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Original Research Paper

Weakly electric fish learn both visual and electrosensory cues in a multisensory object discrimination task

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Weakly electric fish use electrosensory, visual, olfactory and lateral line information to guide foraging and navigation behaviors. In many cases they preferentially rely on electrosensory cues. Do fish also memorize non-electrosensory cues? Here, we trained individuals of gymnotiform weakly electric fish *Apteronotus albifrons* in an object discrimination task. Objects were combinations of differently conductive materials covered with differently colored cotton hoods. By setting visual and electrosensory cues in conflict we analyzed the sensory hierarchy among the electrosensory and the visual sense in object discrimination. Our experiments show that: (i) black ghost knifefish can be trained to solve discrimination tasks similarly to the mormyrid fish; (ii) fish preferentially rely on electrosensory cues for object discrimination; (iii) despite the dominance of the electrosense they still learn the visual cue and use it when electrosensory information is not available; (iv) fish prefer the trained combination of rewarded cues over combinations that match only in a single feature and also memorize the non-rewarded combination.

1. Introduction

Most animals have more than a single sensory system and usually salient objects stimulate more than a single sensory modality. The information provided by the different sensory modalities about the object may be integrated to successfully and robustly guide behavior. Indeed, in the context of foraging, for example, information provided by several senses is combined to improve the system's overall performance (e.g. in barn owl, bat, or fish; Knudsen and Knudsen, 1989; Boonman et al., 2013; von der Emde and Bleckmann, 1998, respectively). Multimodal integration has often been found to be in line with Bayesian optimality, i.e. a weighted combination of the individual modalities in which the weight is proportional to the reliability of the respective senses (e.g. Knill and Pouget, 2004; Ernst and Bülthoff, 2004; Angelaki et al., 2009).

In addition to the visual, olfactory, and mechanosensory senses weakly electric fish possess an electric sense (for review, e.g. Bullock and Heiligenberg, 1986). The electrosensory system is divided into two subsystems, namely the active and the passive system. The passive, or ampullary, electrosensory system is phylogenetically ancient and ampullary electroreceptors respond to low-frequency electric fields as evoked for example by muscle activity of other animate objects in the surrounding (e.g. Kalmijn, 1974). The active, tuberous, system, on the other hand, is tuned to higher frequencies such as the fish's self-generated electric field. With the active electrosense fish sense distortions of their own field that are caused by nearby objects during navigation and prey-detection (e.g Bastian, 1981; Nelson and MacIver, 1999) or originating from interference with electric fields of other electric fish and electrocommunication signals (e.g. Benda et al., 2013).

During foraging weakly electric fish use all their senses depending on availability (von der Emde and Bleckmann, 1998; Nelson and MacIver, 1999, for *Gnathonemus petersii* and *Apteronotus albifrons*, respectively). During shelter tracking behavior, i.e. maintenance of a position within a moving shelter, in both the mormyrid *G. petersii* and the gymnotiform *Eigenmannia virescens* combining visual and electrosensory information enhances behavioral performance (Moller, 2002; Stamper et al., 2012; Sutton et al., 2016; see also Schumacher et al., 2016).

Cognitive abilities of weakly electric fish such as object recognition and discrimination have been studied mainly in the African weakly electric fish, in particular in *G. petersii*. These fish can be trained to distinguish objects of different conductive or capacitive properties (von der Emde and Ringer, 1992; von der Emde and Ronacher, 1994) or to distinguish and recognize spatial properties of objects with their electric sense (von der Emde and Schwarz, 2000; Graff et al., 2004). The electric sense enables these fish to occupy a particular niche characterized by low-visibility and

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nocturnal activity. However, they still use all their senses whenever other sensory information is available (e.g. von der Emde and Bleckmann, 1998; Moller, 2002; Walton and Moller, 2010). The South American weakly electric fish are an established model system for the encoding of sensory information and studies on electrocommunication (e.g. Benda et al., 2013; Krahe and Maler, 2014; Chacron et al., 2011). However, relatively little is known about the cognitive abilities of the South American weakly electric fish (Jun et al., 2014, 2016).

Here we investigate the importance of visual and electrosensory information in an object discrimination task in the South American weakly electric fish *A. albifrons*. Even though the eyes of *A. albifrons* are small compared to other fish (Sas and Maler, 1986), have a rather low density of retinal ganglion cells and relatively poor spatial resolution (Takiyama et al., 2015), visual motion information affects the encoding of moving electrosensory stimuli (Bastian, 1982). In this study we conditioned fish to discriminate objects that carried unique combinations of electrosensory as well as visual cues. By setting both cues in conflict, we assessed the sensory hierarchy of visual and electrosensory information. Our results show that *A. albifrons* can be trained to this type of object-recognition task and that in all tested animals the electrosense dominates over the visual sense. However, they are still able to use visual information if electrosensory cues are not available. We can further show that fish not only learn the rewarded stimulus combination but also learn the non-rewarded combination.

2. Methods

In this study four adult individuals of the black ghost knifefish *Apteronotus albifrons* were conditioned in a discrimination task. Fish were obtained from a commercial fish dealer (Aquarium Glaser, Rodgau, Germany) and were kept in groups of 3 fish per tank. Animals were kept in a 12 h:12 h day – night cycle, water temperatures were 26–27 °C and water conductivity was adjusted to 180–200 $\mu S\,cm^{-1}$. All experimental protocols complied with national and European law and were approved by the Ethics Committee of the Ludwig-Maximilians Universität München (permit no: 55.2-1-54-2531-135-09). Experiments were carried out in the early afternoon during the light cycle in a normally illuminated room. During the entire project (pre-training and experimental days depicted in Fig. 2) animals received food only as a reward.

2.1. Setup

The setup consisted of the experimental tank made of 5 mm strong glass. The tank was subdivided into two compartments by PVC walls (Fig. 1). Outer walls were covered with opaque black plastic sheets to prevent influences from outside the tank. The smaller compartment served as the starting box in which fish waited between trials. A gate allowed the animal to swim from the starting box into the bigger compartment. There, two different objects were presented on approximately 5 cm high pedestals (upturned terracotta flower pots). The objects consisted of a cube (3 cm edge length) covered with a colored cotton cloth hood. Cubes were cut from aluminum, graphite, or PVC. During experiments, the tank was neither heated nor was a filter pump present. Between experimental sessions heater and filter were put back in. Water was regularly (once per week) refreshed from the housing tanks of the fish. We generally tried to maintain a similar water conductivity and temperature as in the housing tanks.

Trials were videotaped using a standard web-cam (Logitech C310, 640 × 480 pixel spatial resolution, 15 Hz frame rate) using custom recording software. Trial durations were estimated from the videos as the time between leaving of the starting box and lowering the feeding pipette into the tank.

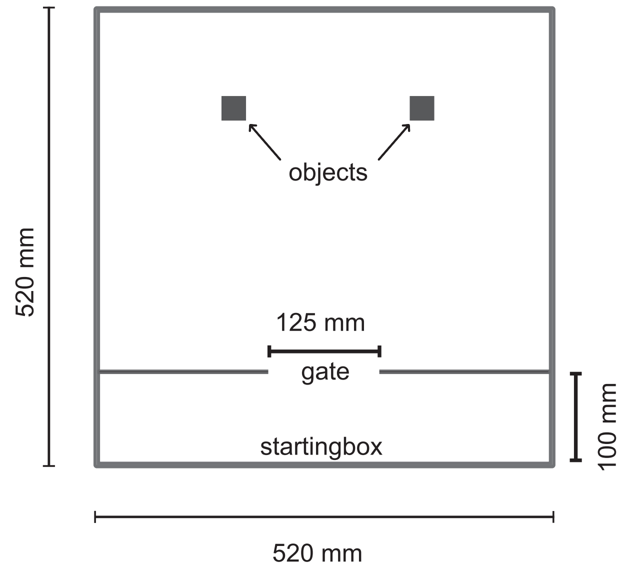

Fig. 1. Experimental tank. Tank was made from glass, dividing wall and gate were made from gray PVC and glued with silicone into the tank. Objects were presented on upturned flower pots that served as pedestals.

2.2. Pre-training phase

Over the course of two weeks (ten experimental days, Monday to Friday, one session per day) the animals were accustomed to the layout of the experimental tank, the feeding with red bloodworms using a pipette, and the general course of the trials. Initially, food rewards were given on top of the rewarded object, and were already present when the gate was opened. After the food reward was found, fish were guided back to the starting box using a net. With ongoing pre-training a delay between the fish's arrival at the rewarded object and the time of the food reward was introduced. Trials were aborted if the fish did not decide within about 60 s after leaving the starting box. In each session that lasted between 40 and 60 min 12–16 bloodworms were offered per individual.

2.3. Training

In the actual training phase neither the pipette nor the food reward were present at the rewarded, S^+, object. Fish had to stay at the selected object in order to be rewarded. The fish indicated a selection by hovering above the selected object. Some fish circled around the object or nibbled at the cloth hood. After the food reward was taken directly from the pipette, the fish were guided back to the starting box and the gate was closed.

Each of the four fish was trained to discriminate between two different combinations of material and color cue (Table 1). The rewarded stimulus consisted of a rewarded color and material, S^+ color and S^+ material, respectively. After each trial the positions of the objects were switched according to a random order (flipping of a coin or throwing a dice). In cases where the arrangement was not changed the objects were moved back and forth to mimic a positional change.

2.4. Tests

After ten training days so-called *test-trials* were randomly interspersed into the training. In these, objects were manipulated

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