Maze Learning and Recall in Weakly Electric Fish,

Mormyrus rume proboscirostris Boulenger 1898 (Teleostei, Mormyridae):

Sensory Bases

by

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Abstract

Maze Learning and Recall in Weakly Electric Fish, *Mormyrus rume proboscirostris* Boulenger 1898 (Teleostei, Mormyridae): Sensory Bases

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Animals use navigational strategies ranging from taxes, landmark and compass orientation, and path integration to cognitive maps. The debate concerning the use of mapping strategies, or less complex mechanisms such as *path integration, landmark orientation,* or *dead reckoning* is far from settled. Nocturnal weakly electric fish (family Mormyridae) leave their daytime hiding places at night to forage for food and return at dawn. Thus, these fish provide an excellent model to explore their navigational strategies.

The present studies explore these strategies using a novel paradigm: after learning to swim through a maze (*acquisition*), the maze barriers are removed and the fish allowed to swim through the empty arena (*recall*).

The recall trajectory is an indicator of the fish s navigational strategy. The role of active electrosensing, sight, and lateral line input is explored. Acquisition deteriorates with the number of senses available. Fish with all three senses intact learned the fastest (7 days), whereas fish with these three senses eliminated needed 18 days suggesting still other sensory modalities at work. Recall, however, is similar across groups suggesting independence of external input. Allowing trained fish to start from a novel location provided evidence for path integration and dead reckoning, but not cognitive mapping.

When the arena provided additional visual or electric landmarks during acquisition, during recall with the maze removed but the landmarks left in place, intact fish did not attend to visual but strongly to electric landmarks (array of Plexiglas and Aluminum cubes). However, when the electric landmarks were relocated or removed prior to recall, fish again became independent of unreliable external cues.

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Glossary

Algorithm a prearranged procedure for solving a given task.

Allothetic describing a method of navigation that is based on external cues (e.g., landmarks) rather than internal/body movement cues.

Allocentric describing a method of navigation based on reference to external cues.

Cognitive Mapping a term first described by Tolman (1948) whereby an animal is able to form a mental image of an environment, derive novel routes between locations, and find its way if displaced to a novel location (see Introduction for alternate definitions).

Cue Any form of sensory information that results in an animal altering its behavior; here, frequently used to mean a physical landmark that the animal uses as a reference point in navigation.

Cue Learning The process of learning the arrangement of landmarks or other cues as the animal navigates the environment.

Cue List A navigational strategy based upon the relationship between oneself and external cues, e.g. landmarks.

Dead Reckoning a strategy of navigation in which the animal attends to and integrates the succession of turns it makes when moving from point A to point B; this method may or may not result a single homebound vector. Dead reckoning is based on purely idiothetic, rather than allothetic cues.

Egocentric describing a method of navigation based on cues relative to oneself (see also ***idiothetic***).

Extramaze Cues outside the experimental maze area (i.e., desk, shelves, the experimenter).

Idiothetic describing a method of navigation based on internal, body-centered cues rather than external cues (landmarks, odor, etc.). (See also *egocentric).*

Internal Representation A mental image of the external environment established by an organism. Note that the way it is used here denotes that it may accompany *any* navigational strategy: it is simply the information that the animal extracts from the surroundings as it navigates.

Intramaze Cues within the experimental maze area (landmarks placed in the tank).

Meander The winding path a fish may take in an empty tank after having been trained to make successive right-left turns in a maze. An complete meander takes into account directions and distances (that is, length of the individual turn itself and distance between turns).

Path Integration a navigation strategy that involves the computation of a single homeward vector from a meandering outbound path. The instantaneous integration of various turns and segments in a route from home creates a homeward vector, and may be based on both allothetic and idiothetic cues.

Place A point in space, generally referenced by idiothetic rather than allothetic cues.

Place Learning The process of learning a point in space as such, rather than in reference to proximal cues.

Template-matching The process of internally matching a currently observed view with a remembered view.

Transfer The relocation of an animal from a learned paradigm to an altered paradigm to examine how the animal will behave in the latter.

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Maze Learning and Recall in Weakly Electric Fish, *Mormyrus rume proboscirostris* Boulenger 1898 (Teleostei, Mormyridae): Sensory Bases

Alice G. Walton

Spatial learning is a behavior that virtually all animals perform in some fashion and accordingly one that has created an area of research that is vast and diverse. Humans make use of spatial navigation every day as we travel from home to work, to new places, and back home. Many nonhuman animals   vertebrates and invertebrates included   carry out searches for food and mates. As an animal covers ground, it must keep track of where it began, where it has traveled (in both direction and distance), and how it may return to its starting point. Many theories of animal navigation have been proposed (for example, Tolman, 1948; Gould, 1986; Collett et al., 2003), and this is one debate in which much discussion has arisen but little consensus has been reached.

The use of mazes has been a long time tool in the study of navigation and spatial memory in animals. Although this approach has led to some discoveries regarding various methods of navigation (path integration, cognitive mapping), we propose a new paradigm, which allows us to address some questions that are as yet unanswered. How do teleost fish navigate? What senses are necessary for learning and recall of a spatial pattern? To answer these questions, we propose a novel technique. After an animal has been exposed to a maze pattern and memorized the route from start to finish, the barriers of the maze will be removed. By analyzing the pattern the animal makes as it travels through the now empty arena, we will be able to determine the navigational strategy the animal utilizes. Comparing the behaviors of normal animals to those who have had one or more senses rendered nonfunctional (either via surgery or by manipulating the sensory environment) will then allow us to make assumptions about the synergism between the senses in the process of learning and recalling this route.

Specific Aims:

1. To determine the navigational strategy of the weakly electric fish *Mormyrus rume proboscirostris*.
2. To establish the role of visual and physical landmarks in navigation.
3. To determine the sensory bases underlying navigation, specifically the role of the active electrosense, vision, and the lateral line.

We hypothesize that (1) mormyrid fish navigate in a manner analogous to cognitive mapping, first described by Edward Tolman (1948); alternatively, they may use internalized cue lists (idiothetic algorithms) to generate required motor routines such as path integration or dead reckoning (concepts to be discussed shortly), or a combination of these strategies. (2) When comparing the behaviors of sighted-discharging fish, sighted-discharging fish under dark conditions, and sighted-nondischarging fish (whose electric organ discharge has been surgically rendered inoperative), there will be categorical differences in the learning or cue acquisition phase, but not in the recall phase. Using a circular maze, we hypothesize that (3) after maze barriers are removed and animals are released from a startbox rotated 90° from the one used in training, fish will attend to extramaze cues and head for the correct goal. (4) Sighted-discharging fish tested under dark conditions may take longer to acquire the task, but will behave similarly to sighted-discharging fish during recall. (5) After learning the maze in the presence of 3-dimentional landmarks, animals will pay no attention to the presence of these landmarks in the recall phase, even when their position in the tank is altered. (6) Electrically silent fish tested in darkness will exhibit the greatest deficit in acquiring the maze pattern; if they are able to learn it even with difficultly, they will perform similarly to other groups in the recall phase of the experiment. (7) Sighted-nondischarging fish tested in the dark *and* without the use of the lateral line system may show behavioral differences both in acquisition and in recall; alternatively, they will take longer to acquire the task, but behave similarly to other groups of fish during recall.

The natural ability of mormyrid fish to swim from a home point, search for food and mates, and return home make these fish prime subjects for studying spatial learning and recall. This inherent routine for the fish is one on which we capitalize as we study their navigation in this new paradigm, and answer these questions that have lacked clear answers.

METHODS OF NAVIGATION

The Questionable Existence of the Cognitive Map

Much research has focused on questions regarding the idea of the cognitive map (for review, see Bennett, 1996) and on the attempt to understand its structure, both behaviorally and neurologically. In his 1948 paper Cognitive Maps in Rats and Men, Edward Tolman presented the concept of the cognitive map in contrast to the narrow strip variety (p. 193) of spatial navigation. In the famous sunburst experiment, rats that had been trained to find food at the end of a simple maze, when suddenly presented with a radial pattern, most frequently favored the arm of the radiation, which was spatially closest to the food location of the previous set-up. Tolman concluded that the rats must have taken on a wider comprehensive map (p. 204), regarding the ability to locate food in a particular point in *space* rather than simply a point in the maze, a dichotomy that O Keefe and Nadel (1978) developed in their own work. Tolman suggested that in a strip-map the given position of the animal is connected by only a relatively simple and single path to the goal. This contrasts with a map representation, where if the starting position of the animal be changed or variations in the specific routes be introduced, this wider map will allow the animal still to choose the appropriate new route (p. 193). Tolman s map variants forecast an ongoing debate in which the very existence of the cognitive map is challenged and the idea that animal navigation may depend on other, simpler strategies is proposed (discussed in detail below).

O Keefe and Nadel (1978) proposed a distinction similar to Tolman s in their book *The Hippocampus as Cognitive Map*, and suggest that there are two methods of navigation that animals may use. The taxon system allows an animal to move from point A to point B with the help of landmarks, but provides a relatively inflexible route; the locale system allows the animal to move from point to point even if landmarks are moved, and it is this dynamic system that mirrors Tolman s wider comprehensive map (p. 204).

> The first striking feature of a map is its flexibility. Whereas a route specifies a starting point, a goal, and a particular direction of movement from the former to the latter, a map specifies none of these, either in its construction or its usage. It can be used with equal facility to get from any particular place to any other. Additional flexibility derives from the freedom from specific objects and behaviors. If one path is blocked another can be easily found and followed (1978).

Like Tolman, O Keefe and Nadel suggest that flexibility is a key feature of the cognitive map: if one passage is unavailable or blocked, the animal is able to derive a novel course through the environment to arrive at its goal.

Gallistel and Cramer s (1996) definition is broader than other authors and proposes that a cognitive map is

> a representation of (at least some) geometric relationships among a home site, terrain surrounding the home site, goals to be visited and the terrain surrounding those goals a representation used for navigation .To navigate, the animal locates itself and its goals within the coordinate framework established by the map. This location enables it to set a course for a goal that it cannot currently perceive by reference to the terrain it can perceive (1996, p. 211).

The perception of geometrical relationships has also been studied in Clark s nutcrackers that navigate long distances to find previously buried seeds in the earth (Kamil and Jones, 1997). These authors found that the birds utilized an abstract geometric relationship between landmarks, as they accurately navigated to a goal located equidistantly between two landmarks, even when distances were changed unpredictably from trial to trial. In an earlier article, Gallistel suggests that in order to utilize a mental map, the animal must make a comparison between what is perceived at present and what is remembered of the environment, i.e. its memory image (Gallistel, 1989). This view is reminiscent of Schuster and Amtsfeld s (2002) proposal for template matching, in which the animal compares the current view with a remembered template of the surroundings (to be discussed shortly).

It was long supposed that the cognitive map theory of spatial navigation applied only to vertebrates, but Gould has suggested that it may apply to other animals as well (Gould, 1986). Gould s team trained honeybees to fly from their hive to a food source at site A (160 m from the hive). When the bees were familiar with this flight pattern, they were caught when leaving the hive and displaced to site B, 160m displaced in a different direction from the hive. Rather than making random paths from site B, flying back to the hive, or continuing as if they had not been displaced patterns which might have been expected if they possessed no true map the bees flew directly to site A. The bees performed identically when this paradigm was tested in a 350m spatial arrangement. As one control, bees returning *to* the hive from site A were caught and released from site B; as expected, this group returned directly to the hive. These results, along other control groups set up by Gould, suggest that honeybees may also possess a mental or cognitive map, similar to the locale map of O Keefe and Nadel (1978).

Other researchers are less convinced of Gould s results and suggest that there are other explanations for the bees apparent mapping behavior. Cartwright and Collett (1987) argue that the bee continuously compares what it currently sees with snapshots of what it has seen. Because this may be difficult or impossible in long-distance navigation, there are two systems the bees use: one snapshot includes landmarks surrounding the goal and the other excludes these landmarks. The latter method is used for approach from long distances; the former is used when the animal is close to the goal. Again there is a suggestion of template recognition or template matching, where the current view is compared with a remembered view. This is a view that will reemerge as we move to the navigational strategies of other animals. (More recent papers have introduced new solutions to bees navigation: e.g., Menzel, Greggers, Smith, Berger, Brandt, Brunke, Bundrock, Hülse, Plümpe, Schaupp, Schüttler, Stach, Stindt, Stollhoff, and Watzl, 2005).

Studies on rat spatial memory have also indicated that the animal s ability to navigate its surroundings may not depend on a mental map after all: Olthof, Sutton, Slumskie, D'addetta, and Roberts (1999) have used a radial arm maze paradigm to demonstrate that rats are unable to form an abstract cognitive map of their environment. In a response to Dallal and Meck s (1990) maze experiment, which suggested that rats possess an internal map of their environment which remains functional even after the maze is rotated, Olthof and colleagues also trained rats to find food in six of 12 arms in a radial arm maze. Once the animals had learned the various food goals, the pattern was rotated by either one arm, two arms, three arms (90°), or by 180°; for control groups, six new arms were randomly chosen and food was placed in this new pattern. If the rats had formed an internal or abstract map of the locations, then the authors expected the experimental rats to find food in the new arrangement more accurately than those in the control group. However, there was no difference in the rates at which each group of rats located the arms containing reinforcement. To test the hypothesis that the animals may need an intramaze anchor (or system of landmarks) to locate the correct arms, Olthof trained rats to locate six arms containing varying amounts of food; previous research has indicated that rats may learn to associate quantity with location (Hulse and O Leary, 1982), therefore the animals may be able to use this anchor as a cue in transfer. There was no evidence, however, that the presence of intramaze cues had any effect on the animal s capacity to identify the reward arms in comparison to those animals in control groups. When extramaze cues were altered during the transfer tests (an exact replication of the Dallal and Meck setup), again no evidence was found that experimental animals had an advantage over control animals. All phases of Olthof et al. s experiment found no evidence for the presence of an abstract mental map in rats, and the authors were unable to replicate the findings of Dallal and Meck (1990). Convincing evidence for the cognitive map theory of spatial navigation is clearly scarce and, when it does exist, inconsistent. It is therefore helpful to explore other theories of navigation, as alternatives or possible compliments to the cognitive map.

Benhamou (1996, 1997) doubts the existence of the cognitive map based on past definitions of the concept: put bluntly, the popular assumption that mammals possess cognitive mapping abilities has never been demonstrated (201). He suggests that the distinction between local and global maps should be recognized (he points out that Gallistel does not separate the varieties of spatial learning), as local navigation can be accounted for without evoking cognitive mapping (202). According to Benhamou

> the concept of a true cognitive map (survey map) over a familiar environment implies the ability of an animal to establish the spatial relationships between salient landmarks beyond perceptible reach. Thereby, the animal would mentally build a kind of global-view-from-above puzzle made of subsets of landmarks that are locally perceptible from given ground-level places (1996).

Given this definition, Benhamou (1996) has argued that rats do not appear to possess a cognitive map. In his study, rats learned the location of a hidden platform in a container of opaque water; within the container was a marquee which created inner and outer areas of water (the goal was hidden in the inner arena). In training, the opening of the marquee was rotated predictably from trial to trial: rats were thus trained to use only extra-maze cues for entry. In testing, the opening of the marquee was changed to a novel orientation; rats were unable to find the hidden platform and searched randomly for it. Benhamou therefore suggests that rats were unable to use a cognitive mapping strategy in navigating the experimental environment, since if they did, their search behavior would have been very different. (Although he does not say that his evidence disputes the existence of the cognitive map, he underlines the fact that it does not support it.)

Bennett (1996) is even less convinced of the existence of the cognitive map. In response to Tolman (1948) and O Keefe and Nadel (1978), who have similar conceptions of the cognitive map regarding the requisite short-cutting, he claims that

> There are two simpler explanations of novel short-cutting which must be eliminated before one can conclude that an animal has a cognitive map, *sensu* Tolman, O Keefe and Nadel. These alternatives are: (1) shortcutting by dead reckoning, and (2) short-cutting by recognition of familiar landmarks from a new angle, followed by movement towards them .I argue that no animals today have been shown to have a cognitive map, *sensu* Tolman, O Keefe and Nadel (1996).

Bennett cites the many studies that either have been unable to support Gould s work or have found simpler explanations for the mapping behavior that Gould suggests (1986). Bennett discusses the alternative explanations to cognitive mapping in some detail: path integration or dead reckoning could explain the bees behavior, as could simply a familiarity with the vicinity of the experimental area. Specifically, he suggests that since the bees were acquainted with the space surrounding the experimental V shape, then when at site A they could conceivably recognize landmarks at site B and move towards them. After having arrived in the vicinity of site B the bees could rely on whatever method of navigation they use to locate the goal itself. This explanation for the mapping behavior of the honeybees may be a viable one, and it is certainly worth reconsidering at the conclusion of the present experiments.

Clearly many writers have pondered the existence of the cognitive map and advocate it in varying degrees. The definition that we will adhere to in the present study is that of O Keefe and Nadel (1978) and Tolman (1948): namely, that the cognitive map is defined by *the ability to navigate to a goal even if displaced to a novel location.*

Path Integration, Dead Reckoning, and Landmarks

The ability of certain arthropods to make a straight line back to a starting spot after a meandering outbound trip, usually termed path integration, is another navigation system, which may or may not depend on the presence of landmarks (Mittelstaedt and Mittelstaedt, 1982; Görner and Moller, 2001; Collett, Collett, Chameron, and Wehner, 2003). According to Benhamou (1996), path integration is an updating process that enables a moving animal to keep track in its memory of the location of the starting point of its outward path with respect to its current position by continuously processing route-based information (p. 321, 1996). Kohler and Wehner (2005) have found that when ants familiarized with a home-goal route are displaced back to the goal as they are entering home, they are able to find home reliably; when displaced from home *or* the goal to an arbitrary spot midway through the learned route, they are able to pick up the route from this location and consistently arrive home. They are also able to arrive home after being displaced to a novel point located laterally to the normal route, although more successfully if they are moved from the *goal* rather than from home. These results suggest that there may be a separation between landmark memories and path integration, since ants are able to find their way home when displaced to a point midway in the route (so that landmarks are now out of phase with the path integrator or vector states, p. 10). It is possible that the ants may have been attending to larger external landmarks such as trees to guide them back onto the learned route. If landmark memory is what is being used by these animals, the results seem to suggest that this form of memory can be dissociated from the path integration vector with which it is normally paired and used to reroute the animal back onto the learned route/vector (Kohler and Wehner, 2005).

Shettleworth and Sutton (2005) stress that although the terms path integration and dead reckoning are usually used interchangeably in the literature, the terms should not necessarily be regarded this way. Dead reckoning, they suggest, is based on idiothetic or self-generated (p. 125) cues, whereas path integration involves integrating a meandering outbound trip into a return vector in a more literal sense and may not involve the same internal mechanisms as dead reckoning. In their study, the authors ask the question, how does dead reckoning relate to the presence of landmarks or beacons? Do the two forms of input compete with one another or work synergistically? They found that rats trained in an arena to move from home to collect food and back home made shorter paths when a beacon was present to signify home. When rats were tested without the beacon present, groups that had been trained with or without the beacon did not differ significantly from one another. When, however, the beacon was moved 45°, almost all beacon-trained rats approached the beacon rather than actual home. These data suggest that animals can use dead reckoning successfully regardless of experience, but that there may be a hierarchy of methods of navigation. In another phase of the experiment, animals were trained in the presence of a beacon that represented home or one that moved around the arena randomly; testing revealed that both groups homed accurately when no beacon was present, but when the beacon was moved to a novel location, rats trained in the random group homed more accurately than those in the beacon group. Shettleworth and Sutton suggest that these data indicate that the two forms of navigation act in parallel, rather than in competition, with each other.

Collett et al. (2003) trained ants to navigate an L-shaped path from home to a food goal; the path was then either shortened or lengthened and ants navigated this new configuration once. Landmarks around the food source were present and remained constant throughout the experiment. When the ants were released from a novel test site, they homed not according to the route with which they had been trained, but according to the shortened or lengthened route to which they had been exposed most recently (and only once). The landmarks associated with the food source did not therefore trigger the accustomed homeward route; rather the preceding (novel) journey was what triggered the homing performance under test conditions. The authors suggest that landmarks do not elicit global path integration memories (the familiar route), but may help in the recall of local memories, i.e. the next immediate portion of the path. Whether this hypothesis is applicable not only to ants but for other navigating animals remains to be seen; some work has addressed similar questions in teleost fish (Schuster and Amtsfeld, 2002).

Kamil and Cheng (2001) suggest that Clark s nutcrackers, birds that bury tens of thousands of seeds and later return to the sites with remarkable accuracy, make use of multiple landmarks to increase accuracy when recovering food. Accuracy is dependent upon the number of available landmarks. The authors also cite evidence that evaluating direction is more important for accuracy than is distance. Whether a similar multiple bearings hypothesis might be applicable to other animals particularly fish is unclear (for one reason, the high level of precision required for nutcrackers may be unnecessary in other navigation situations). Most fish appear to employ multiple senses rather than multiple bearings, although it is certainly feasible that the latter may also be important in fish navigation.

TELEOST FISH: SPATIAL LEARNING

Several studies have demonstrated that teleost fish have a robust capacity to learn new spatial arrangements and to navigate between points using various methods of navigation mapping, path integration, dead reckoning based on egocentric and allocentric information (Rodriguez, Duran, Vargas, Torres, and Salas, 1994; Lopez, Bingman, Rodriguez, Gomez, and Salas, 2000; Odling-Smee and Braithwaite, 2003). These animals are excellent subjects not only for studying their navigation systems but also in the interest of making behavioral and/or neuroanatomical comparisons across taxa. Rodriguez et al. (1994) looked at the ability of goldfish to use allocentric (external) and/or egocentric (internal) cues to find a food source in a four-arm maze. Fish were trained in one of four groups: 1) the allocentric group could use only cues external to the maze to locate the correct arm (i.e., the direction of turn varied, but the absolute location of the food relative to room cues remained constant); 2) the egocentric group used only body-centered cues (i.e., only a left or right turn led to the correct arm, and the location of this arm relative to the room varied from trial to trial); 3) the ego-allocentric group of fish was trained to use *both* external and internal cues; and 4) cues in the control group were entirely unreliable. When transfer tests were run in which fish were either released from a novel arm in the maze or the maze itself was rotated, the researchers found that fish trained in the allocentric group most commonly favored the arm that corresponded to the *place* in which they had been reinforced, whereas fish trained in the egocentric group most frequently favored the arm that corresponded to the *response* (left or right turn) that they were accustomed to making. Those animals in the ego-allocentric group chose egocentric and allothetic arms almost equally, but almost never chose the remaining third arm, indicating that they are able to use both strategies, but were forced to choose when the conflicting cues were presented. The authors note that since in nature ego- and allocentric cues are not usually at odds with one another, it is likely that these methods work in concert and animals attend to both when learning a spatial arrangement, which is in accordance with O Keefe and Nadel s suggestion that the two systems are not exclusive of one another (O Keefe and Nadel, 1978). Although it is unclear why individual animals choose differently in this forced-choice paradigm, this discrepancy in behavior may simply underline the equality of salience in the two forms of cue involved in spatial learning. Perhaps it is not surprising that animals are able to attend to various cues and, therefore, utilize more than one strategy of navigation. Just as animals use all available senses to learn about their environments, it follows that they may have available more than one navigational strategy.

Odling-Smee and Braithwaite (2003) looked at spatial learning in two groups of three-spined stickleback fish, one from pond areas and one from rapid river areas. The fish were trained to find food in one arm of a T-maze using either idiothetic cues (turn left or right) or landmark cues (plants). When the cues were at odds with one another in the test phase, the pond fish used landmarks to navigate more than did the river fish. The researchers suggest that this discrepancy may be due to different adaptations to the animals environments, which may have independently shaped the salient cues for each group. Whether the difference is hardwired in the brain or a function of environmental experience (conditional strategy) is still unclear, but in any event various populations of the same species rely differently on available cues.

Other research has focused not only on the cues necessary for spatial learning, but also on the brain regions that may control navigation (idiothetic, allothetic). These studies are particularly desirable for their comparative value; making comparisons across taxa of both the spatial behaviors and the underlying neuroanatomy allows us to understand the evolution and divergence of these mechanisms. Lopez et al. (2000) looked at the differences in navigation methods used by intact and telencephalon-ablated goldfish when navigating a four-arm maze tank. Fish were trained to find food in a goal box on one arm of the maze; visual landmarks were provided during training and consisted of both intramaze and extramaze cues. In probe trials, (a) intra- and extramaze cues were placed in conflict with one another, (b) extramaze cues were absent, or (c) intramaze cues were absent. In (a) trials, intact fish chose arms corresponding to intra- and extramaze cues with equal frequency; telencephalon-ablated fish responded significantly more to intra-maze cues than to place cues. When extramaze cues were absent (b), all fish chose the arm corresponding to the intra-maze cue associated with the goal arm. When intramaze cues were absent (c), intact fish chose the arm corresponding to the correct place, but telencephalon-ablated fish chose all three arms equally. The results seem to indicate that intact fish are able to use both kinds of cues, similar to Rodriguez et al. (1994), and when placed in conflict with one another, the fish had to make a choice (that varied among individuals). The data also indicate that the telencephalon is important in learning about *place* as opposed to *cues*, and the authors suggest that this brain region is equivalent to the hippocampus in mammals and birds (Lopez et al., 2000). (The ablation of the telencephalon also led to faster learning and fewer errors during training. The authors suggest that learning may be accelerated because animals are only able to attend to one type of cue and therefore develop only one spatial representation of the environment.) What is of particular interest here is the suggestion that fish are able to use both methods of navigation   in effect switch back and forth between the two   and that these methods have a well-understood physiological basis. Further, this research supports O Keefe and Nadel s (1978) suggestion that the two systems are not exclusive of each other but rather can function alongside one another, and that this dual operation may support the presence of the cognitive map (Lopez et al., 2000).

WHY WEAKLY ELECTRIC FISH?

The mormyrid fish model of navigation is one that has commanded increasing scientific interest for its explanatory value, particularly regarding the role of multisensory processing in learning and memory. Weakly electric fish navigate their environments by means of active and passive electric senses, vision, and hydrostatic pressure cues (for a review, see Moller 1995, 2002; Cain, 1995; von der Emde, 1998). The active electrosense appears to be the principle means by which these fish navigate, in concert with their ability to generate electric organ discharge though a specialized electric organ (Bennett, 1971a; Bass, 1986). The electrosense also enables them to interact with their inanimate surroundings and communicate with conspecifics. It has been shown that when schooling, certain social behaviors are absent in electrically silent fish (parallel and single-file swimming), indicating that the active electric sense is particularly important in social-spatial behaviors (Moller, Serrier, Squire, and Boudinot, 1982). Few studies have addressed the precise degree to which mormyrids use vision for navigation, social interaction, and object location, although it is known that these fish are able to differentiate two-dimensional patterns under low levels of light (Schuster and Amtsfeld, 2002).

Mormyrus rume proboscirostris is a nocturnal species indigenous to Central African rivers. In the dark they use their active electric sense to find their way when foraging and searching for mates or food by evaluating distortions of their self-generated electric field, which are perceived by specialized cutaneous electroreceptors (Bennett, 1971b; Zakon, 1986; von der Emde, 1998, 2002). By evaluating the electric images cast on its skin by objects in its vicinity, the fish is able to determine an object s electric properties by evaluating the capacitive or ohmic resistance, and thus whether the object is animate or inanimate (von der Emde, 1998). The fact that these fish are naturally adept navigators their large cerebellum result in a brain-body weight ratio which approaches that of humans (Bennett, 1971b) and that they possess this additional sensory capacity makes them particularly desirable subjects for studies regarding spatial learning and sensory interplay.

MULTI-SENSORY INTEGRATION

In the area of spatial learning in mormyrids, a key question regards the roles of and possible interplay between the senses the active and passive electric senses and vision. Rojas and Moller (2002) examined the ways in which *Gnathonemus petersii* are able to maintain proximity to tubular shelters by integrating vision and the active electric sense (or using only one when the other is rendered inoperative). An important question in this study was, what is the nature of the relationship between the senses? In other words, is the association of the senses synergistic, or, as the authors ask, do they conversely act as back-up, or redundant input (p. 212) for one another? By moving a shelter along a linear course and measuring the degree to which animals were able to maintain proximity to the shelter, these questions were largely answered. The authors found that when one sense was absent, the deficits in performance that were initially observed often diminished with practice, depending on the material of the shelter (i.e. high conductivity aluminum). However, when one or more senses were missing, intermediate levels of performance generally occurred in response to shelters of lower conductivity (Plexiglas). The authors suggest that some kind of sensory synergism is involved in mormyrid navigation, since there is a positive correlation between number of senses and the degree to which fish were able to maintain shelter proximity. Given the compensation of one sense for the loss of others and the fact that after 6th-day elimination of the active electric sense the (previously sighted-discharging) fish still performed as if they had all three senses, there seems to be a degree of redundancy among senses. Such an adaptation would be evolutionarily advantageous, as it allows the animals to rely on a less expensive sense instead of the energetically costly active electric sense (Rojas and Moller, 2002).

There is also evidence that fish use landmarks to negotiate their environment. Cain and Malwal (2002) looked at the employment of landmarks in the ability of *G. petersii* to navigate surroundings, by manipulating the size of the underwater landmark after the fish had learned the relationship between a given marker and the aperture through which it swam (i.e., after it had established an internal representation [p. 3916]). When hydrostatic pressure stayed the same but the landmark size increased or decreased, the trajectory of the fish was not disrupted, but rather the fish oriented to the current stable cue: water pressure. These results indicate that the fish uses an internal representation of its environment, but when there are changes to the remembered points of reference (hydrostatic pressure or landmark size), the fish integrates these variations into its internal representation (Cain and Malwal, 2002).

Cain s earlier work (Cain, 1995; Cain, Gerin, and Moller, 1994) looked into the development of an internal representation of a familiar environment by examining the ability of sighted-discharging, blind-discharging, silent, or sham-operated *G. petersii* to find a remembered aperture location after either it or the water level had been raised. He found that blind-discharging and sham-operated fish found the aperture faster than sighted-discharging fish, although the former groups had more difficulty locating it after it had been raised 10 cm. Cain suggests that the difference between the groups of fish may be due to higher initial-discharge rates of the blind-discharging and sham-operated fish. After the fish learned the location of the aperture, however, discharge rates of the blind-discharging and sham-operated fish dropped, and these groups took longer to locate the raised aperture than did sighted-discharging fish. The author suggests that after the internal representation is constructed, other sensory cues take over, a concept that is very much related to that of Rojas and Moller (2002), discussed above. Griffin (1958) found that after bats have become familiar with a path to a food source, their vocalizations decrease and they seem to rely less on electrolocation. When the obstacles in the path are shifted, the bats collide with these obstacles (Griffin, 1958). In the same way, as Cain s fish learned the location of the aperture, they relied less on the energetically costly (Rojas and Moller, 2002) active electrosense (in effect, they got lazy). When the water level was raised 10 cm, all fish contacted the wall at an increased height, suggesting that despite the absence of one sense, all fish attended to hydrostatic pressure. It is also important that the silent fish used purely visual cues to locate the aperture: after it was raised, the time it took electrically silent fish to find the opening was not significantly different from that of the blind-discharging and sighted-discharging fish. Therefore it is also possible that an internal representation may also be formed with only visual cues, rather than the necessity for both visual and electric input.

There is evidence that *G. petersii* are able to distinguish between visual patterns viewed from a set vantage point (Schuster and Amtsfeld, 2002) under very low light (approximately 10 lux). These authors also showed that this species may use a template-matching mechanism to compare novel visual patterns with remembered visual patterns. The study indicates that *G. petersii* have sufficient visual acuity under low light intensities to acquire memories based on purely visual stimuli, and that they are able, in effect, to make visual decisions. The authors suggest that the ability to template match may have relevance in the natural habitat of *G. petersii*, when they embark on foraging outings in which they move from their usual defined (p. 556) hiding place to new locations. If the fish makes a series of moves from hiding place to hiding place, the authors suggest, it may be that the storage of distinct visual templates, each activated at the corresponding point in the excursion, is what drives the movement. If the viewing angle is fixed (p. 556) from each point to the next visual landmark, the template system may indeed have relevance to the natural environment of *G. petersii*, a concept that is related to Collett et al. s (2003) hypothesis that landmarks may be important in recalling local memories in desert ants. This study also suggests that *G. petersii* may have sufficient visual acuity for spatial memory based on purely visual cues.

Mormyrids are ideally suited for studying vertebrate spatial memory because the fish are naturally exploratory in a new environment and will readily navigate to a reinforcing goal. Under natural conditions, these fish use all available senses to orient and navigate; under laboratory conditions, we will be able to determine the interaction between the senses by manipulating which senses are available to them. If we can determine not only the relative importance of the two major senses vision and the active electric sense but also the fish s navigational strategy, mormyrid fish will be an excellent vertebrate model to investigate learning and recall, and will rival that of the rat.

All experiments comply with the standards of the Institutional Animal Care and Use Committee, Protocol # PM-11/07-02.

Part I: NAVIGATION IN A RECTANGULAR MAZE

Experiment 1: Memory and Recall of a Maze Pattern

The first goal of this study was to determine the role of the fish s active electric sense and vision in the acquisition and recall phases of memory of a traveled path. No prior studies have investigated maze learning and recall using this maze-removal paradigm. Using this procedure, it is possible to understand not only what navigational strategies the fish utilize during the training period, but also what senses are involved. What kind of spatial representation has been formed? In the first part of the experiment, the acquisition or learning phase was studied; the second part examined recall, when the maze barriers were removed. After the fish has learned the maze pattern (evidenced by a significant decrease in time to complete the maze), and the barriers have been removed, what will be the fish s trajectory? Will it complete the maze as if the barriers are still present? This behavior, in which the specific pattern of left and right turns is remembered, would indicate dead reckoning. Will it make a beeline from Start Box to Goal? The internal integration of the meander path learned during acquisition would suggest path integration. Or will it swim an intermediate pattern or hug the wall (thigmotaxis)? Finally, which senses are necessary to acquire and recall this task?

Method

Subjects

Ten female and eight male laboratory-bred *M. r. proboscirostris* were obtained from F. Kirschbaum (Humboldt University, Berlin, Germany) and raised at Hunter College. Fish were housed in individual 18.5-liter tanks, or in a 515-liter tank divided by white mesh plastic screens that allowed the fish to electrically sense and/or see their tank-mates. All tanks/sections were equipped with several shelters and standard aeration and filtration systems. Fish were fed live blackworms twice weekly and tanks were cleaned weekly. Fish were maintained on a 12:12 photoperiod with lights on at 9:00 a.m. Water temperature ranged between 23.4 and 25.0 °C and conductivity ranged between 173.1 and 200 µS/cm. Six sighted-nondischarging, six blind-discharging, and six sighted-discharging fish, all naïve to maze navigation, were used. Each group was staggered regarding the date when they began training/testing so that the entire experiment ran for ten weeks. Sex was identified by radiography after testing was complete (basal anal-fin rays are expanded in males; Brown, Benveniste, and Moller, 1996).

Electric Organ Discharge Elimination

Six *M. r. proboscirostris* underwent Electric Organ Discharge (EOD) elimination (silencing). Fish were removed from their home tanks individually and lightly anesthetized with tricaine methanesulfonate (MS 222, 20 mg/L water), but were still capable of discharging, which was necessary to evaluate the success of the surgery. Each fish was placed on a moist towel with damp sponges covering the upper body to assure proper moisture level throughout surgery. Two or three scales were removed from the most rostral part of the tail along the midline. The motor neuron, which lies dorsal to the spinal column, was severed with the incision of a pointed needle anterior to the caudal peduncle. Monitoring the discharge rate by way of electrodes placed around the fish's body allowed us to determine permanent cessation of the electric discharge. During recovery, each fish was placed back in its home tank with tetracycline (250 mg/18 L water) and closely watched for discomfort, infection, or otherwise erratic behavior. After three to ten days' recovery the fish began maze learning.

Enucleation

Six *M. r. proboscirostris* underwent bilateral enucleation. Each of these fish was similarly anesthetized with MS 222, although a deeper level of anesthetization was allowed (50 mg/L water). The mucus layer that naturally covers the eye was removed, and by asserting pressure to the skull so that the eye protruded, the optic nerve was severed and the eye excised. This blind-discharging group was allowed ten days to recover, and as above, these subjects were watched closely for infection and discomfort.

Maze Tank

The experimental all glass maze tank measured 306 (L) x 61 (H) x 61 (W) cm. The five removable perforated Plexiglas dividers acted as obstacles around which the fish had to navigate (the holes in the dividers were evenly spaced at 5 cm apart and were approximately 0.75 cm in diameter). The holes in the dividers would not affect the fish's navigation by the active electrosense, since when nearing the divider, fish would be able to discern the edge—the difference between fundamentally solid matter and water, thus the associated electric impedance differences. At each end of the tank a small Plexiglas box served as Start Box and Goal respectively. A Plexiglas gate was raised via a pulley system to allow the fish to enter the maze through a 10.2 (H) x 7.6 (W) cm aperture. As mormyrid fish are nocturnal, a blackened Goal box was sufficiently rewarding for fish to seek shelter there. Objects outside the tank remained unchanged throughout the course of the study (cabinets, tanks, location of the experimenter). Differences in the behavior of the sighted-discharging and blind-discharging fish would suggest that extramaze landmarks were being attended to.

Learning

Each fish was trained in the maze three times a day for 19 days, with two two-day breaks between training days five and eight and days 12 and 15. A subject was transferred from its home tank to the release compartment of the experimental maze tank where after a minute of acclimation the start gate was raised, allowing the fish to swim through an opening into the maze. If the fish did not leave the release compartment within five minutes, it was gently coaxed to leave by the experimenter waving her hand in the water. The fish was timed from the moment its midline crossed the threshold of the release box to that when its midline crossed the Goal threshold. If the subject did not reach the Goal within five minutes, the time for that run was stopped and recorded as 300 sec, and the fish returned to the release box for the next run.

Recall

Maze learning was considered to have occurred for each group if a) the time to find the Goal decreased as a function of number of trials, and b) when this time stabilized for three consecutive days at the decreased level. On the nineteenth day, the partitions were removed from the tank and the fish was released as described above (three trials were likewise administered on this day). The trials on the nineteenth day were recorded on videotape, and timed as before.

Analysis

The data were analyzed via two-way factorial analysis of variance (ANOVA) whereby the effect of the day (1-19) and condition (sighted-discharging, blind-discharging, and sighted-nondischarging fish) on time to complete the maze was analyzed. Post hoc Newman-Keuls tests ($\alpha=0.05$) were performed to determine a) the effect of time (days 1-19) across conditions, b) the effect of condition across days, c) whether fish s times to complete the maze on day 18 were significantly different from those on day 19, and d) the effect the two-day weekend breaks in practice might have had on time to complete the maze for all groups (i.e., d 5-8 and d 12-15).

Quantifying the Number of Correct Turns on Test Day (Recall)

To determine whether the fish followed the trained path (Right-Left-Right-Left-Right turn sequence), the fish s trajectory when the maze barriers were removed on day 19 was analyzed. Each trial was recorded on videotape, and the number of turns that would have been sequentially correct had the barriers still been present was counted. For this measure, a matrix was created for each fish and for each trial on the test day. A value of 1 was assigned if the turn was viewed as complete (fish swims to edge of virtual barrier); 0.5 was assigned if the turn was considered partial or incomplete (fish makes the correct turn but does not swim to the edge of the virtual barrier); and 0 was assigned if the turn was absent or made in the wrong direction. For example, a fish who performed the first two turns correctly, the third turn partially, and the last two not at all would receive a value of 2.5 (1.0 + 1.0 + 0.5 + 0 + 0). An additional matrix was created with letter values for each turn, with capital letters representing correct turns and lower-case representing incomplete turns, to preserve the sequence of the maze: for instance, the hypothetical fish above would receive a score of ABc00. In this study the length of each leg was not considered. This measure (direction and distance) may be an additional parameter to evaluate the fish s acquisition strategy.

Results

During the 18 days of training, fish in all groups quickly decreased the time to complete the maze (Fig. 1). There were highly significant effects of condition and day upon time to complete the maze (ANOVA 1, see appendix A), and a significant interaction. Post-hoc Newman-Keuls tests revealed that on day 1, the time to complete the maze of the sighted-nondischarging fish varied significantly from both the blind-discharging and the sighted-discharging fish on d1 ($p<<.001$ for both comparisons, Fig. 1), but the blind-discharging fish did not differ from the sighted-discharging fish on d1 ($p=.707$). By day two (and through day 19), there was no difference between the groups in time to complete the maze. On d1, sighted-nondischarging fish took significantly longer to complete the maze than on all following days. For blind-discharging and sighted-discharging fish the time to complete the maze on day 1 did not differ significantly for the performance on days 2-19.

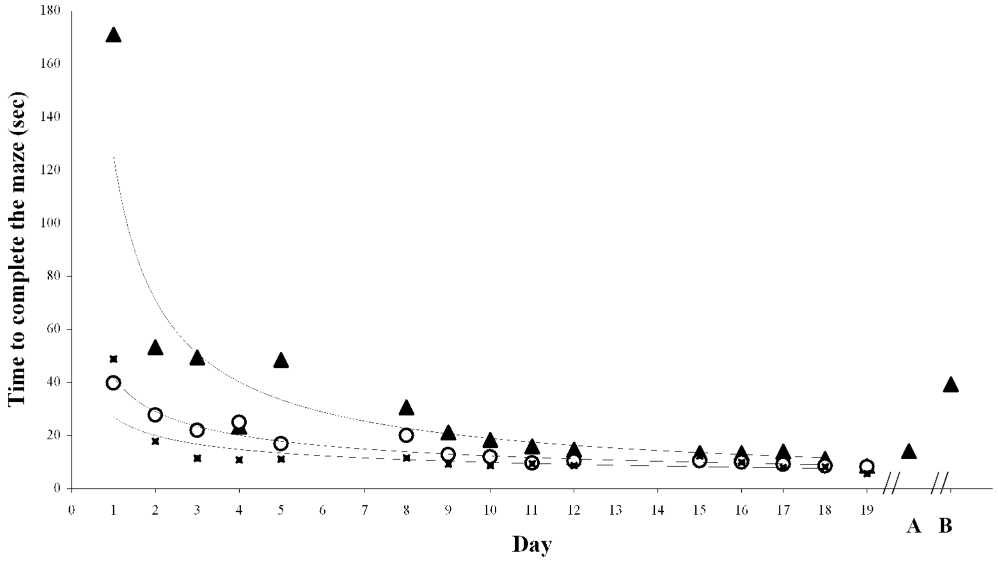

Figure 1. Mean time to complete the maze (sec) in sighted-nondischarging, blind-discharging, and sighted-discharging fish as a function of time (days). Only on day 1 did sighted-nondischarging fish take significantly longer than both blind-discharging and sighted-discharging fish to complete the task (n=8). On day 19, when maze barriers were removed; there was no difference in performance between days 18 and 19 for any group. (Black ■ represents sighted-discharging group, white o represents blind-discharging group, and black ▲represents sighted-nondischarging group.) When the sighted-nondischarging fish were retested after 7 days (denoted as A) or 7 weeks (denoted as B), some increase in time was evident, although neither was statistically different from the last day of training (d 18).

There was also no difference in any of the groups between the last day of training, day 18, and the test day, day 19, when the maze barriers were removed, although performances on day 19 were noticeably shorter than those on day 18 for almost all fish, probably due to the absence of the barriers. The absence of training on days 6-7 and days 13-14 had no effect on the steady decline in performance time.

In comparing the number of correct turns via analyses of variance, we found no significant difference between the three groups. There was a significant change across the three trials for all three groups (F=3.353, p=.044) (Fig. 2), which suggested that fish made straighter trajectories to the Goal across trials. The performance of the sighted-discharging and sighted-nondischarging fish during recall indicated that fish, having been tested once, may have learned the new direct path during the successive two recall trials (the number of correct turns decreased for both groups across trials). This suggests that fish could have attended to external visual cues during learning. Further, the proportion of correct turns for the blind-discharging fish was lower than for the other groups on the first trial and did not appear to decrease over trials. These findings suggest that blind-discharging fish may have attended to the geometry of the tank during learning more than the other groups of sighted fish. It should be noted that fish in the circular tank (Exp. 2) did not attend to 2-dimensional visual landmarks provided on the outside of the tank walls.

To test whether fish could retain their learned trajectory in this maze over longer periods of time, the sighted-nondischarging fish   the only group of fish to significantly decrease their times during training   was tested in the maze (a) one week and (b) seven weeks following completion of all the recall experiments. After one week the fish were still as fast as they were after training; after seven weeks, their time to complete the maze increased, though not significantly (Fig. 1, A and B, respectively).

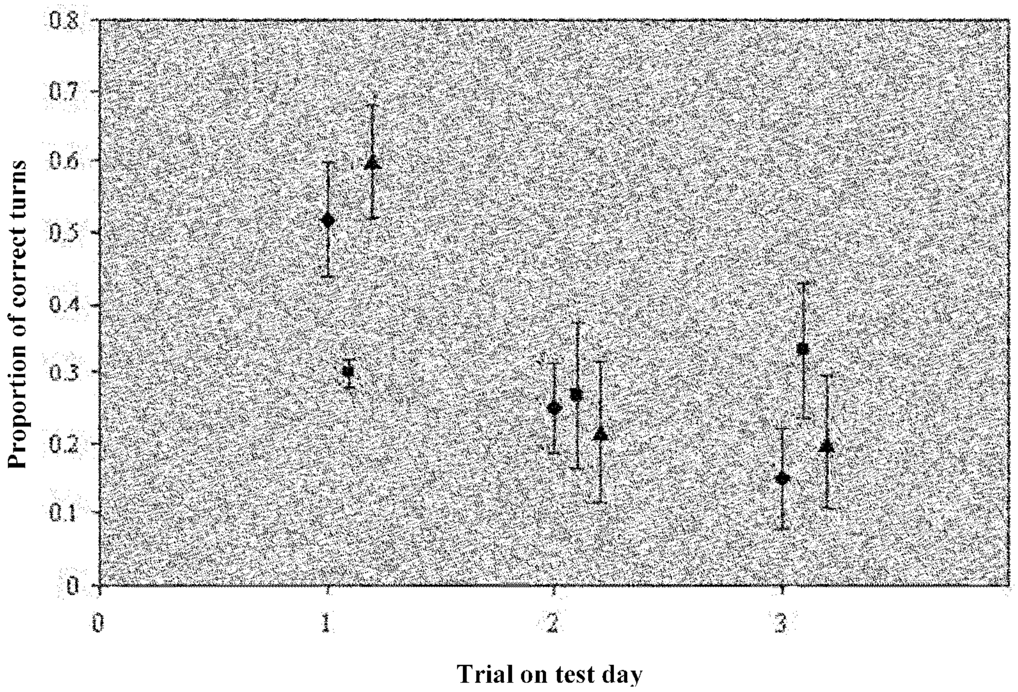

Figure 2. Measure of correct turns (meanders) over three consecutive trials when maze barriers were removed on d19. ♦ - sighted-discharging, ■ blind-discharging, and ▲ sighted-nondischarging fish. Error bars represent 1SD. Note that the number of correct turns for blind-discharging fish started lower than the other two groups and did not appear to decrease over trials. The sighted-discharging and sighted-nondischarging fish may have attended to visual cues during recall, and so learned over the three recall trials. The blind-discharging fish may have attended to the geometry of the maze more than other groups in learning, since they did not have sight available.

PART II NAVIGATION IN A CIRCULAR OPEN FIELD MAZE

Experiment 1 showed that on the first day of training, sighted-nondischarging fish took significantly longer to complete the maze (acquisition phase) than sighted-discharging and blind-discharging fish, but on subsequent days, this difference between groups disappeared. On test day (recall), all groups performed similarly, suggesting that after a path has been acquired, despite the lack of one sense during acquisition, recall is accomplished independent of available sensory cues. These results suggested that fish may use path integration as a navigational strategy, rather than dead reckoning, since fish made increasingly straighter paths towards the Goal on test day. This study does not, however, allow for differentiation between path integration and cognitive mapping, since either strategy could explain the present results.

To determine whether fish rely on cue listing (path integration, dead reckoning) or cognitive mapping, it is necessary to displace the animal from its familiar location in the field to a novel location (see Tolman, 1948; O Keefe and Nadel, 1978). If the animal is able to make its way from the novel location to the goal, then one may say that it uses a cognitive mapping strategy; if it is not able to arrive at the goal, this behavior points to path integration. The geometry of the rectangular maze tank did not allow for the displacement of a fish to a new start area, by virtue of the shape of the tank. In order to start the fish from a novel Start Box after acquisition, a symmetrical tank was required. A circular open field maze was therefore constructed to conduct the following experiments.

EXPERIMENTAL LOGIC

These experiments abide by the following logic. Fish were trained to swim from Start Box A to the Goal. After the fish s time to complete the maze reached asymptote in training (defined in Exp. 2), it was placed either in Start Box A or in Start Box B, rotated 90° from A. The maze barriers were removed for recall. If, after barriers are removed, the fish released from Start Box A meanders to the goal as if the maze barriers are still in place, it may use a form of navigation akin to dead reckoning, rather than path integration or cognitive mapping. If it moves in a straight line to the goal, however, it may use *either* path integration *or* cognitive mapping. Therefore a straight trajectory from Start Box A to the Goal does not distinguish between path integration and cognitive mapping (Fig. 3a). Releasing the fish from Start Box B will allow a separation of these two forms of navigation. If the fish released from the novel location Start Box B swims towards a virtual Goal on the opposite side of the tank, rather than to the actual Goal, it likely uses path integration. If it swims to the correct Goal, however (which is now 90° off from where it is released), this would suggest that it utilizes a mental/cognitive mapping strategy (Fig. 3b). Experiments 2, 4, 5, 6, 7, 8, and 9 will test these predictions.

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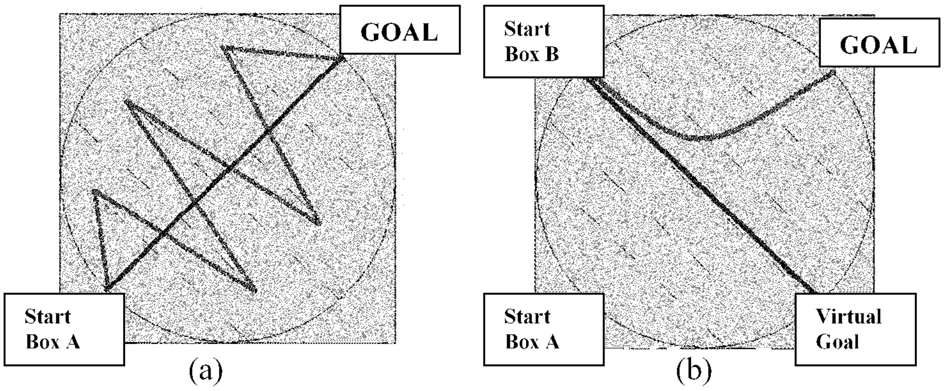


Figure 3. Schema of routes fish would take in recall (after maze barriers have been removed). (a) Release from Start Box A: meander path suggests Dead Reckoning; straight line suggests Path Integration. (b) Release from Start Box B. straight line suggests Path Integration; turn towards Goal suggests Cognitive Mapping.

Circular Open Field Experiments: Summary and Rationale

Independent variables for Experiments 2, 4, 5, 6, 7, and 8: fish condition (sighted-discharging, sighted-nondischarging, blind-discharging, lateral line temporarily eliminated*) and sensory environment, i.e. dark (<1 lux) and light (12-15 lux) during the acquisition phase (learning the maze) and recall phase (maze barriers removed).

Dependent variables for Experiments 2, 4, 5, 6, 7, and 8: (1) Outbound vector (bearings of first straight leg Outbound from Start Box) and Landing vector (direction of contact point with arena periphery), (2) meander accuracy (none, 2-3, or 4-5 meanders); (3) time to reach periphery from Start Box, (4) time to reach Goal from Start Box.

Experiment 2: To determine the navigational strategy of mormyrid fish able to use sight and electrosense under light conditions with visual landmarks present**. Predicted Outcomes:** fish use either cognitive mapping or cue list strategies (dead reckoning or path integration).

Experiment 3 (optomotor response test): To ensure that sighted fish under the applied dark conditions cannot attend to visual cues, the visual capabilities of fish under dark conditions were tested (<1 lux) in an optomotor apparatus. **Predicted outcome**: fish are unable to respond to moving visual patterns under dark conditions.

Experiment 4: To test the differences in navigational strategy under dark conditions. **Predicted outcome**: Fish will take longer to acquire the task, since they lack vision, but will perform similarly to fish under light conditions during recall.

Experiment 5: To determine differences between fish trained and tested under dark conditions (Exp 4) and surgically blinded fish. **Predicted Outcomes**: The two groups will perform similarly; alternatively, differences may be due to the lack of circadian rhythmicity in blind fish (Cobert in Moller, 1995) affecting spatial navigation.

Experiment 6: To test fish that lack both visual input and their active electrosense (sighted-nondischarging fish tested under dark conditions). **Predicted Outcomes:** fish will take longer to acquire the task than fish in Exp. 4, since they lack two major senses, but will perform similarly during recall as fish in experiments 2 and 4.

Experiment 7: To test the role of electric landmarks in learning and recall. **Predicted Outcome:** Fish will acquire the task as quickly as fish in Exp 2. During recall, with fish relying on external cues, there will be differences in swimming patterns depending on the original or manipulated arrays of landmarks. Alternatively, even if landmark positions are manipulated or absent during recall, fish will find the Goal as well as other groups.

Experiment 8: Based on the results of Exp. 6 (sighted-nondischarging fish tested under dark conditions), fish may be using the lateral line sense to navigate. Bathing the fish in Cobalt Chloride will temporarily eliminate the use of this sense: sighted-nondischarging fish will be tested in this manner in the dark. **Predicted outcome:** fish will be at a greater deficit in learning *and* in recall. Alternatively, fish will not exhibit any deficit in recall, similar to fish deprived of sight and active electrosense during the acquisition phase. This would suggest that fish generate a motor routine list based solely on idiothetic cues.

Experiment 9 (Control): Without prior training, sighted-discharging fish will be released from Start Box A with no barriers present. **Predicted outcome:** the initial swimming path will not be directed.

*Note that unless otherwise indicated, fish have an intact lateral line.

Experiment 2: Sighted-Discharging Fish, Visual Landmarks

Using the paradigm described above, the first part of this experiment tested sighted-discharging fish under light conditions (12-15 lux; Schuster and Amtsfeld, 2002) to determine the role of sight and electrosense in cue acquisition and recall. Fish were tested in a sensory environment with visual (17° visual angle) landmarks (one circle, one filled circle, one triangle, and one filled triangle, each 14 cm at the widest point) present on all four walls of the maze tank. Schuster and Amtsfeld, 2002 demonstrated that mormyrid fish can attend to such visual cues.

Method

Subjects

Eight adult *M.r. proboscirostris* were housed individually in 18.9-liter tanks in our laboratory. The animals ranged from 11-20 cm in length. The conductivity of each tank was maintained at approximately 315 μS/cm and temperature was 25 ±1°C; tank water was continuously filtered and aerated. Laboratory lights were kept on a 12:12 photoperiod with lights off at 9 pm. Fish were fed black worms twice weekly. The animals sex was not observed until after the completion of the study.

The maze tank (Fig. 4) was built from 1.3 cm thick slabs of finished glass; the dimensions of the completed tank were 45.7 (H) x 122 (W) x 122 cm (L). A thin clear plastic liner was fitted inside the finished tank to create an arena (122 cm diameter) within the square tank. The space between the circular liner and the corners of the tank served as Start or Goal boxes; openings measuring 10.2 cm (H) by 7.6 cm (W) were cut out of the liner to allow the fish to enter or exit the arena. Plastic doors covering the openings were connected to the top of the circular liner, and were manually operated. The sides of the tank were covered with black corduroy, clamped to the top edge of the tank walls on each side. To provide incentive for fish to learn the maze, a ceramic shelter was placed the Goal box and the Goal was covered with black cloth (as mentioned, mormyrid fish are nocturnal and shelter-seekers, Rojas and Moller, 2002).

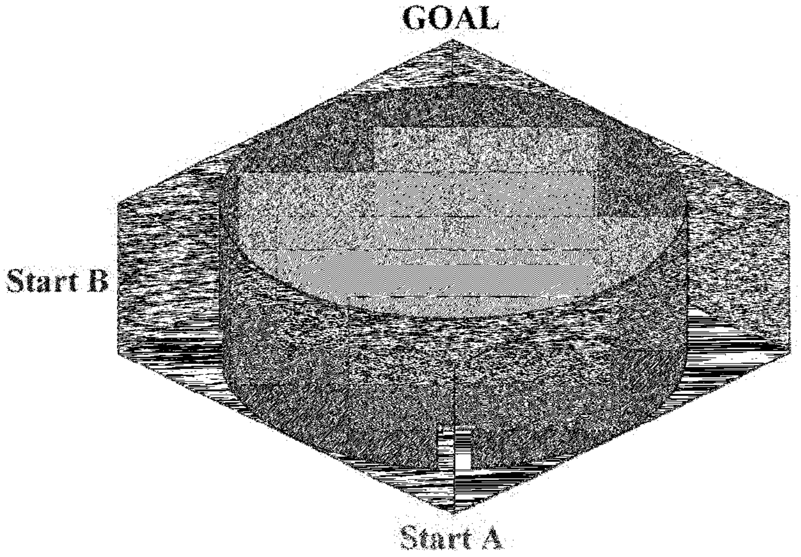


Figure 4. **Circular maze tank**. Dimensions: 45.7 cm (H) x 122 cm (W) x 122 cm (L). Sheet of plastic insert defines core arena with five removable Plexiglas barriers. Diagram is not to scale.

Five pieces of 0.6 cm thick Plexiglas served as maze barriers. These were held in place by a 2.5 cm-thick PVC pipe into which five 0.6 cm notches were cut and which ran diagonally across the tank. Each barrier was separated from the next by 20.3 cm; the distance from the end of each barrier to the circular liner was also 20.3 cm. In the current experiment, visual landmarks (made of black plastic board) were placed on each side of the tank: one circle, one filled circle, one triangle, and one filled triangle, each 14 cm at the widest point (Fig. 5). Landmarks were placed at the center of each side of the tank, against a piece of white paper, and matched the dimensions of those used by Schuster and Amtsfeld (2002). Landmarks were kept in place throughout both phases of the experiment.

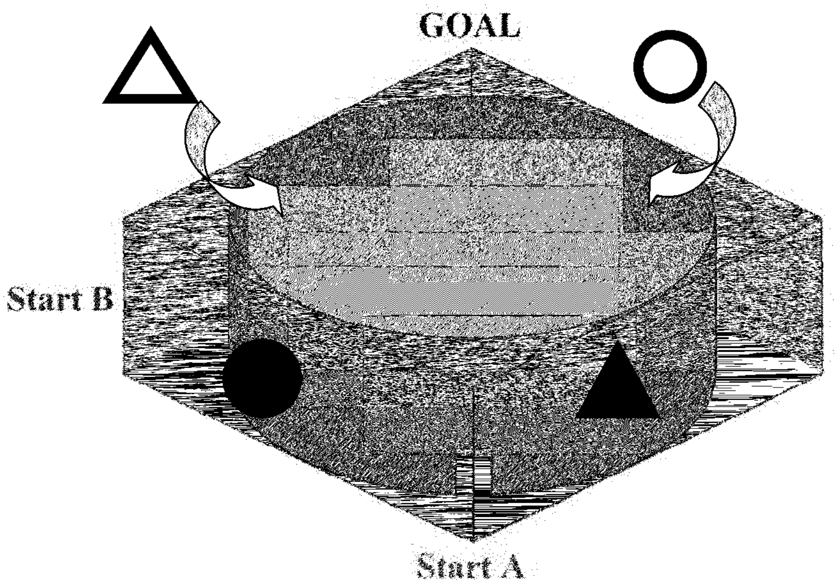


Fig. 5. Illustration of visual landmarks used in this study. Landmarks were placed at the center of each side of the tank, against a piece of white paper (21.6 cm x 21.6 cm).

Training

Light was kept at approximately 12 lux throughout the experiment. Each fish was gently netted and moved from its home tank to Start Box A of the maze tank and allowed to acclimate for 30 seconds. The Start Box door was then lifted and the fish was free to move into the maze arena. Each run was started as the midpoint of the fish s body passed the Start Box A threshold. Timing was stopped as the fish s body crossed the threshold of the Goal. Each fish was given a maximum time of five minutes to complete the maze; if the fish did not reach the Goal within this time, it was placed back in the Start Box and another trial was started (the uncompleted trial was recorded as 300 seconds). Each fish was given three trials per day until the group s mean time to complete the maze no longer decreased. *Asymptote* here represented a 3-day period over which the time (group means) dropped less than 3 sec.

Analysis

Because the tank in Exp. 2-9 was a circular arena, data were analyzed using circular statistics (Zar, 1999). Start Box A was defined as 0° and the Goal box as 180° (arranging the fish s chosen swimming paths in counterclockwise fashion). Start Box B was therefore at 270°. The fish s trajectory after leaving the Start Box was defined by two vectors: a) A Mean Outbound vector was calculated based on the distribution of endpoints defined by the extension of the fish s straight outbound paths (usually up to 25 cm, equivalent to the distance between Start Box A and the leftmost edge of the first barrier) onto the periphery of the tank. Thus, this vector represented a measure of the fish s initial choice during recall to follow the originally acquired path or deviate from it. b) A Mean Landing vector was defined by the distribution of points along the periphery where the fish made first contact. For each group, mean vectors were calculated for fish released in A and/or B, defining mean direction (in degrees) and magnitude (length of the vector, r). An r-value of 0 indicates that the distribution of points was uniformly distributed (i.e., the vector had no direction), and an r-value of 1 indicates that the distribution was directed at a particular point with total precision (see Appendix B).

Recall trials were videotaped with a Sony™ Handycam Digital Video Camera Recorder DCR-HC65, and reviewed on a 21-inch RCA® television. GBC™ SelfSeal Repositionable Laminating Roll was cut to fit and placed over the television screen, and the Outbound vectors for each trial were traced with a Sharpie™ marker. The Outbound vector lines were then extended until they reached the periphery and this point was considered the Outbound vector point. The Landing vector points were defined as the point at which the fish first made contact with the tank wall. In experiments in light conditions, Outbound and Landing points were recorded to the nearest degree.

Two time measures were taken: a) the time it took the fish to make first contact with the periphery, and b) the time to complete the run from Start Box to Goal. (3) To assess the fish s potential dead reckoning strategy during recall, the degree to which fish meandered through the tank was rated where 1=no meander turns (swimming around virtual barriers), 2=2-3 meander turns, and 3=4-5 meander turns. This was the extent to which the remainder of the trial (after the 25 cm of the Outbound Vector) was considered. In order to assess in more detail the possibility of dead reckoning, it would be instructive to analyze each turn the fish made in the Start Box periphery trajectory. Note that for technical reasons it was not possible to obtain the time taken to reach the periphery and the number of meanders.

Mean vectors were analyzed using a commercially available statistics program (Oriana™ designed to accommodate circularly distributed data. Uniformity was determined with the Raleigh test for circular data. Vectors that were *not* uniformly distributed were analyzed in the Mardia-Watson-Wheeler nonparametric test for group comparisons, and those that were uniformly distributed were not considered (see Results: Comparisons for these analyses). Uniform distributions could potentially arise due to underlying bi- or multimodal distributions. Whether the data were uniformly distributed was established using the V-test for circular uniformity under the alternative of non-uniformity and a specified direction (Zar, 1999, p. 618).

Results

Sighted-discharging fish trained and tested in the presence of visual landmarks required seven days to acquire the task, and were tested for recall on the eighth day (Fig. 6). The marked decrease in SEM from day 2 to day 3 may suggest that fish that were initially slower caught up with the faster learners around day 3. The average time to complete the maze on day 7 was 12.2 ± 2.17 sec.

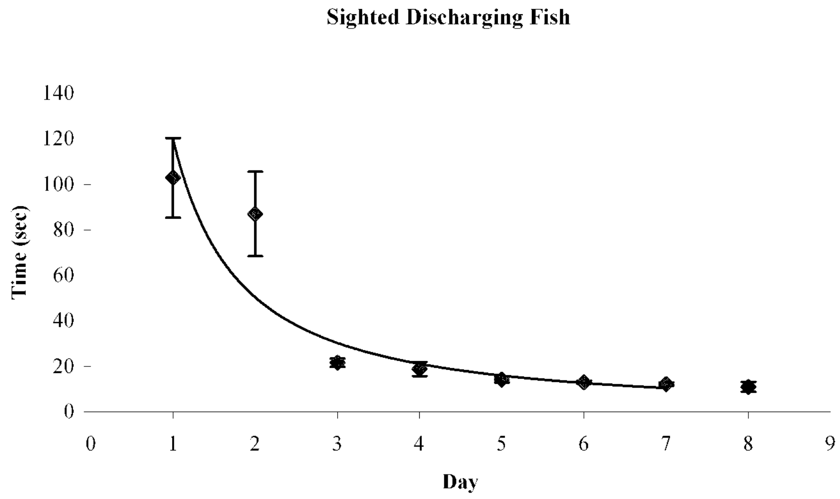


Figure 6. Time to complete the maze for sighted-discharging fish (n=8, Experiment 2). Day 8 represents the test day on which maze barriers were removed. Error bars indicate SEM.

In recall, for fish started from Start Box A, the Outbound vector was θ=199° and the length r=0.825. The Landing vector for this group was θ=181° and r=0.774 (Fig. 7). These data suggest that the Outbound direction was slightly rotated to the left, but the Landing vector was directed at the Goal.

For fish that started from Start Box B (90° off Start Box A), the Outbound vector was $\theta=94^{\circ}$ and $r=0.634$. For this group, an average Landing vector was computed, where $\theta=129^{\circ}$ and $r=0.671$. These results demonstrated that fish initially swam straight out from Start Box B towards the other side of the tank (towards a virtual Goal) but on average, landed on the periphery between the virtual Goal and the actual Goal.

The direction of the Outbound vectors was supported by the meander data that indicated that some fish, started from Start Box A, meandered as if the maze barriers were still present (25% of trials were scored 2, 8.3% of the trials were scored 3, and the remaining trials showed no meanders). In contrast, fish started from Start Box B did not meander at all (100% scored 1), suggesting a possible geometric difference between the two release sites affecting the observed difference in swimming patterns. The discrepancy between the directions of both vectors under the two release site conditions will be discussed in the General Discussion.

Regarding the time taken to reach the periphery from the Start Boxes, no statistical differences were found between fish started from Start Box A and fish started from Start Box B (2.8 sec and 3.2 sec, respectively). These data suggest that although some fish meandered from Start Box A, this swimming pattern did not significantly affect the time it took these fish to fish reach the periphery of the tank.

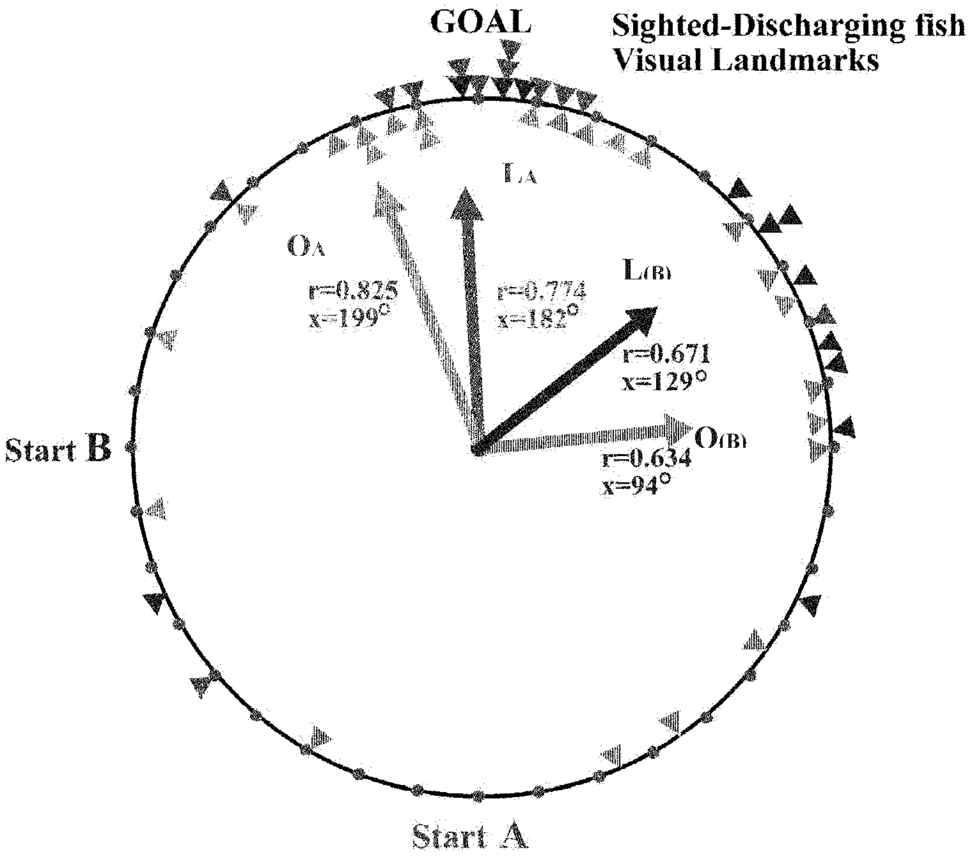


Figure 7. Sighted-discharging fish tested in the presence of visual landmarks. All fish were trained to find the goal when released from Start Box A. Recall: red arrows=fish released from Start Box A; blue arrows=fish released from Start Box B (rotated 90°). Striped arrows: Outbound vectors (O); Solid arrows: Landing vectors (L). Letter in parenthesis following O or L indicates Start Box (A or B).

PART III: NAVIGATION AND SENSORY BASES

Experiment 3: Optomotor response

Before testing the fish under dark conditions and the role of vision in spatial navigation under these conditions could be analyzed, it was necessary to demonstrate that sighted fish do not respond to visual stimuli under dark conditions (<1 lux). An optomotor apparatus was used, similar to that first described in Teyssèdre and Moller

(1982). Eleven sighted-discharging fish and two blind-discharging fish were used in this study.

Method

Fish were individually placed in a glass jar (20 cm diameter x 30 cm height) in 12 cm of water. Conductivity and temperature were consistent with the home tank measurements (315 ± 15μS/cm and 25°C, respectively). The cylinder was surrounded by either striped (1.5 cm black and 1.5 cm white lines) or white paper, which rotated in a clockwise direction around the glass arena by way of a motor underneath the apparatus. The visual angle (17°) of one cycle (one black and one line) corresponded to Schuster and Amtsfeld (2002). The frequency was 0.1 cycles/sec, from Teyssèdre and Moller (1982), which approximates the speed of passing objects as fish swim through their natural environment. Each fish was tested repeatedly, with three variables manipulated: Illumination (normal light or darkness), Visual Cue (stripes or white background), and Motion (Rotation or still). Sessions were videotaped as before with infrared capability for dark trials. Each animal performed six trials per day: three Rotation trials and three still trials in ABABAB pattern, in each of the other conditions (Illumination and Visual Cue). The total time that each fish followed the direction of rotation (clockwise) was counted in each condition and analyzed with Friedman s non-parametric test of repeated measures. For comparison, two blind-discharging fish were also tested for comparison. Under normal light, blind-discharging fish were tested in Motion and Visual-Cue conditions.

Results

Because the distributions were skewed, the median time spent moving in clockwise direction per 120 sec for each condition was determined (Fig. 8).

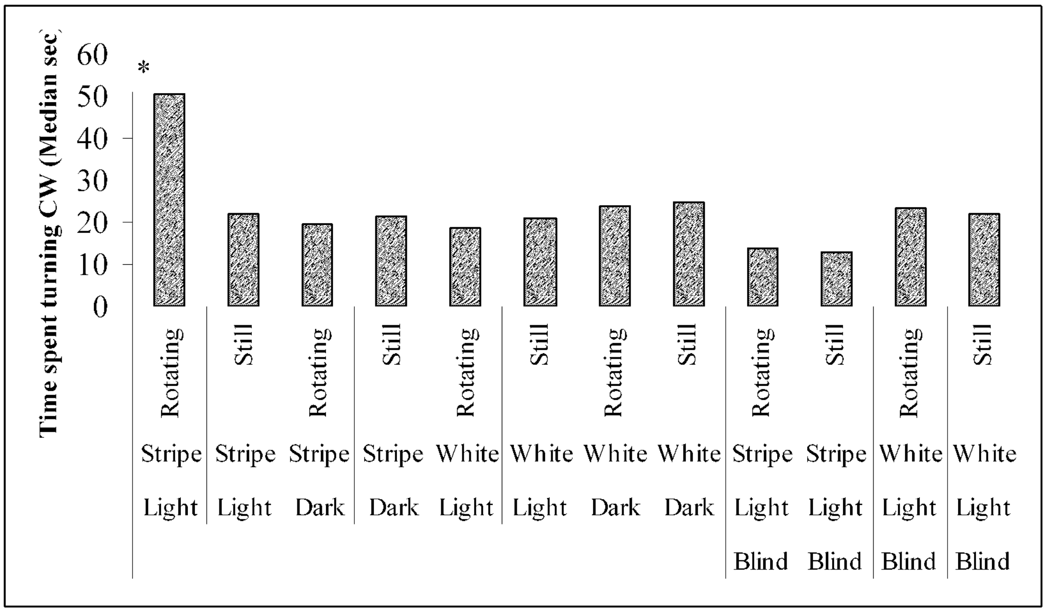


Figure 8. Time (median) sighted-discharging fish spent following a rotating visual pattern in an optomotor apparatus. There was a significant difference between fish tested with the pattern rotating under light conditions and fish in all other conditions. Conditions: Rotating - cylinder turns clockwise, Still - cylinder does not turn; stripe - visual pattern present, white - uniform visual pattern; Normal 12 lux, Dark - <1 lux; Blind blind-discharging fish. * Indicates significant difference (p<.05).

For sighted-discharging animals, Friedman s non-parametric repeated measures K test revealed significant differences between the fish s response to Striped-Rotating-Normal Light condition and all other conditions (p<0.001). In the blind-discharging fish, there were no significant differences between any of the four groups. The results established that under the present lighting conditions, *M. r. proboscirostris* do not respond to visual stimuli below 1 lux illumination. Testing sighted fish in darkness is therefore a viable alternative to enucleation.

Experiment 4: Fish Tested in Darkness

This study was designed to determine the contribution of the electrosense in cue acquisition and recall. Although fish in this experiment were sighted, they were, as established in Experiment 3, functionally blind under <1 lux illumination,

Method

Training was identical to that outlined in Experiment 2, with the exception that under this condition light was kept <1 lux in the entire maze tank throughout training and testing. Although fish were functionally blind, the ceramic shelter could still provide incentive for reaching the Goal (perceived by the active electrosense, as well as lateral line and touch). Conductivity and temperature were kept at 315±15 μS/cm and 25° C, respectively, both in the home tanks and the maze tank. Eight sighted-discharging animals were used in this experiment. During testing, four fish were started from Start Box A and four fish were started from Start Box B, rotated 90° degrees off the original.

Because the water was too deep for the camera to record in the dark, small paper tags were placed at each 10° interval around the tank. The experimenter observed and recorded the Outbound and Landing points of each fish, to the nearest 10° mark, or, if possible, interpolated to 5°.

Results

During acquisition, fish required 11 days to learn the task and were tested for recall on day 12 (Fig. 9). The average time to complete the maze on day 11 was 26.9±6.98 sec. There was an outlier, however, and when this fish was removed from the group s analysis, the average was 20.9±3.95 sec. Still, though, the fish in this group required 11 days to learn the task.

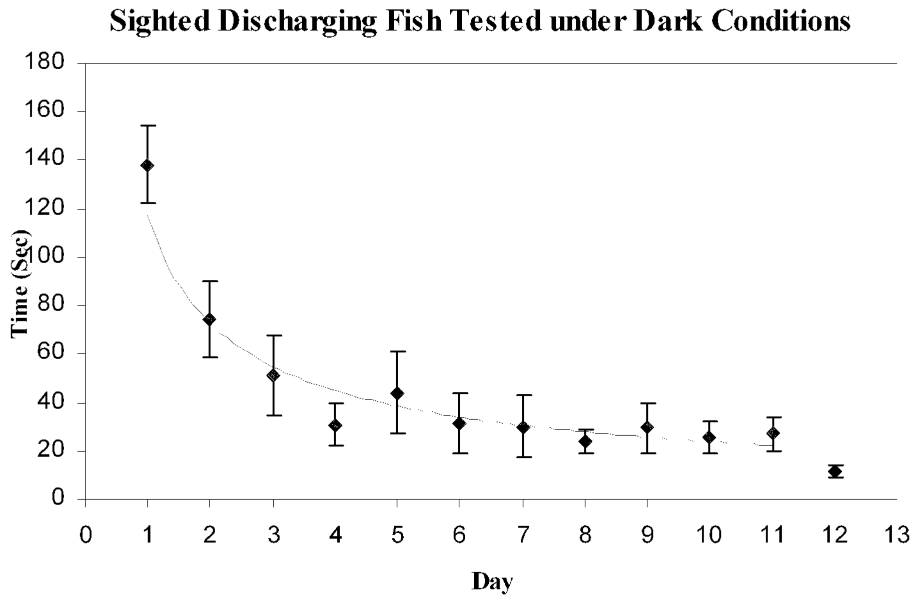


Figure 9. Time to complete the maze for sighted-discharging fish tested under dark (<1 lux) conditions (n=8, Experiment 4). Day 12 represents test day (recall). Error bars represent SEM.

During recall, for fish that started from Box A, the directions of both the Outbound (θ=230°, r=0.867) and Landing vectors (θ=221°, r=0.595) were similar (Fig. 10). This suggests that both the initial bearings and the Landing points on the periphery were rotated counterclockwise. However, as the short length of the Landing vector indicates, the data were scattered around the 2nd and 3rd quadrants of the tank. During recall, for fish that started from Start Box B (Fig. 10), the Outbound vector was θ=81° (r=0.845). The Landing vector was θ=113° (r=0.701). These results indicate that, as in Experiment 2 (release from Box B), the fish s initial bearings were directed towards the opposite side of the tank (virtual Goal), but the peripheral Landing was slightly shifted towards the actual Goal. Note that as in Experiment 2, in three out of 12 trials fish reached the actual Goal.

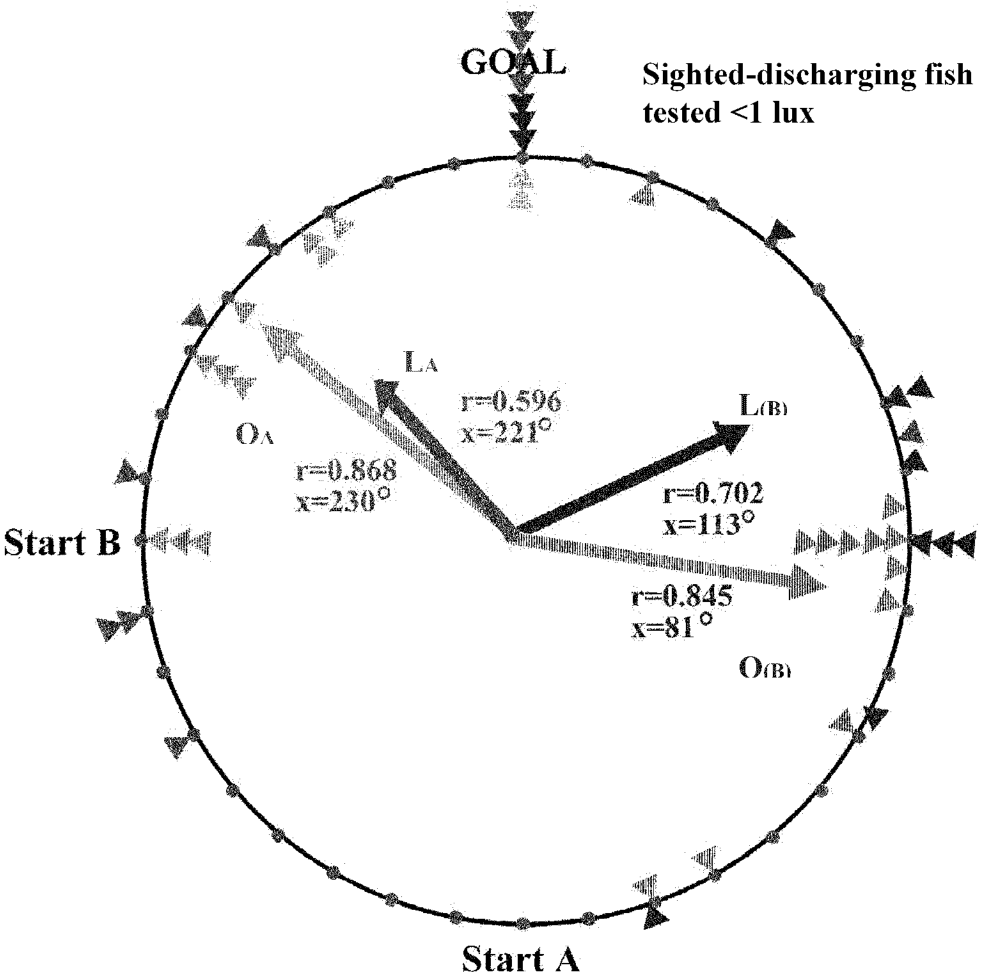


Figure 10. Sighted-discharging fish trained and tested under <1 lux illumination. All fish were trained to find the Goal when released in Start Box A. **Recall**: red arrows=fish released from Start Box A; blue arrows=fish released from Start Box B (rotated 90°). Striped arrows: Outbound vectors (O); solid arrows: Landing vectors (L). Letter in parenthesis following O or L indicates Start Box (A or B). r=length of vector; x=θ (angle).

Fish that started from Start Box A scored 2 on the meander criterion (2 or 3 meanders) in 25% of the trials; in all other trials fish did not meander. When they started from Start Box B, none of them meandered, again indicating that fish meander only from the original Start Box. This difference in the initial path will be discussed below. (No data were recorded for time taken to reach the periphery.)

Experiment 5: Blind-Discharging Fish

This experiment was designed to compare the orientation behavior in sighted-discharging fish tested under dark conditions (Experiment 4) and fish that were surgically rendered blind (blind-discharging fish). For these fish, since all cues during acquisition and recall were identical, no difference in performance was predicted. Note that experiment 3 had established that sighted-discharging fish do not respond to visual cues under *smaller* than 1 lux conditions; thus any differences between fish tested in the dark and blind-discharging fish would be due to factors other than visual input.

Method

Subjects

Four blind-discharging fish from Experiment 1 were used in this study. In testing, all subjects started from the Start Box B. Adding a group to be released from Start Box A seemed unnecessary since only the trajectory taken from Box B would allow a distinction between cue listing and mapping. As with sighted-discharging fish in dark conditions, the incentive for swimming to the Goal would be based on the active electrosense as well as the other remaining senses.

Training and Testing

Training and testing were executed exactly as described in Experiment 2.

Results

Blind-discharging fish required 12 days to learn the maze and were tested for recall on day 13 (Fig. 11). These results are comparable to those obtained for sighted-discharging fish trained under dark conditions. The average time to complete the maze on day 12 was 42.6±21.9 sec, largely due to an outlier. When this fish was omitted from the analysis, the average time was 21.1 ±5.8 sec, which was similar to the time it took sighted-discharging fish under dark conditions.

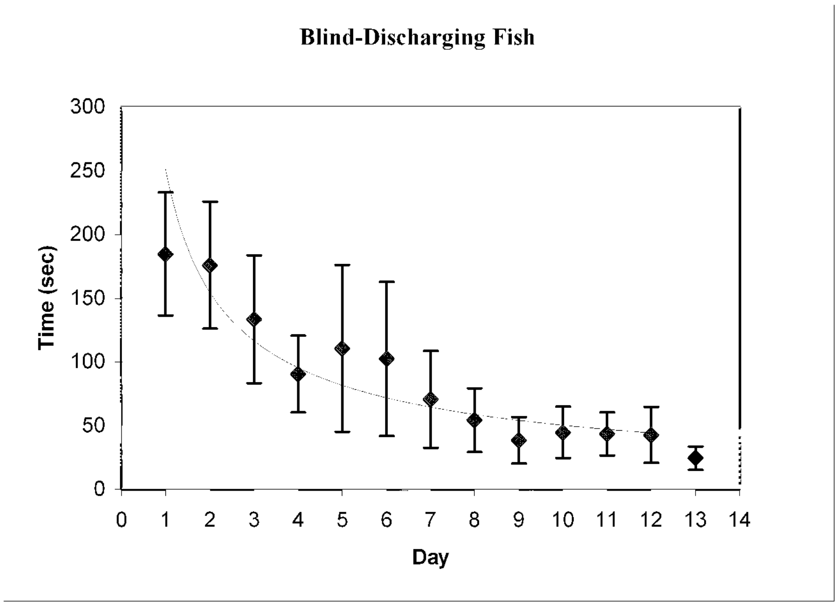


Figure 11. Time to complete the maze for blind-discharging fish (n=4). Day 13 represents test day (recall). Error bars indicate SEM.

The fish s swimming paths during recall were somewhat unexpected although both Outbound and Landing vectors were not significantly different from a uniform distribution. The mean Outbound vector for this group was θ=138° (r=0.456) and the Landing vector was θ=267° (r=0.410). Fish in this blind-discharging group scored 2 on 8.3% of trials and 1 on the remaining 91.7%, indicating that only a few fish meandered from Start Box B. The time taken to reach periphery (3.04 sec) may reflect the fish s mean Landing vector, which pointed back in the direction of the release site. The discrepancy between blinded discharging and sighted discharging fish tested under dark conditions will be addressed in the General Discussion.

Experiment 6: Sighted-Nondischarging Fish Tested Under Dark Conditions

The goal of this experiment was to test fish that have neither the use of sight nor the active electrosense. It was hypothesized that when lacking these two senses, fish would be at a disadvantage during acquisition, possibly more so than the sighted-discharging fish tested under dark conditions, and would be at a complete loss in finding the Goal swimming randomly in the arena. Alternatively, if these fish, lacking sight and electrosense, were still able to acquire and recall the task, they would have to rely on other input such as mechanical stimulation through the lateral line and/or idiothetic, i.e. some form of proprioceptive input to generate an appropriate cue list.

Method

Eight naïve fish were silenced in the method described in Experiment 2 and trained and tested in the dark conditions described in Experiment 4 (<1 lux). Although fish lacked two senses, it was thought that an incentive for swimming to the Goal was the perception of the shelter via the lateral line and touch. Outbound and Landing points were observed by the experimenter as described in Experiment 4, recorded to the nearest 10° mark or, if possible, interpolated to 5°.

Results

Fish required 11 days to learn the task and were tested for recall on day 12 (Fig. 12). Interestingly, their average time to complete the maze on day 11 was 20.5±2.1 sec:

the time needed to acquire the task and the average time on the last day of testing are both comparable to sighted-discharging fish tested under dark conditions (Experiment 4).

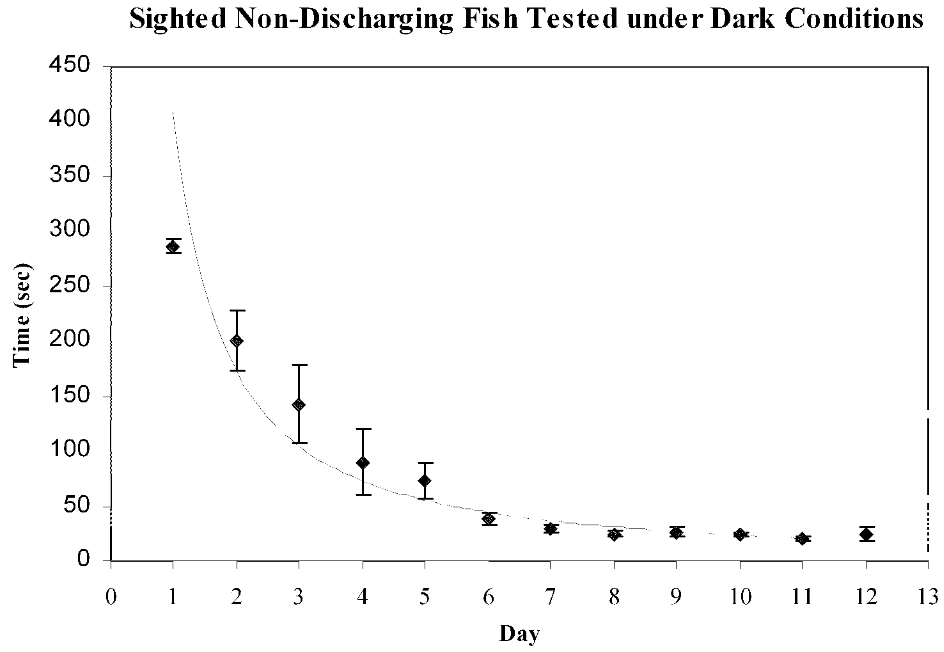


Figure 12. Time to complete the maze for sighted-nondischarging fish tested under dark conditions (<1 lux). Day 12 represents recall (test day). Error bars indicate SEM.

In recall, for the group started from Start Box A, the mean Outbound vector was θ=220°, r=0.583 (Fig. 13). The Landing vector was θ=168°, r=0.809. The Outbound bearings reflect a left turn and the Landing vector is directed at the Goal. The mean Outbound vector for fish started from Start Box B was θ=91°, r=0.720. The Landing vector was θ=103°, where r=0.867. These results suggest that the fish headed straight out from Start Box to a virtual Goal, and landed at the periphery of the tank at points slightly shifted towards the actual Goal.

No meander data were recorded for this group of fish. The times taken by fish released from both Start Box A (6.9 sec) and B (7.1 sec) to reach the periphery of the tank were statistically different from all other groups of fish; however, fish started from Start Box A and those started from Start Box B did not differ from each other in this measure. Implications of these differences will be discussed in the General Discussion.

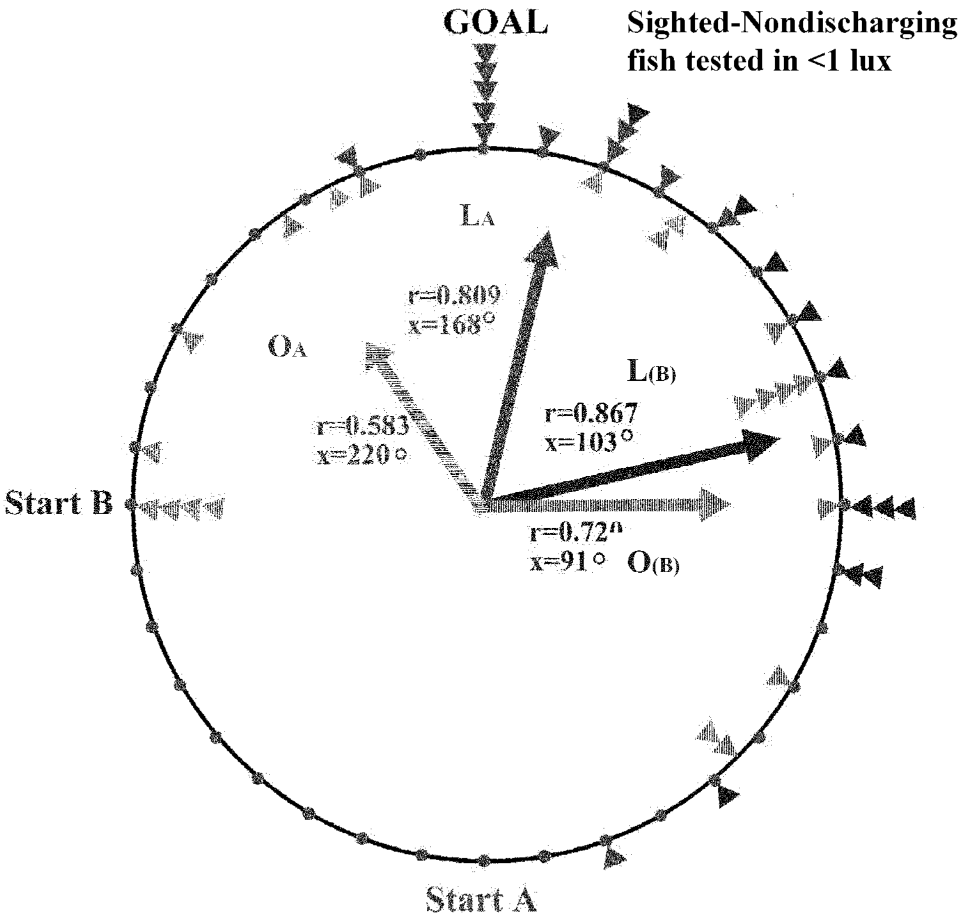


Fig 13. Sighted-nondischarging fish trained and tested under <1 lux illumination conditions. All fish were trained to find the Goal when released in Start Box A. Recall: red arrows=fish released from Start Box A; blue arrows=fish released from Start Box B (rotated 90°). Striped arrows: Outbound vectors (O). Solid arrows: Landing vectors (L). Letter in parenthesis following O or L indicates Start Box (A or B). r=length of vector; x=θ (angle).

Experiment 7: Electric Landmarks

This study was designed to determine the importance of electric landmarks in learning and recall. Experiment 2 suggested that fish do not attend to visual landmarks in this task; it was therefore the goal of this experiment to determine whether electric landmarks play a role in acquisition and recall. Since the active electrosense plays a major role (if not *the* major role) in mormyrid navigation, it was hypothesized that this group of fish would attend to the electric landmarks preferentially. Fish were trained in a manner identical to that described in the previous 3 experiments, except here, rather than visual landmarks, physical landmarks identical in appearance and constructed either of plastic or aluminum cubes (125 cm^3) were employed. The material of the objects (Plexiglas and aluminum) were chosen since other authors have found that mormyrid attend differently to various materials (Push and Moller, 1979; von der Emde, 1998; Rojas and Moller, 2002).

Method

Eleven naïve *M. r. proboscirostris* were trained to learn the maze pattern in the manner described in Experiments 2, 4, and 5, with the addition of physical landmarks in the tank. Ambient light was kept at approximately 12 lux, as in Exp. 2. The aluminum and Plexiglas cubes were placed near the ends of the barriers so that each material signaled a particular turn for the fish (aluminum=right; Plexiglas=left) (Fig. 14). After training was completed and the maze barriers removed, fish were tested in one of three conditions: 1) no change in sequence of landmarks placement; 2) the locations of the aluminum and Plexiglas cubes were reversed; and 3) all landmarks were removed. Since the passive electrosense is important in the perception of metal objects, via the DC field

(Rojas and Moller, 2002), it was hypothesized that since aluminum objects provide active and passive electrosensory cues, fish would pay particular attention to these objects.

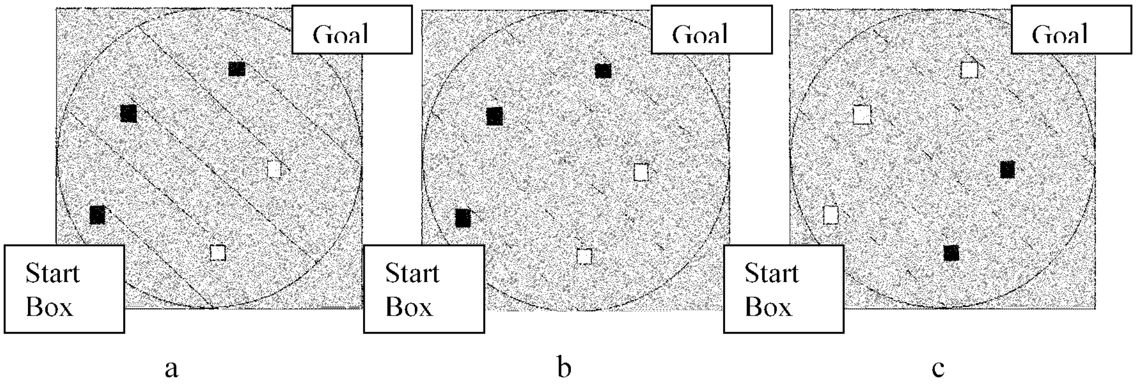


Figure 14. Electric Landmark study. Illustration of maze tank during training (a), and during testing (b & c). (b) represents the setup of the arena during recall in the no change condition (barriers removed, but landmarks remain as they were during training) and (c) represents the setup in the switch condition (barriers removed and modified configuration of electric cues). ■ represents aluminum objects; □ represents Plexiglas objects.

Once fish had reached criterion acquisition time (asymptote), they were divided into three groups: During recall, group A was tested with all electric landmarks left in place in their original order (AL-PL-AL-PL-AL). We hypothesized that if the fish attend to landmarks when executing the maze pattern during recall, then they will also attend to (and therefore meander around) the landmarks even when the maze barriers are removed. If they no longer use the landmarks and make a straight line from Start Box to Goal, then landmarks are clearly unnecessary in the recall phase. Fish in group B encountered all electric landmarks in the original place, but a changed sequence (PL-AL-PL-AL-PL) (Fig. 14). The fish s response to this manipulation would indicate whether they attend to the arrangement of the objects during recall: swapping the two materials would send the animal that attends to the electric nature of the landmark in the opposite direction to it was accustomed to (i.e., now the cue for go left is replaced with that for go right); if fish do not attend to electric landmarks, they should head straight for the Goal. Group C consisted of three fish tested with all landmarks removed and served as control. Recall was tested in all fish released from Start Box A.

Results

During acquisition, fish trained in the presence of electric landmarks required eight days to learn the task and were tested on day 9 (Fig. 15). The average time to complete the maze on the last day of training was 16.5±0.90 sec.

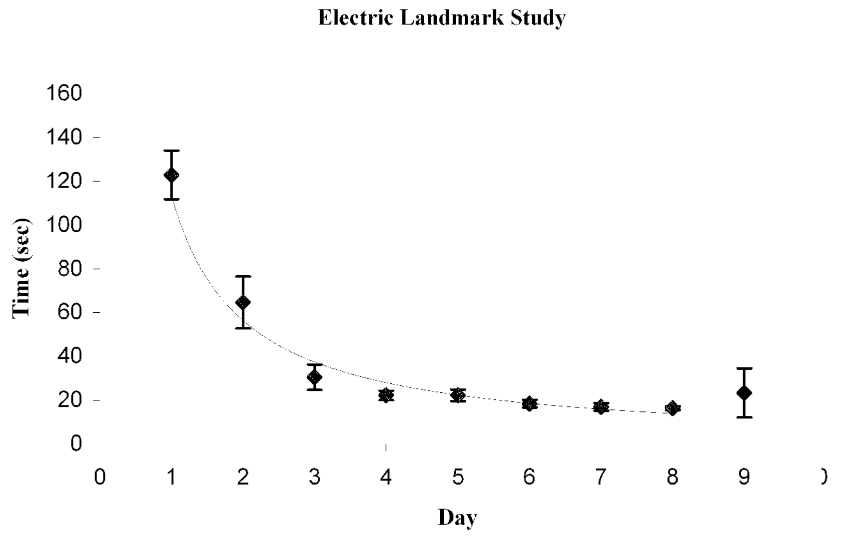


Figure 15. Time to complete the maze for sighted-discharging fish trained with electric landmarks. Day 9 represents recall (test day). Error bars indicate SEM.

The mean Outbound vector for group A (landmarks in their original positions) was θ=256°, r=0.667 (Fig. 16). The Landing vector for this group was 221°, r=0.661. These data represent a strong left-hand turn out of the Start Box and a Landing vector that was also shifted counterclockwise.

The Outbound vector for group B (landmarks in switched positions) was θ=233°, r=0.530; variable initial bearings are indicated by the r-value (Fig. 16). The Landing vector for this group was θ=181°, r=0.849, suggesting that, on average, fish found their way directly to the Goal. The significance of the differences between groups will be discussed in the General Discussion.

Fish in group C (all electric landmarks removed), exhibited nearly perfect goal-directed behavior. The Outbound vector was θ=201° with r=0.736; the Landing vector was θ=177°, with r=0.908 (Fig. 17).

A comparison of the fish s orientation strategies in these three situations clearly demonstrates that electric cues can provide navigational landmark information, but are not required during recall to achieve the acquired task. This is also borne out by the occurrence of meanders during recall. Fish that encountered the unchanged array of landmarks (group A), meandered on 16.7% of the trials (scored 2) whereas fish in the other two groups (B, C) never meandered. There were no significant differences in the time it took the fish in all three groups to reach the periphery (group A, 4.0 sec; group B, 3.7 sec; group C, 3.4 sec).

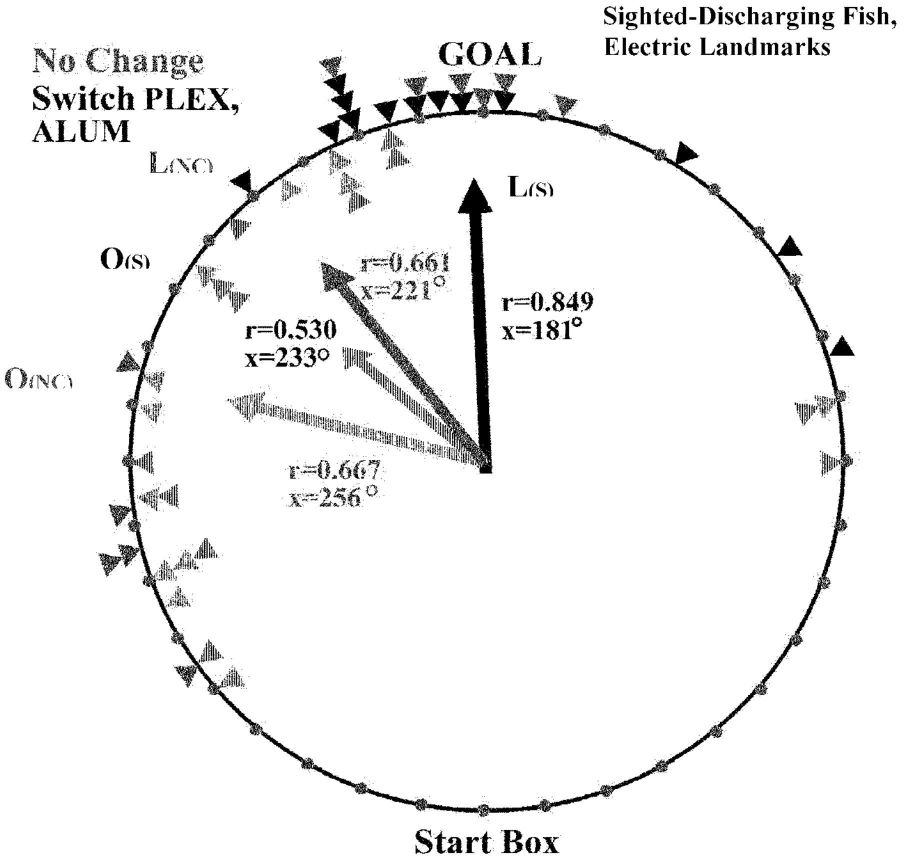


Figure 16. Fish trained in the presence of alternating electric landmarks (Alum, Plex, Alum, Plex, Alum). Recall: Red arrows=no change in landmarks positions between acquisition and recall; black arrows=modified configuration of electric cues (Plex, Alum, Plex, Alum, Plex). Striped arrows=Outbound vectors (O); solid arrows=Landing vectors (L). Letter in parenthesis following O or L indicates Condition (NC, No Change; or S, Switch). r=length of vector; x=θ (angle).

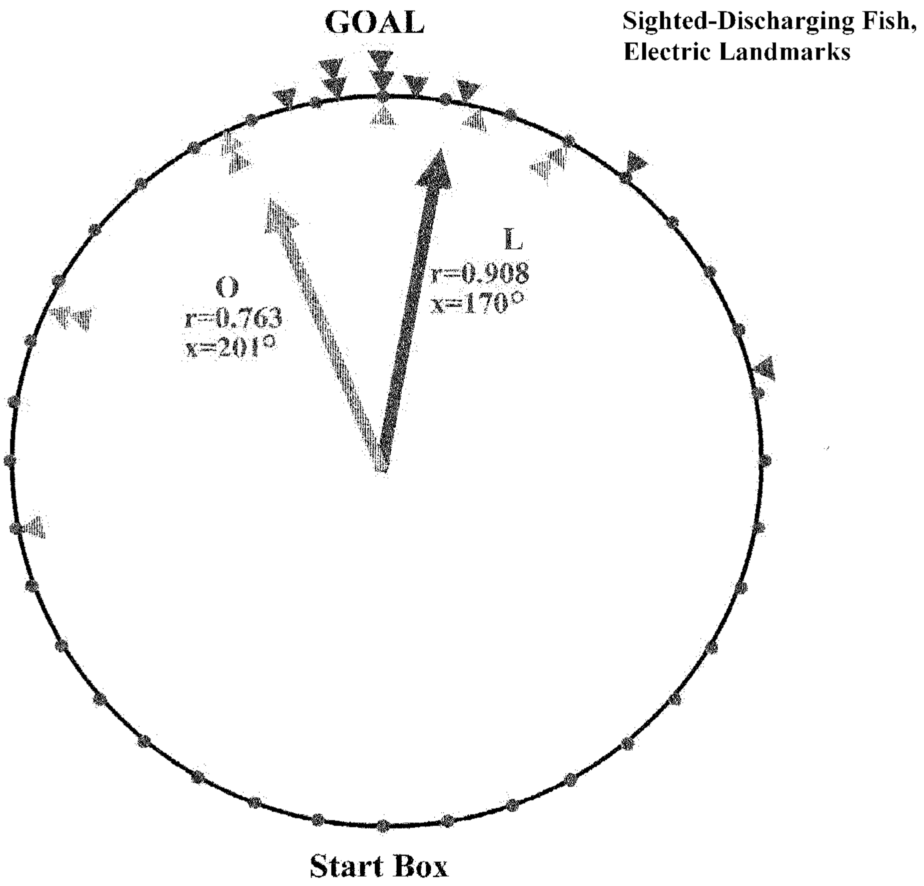


Figure 17. Sighted-discharging fish trained in the presence of alternating electric landmarks (Alum, Plex, Alum, Plex, Alum): Control Group Recall: all landmarks were removed from tank. Striped arrow=Outbound vector (O); solid arrow=Landing vector (L). r=length of vector; x=θ (angle).

Experiment 8: Sighted-Nondischarging Fish Tested Under Dark Conditions with the Temporary Elimination of the Lateral Line

Since fish in previous experiments generally only had a deficit in acquisition, and performed somewhat similarly in recall, the following experiment was designed to determine the role of the lateral line sensory system in navigation. Mormyrid fish possess a lateral line system involved in the detection of slow-moving objects (see review Moller, 1995, p. 367). There have been no studies implicating its role in navigation and sensory integration. Teyke (1988) found that blind cave fish increase their swimming velocity in new environments, perhaps to elicit stronger lateral line cues. The lateral line has been shown to be important in prey capture in non-weakly electric fish (Hoekstra and Janssen, 1985; Pohlmann, Atema, and Breithaupt, 2004), and although largely unstudied in weakly electric fish, it is thought that it may play a complimentary role in prey capture (Coombs, New, and Nelson, 2002). We decided to explore the treatment outlined in Karlsen and Sand (1987) with *M. r. proboscirostris*. It was predicted that fish lacking the use of vision, the active electrosense, and the lateral line would exhibit a deficit not only in recall but in learning the maze as well. Without any of the major senses available, it was hypothesized that the fish would be unable to learn the task.

Before the experimental fish were exposed to cobalt chloride, a sighted-discharging fish was placed in the CoCl tank for approximately 18 hours to insure physical and behavioral well-being. (To date, no studies have successfully used cobalt chloride to eliminate the lateral line sensory system in weakly electric fish. Von der Emde (1998) found that *G. petersii* exhibited negative behavioral effects at a Co^{2+} concentration of 0.1 mMol/L (a concentration found to be effective in the roach; Karlsen and Sand, 1987), which included a lack of motivation toward food, slow swimming, and an appearance that was generally unwell.) After 18 hours in the cobalt chloride, our intact fish appeared well; gill movement was normal as was overall behavior. It was therefore determined that the experimental fish could be exposed and tested in the maze without negative behavioral consequences.

Method

Six naïve fish were used in the present study; one fish was not naïve and had been used five months prior, in Experiment 6. The five naïve fish were silenced in the method described previously. To temporarily eliminate the lateral line sense, a 60-liter tank was prepared with deionized water. A solution of KCl (1.78 mMol/L), KNO_3 (3.57 mMol/L) $NaH_2PO_4\ H_2O$ (3.57 mMol/L), $MgSO_4\ 7H_2O$ (7.14 mMol/L), and NaCl (14.28 mMol/L) (D. Daily, personal communication, January 17, 2006) was added to create an artificial fresh water solution devoid of Ca^{2+} ions, which have been found to reverse the effects of cobalt chloride (Karlsen and Sand, 1987). Cobalt chloride (Sigma-Aldrich) was then added to the water at 0.1 mMol/L concentration (Karlsen and Sand, 1987). Temperature was approximately 25.1 °C, conductivity was 347 µS, and pH was 6.7.

The seven sighted-nondischarging fish were collectively exposed to the cobalt chloride tank for 24 hours prior to training and watched for any changes in behavior. After 24 hours fish were moved to individual 18.5-liter tanks (temperature was approximately 23.1 °C, conductivity was 350 ±5 µS, and pH was 6.7) and were watched for one hour. Fish were then trained through the maze in the dark (<1 lux), as described in Experiments 4 and 6. To ensure that the lateral line sense would be absent for the entirety of the study, fish were trained for seven days (D. Daily, personal communication, January 17, 2006), and the learning curve extrapolated from the power trendline equation. The option to re-expose the fish to cobalt chloride in the midst of the experiment was undesirable, given the stress of transfer to different tanks and the fact that no other groups experienced mid-training changes in routine. Therefore fish were run for seven days and tested on the eighth day. Outbound and Landing points were recorded as described in Experiment 4 (recorded to the nearest 10° mark, or interpolated, if possible, to 5°).

Results

The mean time to complete the maze for six fish (one fish died one day into training) was 108.0±35.8 sec on the last day of training. Fish would have needed approximately 18 days of training to asymptote comparable to fish in the other groups (this was extrapolated from the associated power function; see insert Fig. 18).

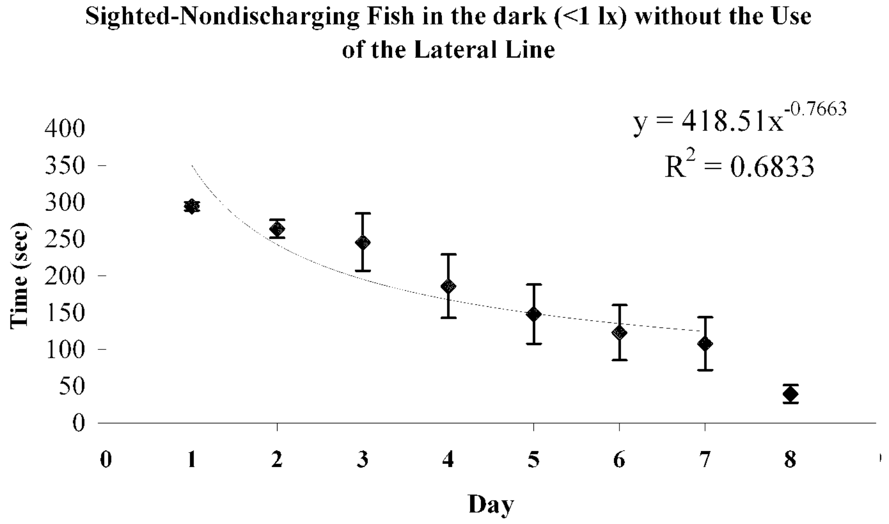


Fig. 18. Time to Complete the Maze for Sighted-Nondischarging Fish Tested Under Dark Conditions with the Temporary Elimination of the Lateral Line. Fish were trained for seven days, to ensure that the cobalt chloride treatment to eliminate the lateral line would remain effective. Extrapolating from the power curve equation, fish would have needed approximately 18 days to asymptote similarly to other groups of fish. Error bars indicate SEM.

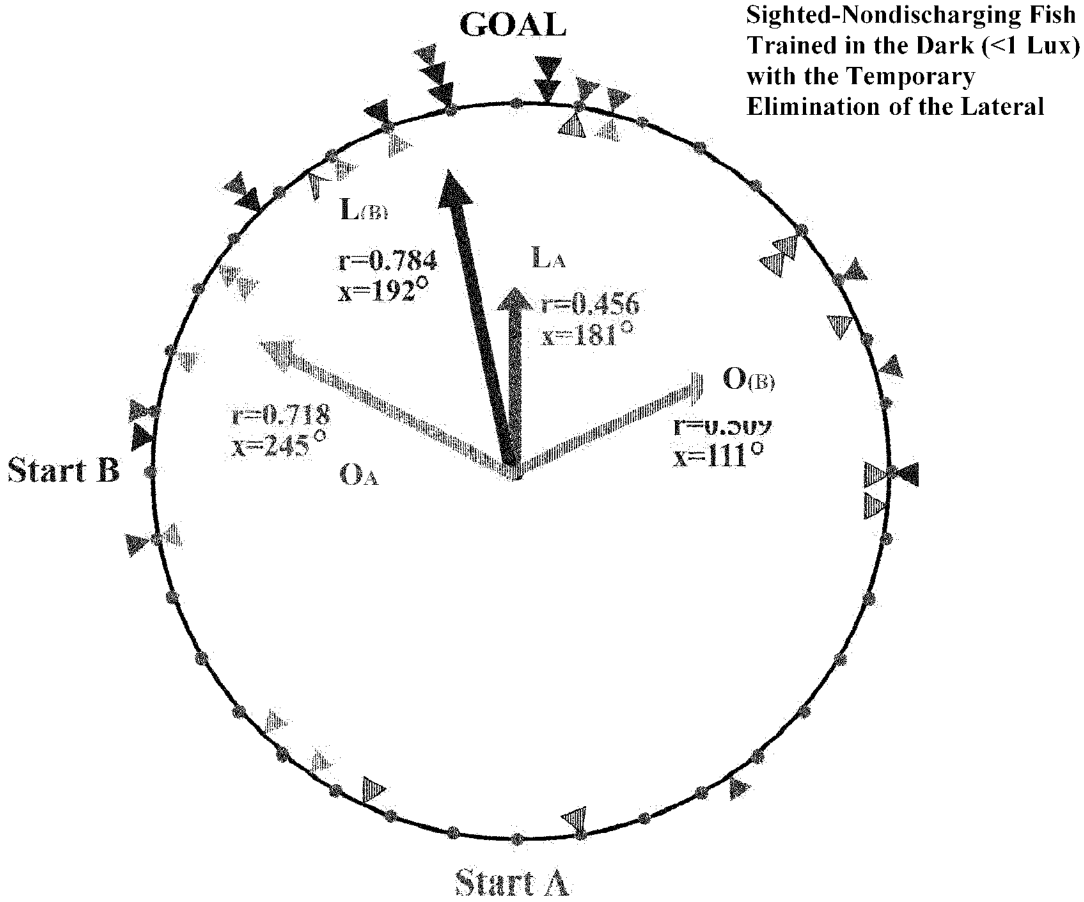


Fig. 19. Sighted-Nondischarging Fish Trained in the Dark (<1 Lux) with the Temporary Elimination of the Lateral Line. All fish were trained to find the Goal when released in Start Box A. Recall: red arrows=fish released from Start Box A; blue arrows=fish released from Start Box B (rotated 90°). Striped arrows: Outbound vectors (O). Solid arrows: Landing vectors (L). Letter in parenthesis following O or L indicates Start Box (A or B). r=length of vector; x=θ (angle).

Most fish released from Start Box A made an initial left-hand turn (Fig. 19): the mean Outbound vector for fish released from Start Box A was 244.9° (r=0.718). The mean Landing vector for this group was 180.9°, although it was uniformly distributed. For fish that were released from Start Box B the mean Outbound vector was uniformly distributed (mean 111.3°) and the Landing vector for these fish was 191.8° (r=0.784), indicating that most fish landed close to the Goal box. No data on meander behavior time taken to reach the periphery were available for this group.

Experiment 9 (Control)

This final experiment was designed as a control for Experiments 2-9 to assess the fish s normal swimming paths when released into the arena without prior maze training. The trajectories of the fish as they were released from Start Box A or Start Box B were recorded and compared to those obtained in the experimental groups. It was hypothesized that fish in this control group would show no directionality in their Outbound and Landing vectors, as indicated by uniform distributions. In one session, eight sighted-discharging fish were run in the empty arena (no maze barriers in place) in light conditions to observe the fish s swimming trajectories in the absence of the maze.

Method

Fish were removed from the 1000-liter tank in which they were housed collectively (conductivity = 400μS/cm, temperature = 26.4 °C, and pH = 6.6), placed in Start Box A or Start Box B, and after 3 trials each, placed in another holding tank (conductivity = 400μS/cm, temperature = 26.1°C, and pH = 6.48) until all fish were run. The session was videotaped as before, and times taken to reach the Goal box were recorded and Outbound and Landing vectors were calculated.

Results

The mean time taken to reach the goal was 42.7±28.7 sec. The Outbound vector of fish released from Start Box A was 208° (r=0.629), and the Landing vector of these fish was uniformly distributed (212°) (Fig. 20). The Outbound vector of fish released from Start Box B was 82.9° (r=0.64), and the Landing vector was 93.4° (r=0.522).

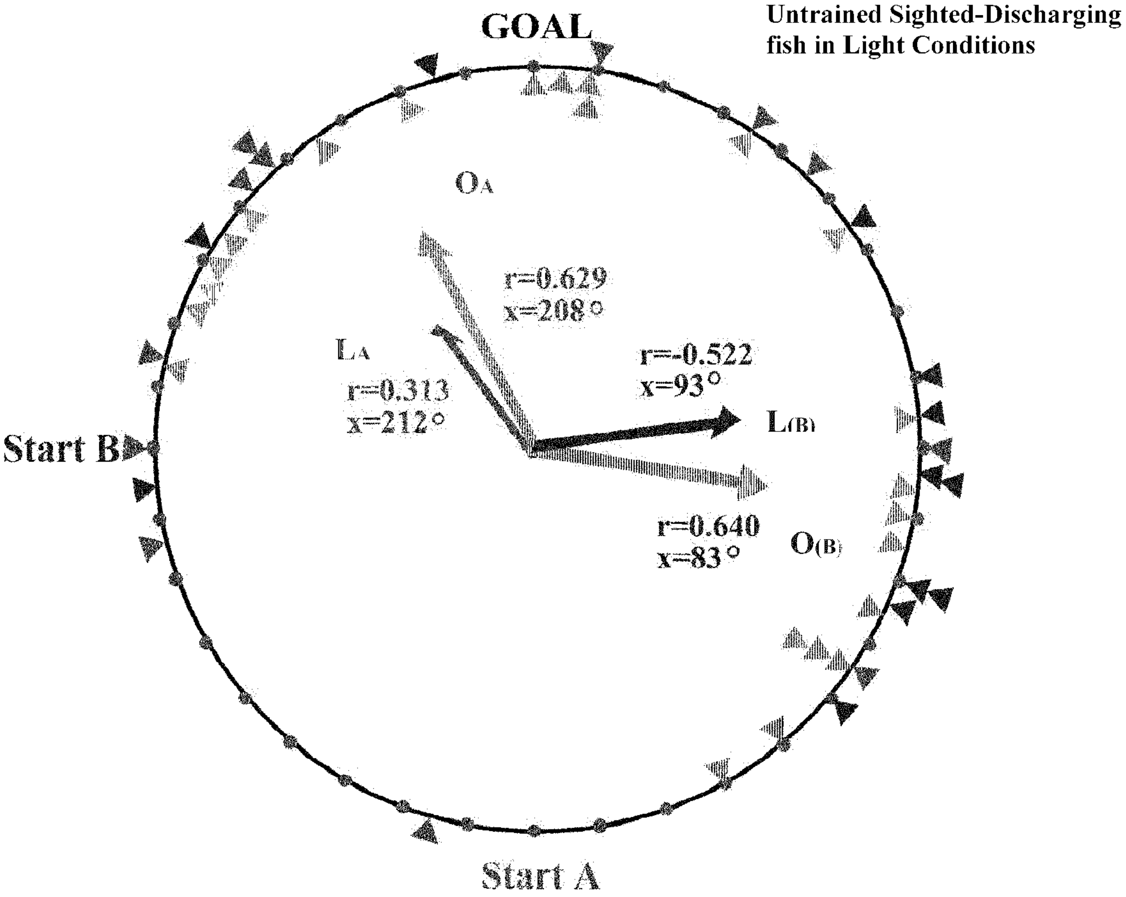


Fig. 20. Untrained Sighted-Discharging Fish under Light Conditions (Control). Fish were not trained through the maze prior to release. Red arrows=fish released from Start Box A; blue arrows=fish released from Start Box B (rotated 90°). Striped arrows: Outbound vectors (O). Solid arrows: Landing vectors (L). Letter in parenthesis following O or L indicates Start Box (A or B). r=length of vector; x=θ (angle).

RESULTS: COMPARISIONS

Table 1 lists each group according to sensory/environmental condition and release site.

Number	Group
1A	Sighted-Discharging, light conditions released from Start Box A
1B	Sighted-Discharging, light conditions released from Start Box B
2A	Sighted-Discharging, dark conditions released from Start Box A
2B	Sighted-Discharging, dark conditions released from Start Box B
3A	Sighted-Nondischarging, dark conditions released from Start Box A
3B	Sighted-Nondischarging, dark conditions released from Start Box B
4A	Sighted-Nondischarging, dark conditions without Lateral Line released from Start A
4B	Sighted-Nondischarging, dark conditions without Lateral Line released from Start B
5	Blind-Discharging, released from Start Box B
6A	Electric Landmarks, No Change (released from Start Box A only)
6B	Electric Landmarks, Switch (released from Start Box A only)
6C	Electric Landmarks, Control (released from Start Box A only)
7A	Control released from Start Box A
7B	Control released from Start Box B

Table 1. Listing of experiments II/2-10. These assignments are used to conserve space in tables only.

A one-way ANOVA [F(5,12) = 153.05, p<.0001] and Newman-Keuls post hoc test revealed that all groups differed significantly from each other in mean asymptote times (Table 2, Fig. 22).

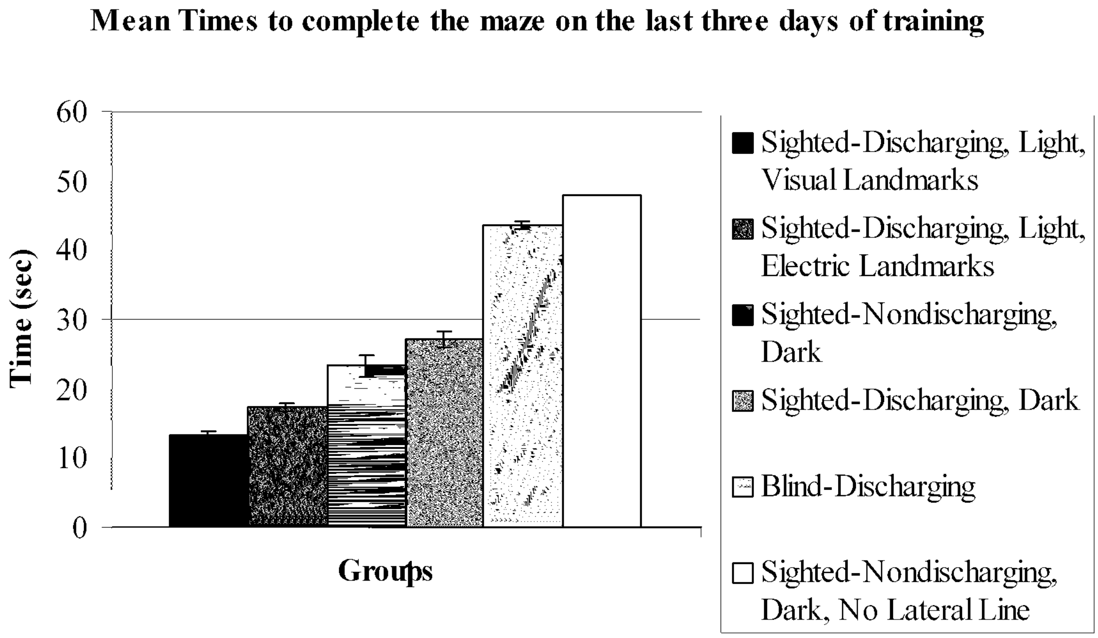


Figure 21. Mean times to complete the maze on the last three days of training for each group (asymptote). Fish were considered to have learned the maze if the mean group time decreased three seconds (or less) per day over three consecutive days. Error bars indicate SEM. Note that for the sighted-discharging fish in the dark with the temporary elimination of lateral line input, the learning curve after day seven was extrapolated from the power curve equation derived from the first seven days of training (thus no error bars).

	Sighted-discharging, Light	Sighted-discharging, Dark	Blind-discharging	Electric Landmarks, light	Sighted-nondischarging, Dark
Mean time (sec)	**13.2**	**27.24**	**43.6**	**17.4**	**23.3**
Sighted-discharging, light					
Sighted-discharging, dark	<0.001				
Blind-discharging	<0.001	<0.001			
Electric Landmarks, light	0.011	<0.001	<0.001		
Sighted-nondischarging, dark	<0.001	0.015	<0.001	0.0014	

Table 2. Between-group comparison (post-hoc Newman-Keuls) of times to complete the maze on the last three days of training (asymptote). P-values are shown. All groups differed significantly from one another in this measure. Note that sighted-nondischarging fish in the dark with the temporary elimination of lateral line input were not compared, since data for this group s asymptote times were extrapolated from the best-fit function.

Mean times to complete the maze during acquisition have been reported above for each group, as have the mean Outbound and Landing vectors, and where possible, times to reach the periphery and number of meanders. Table 3 summarizes acquisition times for each group, mean times on the last three days of training, Outbound and Landing vector directions, and mean times on test day (recall). Comparisons will now be made between and within groups. Tables 4a-d summarize between-group comparisons of Outbound or Landing vectors of fish released from Start Box A or Start Box B (Mardia-Watson-Wheeler tests for nonparametric circular data). Next the Outbound and Landing vectors within groups were compared to each other (Table 5). Finally, to test the two hypotheses (path integration or mapping) the observed vectors were compared with the respective expected vectors (Table 6a-b).

Condition	**Acquisition Time/Mean Time on Last Training Day**	**Outbound Vector**	**Landing Vector**	**Mean time in Recall (three trials)**
1A	7 days/12.23 sec	**199.2°**	**181.2°**	10.99 sec
1B		**93.7°**	**128.5°**	
2A	11 days/26.97 sec	**230.3°**	**221.2°**	11.23 sec
2B		**81.3°**	**112.8°**	
3A	11 days/20.54 sec	**219.5°**	**168°**	24.58 sec
3B		**90.5°**	**102.5°**	
4A	18 days*/45.67*	**244.9°**	180.9°	29.46 sec
4B		111.3°	**191.8°**	
5	12 days/42.61 sec	137.5°	267°	24.48 sec
6A	8 days/16.49 sec	**255.7°**	**220.6°**	23.40 sec
6B		**232.6°**	**180.9°**	
6C		**201.1°**	**169.9°**	
7A	no training	**208°**	212.2°	N/A
7B		**82.9°**	**93.4°**	

Table 3. Number of days required for acquisition, mean time on last day of training, Outbound and Landing Vector for all groups, and mean recall times. Bolded values denote mean directions that are significantly different from a uniform distribution. Non-bolded values represent mean directions of vectors whose length indicated uniform distribution. *Represents the number of days the fish would have required to learn the task, extrapolated from the best-fit power function.

As is evident from Table 3, Outbound and Landing vectors indicate that the trajectories of most fish were directed (with the exception of the Outbound vectors of sighted-nondischarging fish in dark conditions with no lateral line input released from Start Box B and blind-discharging fish, and the Landing vectors of sighted-nondischarging fish in dark conditions with no lateral line input released from Start Box A, blind-discharging fish, and the control group release from Start Box A, as swimming paths were uniformly distributed).

In the following four comparisons, the Outbound or Landing vectors of fish released from Start Box A or Start Box B were compared (i.e., between-groups). Table 4a shows the comparisons of the Outbound vectors of the fish released from Start Box A only, analyzed with the Mardia-Watson-Wheeler test for nonparametric data. It is crucial to note that the Mardi-Watson-Wheeler test takes into account *both* the mean vector and the r-value (the length of the vector), thus groups with similar mean vector angles may be statistically different due to sufficient difference in r-values. The sighted-discharging fish in light conditions released from Start Box A showed differences in direction from sighted-nondischarging fish in dark conditions released from Start Box A and sighted-discharging fish trained with electric landmarks (no change) only. The mean Outbound vector of the sighted-discharging fish in light conditions released from Start Box A was directed at 199.2°; groups for which there was *no* significant difference were sighted-discharging fish in the dark, sighted-nondischarging fish in the dark without the use of the lateral line, sighted-discharging fish in the presence of electric landmarks (switch and removed), and the control group in light conditions released from Start box A. Sighted-nondischarging fish in dark conditions released from Start Box A showed differences from sighted-discharging fish in light conditions released from Start Box A only. There were no differences between the control group (release from Start Box A) and any of the other groups.

Condition	**1A**	**2A**	**3A**	**4A**	**6A**	**6B**	**6C**	**7A**
1A	-	ns	0.015	ns	0.025	ns	ns	ns
2A		-	ns	ns	ns	ns	ns	ns
3A			-	ns	ns	ns	ns	ns
4A				-	ns	ns	ns	ns
6A					-	ns	ns	ns
6B						-	ns	ns
6C							-	ns
7A								-

Table 4a. Outbound Vector comparison between groups of fish released from Start Box A (Mardia-Watson-Wheeler test for differences between groups). ns indicates p >.05.

Between-group comparisons of fish released from Start Box B revealed a significant difference in the Outbound vectors of sighted-discharging fish in light conditions and sighted-discharging fish in dark conditions only (Table 4b).

Condition	**1B**	**2B**	**3B**	**7B**
1B	-	0.021	ns	ns
2B		-	ns	ns
3B			-	ns
7B				-

Table 4b. Outbound Vector comparison for fish released from Start Box B (Mardia-Watson-Wheeler test for differences between groups). ns indicates p >.05.

Table 4c shows the comparisons between Landing vectors for fish released from Start Box A. The only significant differences between these groups were between sighted-nondischarging fish in dark conditions released from Start Box A and sighted-discharging fish in dark conditions, and sighted-nondischarging fish in dark conditions and sighted-discharging fish trained with electric landmarks (switch).

Condition	**1A**	**2A**	**3A**	**6A**	**6B**	**6C**
1A	-	ns	ns	ns	ns	ns
2A		-	0.001	ns	ns	ns
3A			-	ns	.023	ns
6A				-	ns	ns
6B					-	ns
6C						-

Table 4c. P-values for comparisons Landing vectors for fish released from Start Box A (Mardia-Watson-Wheeler test). ns indicates p >.05.

Comparisons of the mean Landing vectors of fish released from Start Box B revealed differences between groups: sighted-nondischarging fish in dark conditions with no lateral line input differed from all other groups, and sighted-discharging fish in light conditions and control (untrained) fish differed from one another (Table 4d).

Condition	**1B**	**2B**	**3B**	**4B**	**7B**
1B	-	ns	ns	0.006	0.029
2B		-	ns	0.016	ns
3B			-	0.001	ns
4B				-	0.008
7B					-

Table 4d. Landing vectors for groups of fish released from Start Box B (Mardia-Watson-Wheeler test); ns indicates p > .05.

Table 5 lists the statistical comparisons between Outbound and Landing vectors within groups. Outbound and Landing vectors differed in sighted-discharging fish in light conditions released from Start Box B, sighted-nondischarging fish in dark conditions released from Start Box A, and sighted-discharging fish trained with electric landmarks (switch).

Condition	Outbound vs. Landing vector
1A	ns
1B	0.001
2A	ns
2B	ns
3A	0.007
3B	ns
6A	ns
6B	0.01
6C	ns
7B	ns

Table 5. Comparison of Outbound vs. Landing vectors for all groups of fish (Mardia-Watson-Wheeler test for differences between groups); ns indicates p >.05.

Table 6a shows the comparisons between the observed vectors and the expected directions fish would have chosen following path integration or mapping strategy, respectively. First, based on the empirically obtained distributions the corresponding 95% confidence intervals were calculated. The null-hypothesis that expected and empirically obtained vectors did not differ from one another is supported when the expected direction falls within this confidence interval (P. Görner, personal communication; November 28, 2005). The results indicated strong support for the path integration hypothesis. In the following the expected direction is set as the direction fish would choose using path integration.

Fish released from Start Box A (at 0°) were expected to swim to the opposite side of the tank (actual Goal, at 180°). Fish in the control group (released from Start Box A) were expected to show undirected swimming from Start Box A. The results show that the expected direction (180°) fell within the 95% confidence intervals for the Outbound vectors of sighted-discharging fish in light conditions released from Start Box A, sighted-nondischarging fish in dark conditions released from Start Box A, sighted-discharging fish trained with electric landmarks , and tested with these landmarks removed , and the control group released from Start Box A. The expected direction also fell within the 95% confidence range for the Landing vectors of all fish released from Start Box A, except for sighted-discharging fish in dark conditions released from Start Box A and sighted-discharging fish trained with electric landmarks (no change).

For fish released from Start Box B (270°), it was expected that experimental fish would swim to the opposite side of the tank, i.e., to a virtual Goal at 90°, assuming a path integration/dead reckoning strategy is used (Table 6b). The expected direction for the cognitive mapping strategy was 180°, in the direction of the actual Goal. Outbound and Landing vectors for the control group were expected to show undirected swimming. The results, however, indicated that *only* the expected direction for path integration fell within the 95% confidence interval, for both Outbound and Landing vectors (except for the Landing vectors of the sighted-discharging fish in light conditions released from Start Box B, which overlapped with neither expected direction). The unexpected result of the control group will be discussed below.

Outbound	**Expected Angle (Path Integration)**	**Expected angle (Mapping)**	**Observed Angle**	**95% Confidence**
1A	180	**NA**	199.2	**±24**
2A	180	**NA**	230.3	±22
3A	180	**NA**	219.5	**±41**
4A	180	**NA**	244.9	±38
6A	180	**NA**	255.7	±33
6B	180	**NA**	232.6	±47
6C	180	**NA**	201.1	**±35**
7A	No direction	**NA**	208	**±36**
Landing	**Expected Angle**		**Observed Angle**	**95% Confidence**
1A	180	**NA**	181.2	**±27**
2A	180	**NA**	230.3	±42
3A	180	**NA**	168.1	**±25**
6A	180	**NA**	220.6	±34
6B	180	**NA**	181	**±23**
6C	180	**NA**	169.8	**±25**

Table 6a. Comparison of observed vectors and expected vectors. For fish released from Start Box A the expected Outbound and Landing vectors were 180°. **Bolded** values indicate angles that fall into the confidence interval and thus are in the expected direction.

Outbound	**Expected Angle (Path Integration)**	**Expected angle (Mapping)**	**Observed Angle**	**95% Confidence (Path Integration)**
1B	90	180	93.7	**±36**
2B	90	180	81.3	**±23**
3B	90	180	90.5	**±30**
7B	90	180	82.9	**±48**
Landing			**Observed Angle**	**95% Confidence (Path Integration)**
1B	90	180	128.5	±33
2B	90	180	112.8	**±31**
3B	90	180	102.5	**±22**
4B	90	180	191.8	**±38**
7B	90	180	93.4	**±48**

Table 6b. Comparison of observed and expected vectors. For fish released from Start Box B the expected Outbound and Landing vectors were 90° (virtual Goal) for path integration, and 180° (actual Goal) for mapping. **Bolded** values indicate angles that fall into the confidence interval and thus are in the expected direction for **path integration**. Note that in no cases did the 95% confidence interval overlap with the expected angle assuming a mapping strategy.

GENERAL DISCUSSION

Rectangular Maze

In Experiment 1, sighted-nondischarging fish exhibited longer times to complete the maze on day 1 than the sighted-discharging and blind-discharging fish. This difference vanished by day 2 and on all subsequent days, all fish performed identically. Sighted-nondischarging fish may be at a minor deficit initially, but they quickly and successfully learn to rely on vision and other senses (e.g., the lateral line). Furthermore, all three groups of fish exhibited similar behavior when allowed to navigate the maze tank in the absence of barriers. The proportions of meanders from Start Box to Goal on the first trials suggests that mormyrid fish may use not path integration as their strategy, although the trajectories did become straighter with increasing trials regardless of condition. Whether these results suggest that fish are able to use one or more strategies is not completely clear from these results. One may argue that the similarity in performance of the three groups on the final day of the experiment was affected by the geometry of the rectangular tank. To control for this possibility, a circular tank was needed to determine what would happen if fish were displaced to a new start point but were unable to use the shape of the tank as a navigational cue. The circular open field offered more options in terms of displacing the fish and therefore teasing apart the navigational strategy utilized, as well as evaluating the differences in acquisition of the task in the learning phase.

Circular Open Field Experiments

According to the hypotheses outlined above, fish may use (a) cognitive mapping, or (b) cue lists path integration or dead reckoning. In the circular open field experiments, sighted-discharging fish with visual landmarks took the shortest amount of time to learn the task (7 days), followed by the sighted-discharging fish with electric landmarks (8 days), and finally sighted-discharging fish in dark conditions, and sighted-nondischarging fish in dark conditions (both groups required 11 days). The fact that sighted-discharging and sighted-nondischarging fish under dark conditions required more time to learn the task may be due to the fact that these groups were lacking one or two sensory modalities. Alternatively, it may be that there was less incentive for these groups to reach the Goal because of the dark conditions: this conjecture may be supported by the fact that the performance in both groups on the last three days of training (asymptote) was longer than for the sighted-discharging fish under light conditions. It was clearly established that the fish learned the task and that the time to complete the maze asymptoted. The similarity in performance across groups in recall further suggests that all fish learned the task during acquisition, and thus the nature of the incentive while obvious for sighted fish is not a major concern for the study. Fish tested under dark conditions may have attended to the non-visual sheltering properties of the goal box. The blind-discharging group required 12 days to learn the maze and thus did not differ in spite of lacking one sensory modality. Sighted-nondischarging fish tested in the dark that also lacked lateral line input, thus missing three modalities, needed 18 days to asymptote (based on extrapolation).

During recall, most fish, regardless of condition, released from Start Box A swam directly to the Goal (Landing vector); fish released from Start Box B swam to a point on the periphery opposite Start Box B (a virtual Goal). These results support hypothesis (b) and suggest that mormyrid fish do not use a form of mapping, but rather use cue lists (dead reckoning and path integration). Meander data add strength to this conclusion, since fish only meandered from Start Box A, suggesting that fish released from Start Box B switched strategies as they may have attended to site-specific cues (see below). Blind-discharging fish were released from Start Box B during recall, but showed no directionality in their Outbound and Landing vectors. This apparent paradoxical behavior was attributed to the fact that long-time blinded fish, lacking circadian rhythmicity, exhibit decreased electric organ discharge activity.

LANDMARKS

As predicted, on test day, the sighted-discharging fish with visual landmarks available started from Start Box A (Exp. 2) swam to the Goal (Outbound vector=199.2°; Landing vector=181.2°). Most fish released from Start Box B swam to a point opposite this new release point, i.e., a virtual Goal (Outbound vector=93.7°). A minority landed at the actual Goal, as is evident in the rotation of the mean Landing vector towards the actual Goal (128.5°). It is interesting that only fish released from Start Box A exhibited meanders, and none released from Start Box B showed this behavior. This may suggest that fish responded quickly to the location change and thus altered their navigational strategy accordingly (i.e., a shift from dead reckoning to path integration). In support of this conjecture is the fact that *only* those fish released from Start Box A showed a counterclockwise shift in their Outbound vectors the direction of the first turn during acquisition (see below for a discussion of this behavior). It is therefore possible that these fish use variable strategies. For example, in one condition they may use dead reckoning, and in another they may use path integration or mapping; these strategies may also vary across individuals. Lopez et al. (2000) showed dissociation between place and cue attention in maze learning in goldfish. It is possible that mormyrid fish are able either to switch between strategies or that the strategy utilized may vary inter-individually: either of these possibilities could explain the results obtained in Experiment 2. For the three trials in which the Landing points were directed at the actual Goal, it is possible that the fish attended to visual landmarks. In general, however, these results suggest that sighted-discharging fish do not use a strategy of navigation akin to cognitive mapping, since they largely headed for the virtual Goal (opposite Start Box B) rather than the actual Goal.

The distinction between the mean Outbound vectors of sighted-discharging fish in light conditions with visual landmarks released from Start Box A and sighted-discharging fish trained with electric landmarks (no change) is interesting, and may be due to the fact that after training with the salient electric landmarks and the continued presence of these landmarks during testing, this group still used the landmarks as external orienting cues. In the absence of the maze barriers one would expect the performance time to decrease, which was borne out in all groups except for the sighted-nondischarging fish under dark conditions and the electric landmark group, the latter by far exceeding the former. This was further evidence that fish attended to these landmarks rather than heading directly for the goal.

The attention to electric landmarks became particularly evident in the meander pattern of this group and the strong left-hand turn visible in the Outbound vector (255.7°), which was the largest first deviation from the Goal direction. Fish trained with electric landmarks were not, by any means, the only group of fish to show this left-hand turn out of Start Box A: All fish showed this behavior. This appears to be due to the initial training path (maze geometry) and not an innate counter-clockwise preference (left-finnedness).

To explore this possibility, another control experiment was run, with non-naïve fish tested in the dark without the use of the lateral line. These fish were treated with cobalt chloride as described in Experiment 8. Fish were then trained in a reversed order maze, i.e., the first mandatory turn was now a right turn instead of a left turn, and so on. When tested without the maze barriers in place, the fish exhibited a strong *right* turn out of Start Box A (155°, r=0.761). When released from Start Box B, an Outbound turn was not apparent, similar to earlier groups; the mean Outbound vector from Start Box B was 128.2° and not significant.

What is important to notice from this experiment is that the first turn during training is remembered and executed during recall (testing) from Start Box A only, no matter what the condition was during training. Not only does this behavior underline the fact that the fish remembered its first acquired turn when released from the original Start Box (but because this behavior is absent when released from the new site), it also indicates that the fish responded to different site-specific cues at the new location.

In the presence of electric landmarks (Experiment 7) during acquisition, fish took one day longer to learn the task than fish in Experiment 2. Fish in Experiment 7 clearly attended to the electric landmarks during acquisition and often stopped to probe the objects, particularly in the early days of training, which may explain the slightly longer average acquisition time. In recall, fish were released *only* from the original Start Box on test day, in one of three conditions: A) with landmarks in their original locations, B) with Aluminum and Plexiglas landmarks in opposite locations, and C) with all landmarks removed from the tank. As discussed above, for fish in group A, the Outbound vector indicated a strong initial left turn out of the Start Box. This may reflect particular attention to added landmarks in this study. The Landing points were largely clustered at the actual Goal, as expected, although five Landing scores were strongly rotated counterclockwise. There were no differences in Outbound or Landing vector for fish in these three groups. This trend suggests that some fish may have hugged the wall after making the first turn and encountering a tank devoid of barriers. For fish in the switch condition, the Outbound vector was more variable, suggesting that the fish may have been startled by the alteration of the electric objects. The mean Landing vector, however, was aimed at the Goal (180.9°), as expected. These results are particularly interesting because they may suggest a change in strategy during navigation from Start Box to Goal. This group of fish may have been initially affected by the change in landmarks, but perceived that electric landmarks *no longer* provided *reliable* cues, and thus switched to internal reliable cues. The analysis of meanders supports this suggestion: some fish in the no change condition meandered, whereas no fish in the switch condition meandered. The lack of meanders in the switch condition suggests that these fish may indeed have switched from relying on unpredictable landmarks to relying on an internal representation. These results may support the earlier suggestion that fish are able to switch strategies when they encounter altered environmental conditions that pose a conflict between acquired internal representation and the geometry of familiar external landmarks. In the current experiments such situations occurred when the fish was displaced from Box A to B (place) or when the surrounding electric landmarks (cue) were displaced. Again, these results support a suggestion by Rodriguez et al. (1994) that there is a fundamental dissociation between place and cue (discussed above).

Finally, for sighted-discharging fish trained with electric landmarks that were removed prior to recall (removed), the Outbound and Landing vectors were both aimed at the Goal, a result which is almost identical to that from fish released from Start Box A in Experiment 2. It is worth noting that fish in the no change condition exhibited the most counterclockwise rotation in the Outbound vector (255.7°), fish in the switch condition exhibited an intermediate amount (232.6°), and fish in the removed condition the least (201.1°); the same is true for the Landing vectors (the mean Landing vector for fish the switch condition was directed most accurately towards the Goal). Interestingly, fish in the switch landmark and removed landmark conditions did not meander. This may suggest that the internal representation established during acquisition (path integration) surpassed electric landmark orientation, underlining the importance of electric cues during learning. Taken together, these data suggest that 1) electric landmarks are more salient than visual landmarks, 2) the *absence* of previously present electric landmarks is less disrupting than unreliable electric landmarks, but that 3) even in the presence of unreliable electric cues, fish can switch strategies and fall back on reliable internalized cues.

SIGHT

Sighted-discharging fish under dark conditions required 11 days to learn the task, 4 days longer than sighted-discharging fish under light conditions. This indicated that under the current experimental conditions vision might play an additive or even synergistic role in learning (see also Rojas and Moller, 2002). The results underline the necessity of allowing each group to asymptote at their own rates in order to observe the difference between conditions (difference between parts I and II). During recall (part II), fish released from Start Box A made strong left-hand turns, reflecting the turn made when the barriers were present. The length of the Landing vector indicated a larger variance in distributions, which was surprising. We would expect the Landing vector from Start Box A to be strongly directed at the Goal as in Experiments 2 and 7. The average Outbound vector for fish released from the Start Box B towards at a virtual Goal, indicating that the fish were headed straight across the tank from this new release point. The Landing points were also clustered around the virtual Goal, although there were three trials in which the fish landed at the location of the actual Goal. This pattern is what we found for the sighted-discharging fish in Experiment 2; again suggesting that mormyrids use a strategy other than cognitive mapping in contrast to goldfish that were shown to use such orientation strategies (Rodriguez et al., (1994). Mormyridae belong to a primitive branch of the Teleostei, the Osteoglossomorpha, whereas goldfish (Cypriniformes, *Carassius auratus*) belong to the Euteleostei. It is plausible because of their taxonomic status to consider cue listing (path integration, dead reckoning) in mormyrids a primitive trait and mapping in goldfish a derived trait.

These results also add strength to the conclusion that visual landmarks in Experiment 2 were not a salient cue during recall for *M.r. proboscirostris*, since fish tested in the dark perform similarly well as fish tested under light conditions. As in Experiment 2, only fish released from Start Box A showed a counterclockwise rotation (left turn) of the Outbound vector (those released from Start Box B headed straight out), indicating that Start Box B fish altered their behavior in response to perceived differences from their original home. Likewise, only fish released from Start Box A meandered, similar to Experiment 2.

In Experiment 5, a control for Experiment 4, the blind-discharging fish required the longest amount of time to learn the maze (12 days). The mean time for the last day of training was 42.6 ± 43.8 sec; this time was largely due to one outlier, but even when this fish was omitted from the data, the blind-discharging group still asymptoted at 12 days. This group of fish was only released from Start Box B during recall. Both Outbound and Landing distributions were uniformly distributed, thus showed no significant directionality. The lack of direction in both vectors may reflect a lack of learning during the acquisition phase. The unexpected performances of the blind-discharging fish may be due to the changes in discharge rates and/or the purported absence of circadian rhythmicity mentioned earlier. Prolonged blindness might have affected lower discharge rates in these fish as compared with sighted-discharging fish measured in the dark. This conjecture would need to be assessed further for a more concrete understanding of their discharge patterns. If blind fish indeed permanently exhibit lower discharge rates, it may result in decrease sensory acuity in these fish (that were blind for over two years when used in the present experiment). Surgically blinded fish display altered circadian rhythms in the laboratory (personal observation) and exhibit a high level of activity outside their shelters during the day as well as at night. The effect of circadian rhythm on navigation has not been previously studied, but may affect the animals ability to home. Cobert (in Moller, 1995, p. 356) showed that *G. petersii* are active during the night and inactive during the day on a 12:12 photoperiod. However, fish kept under constant darkness became arrhythmic after about 48 hours. Until and unless the effects of altered endogenous rhythmicity (e.g. altered general locomotor activity) and its effects on the fish s orientation behavior are clearly established, it is preferable to explore the lack of visual input on mapping and cue listing in these animals by manipulating their *umwelt* rather than through surgical intervention.

ACTIVE ELECTROSENSE

In Experiment 6, sighted-nondischarging fish trained and tested in the dark, fish took the same amount of time to learn the maze as sighted-discharging fish tested in the dark, which suggests, surprisingly, that the lack of the active electrosense does not significantly affect learning. During recall, the fish started from Start Box A (sighted-nondischarging fish in dark conditions released from Start Box A) swam to the left, which again corresponds to the results from experiments 2, 4, and 6; there was no such turn from Start Box B. The Landing vectors indicate that fish swam directly to the opposite side from the Start Box they were released (either to the Goal or virtual Goal). These results suggest not only that these fish use a strategy other than cognitive mapping, but that having neither the active electric sense nor vision available has little effect on navigation of learned spatial pattern. These animals must have developed an internal representation of the maze tank in training despite the absence of the two major senses. On test day they behaved the same way as sighted-discharging fish under light conditions. (No meander data were available for this group of fish.) The similarity in recall between this group of fish, that lack two major senses, and fish in Experiment 2, that have all senses available, is striking, and suggests that reliable spatial memories can be formed in relatively impoverished sensory conditions.

Effects of surgical elimination of the electric organ discharge (silencing)

Surgically silencing fish requires the ablation of the motor neurons that innervate the electric organ through an elaborate stalk system (Bass, 1986). Silencing does not eliminate the motor command, i.e., the signal to discharge is still sent but does not reach the electric organ. Thus, the electric organ discharge corollary response (an internal copy of the motor command; see Bell, 1986) will never be matched by reafference about the actual presence of objects in the fish s vicinity. Thus, to the fish its environment is empty of objects.

The alternative to surgical silencing would be to leave the fish intact and instead jam its active electrosensory system with (electric) white noise fed into the maze tank. Such jamming effectively attenuates a fish s electrolocation ability (Heiligenberg, 1976; von der Emde, 1994). Feeding electric white noise into the tank also mimics natural conditions for *M. r. proboscirostris* since these fish swim in large schools. Under such natural conditions, however, fish have evolved jamming avoidance behaviors that will keep their electrosensory channels free from jamming. Artificial jamming as applied by the above authors in a small aquarium was too strong and frequent and could not be avoided by the fish s evasive electric organ discharge generation. Because of the size of the current maze tank this alternative was technically not feasible in the current paradigm.

LATERAL LINE

The behavior of fish in Experiment 8, lacking vision, active electrosense and lateral line input, emphasizes the stability of the internal representation, constructed in the absence of three major senses in a relatively short amount of time. Although these animals would have needed approximately 18 days to asymptote during training, there was evidence during recall that they were able to learn the spatial surroundings. The Outbound vector of fish released from Start Box A was directed to the left, as it was for all other groups, suggesting that they remembered the first turn from training. The Landing vector was not directed, however. For fish released from Start Box B, the Outbound vector was uniformly distributed, but very surprisingly the Landing vector was directed towards the Goal (191.8°, r=0.784), with remarkable accuracy comparable to fish with additional senses available to them. This may have been a coincidence, or an amplified artifact from training i.e., after finding the first barrier absent, the fish may have continued in the leftward direction until reaching the wall, which happened to be near the Goal box. In any case, more research is needed to determine the role of the lateral line sense in mormyrid navigation, although these studies are clearly difficult to administer over the long run.

GENERAL REMARKS

The current experiments lend strong support to the hypothesis that *M. r. proboscirostris* do not use the form of navigation described by Tolman (1948), the cognitive map, or the related locale system of O Keefe and Nadel (1978). It is more likely that these animals use landmark or cue lists, i.e., path integration or dead reckoning, or a combination thereof. Because most fish, regardless of condition, swam from a novel release site to a virtual Goal on the opposite side of the tank, it must have acquired the Goal location relative to the original release site from which they had been trained. However, because fish did not generally meander from the novel release site, there is some evidence that they responded to the novel site as if they were aware of this move, probably from slight differences in the size or shape of the Start Boxes. Why they did not reorient themselves to the actual Goal is unclear. Perhaps extramaze landmarks were not sufficient to provide relevant cues during training under light conditions.

The present paradigm is somewhat similar to Gould s experiment (1986), on a smaller scale, although fish were not captured mid-route, but rather moved to the novel location before release. It would be interesting to duplicate Gould s paradigm more closely in this way: perhaps the act of removing the fish after release would trigger a greater change in the fish s behavior, causing it to respond differently (or more accurately) to the novel location. The fish s behavior is perhaps analogous to some of the rat navigation experiments detailed earlier: for example, Olthof et al. (1999) found that rats were unable to navigate to the goal when the maze was rotated. Likewise, the extramaze cues that rats were trained to use apparently had no bearing on the test situation when the location of the marquee opening was altered (Benhamou, 1996). However, in Benhamou s experiment rats searched randomly, whereas in ours, fish were generally directed at the location the Goal should be located relative to the trained location. This suggested that mormyrids responded to a change in location, though they were not able to accurately respond to the new release site by locating the actual Goal.

If the fish s behavior is more accurately explained by path integration or dead reckoning, Shettleworth and Sutton s suggestions (2005) may be more relevant, particularly with regard to the Electric Landmark experiment. They found that when beacons were present, the rats used them; when the beacon was moved, rats trained with the beacon still navigated towards it (whereas rats trained to find food without the beacon also found home when the beacon was moved). In our experiment, when electric landmarks were absent in recall (group C), fish were still able to find the Goal accurately. However, when the landmarks were switched around in recall (group B), fish were able to locate the Goal with greater accuracy than fish that encountered landmarks in the same position (A) or with no landmarks present at all (C). This is a major difference in the use of landmarks between Shettleworth and Sutton s (2005) findings and ours, which may have to do with the fact that the beacon generally signaled home in the former experiments. However, Shettleworth and Sutton s rats also learned to ignore unreliable (randomly moved) landmarks during training and to home accurately when the beacon was either (1) moved again during testing or (2) absent. Scenario (1) is similar to the ability of the fish in the switch group to switch from relying on landmarks to relying on an internal representation of the arena, and scenario (2) is again very much like the behavior of the fish in the landmarks removed group. The similarities both in the experimental setups and in the behaviors of rats and fish are interesting, and perhaps more studies on fish should be done to mirror closely paradigms used on other taxa.

The experiments of Rodriguez et al. (1994) have been mentioned earlier regarding the use of electric landmarks, and the possibility that *M. r. proboscirostris* are able to switch from relying on cue lists to an internal representation. To this end, Lopez et al. (2000) suggested that when extra- and intramaze cues are present, goldfish attend to either one when the other is absent; and when these cues are put in conflict with one another, goldfish attend to either cue (which varies inter-individually). Both studies are similar in that they set in opposition two types of cues to determine what cues were attended to in training: in the present study, only the fish was moved, and no physical cues were changed (with the exception of the absence of maze barriers). However, fish did not attend to *extramaze* cues when moved to a novel location which suggests that they may not have attended to them during training, possibly because they were not sufficiently visible, since the tank walls were covered with dark cloth (of course this point is moot for fish tested in the dark). In future studies, it would be instructive to leave the outside tank walls bare, and see if the fish attend to extramaze cues through the tank walls.

The behavior of the sighted-nondischarging fish tested under dark conditions is of particular interest because it is so similar to sighted-discharging fish tested under light conditions. During training, the former group of fish must have used the lateral line, perhaps in concert with the tactile sense. Although it took this group 11 days to learn the task, the memory formed under these impaired sensory conditions was adequate to generate recall behavior that was strikingly similar to sighted-discharging fish tested under light conditions. These results are comparable to data cited earlier in which the loss of one sense was compensated by remaining senses (Cain, 1995; Rojas and Moller, 2002; Cain and Malwal, 2002). The performance in recall of the sighted-nondischarging fish tested in the dark without the use of the lateral line showed that although learning took considerably longer, some representation of the arena was learned during training. An intriguing behavior, not prevalent in the other groups, was observed. Fish now lacking visual, electrosensory (active), and mechanoreceptive input through their lateral line swam closer to the maze barriers and the walls of the tank than fish in the other groups. They exhibited probing behavior using their short mental appendage, which was clearly directed towards the length of the barriers and the turnabouts. Future studies must include this probing behavior in a systematic fashion to assess still another sensory channel, i.e. direct mechanical input through cutaneous mechanoreceptors.

THE PATH OF LEAST RESISTANCE

The results from the control group (Experiment 9) with fish that had no prior maze experience were surprising and raised several questions. Naïve fish (with no senses impaired) were released from both start boxes to observe how untrained fish might behave in an empty arena. It was hypothesized that control groups would swim randomly about and show uniform distributions. However, both Outbound vectors were directed approximately across the tank from the release sites (208° from Start Box A; 82.8° from Start Box B). The Landing vector from Start Box A was uniformly distributed, as expected, but the Landing vector from Start Box B was directed across the tank (93.4°). Under the present condition, intact naïve fish showed directed behavior comparable to that observed in trained fish. Thus, an important question is how these data affect the interpretation of the data obtained from the experimental groups. Obviously, these control trajectories do not reflect path integration or dead reckoning. We will argue that while the similarity between paths taken by control and experimental fish was perplexing, the underlying mechanisms are quite different. First, fish observed under laboratory and field conditions behave differently during night and day (Moller, Serrier, Belbenoit, and Push, 1979; Moller, Serrier, and Bowling, 1989; Rojas and Moller, 2002; Moller, unpublished laboratory observations). During daytime fish seek shelter a behavior that is often characterized by swimming in straight paths, while at night they explore without seeking out a particular destination. Our prediction was that fish released in the empty arena in the dark would explore the environment and not aim towards the opposite side of the tank. To test this hypothesis, nine fish (both naïve and non-naive) were released from Start Box A in the dark (0.0-0.02 lux; conductivity was approximately 566 μS; temperature, 23.3°C; and pH, 7.02). The fish s Outbound and Landing vectors were recorded as before. The length of the outbound vector indicated uniform distribution (θ=221.3°, r=0.268), and the mean Landing vector, directed at 28.44° (r=0.610), pointed in the opposite direction from what was expected and found in trained fish. These data support the suggestion that the trajectories of experimental fish reflect path integration or dead reckoning, while those of the control fish do not reflect any navigational strategy. A second argument in support of different underlying mechanisms lies with the observation that the initial turn during recall was clearly affected by the geometry of the maze (i.e. the arrangement of the Plexiglas barriers) and thus controlled by previous maze learning experience. The control fish tested in the dark showed no direction in their initial turn, which supports this argument.

UNANSWERED QUESTIONS

Several questions remained unanswered in this study. Thus, this short summary is intended to highlight these questions, which in turn may inspire future research. (1) The behavior of fish released from the different start boxes was somewhat surprising: fish released from Start Box A made an initial left turn (the Outbound Vector) *and* meandered, whereas those fish released from Start Box B swam straight, and few or no meanders were recorded. We attributed this difference to as of yet unidentified start box-specific cues or, also, to extramaze cues. (2) The behavior of blind-discharging fish was equally unexpected, since in Experiment 1 they behaved more like sighted-discharging fish (regarding acquisition time) than sighted-nondischarging fish. In Experiment 5, the Outbound and Landing vectors of the blind-discharging fish were uniformly distributed. It was suggested earlier that this lack of direction might indicate a lack of learning during acquisition, since on the last day of training this group s mean time was still significantly longer than other groups. Whether the circadian rhythmicity of the fish was indeed disrupted and thus affected navigation is a possibility, though no data have suggested that arrhythmia might have deleterious effects on spatial learning and memory. (3) The behavior of the control fish was unexpected and has been discussed in some detail above. (4) As is often an issue in research, it is possible that the behavior of animals observed in captivity is not representative of their behavior in nature. The laboratory *M. r. proboscirostris* have never swum out from their shelters for more than 3 m. It is possible that because our animals have never had to depend on a navigational strategy to return home from a long distance they are out of form and do not perform as they would if they constantly navigated a real environment. It would be interesting to compare the navigational strategies of laboratory fish and those raised in the wild. There may be some substantial differences in behaviors, particularly in what cues are attended to, and where the fish actually navigated (virtual Goal or actual Goal).

CONCLUSIONS

What is clear from this series of experiments is that *M. r. proboscirostris* are excellent navigators, with or without all of their natural senses available to them. They do not appear to use the cognitive map strategy of navigation (Tolman, 1948; O Keefe & Nadel, 1978), although increasing amounts of research suggest that few animals do (Olthof et al., 1999; Kamil and Cheng, 2001). The suggestions of researchers that shortcutting via cognitive mapping can be explained accurately by dead reckoning and landmark memory (Benhamou, 1996) is appealing, and provides a perspective by which to compare one s findings. Arguably, the most compelling research for the cognitive map theory mentioned here is that of Rodriguez et al. (1994), which interestingly uses goldfish: the findings of this paper, however, could also be explained by landmark learning, and would require no mention of the cognitive map. The cognitive map theory is certainly an attractive means for humans to explain navigation, but perhaps it is not the right term to conceptualize the representation of geometric relationships in the animal s brain. Perhaps humans are so attached to the concept of the map because it is an apparently parsimonious explanation for a highly complicated behavior (realistically and neurologically, however, it is perhaps the most complex model for navigation). Future studies employing innovative and clever methods are needed to determine whether the cognitive map concept of navigation bears any explanatory power, or is simply an outdated means of thinking about this fundamental and complicated behavior.

APPENDIX A: ANOVA, Exp. 1

ANOVA for the effect of experimental condition (sighted-discharging, blind-discharging, and sighted-nondischarging) and days of practice on time to complete the maze.

1 = Experimental Condition, 2 = Day (1-19)

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	11318.46	225	384.2091	29.45	.000000
2	14	6938.44	225	384.2091	18.05	.000000
12	28	2064.91	225	384.2091	5.37	.000000

Effect of Trials (1-3) and Experimental Condition (sighted-discharging, blind-discharging, and sighted-nondischarging) on number of correct turns after the maze barriers were removed on d 19.

1 = Trial (1-3), 2 = Experimental Condition

	df Effect	MS Effect	df Error	MS Error	F	p-level
1	2	7.625000	45	2.274074	3.353013	.04
2	2	0.263889	45	2.274074	0.116042	.89
12	4	2.305556	45	2.274074	1.013844	.41

APPENDIX B: Circular Statistics

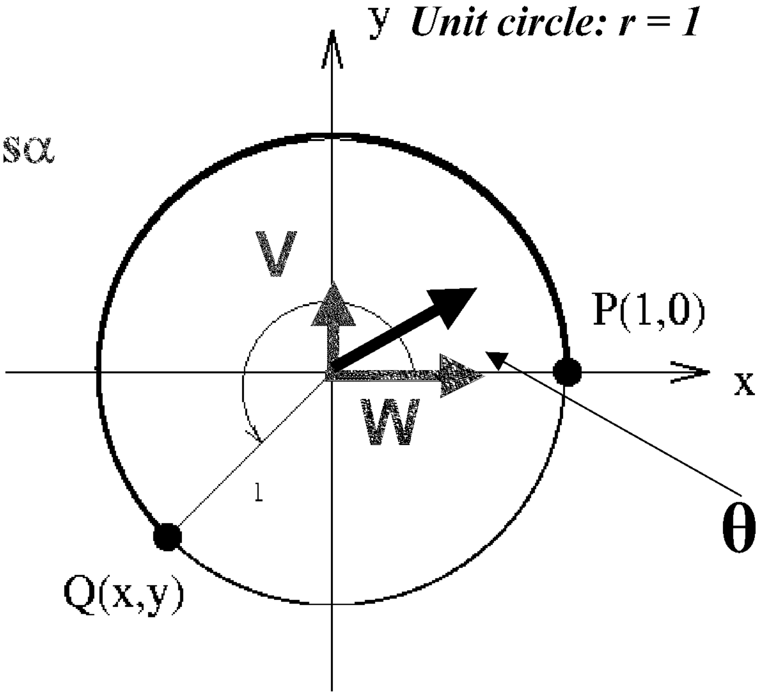
$$\begin{array}{ll}
\sin\alpha_1 & \cos\alpha_1 \\
\sin\alpha_2 & \cos\alpha_2 \\
\sin\alpha_3 & \cos\alpha_3 \\
\underline{\sin\alpha_k} & \underline{\cos\alpha_k} \\
n = k & n = k \\
V = \Sigma\sin\alpha & W = \Sigma\cos\alpha \\
n = 1 & n = 1
\end{array}$$

$$\tan\theta = V/W$$

$$r^2 = V^2 + W^2$$

$$r = \mathrm{sqrt}\,(V^2 + W^2)$$

$$r = 0 \text{ to } 1$$



To find the mean vector in a circular distribution, the sine and cosine values are taken for each data point. The average sine value is equivalent to the y-component of the vector, and average cosine value is equivalent to the x-component. The tangent of the mean direction angle is the average sine value divided by the average cosine value (θ can be found from the inverse tangent function). The r-value representing the length or magnitude of the vector is derived by using the Pythagorean s theorem (see above). r is an indicator of the distribution s density.

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