorter. These changes were especially evident in the two better-performing bats. The number of pulses per group also correlated positively with the performance of the bats. For the same gap size, the better-performing bats always emitted groups with more pulses throughout the whole approach.

With increasing difficulty, the pulse density also increased and was highest in the 10 cm condition. A high pulse density guarantees



**Fig. 7. Pulse density of the four bats from exemplary flights in each condition.** Each bat is shown separately: EF1 (A), EF2 (B), EF3 (C) and EF4 (D). Wire spacings are colour coded: purple, control flight; dark blue, 50 cm; light blue, 40 cm; green, 30 cm; orange, 20 cm; red, 10 cm distance between wires. Diamonds show the mean value for distance bins of 30 cm.

a high information flow. However, this could also be achieved by increasing the number of calls without arranging them into groups. We therefore conclude that the grouping of signals and the number of signals within a group is crucial for the precise localization of obstacles.

In other experiments that addressed somewhat different questions but in which bats also had to perform echolocation tasks of increasing difficulty, the animals also varied the signal patterns and emitted more signals per group with increasing difficulty of the task (Moss and Surlykke, 2001; Moss et al., 2006; Petrites et al., 2009).

Moss et al. (Moss et al., 2006) recorded the echolocation behaviour of *E. fuscus* that captured tethered insects positioned at different distances from background vegetation. They found that the bats produced groups of pulses with rather stable pulse intervals at a higher incidence with increasing difficulty of the task. Moss and Surlykke (Moss and Surlykke, 2001) described similar groups of sounds with relatively stable intervals in bats approaching prey and termed them 'sonar strobe groups'. In both publications, the authors propose that the relatively stable within-group pulse intervals play an important role in the analysis of auditory scenes by *E. fuscus*, being used to sharpen the spatial display of the environment. In our approach sequences, we did not find stable within-group pulse intervals in groups of three or more signals. The within-group pulse interval was continuously reduced within most of the groups and also in succeeding groups when approaching the obstacles (Fig. 4). Because of the changing within-group pulse intervals, the term 'strobe group' is, in our opinion, misleading, because it somehow suggests that bats ensonify their targets like a stroboscope at a constant rate. However, our study and those of Moss and Surlykke (Moss and Surlykke, 2001) and Moss et al. (Moss et al., 2006) share the finding that bats that are challenged by a more difficult echolocation task increase the number of pulses in their pulse groups.

Petrites et al. (Petrites et al., 2009) investigated how the pulse emission pattern of *E. fuscus* was influenced by different clutter conditions when flying through corridors of different width formed by rows of vertically hanging chains. At higher clutter densities the bats had to perform the more challenging motor task of flying through a narrower corridor and avoiding the chains. With increasing difficulty the bats produced more groups with two and three pulses and flew more slowly. The authors assumed that short within-group pulse intervals indicate that bats concentrate on nearby targets whereas long inter-group pulse intervals provide the time to probe farther into the acoustic scene. They pointed out that short pulse intervals have the disadvantage that echoes from far-off targets of a previous call could be assigned to the next call, thus creating an echo ambiguity, which was defined as ambiguity type II by Melcón et al. (Melcón et al., 2011). However, the increase of clutter density has no effect on type II ambiguity, which is only determined by the spatial relationship between the bat and far-off targets (Melcón et al., 2011). Additionally, bats landing at a site with no clutter targets behind it and thus facing no ambiguity problem also produce group patterns typical of an approach. We therefore assume that the bats reacted to the more stringent requirements of localization accuracy to avoid the chains in a narrower corridor by producing more pulse groups with more pulses and by flying more slowly. These data resemble the results of our study.

Moss and Surlykke (Moss and Surlykke, 2001) and Moss et al. (Moss et al., 2006) suggested that stable pulse intervals within the strobe groups may be used by the bats to sharpen the spatial display of its environment. They assumed that 'the grouping of signals would serve to increase the information carried by echoes in discrete

blocks of time' [p. 2224 (Moss and Surlykke, 2001)]. They also pointed out that the bats produce signal groups when a detailed assessment of surroundings may be particularly important for the planning of further motor reactions. Moss et al. (Moss et al., 2006) came up with the hypothesis that 'sonar strobe groups are used to build a spatial representation of the environment from a collection of "snapshots", and information extracted from these successive snapshots guides the update of appropriate motor behaviours' (p. 10). They also assumed that 'the stable pulse intervals of strobe groups may support sharpening of the spatial images carried by echoes' and modulate the activity in populations of neurons encoding spatial information, possibly by affecting echo-delay tuning.

We found that the within-group pulse intervals, especially in groups with many signals, are far less stable and are reduced within groups in relation to the decreasing distance to the obstacle. However, we agree with Moss and Surlykke (Moss and Surlykke, 2001) and Moss et al. (Moss et al., 2006) that grouping likely results in sharpening and stabilizing the neural representation of target distance in an auditory scene. We also agree that bats produce signal groups when a detailed assessment of the surroundings may be particularly important. Thus from the literature as well as from our own data, we conclude that an increase in the number of signals per group probably leads to a more precise localization of targets. This conclusion is supported by the result that in comparable situations the better-performing bats always emitted groups with more signals than the less-skilled bats.

Neurophysiological data may give some hints as to the neural mechanisms that could improve the localization accuracy by integrating and sharpening the spatial information delivered by groups with more signals. Delay-tuned neurons change their best delay in response to either the temporal arrangement and/or the amplitude relationships of stimulus sequences of pulse-echo pairs. Such so-called tracking neurons have been found in the cortical FM-FM area of *Pteronotus parnellii* (O'Neill and Suga, 1982; Suga et al., 1978; Taniguchi et al., 1986) and in the auditory cortex of *M. lucifigus* (Sullivan, 1982; Teng and Wong, 1992; Wong et al., 1992). These data are summarized in Dear et al. (Dear et al., 1993) and Dear and Suga (Dear and Suga, 1995). The neurophysiological data show that delay tuning depends on the temporal and amplitude relationships in sequences or 'packages' of simulated pulse-echo pairs. This suggests that not only the temporal arrangement of signals in groups but also their amplitude patterns may be important for the localization accuracy. There is some evidence from behavioural studies that the amplitudes of pulses within groups are changed systematically. For instance, landing *E. fuscus* increase their pulse amplitude steadily within groups of two to four pulses (Koblitz et al., 2010).

The pulse groups produced by bats resemble the stimuli sequences used in neurophysiological studies (Dear et al., 1993; Dear and Suga, 1995). We hypothesize, therefore, that the series of pulse-echo pairs within each group is processed as a package and that the longer intervals between groups give bats the time to process the information of each package. Our hypothesis is somehow related to the assumption of Moss and Surlykke (Moss and Surlykke, 2001) that 'the grouping of signals would serve to increase the information carried by echoes in discrete blocks of time'. There might be populations of neurons that are specialized to decode the spatial information delivered by the packages of pulse-echo pairs in the different groups found in approach sequences. Such neurons may be tuned to a specific number of pulses, their intervals and amplitudes, and to the approach speed,

in such a way that the reduction in distance to the target during the emission of a group of pulses is compensated by a corresponding reduction in the best delay, thus increasing the localization accuracy. With such highly adapted neurons, bats could track the target of interest. In addition, the localization accuracy may be increased with the number of pulses within a group by an averaging process.

The package hypothesis is supported by data of Koblitz et al. (Koblitz et al., 2010), who showed that in bats approaching a landing bar the emission of pulse groups follows a fixed motor pattern where the information gained from the preceding pulse group determines how many calls will be emitted in the next group. This implies that the information delivered by each package is read out in the interval between groups and is used to adjust the motor behaviour.

We suggest, therefore, that the temporal sequence of pulse groups indicates the update rate for new information on the encountered acoustic scene. Independent of wire distance, the interval between succeeding groups stayed approximately the same at ~70 ms in all bats while flying towards the obstacle. Therefore, the bats would receive a new update every 70 ms, which corresponds to an update rate of approximately 14 Hz. In human vision, single fixations of a visual scene can take 100–600 ms, which would correspond to an update rate of 1.7–10 Hz (Rayner and Pollatsek, 1992). Thus, the update rate for new information in the bat's acoustic scene would be higher than in human vision. The low update rate in humans generates a continuous perception of the world. We therefore assume that bats also perceive the world continuously and not, as often suggested, in a stroboscopic way.

We are aware that our hypothesis is rather speculative, but the database for the generally accepted assumption that echolocating bats gain their information solely by the analysis of single pulse–echo pairs is equally sparse. It does not explain why the response behaviour of neurons changes if they are stimulated with sequences of pulse–echo pairs.

## MATERIALS AND METHODS

### Bats

The experiments were conducted with four adult big brown bats (*E. fuscus*) from February to August 2009 at the University of Tübingen (Germany). The bats were housed under standardized conditions (16.5 h:7.5 h reversed light:dark cycle, 24±2°C temperature, 65±5% humidity) and had free access to water. Bats were kept on a diet of mealworms (*Tenebrio molitor*) supplemented with vitamins (Nutri-Cal®, Albrecht GmbH, Germany) and minerals (Korvimin®, WDT eG, Germany) every second week. On experimental days bats were only fed during the experimental session.

### Experimental setup

We conducted the experiments in a flight room (5.9×3.4×2.9 m) in complete darkness to prevent the use of vision. The walls and the ceiling were covered with sound-absorbing foam to reduce echoes. Bats were trained to fly from a release point (1.20 m above the ground) out of the experimenter's hand to a vertical landing grid (1.30 m above the ground) on the other side of the room. Flight distance between the start and the landing site was 4.0 m; 1.93 m in front of the landing grid, the bats had to pass through an aluminium frame in which an obstacle consisting of vertically stretched wires could be mounted. The frame was movable from left to right and back to prevent the bats from remembering the exact position of the wires. Control flights (six per bat) were conducted without wires. The obstacle consisted of nylon wires with a diameter of 1.5 mm to ensure that the bats were able to detect the wires easily (Schnitzler, 1968; Sümer et al., 2009). The largest distance between the vertical wires was 50 cm, which is far above the ~30 cm wingspan of *E. fuscus*. The distance between the wires was reduced in 10 cm increments to a final gap size of 10 cm.

### Data recording and analysis

The bats' echolocation and flight behaviour were recorded with an infrared video system and a synchronized ultrasonic recording device (PCTape, Department of Animal Physiology, University of Tübingen, Germany). The video system consisted of two infrared cameras (IR CCD Camera, SANYO, Japan; IR 4.8 mm lenses, TV lens, Germany, 50 half-frames s<sup>-1</sup>), positioned behind the bat's release point. Each half frame was illuminated for 1 ms by two infrared flashes mounted onto the cameras. The video data were transferred to two camcorders (DCR-TRV50E, Sony Corporation, Japan) and stored on video tapes. The echolocation signals were picked up through a custom-made ultrasonic microphone (flat frequency response of ±3 dB between 18 and 110 kHz) placed next to the landing grid. The signals were digitized (256 kHz, 16 bit) and stored as .wav files. For analysis, the video recordings were digitized and analysed using commercial software (SemiMotion 6.5, SIMI Reality Motion Systems GmbH). We reconstructed the bats' three-dimensional flight path (reconstruction error ±1.2 cm both in flight direction and height and approximately ±2.2 cm in width) and the position of the landing grid, and calculated the bats' position and their absolute velocity in relation to the x-axis position of the obstacle frame. In the reconstructed flight paths, the position of the aluminium frame was set to x=0 m (Fig. 2). The beginning of flight reaction to the obstacles was defined as the position where the bat's flight path deviated from the flight path in the control condition by more than one standard deviation. Sound recordings were analyzed with custom-written software (Selena, Animal Physiology, University of Tübingen). Signals were displayed as colour spectrograms (FFT 256, Hann) with a dynamic range of 90 dB. Because of auto-padding and interpolation in time, we obtained a frequency resolution of 250 Hz and a time resolution of 0.08 ms. The beginning and end of the calls were measured in the spectrograms and defined as 20 dB below the maximum amplitude. Groups are defined as sequences of two or more calls with distinctly shorter within-group pulse intervals compared with the longer inter-group pulse intervals. Parameters determined in the sequences were pulse number, pulse duration, within-group pulse interval and inter-group pulse interval (defined as the interval between the first call of a group and the last call of the preceding group) and group interval (defined as the interval between the first call of a group and the first call of the preceding group). Moreover, we determined the number of calls in the last long group before passing the obstacle, which we refer to as the terminal group. Sometimes the bats emitted an additional group, usually of two signals before passing the obstacle. These pulses were most likely directed to the landing grid behind the obstacle (Jensen et al., 2005; Surlykke et al., 2009). These signals do not belong to the terminal group and were not included into the statistical analysis. In addition, we calculated pulse density (number of pulses per metre flown) and analyzed the reaction interval, defined as the time interval from the first group with more than two signals until bats passed the obstacle.

### Database and statistics

For each animal and position we recorded at least 15 flights. To determine the obstacle avoidance performance, we analyzed a total of 629 flights and classified them either as flights without contact with the wires or those with contact or collision. For reconstruction of the flight paths and analysis of sound duration, pulse interval and number of calls in the terminal group, we analyzed 10 flights without contact for each bat and condition. The maximum number of correct flights was used under conditions where bats performed fewer than 10 flights without contact. The overall number of flights was 183 with 5742 sounds. In the control condition we analyzed six flights per bat. Group interval and inter-group interval were analyzed for five flights per condition and bat. Pulse density was calculated for one representative flight per bat and condition starting 1.5 m in front of the obstacle. Statistical analyses were performed with Microsoft Excel 2007 (©2006 Microsoft Corporation) and JMP® 7.0.2 for windows (©2007 SAS Institute Inc.). In order to avoid pseudo-replication, we first calculated the average for each of the four bats and then calculated the overall average. For parametric data, we performed Pearson correlation and ANOVA. A *post hoc* Tukey's test was applied to reveal any significant differences between conditions. For non-parametric data, we ran Spearman rank correlation and a Kruskal–Wallis test. *P*-values <0.05 were considered significant.



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**Competing interests**

The authors declare no competing financial interests.

**Author contributions**

A.D., H.-U.S. and S.S. conceived and designed the experiments. S.S. performed the experiments and analyzed the data. H.-U.S. and A.D. contributed methods and analysis tools. S.S., A.D. and H.-U.S. wrote the paper.

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