ORIGINAL PAPER

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Distance discrimination during active electrolocation in the weakly electric fish *Gnathonemus petersii*

Abstract Weakly electric fish use active electrolocation for orientation at night. They emit electric signals (electric organ discharges) which generate an electrical field around their body. By sensing field distortions, fish can detect objects and analyze their properties. It is unclear, however, how accurately they can determine the distance of unknown objects. Four *Gnathonemus petersii* were trained in two-alternative forced-choice procedures to discriminate between two objects differing in their distances to a gate. The fish learned to pass through the gate behind which the corresponding object was farther away. Distance discrimination thresholds for different types of objects were determined. Locomotor and electromotor activity during distance measurement were monitored. Our results revealed that all individuals quickly learned to measure object distance irrespective of size, shape or electrical conductivity of the object material. However, the distances of hollow, water-filled cubes and spheres were consistently misjudged in comparison with solid or more angular objects, being perceived as farther away than they really were. As training continued, fish learned to compensate for these 'electrosensory illusions' and erroneous choices disappeared with time. Distance discrimination thresholds depended on object size and overall object distance. During distance measurement, the fish produced a fast regular rhythm of EOD discharges. A mechanisms for distance determination during active electrolocation is proposed.

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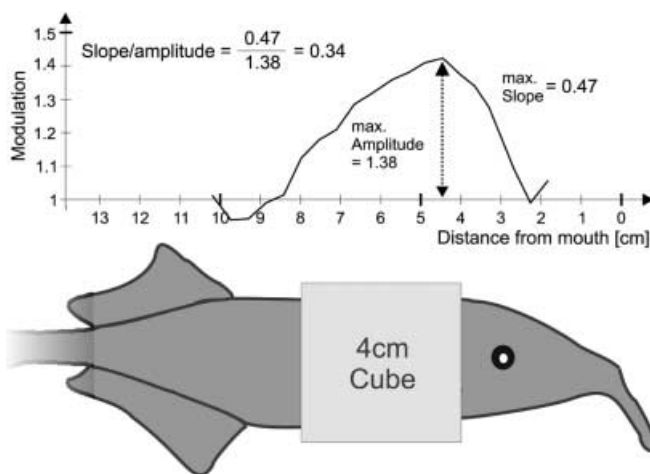Tel.: +1-206-543-2718
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Introduction

A feature of particular importance to animals for orientation within their environment is to know how far away are other animals or objects. Depending on the sensory system, several parameters of the physical input to the sense organs can be used for this necessary distance determination. In echolocating bats, for example, the time delay between emitting a sound and hearing the echo reflected by an object codes object distance (Neuweiler 1990). Visually oriented animals can use the dynamic properties of visual images; for instance, the change of image size or angle of an object projected on the retina during moving (Wagner 1982; Lehrer et al. 1988). In binocular animals with overlapping visual fields of the two eyes, the disparity of the two retinal images depends on object distance and can be used for visual depth perception (Collett and Harkness 1982; Howard and Rogers 1995; Timney and Keil 1999).

In animals which are active during the night, the visual sense is of limited use for orientation. As a substitute for vision, most fish can employ their mechanosensitive lateral line system for object detection in the dark (Bleckmann 1994). In addition, some fishes are electroreceptive and can orient by detecting environmental electric fields (Lissmann and Machin 1958; von der Emde 1998). Weakly electric teleosts use a specialized electric organ to generate their own electrical fields by emitting electric signals (electric organ discharges, EOD). Objects within this field close to the fish cause distortions of the electrical field lines, which are detected by electroreceptors located in the fish's skin. In members of the African Mormyridae, the particular type of

electroreceptor cells thought to be used for object detection (Bell 1990; von der Emde and Bleckmann 1992) are grouped in electroreceptor organs called mormyromasts, which are distributed over most of the body surface of the fish. The process of detecting objects and analyzing their electrical properties by measuring the distortions they cause in a self-produced electrical field has been called *active electrolocation* (Lissmann and Machin 1958; Bastian 1994; von der Emde 1999).

If an object approaches an electrolocating fish, the distortions of the amplitude and waveform of the locally occurring EOD which it causes are greatest at those parts of the skin to which it is closest. Skin areas farther away from the object are less affected and cutaneous electroreceptors beyond a certain distance from the object do not perceive any change in local EOD parameters (Caputi et al. 1998). The electric image of an object (Fig. 1) can thus be defined by the area of the skin where electroreceptors experience an alteration of local EOD parameters relative to the situation without an object present (Rasnow 1996; Caputi et al. 1998; Assad et al. 1999). Electric images of objects depend on object size, shape, distance, and material. If a fish wants to detect, localize, and analyze a nearby object, the electrosensory system must somehow extract this information from the electric image of that object.

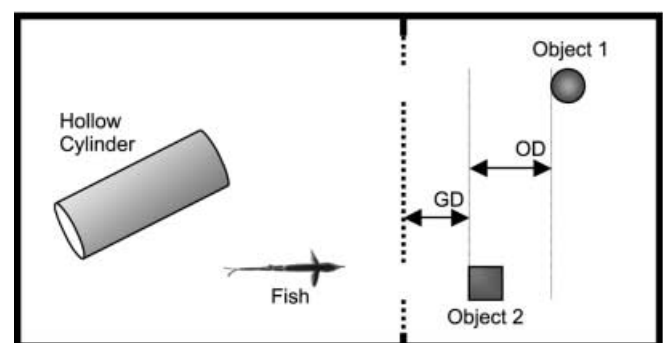The mormyrid *Gnathonemus petersii* is nocturnal and inhabits African freshwater rivers and streams. It was shown that electrolocating *G. petersii* can not only detect an object and analyze its electrical properties (Belbenoit 1970a; von der Emde 1999) but, in addition, they can measure its distance in complete darkness (von der Emde et al. 1998). It is thought that they do so by analyzing the electric image of an object, in particular by determining its ''slope/amplitude ratio'' (Fig. 1). The slope at the edge of the image is a value for the transition from low-amplitude values at the image's rim area to high-amplitude values at its center. It was shown that the rostral slope of the image divided by the maximal image amplitude only depends on the distance of the object and not on other object properties such as size, shape or material (Fig. 1; von der Emde et al. 1998). In the present paper we have further analyzed this unique mechanism for distance determination used by *G. petersii*, by testing how accurately these fish can determine distances of different types of objects using only active electrolocation. Distance discrimination thresholds were measured with various objects, which were placed at different overall distances from the fish. In addition, we monitored both the electromotor and the locomotor behavior of the fish during distance determination. We found that distance discrimination does not depend on the type of object offered, except for hollow, water-filled cubes and ideal spheres. The frequency of EOD production during distance determination is very constant at a high rate of about 80 Hz. Fish can use electroreceptive skin areas on the head or on the dorsal and ventral parts of their flanks for distance determination of objects.

Materials and methods

Animals

Fig. 1 One-dimensional electric image (*above*) of a solid, metal cube which was placed at the side of a *Gnathonemus petersii* at a distance of 22 mm from the skin (*below*). The electric image was measured with two differential electrodes, which were moved along the midline of the fish from the tail towards the mouth. The image is expressed as the modulation of the locally measured amplitude value in the presence of the object relative to the value measured when the object was not present. A value of 1 therefore means that the object did not influence the local amplitude value, while a value of 2 would mean a doubling of the amplitude value in the presence of the object. The *dotted lines* depict the maximal slope at the rostral side of the image and the maximally occurring amplitude modulation value, respectively. The ratio of the slope value and maximal amplitude value is a number that only depends only on the distance of the object, and not on its size, shape or material

Fig. 2 Experimental set-up seen from above. The *thick dotted line* marks the position of the dividing wall, which contained two gates (gaps). The distance between the gate and the closer object was called gate distance (*GD*). The distance between the two objects was called inter-object distance (*OD*). This figure was not drawn to scale

Experimental set-up

Training tanks were divided into two compartments (areas: 60×35 cm^2 and 20×35 cm^2) with the help of a widely perforated, plastic mesh screen (Fig. 2). The bigger of the two compartments was used as the 'living area' for the fish, and contained water plants and a single hollow, water-filled cylinder as a shelter. The fish could swim into the smaller compartment of the tank through two lockable gates (4×4 cm^2) in the dividing wall, only during experimental hours. During training and testing sessions, an object was placed at a certain distance behind each of the two gates. Most experiments were conducted in very dim light, just sufficient for the experimenter to see the fish. However, control experiments (see below) were also conducted in total darkness in order to be sure that the fish were using their electric sense rather than vision. The fish were trained in a food rewarded two-alternative forced-choice procedure to pass through that gate behind which the object was furthest away.

The training procedure

The fish was first required to discriminate between two identical metal cubes placed at different distances behind the gates. In later experiments, several other object combinations were tested. During each training session only a single object combination was used. Before each trial, the objects were first placed at different distances behind the gates and then the trial was started by opening both gates simultaneously. The fish swam to the dividing wall and, after inspecting both objects through the corresponding gates, decided which gate to pass through. If the fish passed through the correct gate, it was rewarded with a worm and then had to swim back into the living compartment. In the case of a wrong decision, the fish was obliged to leave the training compartment immediately. The gates closed again and a new trial was prepared by the experimenter.

The location of the correct gate was determined in a pseudo-random schedule (Gellermann 1933). At the beginning of each training session, inter-object distances (ODs) were large. When the fish had made several correct choices, OD was reduced. The distance of the closer object to its gate (gate distance: GD) was kept constant throughout a single training session but changed between sessions. Depending on the fish, it was possible to conduct between 100 and 120 trials per session (maximum 180 trials).

Threshold determination

After a fish had learned the basic training task with two identical cubes (> 80% correct choices), distance discrimination thresholds were determined. For each object combination and each GD, a separate threshold value was established. During a testing session, the gate-distance of the closer object was kept constant, while the distance of the object located farther away was reduced in steps resulting in progressively smaller ODs. Each testing session started with an OD of 4 cm. After the fish had made more than 90% correct choices in ca. 15 trials, we started to reduce OD.

During threshold determination the fish was not always rewarded for a correct choice in order to avoid reinforcement of subjectively wrong decisions. Before threshold determination started, a rough estimate of the threshold value was established by offering the fish several object combinations covering the whole range of possible distances. After the approximate threshold value was known, the fish was only rewarded for a correct choice when OD was 2 cm or more above this value, i.e., when it was clearly above threshold and it was "easy" for the fish to discriminate the distances of the two objects. For exact threshold determination three trials were conducted in a row with a large OD, during which the fish was rewarded for a correct choice, followed by a single trial with a smaller OD that was closer to threshold and which was not rewarded. This was repeated using three more rewarded trials with large ODs, followed by another test closer to threshold, successively, until a clear threshold was defined. This procedure avoided long frustration periods without a reward and maintained discrimination performance and motivation at a high level.

The distance discrimination threshold was defined as that OD at which a fish discriminated correctly between the two objects in 70% of the trials. Thresholds were determined from the psychophysical functions as the point where a fitted sigmoid line crossed the 70% correct level (see Fig. 3). For each sigmoid, a linear transformation function based on the inverse Boltzmann equation was calculated. By using this transformation function, we transformed each data point and calculated a linear regression line corresponding to the original psychometric function. Different psychometric functions could then be compared using these linear regression lines and applying the appropriate statistical methods (Zar 1984).

Control experiments

It was necessary to be sure that fish were using electroreception rather than visual or mechanoreceptive senses to perform theses tasks. For this control of our results we conducted four types of experiments.

Verification of threshold determination by another experimenter

In these controls, another person conducted a testing session to establish a psychometric function for a given object combination and a given GD.

Experiments in the presence of electrical noise

These experiments were conducted as a positive control to show that the fish were indeed using active electrolocation for measuring object distance. The reafferent sensory signal generated by the fish's own EODs was jammed by playing electrical noise into the tank during a test session. Two carbon electrodes were placed close to and parallel to the dividing wall, on either side of the tank. These were connected to an arbitrary waveform generator (M395, Wavetek) which produced electrical noise with a bandwidth from 200 Hz to 15 kHz, with a minimal amplitude of 2 V cm^{-1}. Mormyromast electroreceptors, which are the only receptor organs used for active electrolocation by *G. petersii*, are sensitive only within this frequency range (Bell 1990; von der Emde and Bleckmann 1992), and we assume that they were completely jammed by this noise. The minimum amplitude of the interfering noise was larger than that of the fish's own EOD, as measured at the pores of the electroreceptor organs on the fish's skin.

Experiments in infrared light

In order to test whether vision played a role during distance determination, experiments were conducted in infrared light (> 880 nm), which is not visible to *G. petersii* (Cialo et al. 1997). The fish was observed with an infrared-sensitive video camera, visualized on a TV screen and a psychometric function was determined.

Experiments with a pane of glass placed between the two objects

An electrically isolating pane of glass (thickness: 2 mm) was placed perpendicular to the dividing wall, between the two objects, in order to prevent interference of the two objects and to prevent the fish from electrolocating both objects simultaneously.

For each type of control experiment, psychometric functions were determined in the same way as during normal sessions. The resulting functions were compared statistically to test for differences during control and regular sessions.

Electrolocation behavior

In order to investigate the electromotor behavior of the fish performing distance measurements, the rhythm of EOD production by
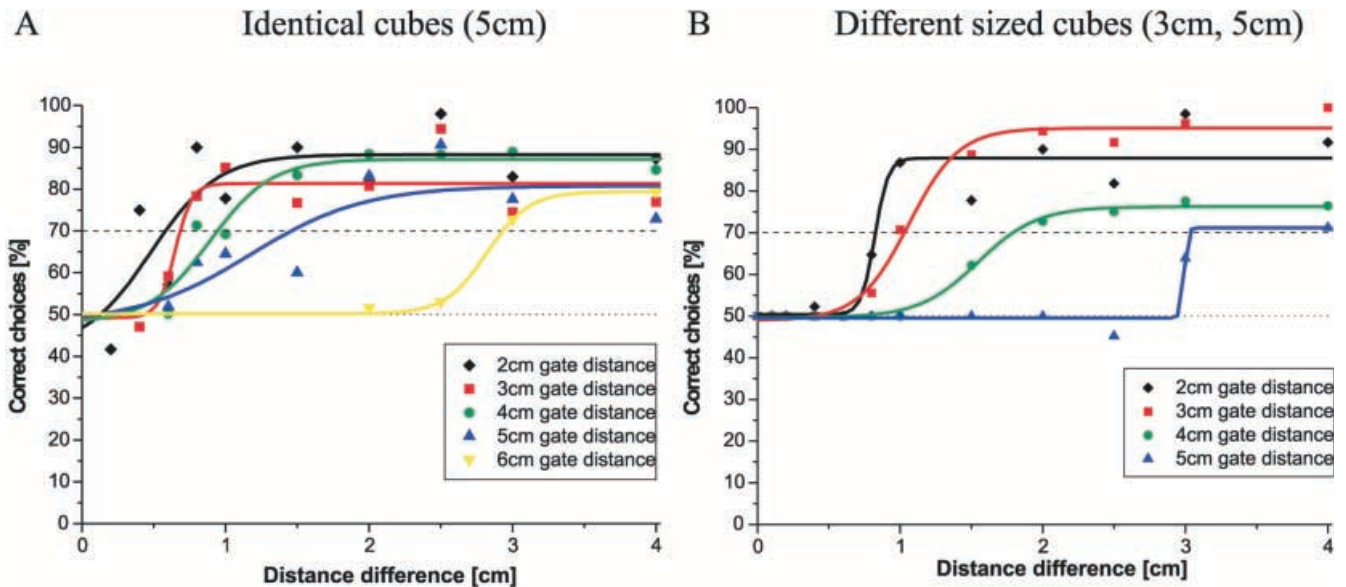
Fig. 3A, B Psychometric functions of a *G. petersii* discriminating between the distances of two metal cubes. Each curve gives the results for a fixed gate distance, i.e., the distance of the closer object to its gate. A sigmoid function was fitted to each data set (*solid lines*). Threshold was defined as that OD where the fitted function crossed the 70% correct level (*dashed lines*). The *dotted lines* mark the chance level of 50% correct choices. **A** Discrimination between two identical metal cubes of a side length of 5 cm. Each data point consists of at least 70 decisions by the animal. **B** Discrimination between two metal cubes of different sizes. The side length of the small cube was 3 cm; the side length of larger cube was 5 cm. Each data point represents at least 50 decisions by the fish

the fish was recorded during entire discrimination trials. Simultaneously, the locomotor behavior of the fish was recorded on videotape with a video camera. Electric signals were recorded with two pairs of electrodes mounted inside the tank with their connecting axes perpendicular to each other, such that they formed a cross. A differential amplifier was connected to each electrode pair. The recorded electrolocation signals of each pair were rectified and added. The resulting signal triggered a TTL-pulse, which was recorded on the audio channel of a videotape. For data analysis, the spike analysis software Spike2 (CED, Cambridge) was used to calculate the instantaneous EOD frequency of the fish versus time. For observation of locomotor behavior of the fish, a video camera was placed above the tank. Entire trials were analyzed offline by playing back the videotape in slow motion and tracking the exact position of the fish and its orientation towards the objects, using the software *Viewer* (BIObserve, Bonn). EOD frequency could thus be synchronized with certain locomotor behaviors of the fish performing the discrimination task.

Results

Training

First, the fish had to learn to pass through either one of the gates in order to receive a worm as a food reward. Depending on the individual, it took 1–2 weeks to learn this. Next, the fish was only rewarded for passing through the gate behind which the object was placed farther away compared to the object behind the other gate. During training we used two identical metal cubes

with a side length of 5 cm, a GD of 3 cm, and an OD of about 4 cm as electrolocation targets. After a period of about 2 weeks, the fish solved this task reliably. To teach the fish to choose objects according to their distance only, we changed GD and OD in a random order during the following 2 weeks. Our goal was that the fish should acquire a general concept of choosing the farther away object, independently of the overall distance and arrangement of the two objects. Depending on the individual fish, it took a period of 5–8 weeks before this concept was learned and testing was started, now using different types of objects as targets for electrolocation.

When new object combinations were used, the fish could always immediately solve the discrimination task and never needed any retraining, no matter what type of objects were used. This indicates that the fish indeed had learned to measure the distances of the objects and not other cues, which depended on the particular types of objects used during training.

Threshold measurements with two identical objects

Metal cubes

In these experiments we used pairs of two identical metal cubes with a side length between 2 cm and 5 cm. Discrimination thresholds were determined for gate distances of 2 cm, 3 cm, 4 cm, and 5 cm. Thresholds were obtained from three fishes. All fish could discriminate between the two objects when ODs were large. When OD was reduced, the fish's performance deteriorated at a certain point, depending on GD. Separate psychometric functions were plotted for each object combination and each GD. For each psychometric function a corresponding regression curve (Boltzmann) was calculated (Fig. 3), from which discrimination thresholds were determined (Figs. 4, 5). Threshold values ranged from values between 0.5 cm and 1 cm for small GDs, to more than 3 cm for larger GDs

(Fig. 4A). Discrimination threshold was inversely correlated with object size (Fig. 4B). In other words, the larger the object and the closer it was located to the fish, the better the fish could determine its distance.

Figure 5 shows relative discrimination thresholds (threshold value ΔD divided by GD) for all fish when tested with two identical metal cubes. Like absolute thresholds, relative thresholds increased with object size. At small GDs up to 3–4 cm, relative thresholds were almost constant. At larger GDs they increased steeply. This increase was more pronounced when the target objects were small.

Plastic cubes

The results obtained in experiments employing two identical plastic cubes (2 cm, 3 cm, 4 cm, and 5 cm side lengths) were qualitatively and quantitatively similar to the results obtained with two identical metal cubes (P always <0.01). Again, threshold values were directly correlated with object size and inversely correlated with GD.

Objects of other shapes

In these experiments, two identical objects of one of three different shapes were offered to the fish. We tested metal pyramids ($5 \times 5 \times 8$ cm^3), metal spheres (diameter $= 4$ cm), and metal plates ($4 \times 4 \times 2$ cm^3). The psychometric functions obtained with different shaped objects were similar to the results found with cubes of corresponding face areas or volumes. Threshold values were almost identical (Fig. 4C; $P < 0.005$ for metal
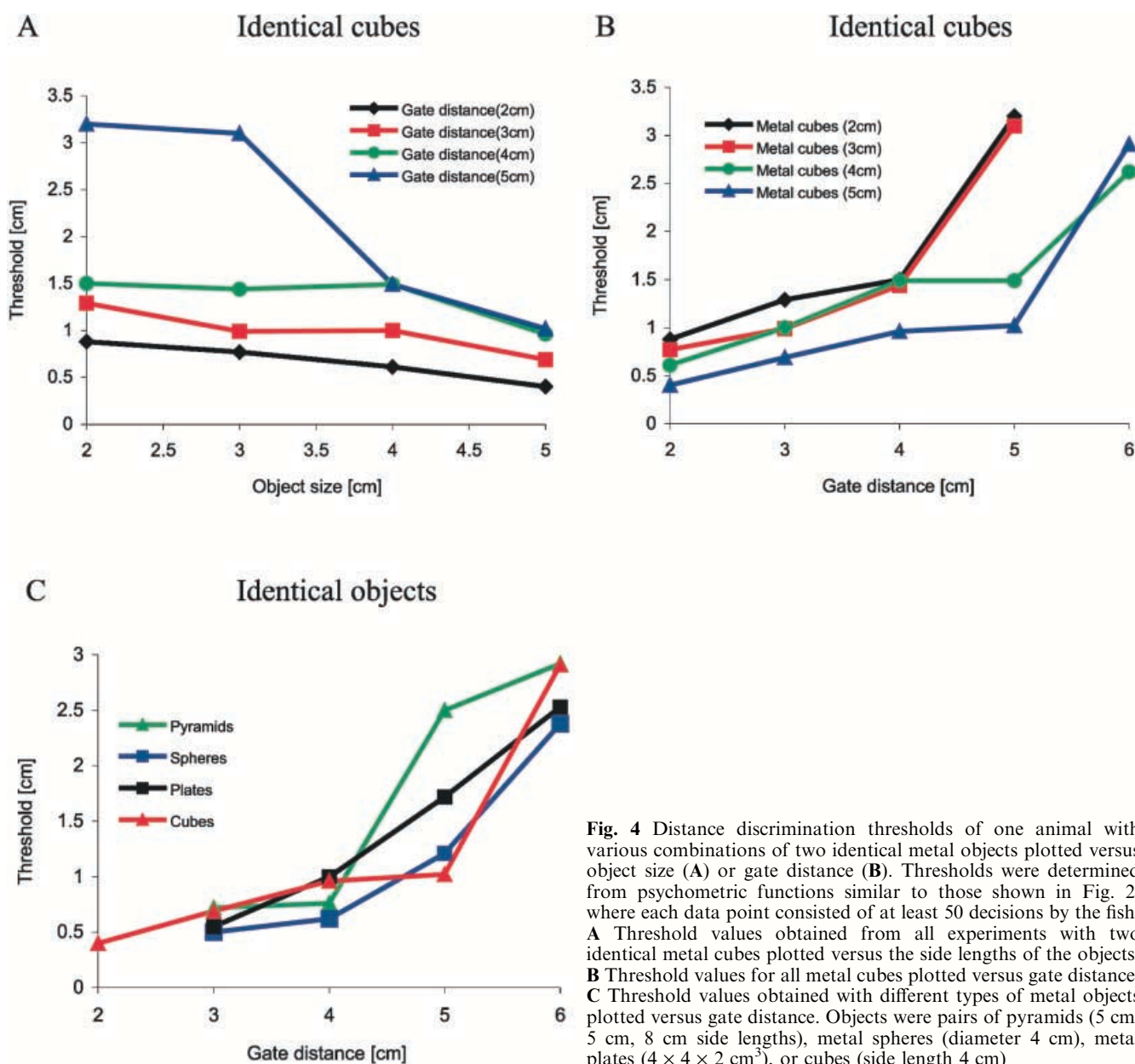
Fig. 4 Distance discrimination thresholds of one animal with various combinations of two identical metal objects plotted versus object size (**A**) or gate distance (**B**). Thresholds were determined from psychometric functions similar to those shown in Fig. 2, where each data point consisted of at least 50 decisions by the fish. **A** Threshold values obtained from all experiments with two identical metal cubes plotted versus the side lengths of the objects. **B** Threshold values for all metal cubes plotted versus gate distance. **C** Threshold values obtained with different types of metal objects plotted versus gate distance. Objects were pairs of pyramids (5 cm, 5 cm, 8 cm side lengths), metal spheres (diameter 4 cm), metal plates ($4 \times 4 \times 2$ cm^3), or cubes (side length 4 cm)
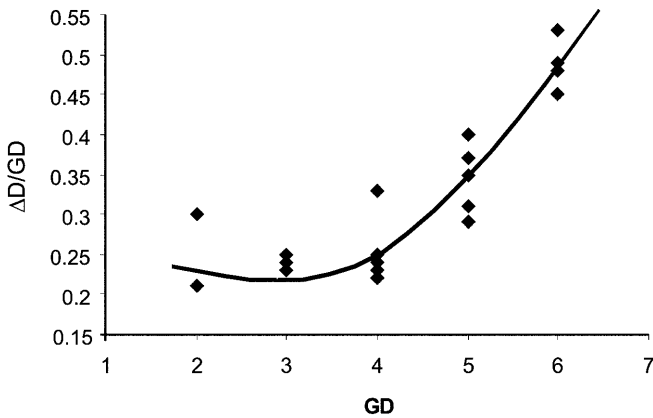
Fig. 5 Relative thresholds ("Weber-ratios", $\Delta D/\mathrm{GD}$) plotted versus GD for experiments performed with a pair of identical metal cubes with a side length of 5 cm. ΔD is the threshold value at a given gate distance. Each point gives the result determined from a psychometric function obtained during one session of a single fish

spheres, $P < 0.005$ for metal plates, $P < 0.01$ for metal pyramids, $n = 2$).

Threshold measurements with two non-identical objects

Cubes of different sizes

Two fish were tested with cubes of the same material but of different sizes (3 cm and 5 cm side lengths). Without further training both animals could discriminate immediately between the two objects when the OD was above threshold. Threshold values were between the values obtained in tests with two identical cubes of corresponding sizes (Fig. 6; P always < 0.01).

Cubes of different material and equal size

The psychometric functions obtained with a pair of equally sized cubes (3 cm and 5 cm side length) made out of different material (metal and plastic) did not differ from those obtained with a pair of identical objects both made of the same material (P always < 0.01).

Cubes of different sizes and different material

Figure 6 shows that the fish's general ability to discriminate distance remained equally good when the objects placed behind the gates were of different size and also made of different materials. The results illustrated were obtained with two cubes of 3 cm and 5 cm and made out of plastic or metal, respectively. The fish's discrimination ability with these object pairs was similar to that observed using different sized cubes made of the same material ($P < 0.05$, $n = 2$; Fig. 6).
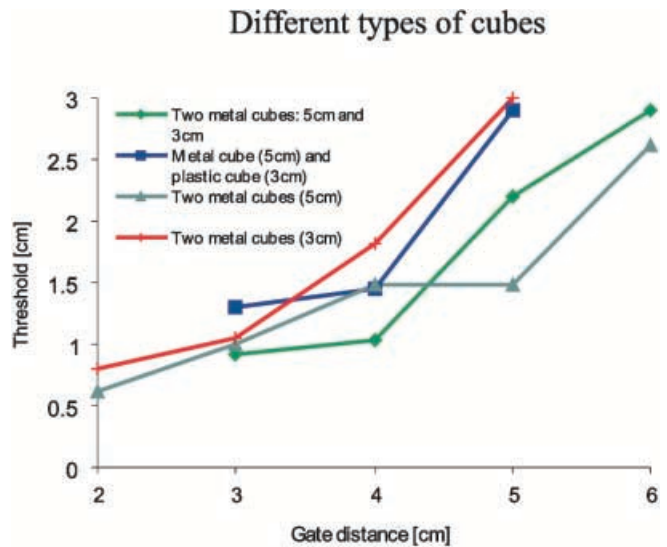

Fig. 6 Distance discrimination thresholds determined from the psychometric functions obtained with one animal using different combinations of cubes. Each curve shows the results for a certain combination of objects, whose size and material varied. Either two identical objects, two metal cubes of 5 cm (*triangles*) or two metal cubes of 3 cm (*crosses*), or a pair of two different objects were used. For the latter, a metal cube with a side length of 5 cm was paired with a metal cube of 3 cm (*diamonds*) or with a plastic cube of 3 cm (*squares*)

Objects of different shapes

We offered to the fish either a metal cube (5 cm side length) together with a metal pyramid (5 cm, 5 cm, 8 cm side lengths; $n = 2$) or a metal cube together with a metal plate of the same 'face' area (4×4 cm²; $n = 2$). Our results showed that the fish did not have any problem to discriminate the distances of these objects despite their different shape. The psychometric functions that resulted from these experiments looked very similar to those obtained with two identical metal cubes, and threshold values were also similar ($P < 0.05$, $n = 2$; Fig. 7). Our results obtained with a pair of a metal cube and a plate also indicate that our fish measured object distance as the distance towards the surface of the object and not towards its center.

The situation changed when either a hollow, water-filled metal cube (4 cm side length) or a metal sphere (diameter: 4 cm) were offered together with a solid metal cube (4 cm). The hollow metal cube had six solid metal faces made out of 1- to 2-mm-thick aluminum, which were soldered together in such a way that tiny holes remained to allow aquarium water to flow in. With these two object combinations, fish started to make mistakes at ODs at which they had previously easily discriminated the distances of two identical solid cubes or two identical metal spheres. To show these results more clearly, the data was presented in another way in Fig. 8. In each graph, two different cases were plotted separately: (1) one curve shows the discrimination performance of the fish for those cases in which the sphere or the hollow,

Two objects of different shape
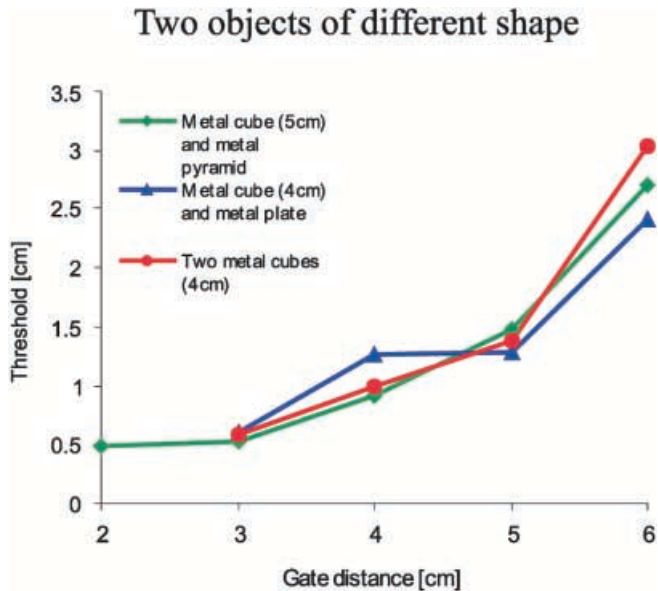
Fig. 7 Threshold values of one fish obtained with objects of different shapes plotted versus GD. A metal cube with a side length of 4 cm was paired with a metal pyramid (*diamonds*), a metal plate (*triangles*), or with another identical metal cube (*squares*)

water-filled cube was located farther from the corresponding gate than the solid cube (open symbols), and (2) another curve shows the results when the solid cube was located farther away than the other object (filled symbols).

When the hollow, water-filled cube or the sphere were positioned farther away, the fish's performance was always around 90% correct choices, no matter how small the inter-object distance was. Even when the two object were placed at identical distances from their gates the fish continued to chose the hollow, water-filled cube or the sphere, respectively, and not the solid cube (distance difference = 0 in Fig. 8A, B).

When the solid cube was placed farther away than the sphere or the hollow, water-filled cube, fish started to make mistakes even at relatively large ODs. When distance differences were reduced, the percentage of correct choices dropped below the chance level (50% correct choices) and finally reached values between 10% and 20% correct choices (Fig. 8A, B). In this situation, the fish chose the sphere (or the hollow, water-filled cube) even though the choice of the solid cube would have been correct. This means that the sphere (or the hollow, water-filled cube) appeared to the fish to be farther away than the solid cube even though the opposite was true. In analogy to optical illusions we have called this phenomenon an "*electrical illusion*". In our experiments, electrical illusions never occurred when objects other than hollow, water-filled cubes or spheres were used (e.g., Fig. 8C).

In order to test whether another function than a sigmoid function fitted these data (when the cube was placed farther away than the alternative object – filled squares in Fig. 8) better, we also tried to fit the data set with two sigmoid functions rather than one. To do this,

we assumed additional data points with a y value of 50% correct choices. For the function running from ca. 90% to 50% correct choices, six additional 50% values were assumed at the next x values following the smallest x value with a performance of > 50% correct choices. For the sigmoid running from ca. 10–50% correct choices, five additional 50% data points were assumed at the x values following the last x value with a performance of < 50% correct choices. However, as can be seen from the example shown in Fig. 8A, no better fit could be obtained with two sigmoid functions, rather than just a single sigmoid curve. In particular, in no case was there an obvious "plateau phase" at 50% correct choices, which is the distance difference where the two objects appeared to the fish to be at the same distance (see Discussion).

When we continued to train the fish to discriminate between the distances of a sphere and a cube for about 2 weeks, the electrical illusion shown in Fig. 8B disappeared (Fig. 8D), when the fish was always rewarded for a correct decision. After this time, the fish no longer erroneously judged the sphere to be farther away than the cube, and discrimination thresholds were identical to those obtained with other types of objects. Even when fish were tested with other types of objects (metal pyramids, plates, spheres, ellipsoids) for a period of several months, the illusion did not reappear, indicating that a permanent learning process had taken place.
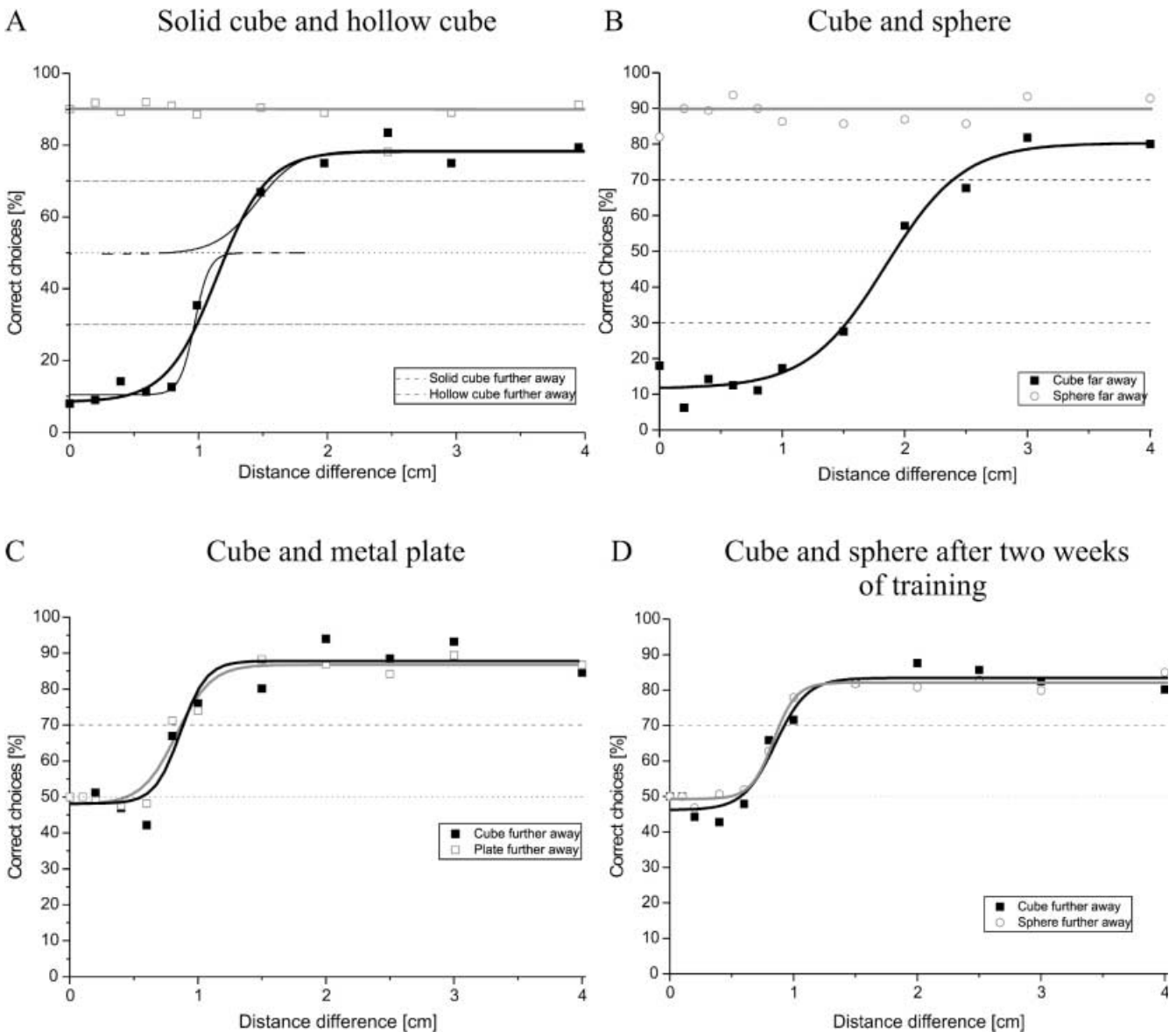
Control experiments

Another experimenter

Results obtained when another person conducted the experiments and determined a psychometric function did not differ from those obtained during regular sessions ($P < 0.05$, $n = 1$). This shows that no involuntarily given cues by the experimenter influenced our threshold measurements.

Experiments in complete darkness

In the presence of infrared light the psychometric functions obtained were very similar to the functions measured in visible light. The resulting threshold values were not different under the two conditions ($P < 0.05$, $n = 2$; Fig. 9A). This means that the fish did not rely on visible cues for distance discriminating when they were able to use active electrolocation. If, however, active electrolocation was prevented by the presence of masking electrical noise, fish did use visual cues for distance measurement (last column in Fig. 9B).

Electrical noise

Introduction of electrical noise into the testing tank prevented the fish from using active electrolocation be-

A Solid cube and hollow cube

B Cube and sphere

C Cube and metal plate

D Cube and sphere after two weeks of training

Fig. 8 Psychometric functions for one fish discriminating between a solid metal cube (side length 4 cm) and **A** a hollow, water-filled metal cube (side length 4 cm), **B** and **D** a metal sphere, and **C** a metal plate. GD (distance of the closer object) was 2 cm for **A** and **C** and 3 cm for **B** and **D**. In each graph, the results were split into two cases: *filled symbols* (*gray lines*) depict the discrimination performance for those trials during which the solid metal cube was placed farther away from its gate than the alternative object. In this case, the choice of the solid cube was correct. *Open symbols* (*black lines*) show the results obtained when the alternative object (hollow, water-filled cube = squares; sphere = circles; plate = square) was placed farther away than the solid cube. Hence, choices of these alternative objects were correct. Each data point consists of a minimum of 50 decisions by the fish

cause the noise masked the electric signals produced by the fish. When the experiments were performed in darkness, fish could no longer discriminate between the two objects located at different distances. They could, however, solve the task with a high percentage of correct choices when the noise was turned off ($n=1$; Fig. 9B).

This shows that the fish indeed used active electrolocation for distance measurements.

When the electrical noise was presented and the lights were turned on, distance measurements by the fish were again possible (last column in Fig. 9B). In this case, the fish probably relied on visual cues to determine the distance of the objects.

A non-conducting barrier between the two objects

A pane of glass placed between the two objects prevented the fish from electrolocating both objects simultaneously because of its electrically isolating properties. In the presence of the glass pane, the fish's discrimination performance was in most cases identical to that in its absence ($P<0.01$; $n=2$) (Fig. 9C). This shows that in order to compare the distances of the two

A — visible light - darkness

B — Identical metal cubes

C — Dividing glas
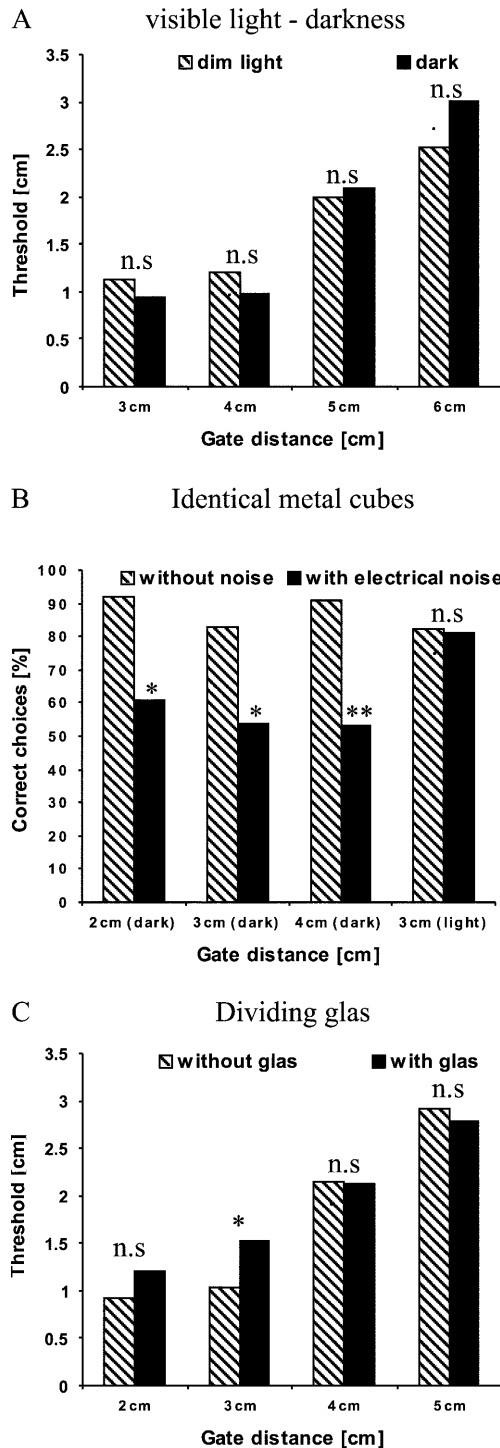
Fig. 9A–C Control experiments. A fish had to discriminate between the distances of two identical metal cubes (n.s. = non significant, *P < 0.05, **P < 0.01). Each data point of the psychometric functions consisted of at least 40 decisions by the fish. A Thresholds obtained in complete darkness (only IR light present) compared with threshold values obtained in the presence of dim visible light. The fish had to discriminate the distances of a large (side length 5 cm) and a small metal cube (3 cm). B Performance of a *G. petersii* discriminating between the distances of two identical metal cubes in the presence and the absence of jamming electrical noise. The closer object was placed at the GD given at the abscissa, the other was located 1.5 cm farther away from its gate. All experiments were conducted in complete darkness, except for that depicted in the last column, which shows the results in the presence of electrical noise with a dim visible light turned on (GD = 3 cm). C Same objects as in A. Threshold values obtained in the presence of a pane of glass positioned between the two objects compared with the usual situation in the absence of the glass

objects, the fish measured the distance of each object separately and compared their distances mentally.

Swimming behavior during the discrimination task

Between each trial the fish stayed in the cylinder provided as shelter in the living compartment of the training tank. When the gates were opened, the fish swam quickly towards the dividing wall. Close to the wall, the fish decreased its swimming speed and approached one of the gates. Close to the gate, the fish stopped for about 1 s directly facing the object behind the gate. Because the fish was not allowed to pass the gate before making a decision, it stopped with its head at the gate opening such that its mouth almost touched the gate's frame. After a short backward movement, the fish turned 90 degrees towards the other gate, placing the gate and the object at its lateral body side. The fish then swam towards the other gate and inspected the object behind it in the same manner as at the first gate. After inspecting each object's distance at least once, the fish finally made a decision by swimming through one of the gates. A correct choice was rewarded with food in the form of a chironomid larva given in the training area. When the fish had eaten the reward it swam back through one of the gates into its living compartment. Some fish continued to search for food in the training compartment. When this happened, the fish was gently chased back to the living area using a plastic rod to cause local turbulence.

When ODs were small, the number of alterations between the two gates was larger than in trials with a large distance difference between the two objects. Near threshold, fish sometimes alternated between the two gate more than ten times before finally making a decision.

Electromotor behavior during the discrimination task

The rhythm of EOD production was monitored in several trials during the discrimination task. The normal resting EOD frequency between trials was low (0.5–15 Hz, corresponding to inter-EOD intervals of 65–2000 ms) and irregular. When the fish approached the gates, frequency increased to 65–70 Hz (intervals between 13 ms and 15 ms). When the fish stopped in front of the gates, EOD rate was high (around 80 Hz, intervals 12.5 ms) and very regular. After passing through a gate

and entering the training compartment where the fish searched for and ate the reward, the EOD frequency decreased somewhat but remained high between 60 Hz and 70 Hz (intervals between 13 ms and 17 ms). When the fish was chased back out of the training area, the EOD frequency increased again up to about 80 Hz. Immediately after leaving the training area and hiding in the shelter, the EOD rhythm slowed to the resting rhythm of about 0.5–15 Hz (Fig. 10).

Discussion

Our results show that during active electrolocation *G. petersii* can use its electric sense to discriminate the relative distance of two objects independently of material, size, or shape of the objects. Once the fish had learned to solve the training task involving two metal cubes, they could immediately also solve similar tasks with other types of objects. This indicates that the fish had indeed learned a concept involving the *distance* of the objects. Thus, *G. petersii* has a true three-dimensional depth perception during active electrolocation.

When comparing object distances, our fish measured the distance towards the surface of an object and not towards its center, as previously proposed (Rasnow 1996).

Fig. 10 Inter-electric organ discharge (EOD) intervals (*thick line*) of a *G. petersii* during a single discrimination trail plotted versus time. The second *y*-axis on the right gives the corresponding instantaneous frequency of EOD emission. *Thin lines around the thick line* depict one standard deviation each. The fish had to discriminate between two identical metal cubes (side length 5 cm). *Vertical lines* mark the occurrence of certain events during the trial. Time periods *A–G* indicate periods where certain behaviors occurred. The first line at about 6.5 s marks the instant of opening the gates, which was the signal for the fish to swim towards them. Before this, the fish was in the hiding tube waiting for the trial to start (*A*). During period *B*, the fish was inspecting the objects. During period *C* the fish had made up its mind and was passing through one of the gates. Subsequently, it was searching for and eating the reward (*D*). Because this individual did not swim back on its own, it was chased back into the other compartment during *E*. During *F*, the fish was leaving the training compartment and stayed in *G* inside its hiding tube to wait for the next trail

### Use of active electrolocation for distance determination

The results of control experiments showed that even when vision was possible, the fish did not use it for distance discrimination. However, if active electrolocation was prevented by introducing jamming electrical noise into the testing tank, fish changed to vision to solve the task. This shows that fish could have used vision to estimate the distance of objects, but did not utilize this ability in our experiments, maybe because depth perception is more accurately performed through active electrolocation. In the wild, however, visual and electric cues might be utilized simultaneously for the various orientation tasks encountered under natural conditions.

The fact that our fish indeed used only active electrolocation to measure the distance of objects is shown convincingly by the experiments with electrical noise. The animals were completely unable to solve the task in the presence of electrical noise with a frequency between 200 Hz and 15 kHz that prevented the perception of their own electrolocation signals (Fig. 9).

Passive electrolocation of objects relies on the perception of low-frequency (< 200 Hz) electric signals not actively emitted by the fish itself (Wilkens et al. 1997; von der Emde 1998). There was a slight chance that distance determination might have been possible by perception of such low frequency signals through ampullary electroreceptors (Zakon 1987). We can exclude, however, that our fish used such cues, because the fish could no longer solved the discrimination task when jamming high-frequency electric noise was present (Fig. 9), despite the fact that low-frequency signals could still be perceived. Similarly, the involvement of other senses, e.g., of the mechanosensory lateral line system (Bleckmann 1994), can be excluded because electrical noise does not prevent its utilization. Taken together our results show that neither the mechanosensory lateral line system, the ampullary system, nor the visual sense did play a role for distance discrimination in our experiments.

Cues for solving the distance discrimination task

Our findings indicate that fish indeed measured the distance of the objects and not some other indirect cues
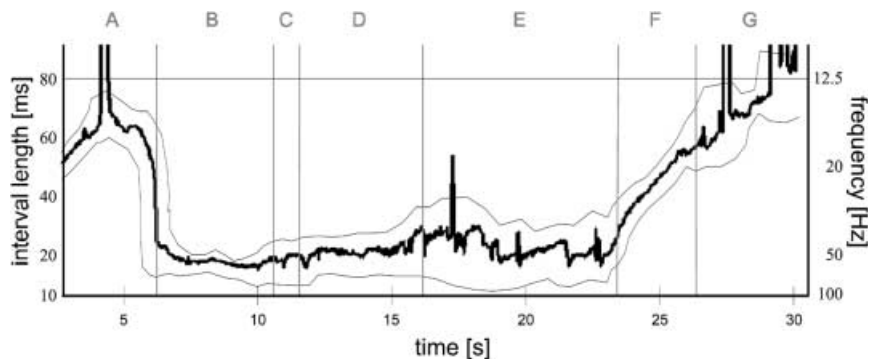
to solve the task. For example, it was not sufficient for the fish to just measure the change of amplitude of the self-produced electric signal caused by the presence of the objects. Signal amplitude depends not only on object distance but also on object size and material. This multiple dependency would lead to ambiguities during distance measurement when two different types of objects are evaluated by the fish (von der Emde et al. 1998). An example might illustrate this point: a large cube located far away from the fish can induce the same maximum amplitude change as a small cube located at a closer distance. Thus, if the fish would only measure the maximal amplitude change for distance determination, it would chose the wrong gate when comparing the distance of a large cube nearby and a small cube farther away. This, however, did not happen in our experiments (Fig. 3B).

Similar problems would occur when a fish only measured the width of the electric image (Fig. 1) which an object projects onto its electroreceptive surface (Rasnow 1996; von der Emde et al. 1998). The electric image of an object increases in width when the object moves away from the fish. However, image width also depends on the size of the object. Large objects produce wider electric images than small objects. This ambiguity would lead to errors in the present task if the distances of two objects of different sizes are compared (Caputi et al. 1998; von der Emde et al. 1998). Because distance discrimination thresholds were not affected under these conditions (Fig. 6), it is very unlikely that image width served as the only cue for distance determination in our fish.

We tested several other *single* parameters of the electric image, such as the slope of the electric image or the integral over its amplitude curve. None of them could have served as a reliable indicator for the distance of an object (von der Emde et al. 1998). Because single parameters did not work, we tested several *combinations* of image parameters such as the relative electric image width, i.e., the ratio of the size of the electric image over its maximum amplitude (Rasnow and Bower 1997). However, our results show that this parameter combination does not provide the fish with unambiguous distance cues with all object combinations. It is affected by a metal plate (such as that used in Fig. 7) in a different way than by a metal cubes (S. Schwarz, unpublished observations). At the same distance from the fish, metal plates and metal cubes give different values. Thus, if our fish would have used relative image width for distance comparison, they wouldn't have been able to correctly discriminate the distances of these two objects (Fig. 7).

The only parameter combination calculated from the electric images which provided the fish with unambiguous distance information for all types of objects used in our experiments was the ratio of the maximal slope of the image over its maximal amplitude (Fig. 1; von der Emde et al. 1998). This *slope/amplitude ratio* can explain all the behavioral results reported in this paper. Moreover, only the use of the slope/amplitude ratio can explain the "electrical illusions" which occur when metal spheres are electrolocated (Fig. 8A, B; von der Emde et al. 1998).

The praying mantis determines the distance of a prey object by watching it from different angles and comparing the images using motion parallax (Poteser and Kral 1995). Our behavioral data indicate that theoretically *G. petersii* could also have used such a mechanism during active electrolocation. This was not very likely, however, because of the lack of typically peering movements as observed for example in the praying mantis (Poteser and Kral 1995). An exact analysis of the swimming behavior when approaching an object in combination with measurements of the electric images showed that such a mechanism does not play a crucial role in *G. petersii*. The fish did not always approach the objects in such a way as to provide them with "views" of the object from different angles. We also have no evidence that during active electrolocation the angle of an object relative to the fish can provide any distance cues. In addition, the use of motion parallax can not explain why the electrical illusions occurred when the fish measured the distances of metal spheres.

Some insects can determine their distance to objects by using position-dependent relative changes in the size of the object's retinal image during approach. This mechanism critically depends on motion and is known as the flow field theory in flies (Wagner 1982) and bees (Lehrer et al. 1988). A condition for this mechanism to work with unknown objects is that image width (or relative image width) changes in proportion to distance, because only then the mechanism is independent of other object parameters. The results of the electric image measurements showed that this is not the case for all object types used in our behavioral experiments (e.g., for the object combination shown in Fig. 7; von der Emde et al. 1998). Therefore, our fish could not have used dynamic changes of these or other image parameters to determine object distance while moving.

Electrical illusions

The occurrence of the electrical illusions showed that for the fish a metal sphere (or a hollow, water-filled cube) appeared to be farther away than it actually was. For example, in the experiment shown in Fig. 8B, the fish chose a sphere located at a distance of 3 cm as often (50% correct choices) as a solid cube placed at a distance of about 5 cm. For the fish, the sphere appeared to be at the same distance as the cube. When the cube was placed at a distance larger than 5 cm, it was judged to be farther away than the sphere. However, when it was placed between 3 cm and 5 cm (e.g., at 4 cm, which is objectively still farther away than the sphere) it was judged to be closer to the fish than the sphere. The fish experienced the experiment shown in Fig. 8B as if a cube was moved from larger distances than the sphere to an equal distance (crossing of 50% line) and finally to closer distances.

Because of the shapes of the curves shown in Fig. 3, which show several data points at 50% correct choices close to a distance difference of 0 cm, it might have been possible that the data set (solid squares) in Fig. 8B would show a better fit to a function displaying a plateau phase at a value of 50% correct choices. However, when we tried to mimic such a function containing a plateau by fitting two sigmoid functions (one from 90% to 50% and one from 10% to 50%) to the data, it turned out that in none out of five cases a better fit could be obtained with two sigmoids compared to a single sigmoid function. Therefore, we decided to fit single sigmoids to all our data sets, which is the usual procedure as described in the psychophysical literature (e.g., Gescheider 1985).

Interestingly, the "electrical illusions", which occurred when the fish measured the distance of a sphere, disappeared after 2 weeks of continuous training (with rewards) of the fish using spheres and cubes as electrolocation targets (Fig. 8D). Presumably, the fish had learned that spheres give wrong distance values and were able to correct for this error while performing the discrimination task. However, in order to do so, they must have been able to recognize the sphere, i.e., to determine object shape. Thus, we hypothesize that *G. petersii* is able to perceive the shape of certain objects during active electrolocation.

Electrolocation behavior during distance determination

When solving the discrimination task fish usually approached each of the two objects repeatedly before making a decision. Close to the gate in front of an object, the fish briefly stopped and then turned to move to the alternative object. Thus, objects were inspected with two electroreceptive surfaces: first with the "forehead", an area above the mouth, which is densely covered with electroreceptor organs (Harder 1972). Secondly, the object was often positioned facing the lateral side of the body. Electroreceptor organs can be found on dorsal and ventral skin areas of the fish's side, but not in a center region close to the lateral line canal. Both on the forehead and on the fish's side electric images of the object might have formed during object inspection, but we can not tell from our experiments whether both or only one of them were used in order to determine object distance.

The rhythm of electric pulse production was very high (ca. 80 Hz, corresponding to an interval length of less than 13 ms) and very regular (standard deviation around 3–7 Hz) when the fish was close to the gate and presumably in the process of determining the distance of the object (Fig. 10). A very similar high rate of EOD emission, also accompanied by a high regularization, was reported in another study of active electrolocation in *G. petersii*, which involved a trained discrimination of the electric properties of objects (von der Emde 1992). The high and regular discharge rhythm during active electrolocation contrast with a very variable and on the average much slower EOD rate during other behaviors, such as during swimming, feeding, or resting. Other species of mormyrids show a very similar regularization of EOD production at a high level during active electrolocation (Moller et al. 1989; von der Emde 1992). In pure electrolocation tasks, it is obviously advantageous for the fish to probe their targets with as many signals as possible. One can only speculate about the reasons for a regular discharge rate.

The spatial limits of active electrolocation

What are the spatial limits of active electrolocation? Our results show that the resolution of distance perception rapidly deteriorates with increasing overall object distance (Fig. 4). At an object distance of 6 cm, the relative discrimination threshold is at or larger than 50% (Fig. 5). Additional experiments performed in order to find the detection threshold of a 5-cm metal cube revealed that this object "disappeared" (was no longer detected) when it was placed at a distance of between 10 cm and 11 cm from the gate (Schwarz 1997). Because the fish directly approached the gate we can say that a 5-cm cube can be detected by a 12- to 14-cm-long *G. petersii* up to a distance of 10–11 cm. This number corresponds well to those reported by other authors, which used other fish species and other types of objects (Belbenoit 1970b; Heiligenberg 1975; Bastian 1976; Rasnow and Bower 1997). A general rule, indicating how far away an electric fish can electrolocate is difficult to formulate because the maximum detection distance critically depends on several parameters, the most important ones being object size (Figs. 3, 5) and water conductivity (von der Emde 1993). As a rule of thumb, one can say that the detection thresholds for medium sized objects in the centimeter range in water of natural conductivity (ca. 100 μS cm^{-1}) are a little less than one standard length of the fish.

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