Fish can encode order in their spatial map

Theresa Burt de Perera

Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK (theresa.burt@zoo.ox.ac.uk)

Animals must often orient through areas that are larger than their perceptual range. The blind Mexican cave fish, *Astyanax fasciatus*, depends on detecting self-induced near-field wave perturbations by objects via the use of its lateral line organ. Its perceptual range (less than or equal to 0.05 m) is greatly exceeded by its ecological ranging requirements (ca. 30 m). Although known to possess a spatial map of its environment, it is not known how this fish links places (or the area over which the perceptual range extends) together. Using the blind cave fish's propensity to accelerate when faced with objects or environments that are recognizably different, I used a behavioural assay to test whether fishes can learn and remember the order of a landmark sequence. I show, to my knowledge for the first time, that blind Mexican cave fish can encode order in their spatial map. The ability to represent the order in which a series of places are spatially linked is a powerful tool for animals that must orient beyond the limit of their perceptual range. The resulting spatial map would be analogous to a jigsaw puzzle, where each piece represents a place whose size is constrained by the animal's perceptual range.

Keywords: spatial map; order; blind cave fish

1. INTRODUCTION

Many animals orient over distances that are greater than their perceptual range. We do not yet know how they are able to travel through space efficiently if they are unable either to follow trails, or to use distant markers to guide them. The blind Mexican cave fish (*Astyanax fasciatus*) exemplifies this problem because its perceptual range (which is no greater than 5 cm; Hassan 1985, 1989) is greatly exceeded by its ecological ranging requirements (ca. 30 m diameter; Mitchell *et al.* 1977).

Until very recently, fish were assumed to be instinct-driven with little or no cognitive ability (Laland *et al.* 2003). However, recent research has challenged this view and we now know that fishes are capable of building long-term memories (Teyke 1989; Warburton 2003) and learning complex cues (Odling-Smee & Braithwaite 2003; Burt de Perera 2004). Although there has been a recent effort to understand the spatial abilities of fishes, our knowledge is still scant in relation to terrestrial animals (Healy 1998). However, the brain circuits involved in spatial cognition appear to be homologous to those of birds and mammals (Broglia *et al.* 2003) and so it would be expected that spatial behaviour in fishes would be as complex as that in land vertebrates. There is evidence that fishes can encode information about their surroundings into a cognitive map (Reese 1989; Teyke 1989; Rodriguez *et al.* 1994). The critical question is how they are able to form a map that is spatially larger than the fish's perceptual range. An ability to encode the order of distinct landmarks would provide fishes with a solution to this problem, allowing them to represent familiar space by linking places together.

Many terrestrial animals are able to learn and remember order, including rats (Compton 1991), monkeys (Swartz *et al.* 1991), pigeons (Terrace 1993), bees (Collett *et al.* 1993), chimpanzees (Biro & Matsuzawa 1999) and humans (Melan *et al.* 1998). However, an understanding of order has never, to my knowledge been tested in fishes. The aim of this study is to test whether blind Mexican

cave fish can learn landmark sequences by encoding serial order into their familiar area map.

As blind Mexican cave fish swim they create a near-field particle displacement wave ahead of them which is distorted by physical structures in the fish's path. The resulting change in the flow field around the fish is detected by superficial lateral line neuromasts (Montgomery *et al.* 2003). The cave fish respond to perceived changes in their environment by temporarily accelerating which extends the bow wave and hence the near-field range of the lateral line system. They eventually habituate to the change and reduce their swimming speed as they become familiar with their environment (Teyke 1988; Burt de Perera 2004). Removal from and later return to the same environment causes dishabituation, but with peak velocity reducing over consecutive iterations reaching a relatively stable level which indicates familiarity. Once stable, the fish's ability to perceive subsequent experimental changes to the environment can be tracked by measuring any increase in the level of dishabituation. By allowing the fish to learn an array before changing the order of the landmarks, it is possible to test whether order is encoded in the fish's spatial map. The hypothesis is that blind Mexican cave fish can learn and remember the order of places in their environment and use this internal representation to recognize a change in the order of a landmark sequence.

The aim of this study was to test the hypothesis that blind Mexican cave fish can encode the order of landmark sequences in their internal representation of space.

2. MATERIAL AND METHODS

(a) Fish

Blind Mexican cave fish, bought from a local supplier, were kept in aerated and filtered aquaria at room temperature (22–23 °C) with a regular supply of TetraMin dried food.

Figure 1. Mean swimming velocity of 10 fish over a 10 min period in pilot test ($\pm$ s.e.). Peak swimming speed is reached between 2 and 5 min. As the fish become familiar with their environment their swimming speed reduces to a low and relatively stable level.

(b) Apparatus

A circular arena (29 cm in diameter) containing a smaller circular holding area (11.5 cm in diameter) was placed within a rectangular aquarium (60 cm $\times$ 30 cm $\times$ 30 cm). The placing of the holding area created an outer annular channel through which the fish could swim and weighted, symmetrical, geometrically different landmarks were positioned into this channel. Pilot studies showed that the fish could discriminate between each possible pairing of the four landmarks selected for the experiment and that there were no differences in their ability to discriminate between these pairings. The constraints of the channel meant that the fish were forced to swim along the landmark sequence rather than across the centre. During each trial the fish were given free access to these landmarks and between trials the fish were caught within the holding area, preventing access to the array. Between each trial the landmarks were raised out of the water to control for experimental manipulation. The sequence remained the same during each set of training trials but was varied at random between fish and treatments. Hence, the two landmarks that were switched between the base and test trials also varied between fish and treatments. The tank was lit overhead by fluorescent lighting and noise was kept to a minimum. The water level was maintained at 7 cm to restrict vertical movement. The starting times of each sequence of training and test trials were varied over the day to control for diurnal rhythms in swimming velocity. Hence, the test trials were performed at different times of the day over the experiment.

(c) Training and testing

Pilot studies showed that the fish habituated to the experimental apparatus within a 10 min period and that they reached peak velocity between 2 and 5 min after introduction (figure 1). The fish required six to eight repeated trials to learn the experimental arena and for their peak velocity to decrease to a relatively stable value (figure 2). Thus, each fish was subjected to eight training trials and on the ninth trial the positions of two landmarks were switched during the inter-trial interval. Mean swimming velocities in minutes two to five (where swimming velocity reached a peak) were compared between trials eight (baseline trial) and nine (test trial). Swimming velocities were measured in these critical trials using a digital camera and an automated tracking programme developed using IMAGEJ.

Figure 2. Mean peak swimming velocity of 10 fish over eight repeated trials in four treatments ($\pm$ s.e.). Treatment 1: light grey dotted and dashed line; treatment 2: black dashed line; treatment 3: black dotted line; treatment 4: solid line. Removal and later return to the same environment causes dishabituation in each fish. However, peak velocity decreases over consecutive trials until it reaches a relatively stable level, indicating that the fish are familiar with the environment. The fish become familiar with an artificial landmark array of four weighted plastic bricks (2 cm $\times$ 1.5 cm) after 6–8 trials.

To test whether fish can encode order, I used a four-step protocol in which potentially alternative position or spatial cues for encoding familiarity with a landmark array were sequentially removed. Over the four steps, the number of cues available that could give the fish information about relative landmark position in space was decreased until the only available cue was the order of the landmark sequence. In step 1, I tested whether the fish were capable of perceiving a simple change in the array by switching two adjacent landmarks between the eighth (base) and ninth (test) trials. A resurgence in dishabituated swimming velocities between the base and test trials would show that fish are capable of recognizing the landmark array potentially using one of the following cues: absolute position of landmarks in relation to global cues; relative distance of landmarks to each other; the sequence of landmarks in relation to each other (order). In step 2, absolute position cues in relation to the surroundings were scrambled during training, to make them uninformative. Dishabituation to the position would demonstrate that absolute position is not a necessary cue for spatial representation. In step 3, I used a similar training procedure to remove the relevance of position, but now tested fish with a landmark swap that preserved the relative distance of all landmarks to each other, but altering order with respect to the swimming direction (clockwise or anticlockwise). However, the test treatment in step 3 also involved, on average, a larger rotation of position (180°) than that encountered during the training phase (on average 90°). To confirm whether order was in fact the cue encoded, I performed a fourth step with just three landmarks in an isosceles triangle. During training, landmarks were rotated an average of 90° , but on the test trial, the landmark swap of the two bottom landmarks in the triangle leading to a change in order involved only a 60° movement so that the fish could not have responded to an increase in the amount of movement between training and testing. In this final treatment, order was changed but no other cue could give information. Dishabituation would demonstrate unequivocally that the fish had perceived the change and that the fish were able to encode order in their spatial map.

3. RESULTS

In step 1, mean peak swimming velocities increased between the base and test trials (figure 3a; paired t -test: $t_{10} = 5.49$; $p < 0.01$) showing that fish are capable of recognizing the landmark array using absolute position of landmarks in relation to global cues, relative distance of landmarks to each other or the sequence of landmarks in relation to each other (order). In step 2, the fish dishabituated to a position swap (figure 3b; paired t -test: $t_{10} = 3.73$; $p < 0.01$), demonstrating that absolute position is not a necessary cue for spatial representation. In step 3, the fish again showed that they perceived the change (figure 3c; paired t -test: $t_{10} = 0.236$; $p = 0.021$) using cues either from order or from the amount of landmark movement. To confirm whether order was in fact the cue encoded, I performed a fourth step with just three landmarks in an isosceles triangle. The fishes demonstrated unequivocally that they had perceived the change (figure 3d; paired t -test: $t_{10} = 2.96$; $p < 0.01$), showing that they do have an internal representation of order.

4. DISCUSSION

Without the facility for leaving geocentrically fixed markers (such as pheromone trails) or for long-distance perception (such as vision), the problem of connecting relatively remote locations in familiar space becomes particularly acute. The ability to encode the order of distinct landmarks provides the blind Mexican cave fish with a solution to this problem, allowing them to represent familiar space by linking places together.

To my knowledge, this is the first time that order sensitivity has been directly tested in a fish, although a study by Reese (1989) suggests that a representation of order may be important in the wild. Butterflyfishes (family Chaetontidae) follow very predictable paths as they swim between feeding patches within their home area and they use these paths repeatedly during the day. After displacing part of the coral reef the fish stopped swimming along the path and searched for the missing coral head, suggesting that they are using a form of pilotage reliant on order. Similar abilities have been found non-trail following insects such as honeybees (*Apis mellifera*), which also orient well beyond their perceptual range (Collett *et al.* 1993). The learnt routes of honeybees consist of associations between landmarks and specific, local vectors (based on path integration with a sun compass and optic flow) that link one landmark to the next forming a path segment (Wehner & Rosell 1985; Srinivasan *et al.* 1996, 1997). As with bees, it is not known whether fishes are able to represent the entire landmark sequence in their memory as do primates, or whether they associate pairs of landmarks as do pigeons (Terrace 1993).

Blind Mexican cave fish use their lateral line system to perceive local structures in their path. At any point in space a cave fish can perceive a certain number of these local structures, and the set of structures within the perceptual range could be used by the cave fish to define a particular place. The results of this study show that the order of the sequence of places could be learnt and stored as an internal representation of space. The fish would be able to orient efficiently by sensing its surroundings using the lateral line system to identify its current location, and then remember

Figure 3. Mean peak swimming velocity of the 10 fishes in each of the four treatments (a–d) in the base and test trials ($\pm$ s.e.). An increase in swimming velocity indicates dishabituation to the environment and hence that the fishes had recognized a change. The levels of dishabituation between the base and test trials were statistically significant in all four treatments (see § 3 for details). Inserts show plan of experimental enclosure (not to scale); arrows indicate landmark switch.

the place that follows. The spatial map would therefore be based upon ordered lists of learnt places through which the

fish could pilot. The actual mechanism by which the cave fish progress between places is not yet known. Rather than discrete landmarks being linked together in a chain, it is likely that the fish's representation of space is more continuous, and is based upon sequences of places that are within the fish's perceptual range at any one time. As it swims across this range, at a defined point, it would begin to perceive the next place and it is the order of these places that is critical to the formation of its spatial map.

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