Catfish with distaste for preservatives

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ABSTRACT. The effect of food quality on the electrodetection performance of two catfish, *Ictalurus nebulosus* (LeSueur, 1819), was assessed in a two-alternative forced-choice experiment while the fish were performing at threshold level. Three different types of food were administered, namely minced beef paste (N), minced beef paste with 0.7% Butylparaben (B), and fish food pellets (P). When fed and rewarded with minced beef paste the fish made a maximum number of correct choices. Minced beef paste with 0.7% Butylparaben reduced the response performance to approximately 50 %. When fish food pellets were used, the performance returned to maximum again. It is concluded that such conditioning experiments are suitable for quantifying the aversiveness of food.

KEY WORDS : *Ictalurus nebulosus*, electroreception, preservative, automated feeding, welfare.

INTRODUCTION

This experiment deals with the effect of automated feeding dispensers on the response behaviour of electrosensitive catfish, *Ictalurus nebulosus*, in a two-alternative forced-choice experiment. In earlier experiments on the detection threshold of the electrosensory system in catfish, the reward was delivered as protein-rich paste, distributed via a peristaltic pump through a silicon tube with a diameter of 3 mm (PETERS et al., 1995a; PETERS et al., 1995b; PETERS et al., 1996; PETERS et al., 1999; EEUWES et al., 2001). The advantage of this system is that food can be delivered under water in any quantity needed. However, the drawback is that the food spoils rather fast and has to be refreshed every one or two days. In order to improve the manner of food distribution, we tested the effect of adding a preservative to the protein-rich paste, and the administration of dry fish food pellets.

MATERIAL AND METHODS

Animals

Two specimens of freshwater catfish (*Ictalurus nebulosus)*, one female (115 gram and 180 mm, referred to as fish BB) and one male (73 gram and 160 mm, referred to as fish BC), were the subjects of this experiment. The catfish were obtained from Visplant (Numansdorp, The Netherlands). They were kept in glass tanks filled with copper-free tap water at Utrecht University until the experiments commenced. During the experiments fish BB was kept in a glass aquarium (91 x 28 cm, water height 9 cm) and fish BC was housed in a similar glass aquarium (91 x 28 cm, water height 8.5 cm). These aquaria were connected to a buffer tank, from which water was circulated and filtered. The total water volume was 180 litres. Once a week, the water was partially refreshed. The tanks were placed in an air-conditioned room, and the temperature of the water was kept at 17± 2˚C with a cooling device. Initially the tanks were filled with copper-free tap water, conductivity 0.25-0.33 mS. The conductivity increased by approximately 0.01 mS in the course of the experiments due to excretions of the fish and feeding. A 12h dark-12h light regime ensured that experiments were only performed during the most active period of the fish, i.e. at night.

Protocol

The electrodetection threshold was determined for each fish in a number of threshold sessions. The catfish were subjected to one session a night. A single session consisted of 100 trials. At the beginning of each trial a light bulb above the test tank was switched on, which caused the fish to seek shelter underneath a PVC strip, approximately the same size as the fish, attached to the wall of the tank (Fig. 1). If the fish stayed underneath its shelter for two seconds, the light was switched off and a weak uniform direct current field was presented.

In the uniform fields, the side at which the anode was located was alternated semi-randomly. If the fish interrupted the infrared bundle nearest to the cathode, food was distributed by the food dispenser at that site, followed by 30 s of dark feeding time. If the fish interrupted the infrared bundle nearest to the anode, the light bulb above the tank was switched on immediately and no food was offered.

At all times, the light above the test tank operated as a negative reinforcer. After a correct choice (choosing the cathode), the strength of the following stimulus was decreased by 1 dB. After a false choice the following stimulus was increased by 3 dB. A trial was marked a no-go trial when fish did not make a choice within ten minutes. In this case, the stimulus did not change in strength. The steps up and down were not equal (respectively 3 dB and 1 dB) because if so, the stimulus would remain undetectable for a long period near the threshold value, and the fish would become less motivated. This so-called staircase method eventually reveals the electrodetection threshold in orientation in catfish (PETERS et al., 1995b).

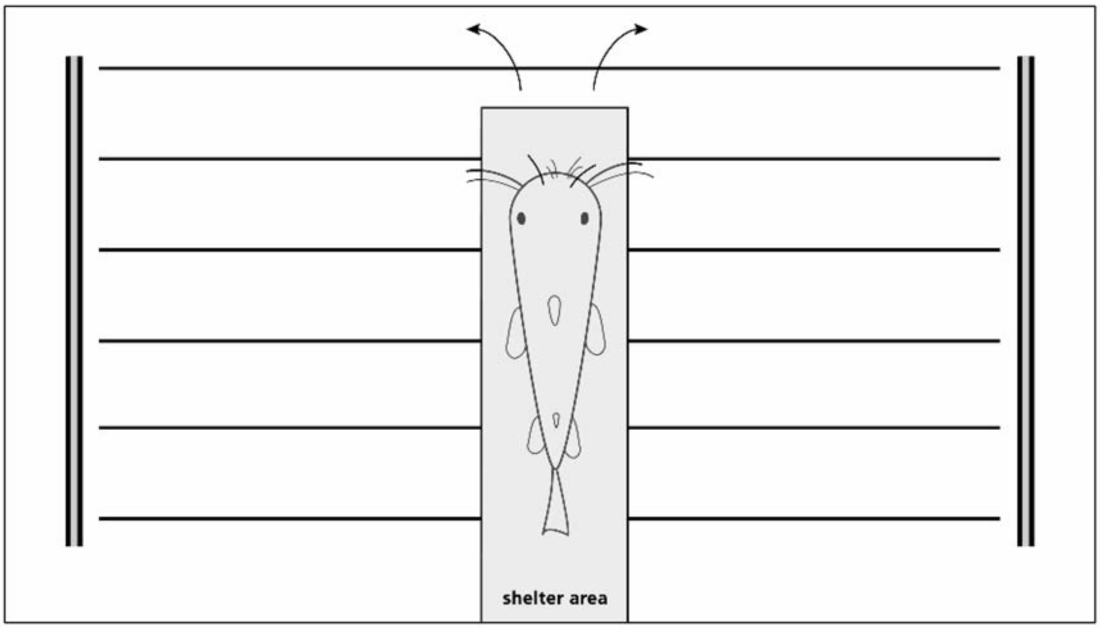

Fig. 1. – Schematic drawing of the experimental tank, top view. Vertical bars represent the strip electrodes. Horizontal lines represent the field lines during a trial. The shelter area provides protection for top lights (not shown) and serves as a dwelling space between trials. Several centimetres from the electrodes infrared detectors are placed on the outside of the tank. At the same position a plastic bar is placed at the bottom of the tank to provide a tactile stimulus for the catfish. This position is marked as the decision point. Food dispensers are placed between decision point and electrodes.

Stimulation

Stimuli were generated by a LAB-PC data acquisition card (National Instruments). The stimulus was fed into a homemade voltage-to-current-converter (VCC), powered by floating power supplies. To generate uniform fields the VCC was connected to a pair of electrodes made of a strip of Perspex (15 x 30 cm) and silver wire.

Shaping

Before the actual testing started, the fish was subjected to a period of shaping. In this period the stimulus protocol differed. The anode location was switched from one side to the other after each trial. The field strength (60-350 µV/cm) was certainly within the perceptive range. As soon as the fish performed at a 90% level, the anode location in uniform fields was randomised with a maximum of three in succession at the same side. When the level of correct choices was 90% or over, the experiment was initiated (PETERS & VAN WIJLAND, 1974).

Data analysis

Threshold has been defined as the stimulus strength that could be maintained by the fish for a certain period of time. As the steps up and down were unequal, every false choice had to be compensated for by three correct choices in order to maintain the same overall stimulus strength. To determine whether the catfish had reached its threshold or was still changing its performance, the running average over twelve successive trials was calculated. If the running average stayed the same for four successive calculations, this value was accepted as the threshold value. This means the false-correct-correct-correct sequence had to be repeated at least three times, in which the order of the false and correct choices is irrelevant as long as the initiation point of the sequence is preceded by more than three correct choices. If a single session yielded more than one threshold value, only the lowest of these values was used in further analysis.

Statistical analysis was performed per individual, since the variance between the two specimens was large. Hence, each catfish was used as its own control. Differences in threshold values, correct choices, false choices, and no-go trials between minced beef paste with (B) and without Butylparaben (N) were analysed using the Mann-Whitney U test.

Composition of food

Three types of food were tested : (i) Food paste composed of 60 g beef, 1.5 g Trouvit elite response fish food, 1.5 g agar-bacto/gelatin (1:1), and 125 ml water. This minced beef paste has been used on a regular basis in conditioning experiments. (ii) The same concoction with 0.7% Butylparaben (from 10% in 95% alcohol). Butylparaben is an antimicrobial and antifungal preservative commonly used in cosmetics, foods, and pharmaceuticals. The used concentration was based on a preliminary study (KLAVER, unpubl.). (iii) Trouvit fish food pellets, eel 2 mm (Trouw, Putten-NL). The minced beef paste, both with (B) and without Butylparaben (N), was delivered via peristaltic pumps and plastic tubing, and a hydraulically-driven syringe delivered the fish food pellets (P). The pellet distribution mechanism showed some imperfections and therefore not all correct choices of the catfish were rewarded with a fish food pellet. However, in such an instance, the succeeding correct choice was properly rewarded.

RESULTS

The responses of fish BB and BC to changing food types and distribution system are presented in Fig. 2. In both fish, the effects of these changes are seen best in the percentage of correct choices and no-go trials.

In fish BB, every deviation from the ‘normal’ food type, i.e. that used during shaping, resulted in an increase of no-go trials and decrease of correct choices ($p \ll 0.001$, N compared to B). Performance was immediately restored when the fish was rewarded with food (N) again. The electrodetection threshold slowly rose ($p=0.002$, N compared to B) when Butylparaben was added to the food, whereas the number of correct choices immediately decreased. Apparently, the number of no-go trials increased at the cost of correct choices. This pattern was repeated at the second administration of Butylparaben (B). As the food type and distribution were changed more radically; the fish pellet dispenser was installed, the electrodetection threshold instantly rose by a factor of twenty-five. Initially, the percentage of no-go trials barely increased, and the percentage of correct choices dropped dramatically. However, after six sessions**,** it performed at its usual threshold level again. This indicates that the fish experienced difficulties in understanding the new set-up.

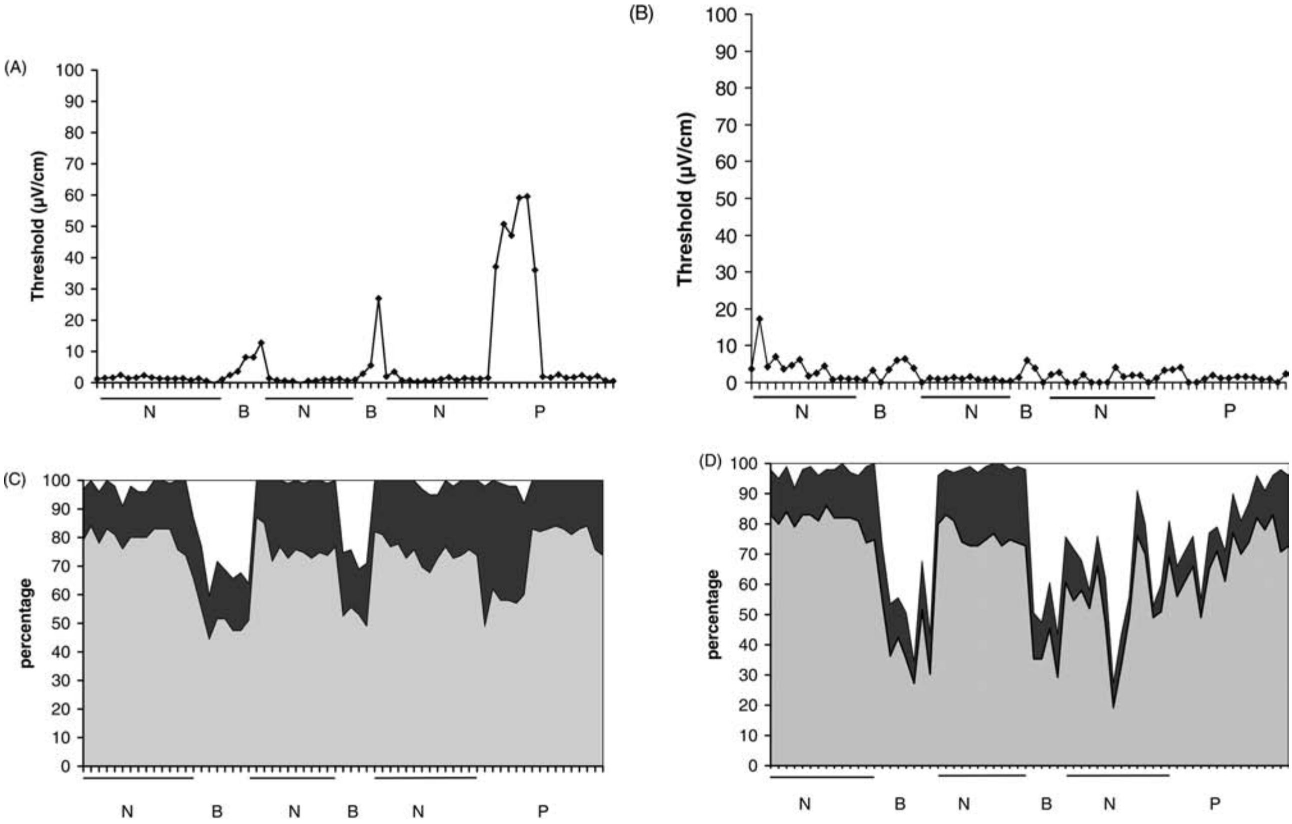

Fig. 2. – The effect of three different types of food on electrodetection performance in catfish. **N**. normal food **B**. food with 0.7% Butylparaben **P.** fish food pellets Trouvit eel 2 mm. (A) Electrodetection thresholds of fish BB. (B) Electrodetection threshold of fish BC. (C) Percentage of correct choices, false choices, and no-go trials of fish BB in a two alternative forced-choice experiment set-up. (D) Percentage of correct choices, false choices, and no-go trials of fish BC in a two alternative forced-choice experiment set-up. In figure C and D, correct choices are represented by the shaded areas, false choices by the black areas and no-go trials by the white areas.

Fish BC showed a similar response pattern when fed with minced beef with Butylparaben (B). An increase in no-go trials and decrease in correct choices were seen ($p \ll 0.001$, N compared to B) with little influence on the electrodetection threshold ($p=0.073$, N compared to B). However, after the second set of experiments with food additives, the performances of the fish were never fully restored. Thus, although fish BC was still capable of performing at threshold level, it seemed to have lost its motivation to perform.

DISCUSSION

Since both catfish were capable of performing at a threshold level comparable to that of previous experiments in the same species (e.g. Eeuwes et al., 2001), several conclusions can be drawn from the collected data.

When Butylparaben was added to the food, the threshold level increased in both fish (borderline significance in fish BC). This implies that the fish either had more difficulties perceiving the fields or did not make enough choices to reach its final threshold. If the catfish were experiencing difficulties in perceiving the fields, one would expect an increase of false choices at the cost of correct choices. However, the number of false choices did not increase in either fish ($p=0.128$ for BB and $p=0.366$ for BC, N compared to B). Therefore the decreasing percentage of correct choices can be explained by the increase of no-go trials. This indicates that the fish was capable of performing the required task and still had full control over its electroreceptive system. During testing with Butylparaben, food was not eaten on a regular basis. This fact, in combination with the response behaviour, leads us to conclude that the catfish is not willing to perform when fed with Butylparaben food. Apparently, food with Butylparaben is not considered to be a proper reward by the catfish, and we might even go so far as to state that our fish have 'taste'.

The sudden increase in the electrodetection threshold of fish BB after changing the set-up to one with a food pellet dispenser indicates difficulties in understanding the set-up. One of the main reasons for these difficulties was determined by video-analysis of the experiments; the food pellets floated on the water-surface whereas the fish had been trained to retrieve its reward near the bottom of the tank. Thus, the fish had to relearn where to get its reward. In spite of this and some imperfections of our food distribution device, food pellets proved suitable as a reinforcer or reward in conditioning experiments. Obviously, a fine-tuned system is to be preferred.

The last conclusion that may be drawn is that this kind of experiment is suited for welfare studies. The course of the detection threshold tells us whether or not the fish has control of its sensory system. The number of no-go trials, on the other hand, can be used as a measure to quantify the extent to which the fish dislike the food presented. Since fish generally are willing to work for food, the balance between a fish's appetite and the aversiveness of additives (or other aversive stimuli) gives us a valuable tool for quantifying the aversiveness of a stimulus.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technical support of Gerard Stooker, Ed van der Veen, Rob Loots, and Rob van Weerden.

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