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Sensory Performance of Blind Mexican Cave Fish After Destruction of the Canal Neuromasts

H. Abdel-Latif, E. S. Hassan and C. von Campenhausen
 Institut für Zoologie der Universität, D-6500 Mainz

The lateral line system (LLS) of fish consists of sense organs distributed all over the body surface that respond to water movements along the skin (free neuromasts), and others found in sub-epidermal tubes with pores opening to the outside (canal neuromasts).

In the present study three kinds of behavior of the Mexican cave fish *Anoptichthys jordani* were recorded quantitatively after the canal organs had been destroyed. Hairs were pushed through the pores until the lumen of all canals was filled completely as shown by methylene blue (0.01%), which readily enters normal canals but not those stuffed with hairs. Care was taken not to damage the free neuromasts. In addition, a regeneration time of 10 days was allowed to make sure that only the canal neuromasts were inactivated.

First, the spontaneous swimming behavior was recorded in an aquarium (19 × 28 cm, depth of water 10 cm) by means of a TV camera connected to a computer, which transformed the image of the fish into a point, the coordinates of which were stored at intervals of 0.5 s. From these data, the

swimming velocity was computed and averaged. The fish were transferred to the experimental aquarium for the recordings. Figure 1 shows the averaged velocity of six normal fish (empty symbols) and the same fish after stuffing the canals with hairs (filled symbols). It is obvious that the latter fish swim more slowly.

The normal fish swam along the walls more often than the operated ones.

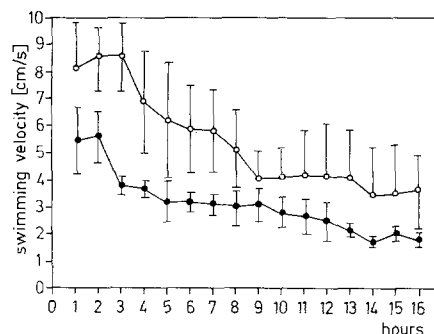


Fig. 1. Swimming velocity of 6 normal fish (○) and 6 fish with canals stuffed with hairs (●) vs time. Velocity computed from recordings during the first 15 min (2/s) of each hour

This can be seen in Fig. 2. Here, the number of recordings in the peripheral (a) and in the central part of the aquarium (b) were counted to compute the distribution factor $k = (a - b)/(a + b)$ which becomes $k = 1$ if the fish swam along the walls all the time, and $k = -1$ if they stayed in the central area exclusively. The central area covered three-quarters of the whole aquarium floor. As one can see, the operated fish (filled symbols) did not swim along the walls as often as the normal ones (empty symbols).

In the second experiment, the capability of the fish to detect a grid pattern at the wall was investigated using the experimental apparatus described in [4]. The experimental aquarium, outlined in Fig. 3, was subdivided by a wall with two openings. Fine rods (diameter 1 mm, distance between the rods

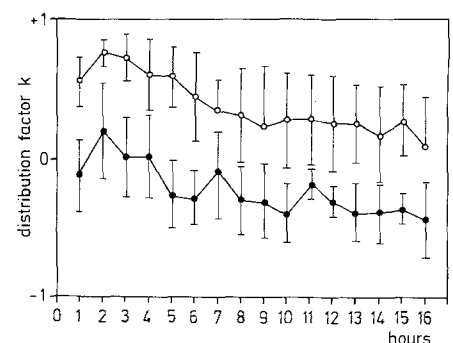


Fig. 2. Distribution factor k (see text) versus time, average of 6 normal fish (○) and 6 fish with canals stuffed with hairs (●). k computed from 2 recordings per s during the first period of 15 min in each hour

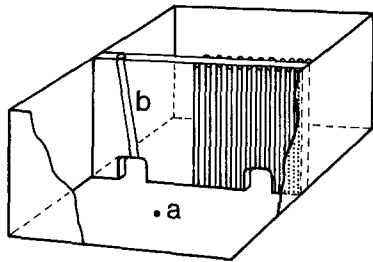


Fig. 3. Experimental aquarium for the second experiment, details in [4]. *a* Feeding place, *b* canal for punishing water jet

10 mm) were fixed to one-half of the wall. The shadow of the fish swimming through an opening was recorded by light detectors below the aquarium floor and the signal was transferred to a computer. The fish were trained to use the opening at the patterned half of the wall and to avoid the other or vice versa. On swimming through the correct opening, the fish triggered an acoustic signal which indicated that food was available at the feeder for a few seconds. On swimming through the wrong door, a sudden water jet was triggered which hit the fish from above. The computer controlled and recorded these events and an electric motor swapped the left and right side of the subdividing wall to prevent place learning. The fish were free to pass from one compartment to the other as frequently as they wanted.

The two upper curves in Fig. 4 give the percentage of correct choices for two normal fish. Each point was calculated from 40 consecutive choices. Fish A needed 9 days and B 7 days for the 600 choices. The averaged values are all

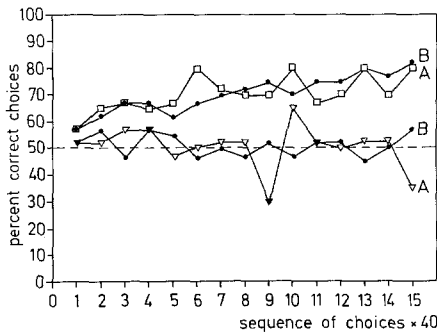


Fig. 4. Detection of the grid at the wall for 2 normal fish (upper curves) and the same 2 fish with canals stuffed with hairs in percent of correct choices. Each point is computed from 40 consecutive choices

above chance level, going up to 80 % correct choices in some recordings. The two other curves show the corresponding data after destruction of the canal neuromasts. In this case, fish A needed 20 days and fish B 14 days for the 600 decisions. These curves indicate no capability of discrimination. The percentage of correct choices was 51.13 % (SD 3.91) in fish A and 50.33 % (SD 8.09) in fish B. The altered swimming behavior was obvious in this experiment. The fish were rarely observed to glide along the wall, which is necessary for the perception of the pattern [7]. Most of the time they stayed on the spot or moved slowly through the open water.

It is obvious from these recordings that elimination of the canal organs does change the behavior. The altered behavior can be interpreted on the basis of earlier results [1, 3–6]. It has been shown that, in a new environment, the cave fish swims fast so as to produce a flow field around itself and glides along the walls and other stationary objects more frequently. According to the flow-field hypothesis, the LLS picks up the modification of the flow field caused by the objects the fish passes. When the fish has become familiar with its surroundings, the swimming behavior changes. The velocity becomes more variable and slower on the average, and the fish swim along the walls less often. The recordings confirm earlier results concerning the decrease in velocity (Fig. 1). In addition, the data indicate that the characteristic swimming behavior, on which the flow-field hypothesis is based, is largely changed: without functional canal organs the fish swim more slowly (Fig. 1), less often along the walls (Fig. 2), and do not discriminate between grid and no-grid pattern (Fig. 4).

No change of behavior was observed in the third experiment, in which the capability to find an oscillating object in the water was tested. A plastic sphere (diameter 5 mm) connected with a steel rod (diameter 2 mm) to an electronically controlled shaker (4810 G Minishaker, amplitude recording with 4371 Accelerometer, both Brüel & Kjaer) above the water was moved up and down sinusoidally at various frequencies (10, 30, 50, 70, 90 Hz) and amplitudes (0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4 mm). The fish were transferred to

the experimental aquarium (20 × 30 × 20 cm) 24 h before the oscillator was lowered into the water. When the fish happened to be at maximal distance from the sphere (about 20 cm) oscillation was switched on and the time until the fish began biting into the sphere was recorded. Then the fish were fed with a food paste of low caloric value presented through a fine plastic tube near the sphere. Usually the fish attacked the oscillating sphere immediately. If they did not approach the sphere within 1 min, this was counted as a negative result.

Figure 5 indicates for seven fish at two frequencies and seven amplitudes that there was no significant difference between normal and operated fish. This result should not be interpreted as counting against the significance of the test concerning the LLS. In other experiments of this type, which will be reported elsewhere, positive responses were reduced and even extinguished when the sensitivity of the sensory cells of the LLS was reduced by cobalt ions in the water. It can be concluded, therefore, that the moving object was found by means of the free neuromasts and that the canal neuromasts are not necessary for this task.

The canal neuromasts appear to be of importance in connection with the flow-field hypothesis. If a blind fish gains information on stationary objects while passing them, it needs information on its own position in space continuously, and this may be derived from acceleration and deceleration as recorded in the vestibular organ. But the

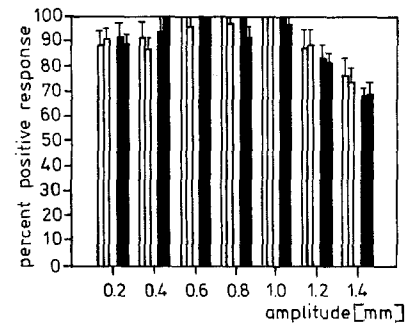


Fig. 5. Percentage of positive responses to the oscillating sphere by 7 fish versus amplitude of oscillation. Four columns for each frequency, the left one of each pair for normal fish, the right one for the same fish after destruction of the canal organs. Empty columns: 50 Hz, filled columns: 70 Hz

amplitude of the hydrodynamic stimulus to the LLS depends on the relative velocity between fish and water [3]. The sensory signal of the LLS, therefore, has to be processed with respect to velocity in space and also with respect to the velocity relative to the water, since the water may be moving itself. The canal neuromasts are ideally suited for recording the relative velocity. The velocity of the water along the skin of the fish gliding through the water will vary locally due to the shape of the fish. According to the Bernoulli equation, different velocities imply pressure differences. This is true for steady flows. If the velocity changes, an additional pressure change proportional to the acceleration will occur [2]. Different pressures along the skin may act at the pores of the canal system and produce the stimuli to the canal neuromasts. If a fish model with artificial canals filled with ink is held in a laminar flow of water, the ink can be seen to leave the model at those pores that are exposed

to the highest water velocity, demonstrating the possibility that canal neuromasts provide information on the relative water velocities along the surface of fish gliding through the water. Whether the stimulation of canal neuromasts by different but steady water velocities at the pores is physically possible is not known, since recordings of the sensory response under appropriate conditions are lacking for cave fish. The question can hardly be answered by calculation because too many physical parameters are not known accurately enough. The canals, for instance, are filled with a jelly of unknown properties. This mass can be observed sticking to hairs when they are pulled out of a canal slowly. It is not known in which way the canal neuromasts are mechanically connected to this mass and how it may improve or impair the recording of steady pressure differences. In the Mexican cave fish, the canal neuromasts have been shown to be nec-

essary for sensory tasks involving the self-produced flow field but not for the task of finding a moving object. The hypothesis is proposed that the canal neuromasts provide information on the water velocities with respect to the fish, which is needed to process the sensory signals of the free neuromasts when the fish is investigating the surroundings by means of the flow field.

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Telencephalic Structures Are Involved in Learning and Memory in the Newt *Triturus alpestris*

E. Wenz and W. Himstedt

Institut für Zoologie, Technische Hochschule, D-6100 Darmstadt

The neurobiology of learning is one of the most central and challenging frontiers of life science. The multivariate nature of the systems involved has made conceptual and analytic progress rather slow. In the search for animal models suitable for studying the neurobiology of learning, amphibians have not received much attention. This neglect is largely due to the fact that compared to other vertebrates amphibians are generally considered to show mainly stereotype, inborn behavior patterns that are hardly modified by learning processes. However, in recent years it has become evident that statements which suggest that amphibians are unable to learn (e.g., [1]) are unjustified. Feeding behavior in toads and clawed frogs can be easily modified by

conditioning [2, 3]. A possible role of telencephalic structures in learning in toads [4] has recently been demonstrated. Little is known, however, about learning and forebrain functions in urodeles.

In order to investigate forebrain involvement in learning in the newt *Triturus alpestris*, conditioning experiments combined with forebrain lesions were initiated. Forty newts were trained to raise their heads expecting food reward when presented with a predator dummy (large black moving rectangle of 100 × 60 mm) which normally elicits avoidance behavior. Conditioned responses (CR, head lifting) occurred regularly in 70 % of the animals after 20 to 30 training trials. Only successfully trained subjects were used for the

subsequent lesion experiments. After opening the skull under anesthesia parts of the telencephalon were removed bilaterally by knife cuts or destroyed by thermocoagulation. Sham operations in three animals did not impair learning and memory.

Total ablation of the telencephalon ($n = 3$) was carried out to see whether this brain structure is necessary for learning. In fact, total removal of both telencephalic hemispheres led to avoidance behavior in previously conditioned animals. Additionally, the level of general activity decreased, and normal feeding behavior was disrupted. In order to localize the learning-related neural site various parts of the telencephalic hemispheres were selectively lesioned bilaterally.

Lesions in the dorsal part of the medial pallium ($n = 4$) did not substantially impair the CR to the stimulus. One animal, however, did not regain the previous level of CR which was 100 % before but dropped to 40 % after lesioning the medial pallium.

Lesions in the dorsal pallium ($n = 4$) caused an initial decrease in CR, but