

City University of New York (CUNY)

CUNY Academic Works

School of Arts & Sciences Theses

Hunter College

Spring 5-5-2018

Darkness as a Positive Reinforcer in Weakly Electric Fish

Amber Lea D. Kincaid

Hunter College

[How does access to this work benefit you? Let us know!](#)

More information about this work at: https://academicworks.cuny.edu/hc_sas_etds/338

Discover additional works at: <https://academicworks.cuny.edu>

This work is made publicly available by the City University of New York (CUNY).

Contact: AcademicWorks@cuny.edu

Darkness as a Positive Reinforcer in Weakly Electric Fish

By

Amber Lea D. Kincaid

Submitted in partial fulfillment
of the requirements for the degree of
Master of Arts in Animal Behavior and Conservation
Hunter College of the City of New York

2018

Thesis Sponsor:

May 5, 2018
Date

Peter Moller, PhD
First Reader

May 5, 2018
Date

James Gordon, PhD
Second Reader

Acknowledgments

This research was funded by the Hunter College Animal Behavior and Conservation Committee Award and the Hunter College School of the Arts and Sciences Master's Thesis Support Grant.

The author wishes to express unending gratitude to Dr. Peter Moller for a monumental amount of guidance, education, and humor, and to Dr. James Gordon for his contributions and collaboration. Thanks also to Paul Swedis, Elizabeth Jaeger, Leslie Zhen, Tara Anne McCloskey, and Dr. Sheila Chase for their assistance, input and support.

Abstract

Operant conditioning in non-human animals commonly relies on food as a positive reinforcer. This presents a problem in subjects who are not food-motivated or who have dietary or caloric concerns. Darkness was used as a positive reinforcer in 14 adult and subadult elephantnose fish (*Gnathonemus petersii*) in a two-alternative forced-choice object discrimination task. Of the 14 subjects, one performed above chance levels and showed a significant learning response to stimuli. This study provides preliminary support for the use of darkness as positive reinforcement in weakly electric fish and presents potential areas of interest for future study.

Introduction

The elephantnose fish (*Gnathonemus petersii*), a mormyrid native to freshwater streams and rivers in sub-Saharan central and western Africa, integrates vision, olfaction, the lateral line, and electrosense to perceive its environment. *G. petersii* are nocturnal and occupy dark, silty waters; they are therefore very well adapted to life in a dark environment. They have a highly developed and complex retina equipped with an integrated tapetum lucidum that allows them to see quite well under dim light conditions (Landsberger et al., 2008). While the integration of multiple sensory systems may synergistically improve these fishes' ability to gather such information as substrate, schooling activity, predator location, obstacles, and prey availability (Moller, 2002), it is believed that they primarily rely on their electrosense (Push et al., 2013). By emitting weak electric pulses from an electric organ located in the caudal peduncle and perceiving disturbances in its self-generated electric field with receptors in its skin, *G. petersii* creates an electric image of objects in its environment (von der Emde et al., 2008) and is able to determine various characteristics of those objects up to approximately two body lengths away (Moller, 1995, p. 112). When *G. petersii* encounters a novel object, it will often produce a "novelty response," in which it increases the rate of electric organ discharge (EOD) (Post & von der Emde, 1999), or it may regularize the EOD rate (Moller, 1995, p. 115). This allows these fish to gather more information about the object and has been used as an indicator of learning in behavioral studies (Moller, 1995, p. 123). Along with an increased rate of EOD, *G. petersii* may also exhibit "probing motor acts," such as swimming back and forth near the object it is investigating, or probing the object with its elongated chin (Moller, 1995, p. 122). Chin probing in particular is likely to provide the fish with a wealth of information, as there is a concentration of electroreceptors on the chin that acts as an electric fovea, similar to the fovea in the eye (von

der Emde, 2006).

The electrosense in *G. petersii* is highly sensitive and capable of conveying a wealth of information to the fish's brain, even when other senses are not available. The electrosense may be more useful alone than any other single sense: *G. petersii* that were blinded performed better at finding an aluminum shelter using electrolocation than did fish that were electrically silenced and relying on vision, even though the shelter was opaque and therefore easily visible (Moller, 2002). Aside from discriminating between objects' electrical resistance, *G. petersii* can use active electrolocation to perceive shape, size, electric nature, and distance of nearby objects, even if the objects' positions in space change (von der Emde et al., 2010). Schwarz and von der Emde (2001) showed that *G. petersii* were able to determine the relative distance between two objects, regardless of the individual objects' properties. Furthermore, von der Emde and Fetz (2007) found that these fish can distinguish several relative differences in objects' properties, such as size, shape, volume, and contour. Perhaps most impressive is that, even though sensing and perceiving many parameters of one or more objects at once is a complex task, these fish process this information quickly, as evidenced by their ability to move through their environment in total darkness just as they would in conditions with enough light for them to see (von der Emde, 2006). Detecting prey, predators, obstacles, and shelter are necessary for any animal to live and locomote through its environment. The ability of *G. petersii* to determine a wealth of object characteristics using their electrosense is adaptive to their naturally dark habitats.

Nearly all of the studies on object discrimination in *G. petersii* have used operant conditioning to train the fish in their respective tasks. This is common practice in animal behavior studies, most often using food as a positive reinforcement for correct behaviors during training, following procedures initially developed by Thorndike and Skinner (Castro &

Wasserman, 2010). While food has proven an effective positive reinforcement, it can limit training to animals that are hungry, often necessitating underfeeding prior to training. It also can lead to overweight animals if they continue to work for food rewards after they are satiated. Additionally, food rewards may limit the time of day and for how long training can be conducted. For example, fish do not tend to have the same physiological response to food deprivation as most mammals and birds, which can result in very few trials per day before the fish lose interest, therefore drawing experiments out over long periods of time (Agrillo, Petrazzini, Piffer, Dadda, & Bisazza, 2012). This is not to say food has not been successful in training electric fish; in the first study showing that mormyrids can discriminate between objects using electrosense exclusively, Lissmann and Machin (1958) conditioned *Gymnarchus niloticus* to choose a porous porcelain tube filled with water by positively reinforcing that choice with food and punishing them for choosing an identical tube filled with wax by chasing them away with a wire fork. More recently, food has been used as a reward for several experiments on object discrimination in *G. petersii* (von der Emde, Schwarz, Gomez, Budelli, & Grant, 1998; Schwarz & von der Emde, 2001; von der Emde & Fetz, 2007; Schuster & Amtsfeld, 2001; von der Emde, 2004; von der Emde et al., 2010). However, von der Emde has also successfully used recorded electric organ discharges (EODs) of conspecifics as positive reinforcement to condition *G. petersii* to determine the shapes of different objects using just their electrosense. Agrillo et al. (2012) discussed several recent studies in which fish of various species were operantly conditioned with access to conspecifics. Sovrano, Bisazza, and Vallortigara (2002) trained redbtail splitfin (*Xenotoca eiseni*) to locate and open a door to rejoin a social group. In a similar experiment, Agrillo, Dadda, Serena, and Bisazza (2009) demonstrated the ability to learn numerosity in mosquitofish (*Gambusia holbrooki*) with social reinstatement as a reward for

passing through the correct door. The subjects in both of these studies could see the other fish during the experiment, so this design could constitute baiting rather than true reinforcement, but the fish worked for access to conspecifics nonetheless. In an associative learning task, Al-Imari and Gerlai (2008) discovered that zebrafish (*Danio rerio*) spent more time near a red card that had previously been presented next to a conspecific than one that had not, and they make the argument that social interaction may be a preferable reinforcer over food for zebrafish and other fish species that are physiologically less food-motivated than mammals.

Though perhaps less salient and less studied than food rewards, other forms of positive reinforcement have been successful in conditioning various avian and mammalian species. Food is successful as a reinforcer in part because an individual's homeostasis depends on it, but sensory reinforcement, not having to do directly with homeostasis, has been a successful reinforcer in many species (Baldwin & Start, 1981; Hogan & Roper, 1978). For example, Roper (1975) concluded that nesting material is equally effective as food in teaching mice to press a key. Access to litter proved to be an effective reward for hens when they were asked to break a photobeam (Dawkins & Beardsley, 1986). After initial training with food as a reward, pigeons continued to peck at key lights to gain access to views of their mates (Gilbertson, 1975). Blue foxes were trained to press a lever with earth flooring as a positive reinforcer (Koistinen, Ahola, & Mononen, 2007). Squirrel monkeys were trained to press a lever after being kept in total darkness by using light as a positive reinforcer (Parker, 1996). Many studies using non-food reinforcement have used operant conditioning specifically to test whether animals will work for these other rewards (Baldwin & Start 1981; review: Cooper & Mason, 2001; Perret et al., 2015), though these studies often use food as the primary form of positive reinforcement when first training the animals to perform the desired behavior (Lee, Floyd, Erb, & Houpt, 2011;

Gilbertson, 1975). Welfare and practicality can both be positively affected by finding successful positive reinforcement other than food.

Surprisingly, darkness has never been used as positive reinforcement for a nocturnal animal. Weakly electric fish are nocturnal and are highly motivated to seek out darkness, especially as a protection against predators (Kareklas, Elwood, & Holland, 2017). Walton and Moller (2010) capitalized on this by using an opaque box as the goal for mormyrids learning a maze. In an experiment to determine effectiveness of different senses in mormyrids, subjects maintained closer contact with opaque shelters (Rojas & Moller, 2002). Cain, Gerin, and Moller (1994) successfully used darkness to lure fish out of an illuminated compartment through an aperture to test their ability to detect such apertures. The fish's visual responsiveness to selected spectral wavelength was determined by Ciali, Gordon, & Moller (1997). We therefore hypothesize that darkness will serve as an effective positive reinforcer for weakly electric fish in a two-alternative, forced-choice object discrimination task.

Method

Subjects

Fourteen subadult elephantnose fish (*Gnathonemus petersii*) were tested. The fish ranged in length from 110 mm to 160 mm; sex was unable to be determined. Fish were group-housed until one day prior to testing at which time they were moved to individual tanks. Fish were on a 12:12h light:dark cycle with lights on at 800h. All procedures were approved by the Institutional Animal Care and Use Committee of Hunter College.

Materials and Procedure

Each fish was trained for four days and tested on the fifth day in a two-alternative forced-choice experimental tank. The experimental tank consisted of a 20cm x 30cm holding area, and

a 10cm-wide pulley door that led to a 28 cm x 30 cm chamber divided into two equal halves (see figure 1). Distance from the nearest edge of the door to the dividing wall was approximately 7 cm. On each side of the dividing wall was a porous porcelain cylinder, 4cm in diameter and 20 cm in height. The cylinders were equidistant from the dividing wall and the tank wall, and the center of each cylinder was 10 cm from the near edge of the holding area. A stainless steel electrode was partially submerged at each end of the tank and connected to a Dell Inspiron laptop computer to monitor the fish's electric organ discharge activity during each trial, which was recorded on the computer. Three utility clamp work lights were placed over the tank, each with a different strength light bulb. One lamp was designated for the neutral light condition in the holding area and its illuminance ranged from 100 to 400 lx. One lamp served as the positive reinforcement, with illuminance kept below 5 lx. The third lamp served as the punishment with illuminance ranging from 500 to 2900 lx.

The water pH was kept between 6 and 8, the temperature was kept between 20 and 25 degrees Celsius, and the conductivity was kept between 150 and 750 $\mu\text{S}/\text{cm}$.

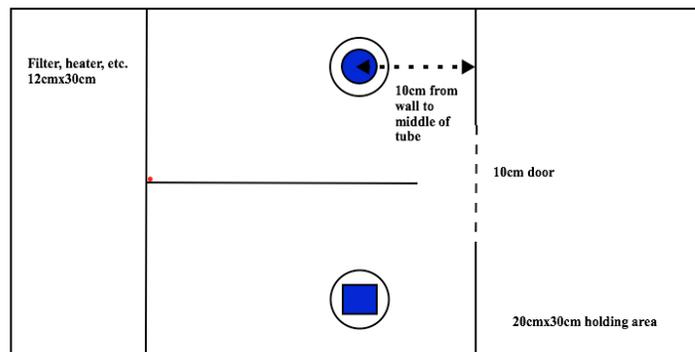


Figure 1. Experimental tank diagram. The blue shapes represent the stimulus objects, surrounded by the porcelain cylinders. Two red dots represent the electrodes.

Fish were pseudo-randomly assigned a positive and negative stimulus. The stimuli were a 1cm² aluminum cube, a 1cm² acrylic cube, and a 1cm-diameter aluminum sphere. One object was placed inside each porcelain cylinder, blocking the stimuli from sight but not from

electrosense (Lissmann, 1963). The objects were lowered into and raised out of the tubes with gauze, which is electrically transparent. The positive stimulus was randomly placed in either the left or right cylinder on day one, with the negative stimulus placed in the other cylinder. The objects were switched each day, so that they were in the same cylinders on days one and three, and in the opposite cylinders on days two and four, thus avoiding a site preference during the training phase. On day five, the objects were switched between cylinders so that the positive stimulus was on the right for seven or eight trials, and on the left for seven or eight trials (out of 15 total trials). The location of the objects was decided by using a random number generator found at www.randomizer.org. The objects were switched between each trial on days three and four for the first four fish, but their poor performance on training and testing days indicated this may be a hindrance to learning; this problem was addressed by using the above-stated method for the remaining 10 fish.

On each of the five days, the fish was gently netted from its individual tank and placed into the holding area of the experimental tank, where it was left for three minutes to acclimate. After three minutes, the pulley door was opened, and the fish was allowed to swim through the door. Once the fish had swum through the door, the divider wall forced it to swim either left or right, and the light condition was changed based on whether the fish swam toward the positive stimulus or negative stimulus. If the fish swam toward the positive stimulus, the neutral light was turned off and the positive reinforcement light was turned on, putting the fish in almost complete darkness. If the fish swam toward the negative stimulus, the neutral light was turned off and the punishment light was turned on, brightly illuminating the tank. If the fish failed to swim through the opening on its own, it was gently nudged with a net toward the door, at which point it was allowed to choose to which side of the divider wall it would swim. The first six fish

were allowed to swim freely through the side of the tank with the objects for one minute once they had made a choice and the resultant light had been turned on. The fish was then gently netted and placed back into the holding area of the tank, and the light was returned to neutral. The final eight fish were prevented from swimming to the other side of the divider wall by blocking the path with a net. Once they had made their choice and been rewarded or punished, the door was opened for them to swim through to the holding area. If they did not swim through on their own, they were gently netted and returned to the holding area. There was one minute between each trial. The threshold for successful training was set at 70% of trials on day five.

All trials were video recorded using a Sony Handycam HDR-CX900 for behavioral analysis, which was conducted using Griffin Video Coding Program developed by Ragir and Singh (2014). The fish's electric output was recorded using the two stainless steel electrodes, which were connected to a Dell Inspiron laptop running g-PRIME software (Physiology Recording & Identification of Multiple Events, G.K. Lott Cornell University, 2007).

Results

Fish 1 was excluded from analysis because it failed to swim through the door of the experimental tank on over 75% of trials. Fish 2 was excluded due to a software error that rendered its EOD data unviewable. Of the remaining 12 fish, only fish 7 performed above the 70% correct choice threshold indicative of successful object discrimination training (11 out of 15 trials) (see figure 2). Trials were analyzed by day for indications of learning based on temporal improvement in performance. While there were overall trends on each day, there were no significant improvements in performance from the first to the 15th trial on each day (see figure 3). A Pearson's correlation coefficient was calculated for overall changes in collective performance for each day. Improvement on Day 1 approached significance, $r(13) = .433, p =$

.053. The remaining four days did not show statistically significant improvement or decline in performance: Day 2: $r(13) = .401, p = .069$; Day 3: $r(13) = .053, p = .426$; Day 4: $r(13) = .316, p = .126$; Day 5: $r(13) = .031, p = .456$.

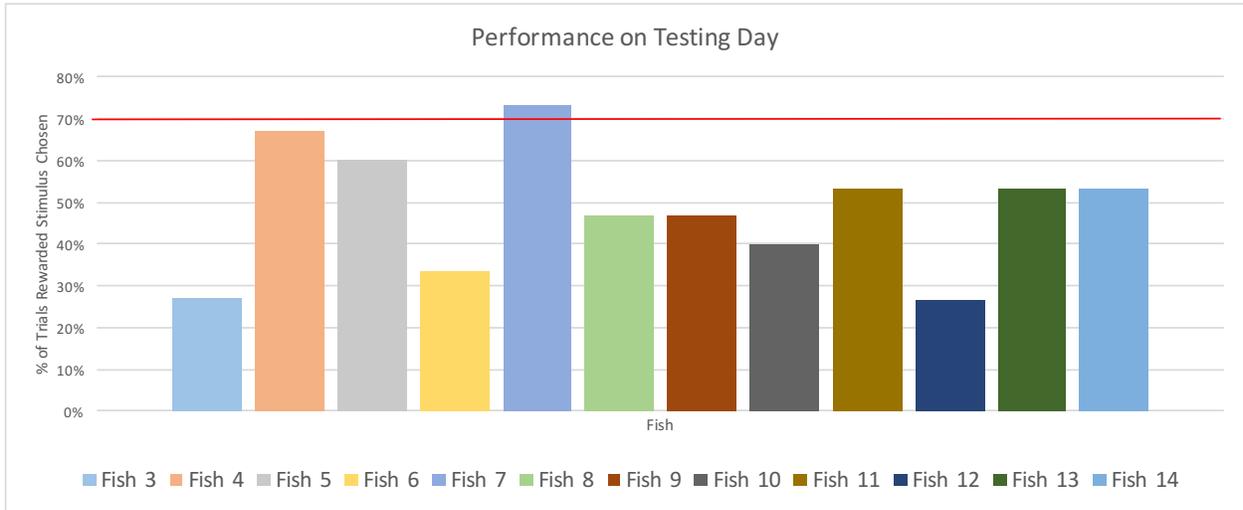


Figure 2. Percentage of trials each fish swam to the rewarded stimulus on Day 5

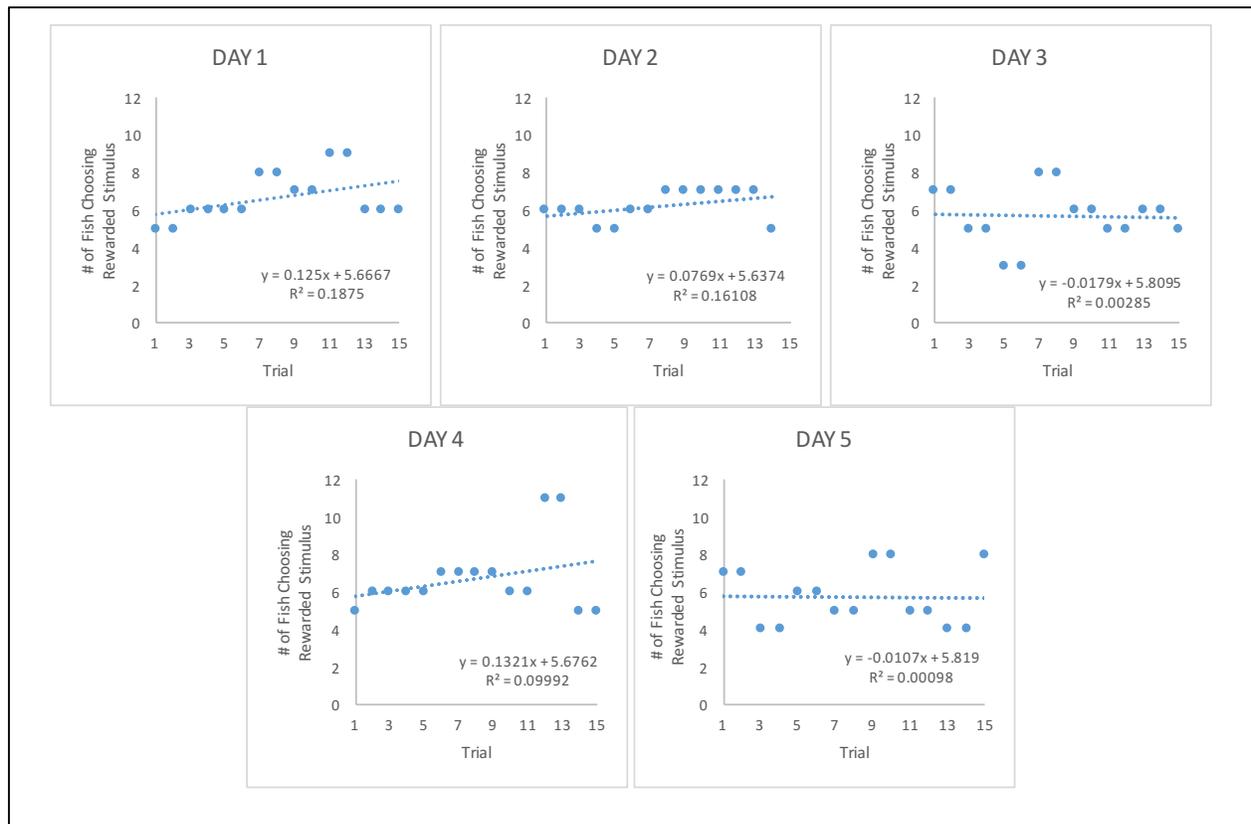


Figure 3. Total number of subjects who chose the rewarded stimulus per trial. Day 1 showed improvement that approached significance, but overall changes on a given day were not above chance levels.

Microsoft Excel was used to analyze EOD data by converting EOD recordings into .csv files such that each discharge received a timestamp. Subsequently, scatterplots could be graphed to show the inter-discharge intervals (IDIs) of each fish on each trial to visually analyze EODs for evidence of a novelty response (see figure 4). From this data, the EOD activity during first five seconds after the fish passed through the door in each of the first 10 trials on each day were consolidated into average IDI per second. This measure was chosen because the fish are most likely to gather pertinent information in the decision-making process immediately as they pass through the door, and the first 10 out of 15 trials were deemed sufficiently representative of overall performance. These averages were compared to control averages from five seconds of recordings while the fish were in the holding area of the experimental tank prior to each trial (on some trials, fish were only recorded for four seconds prior to swimming through the door, in which case the EODs recorded in those four seconds were used as the control).

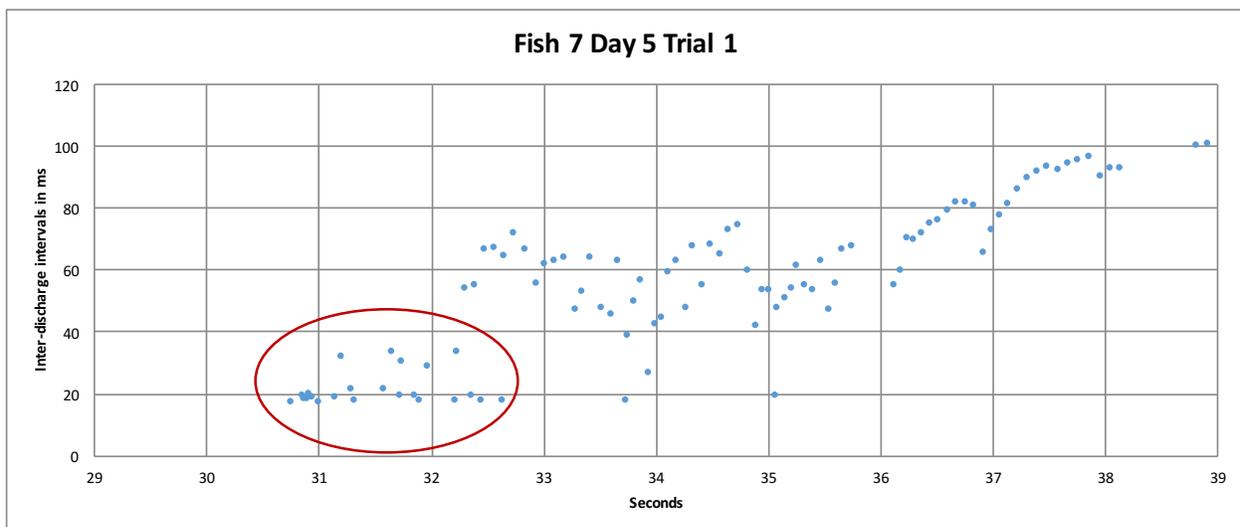


Figure 4. EOD output by fish 7 during the first 10 seconds after passing through the door of the experimental tank. The time course of IDI duration illustrates the startle response shortly after entering the door (between seconds 30 and 33).

To control for inter-individual differences in baseline EOD activity, response index (RI) was created by dividing the experimental averages by the control averages. An RI of 1 indicates

the same amount of EOD activity, i.e. the comparable average IDI, during the experimental condition as during the control condition. An RI below 1 indicates a smaller IDI during the experimental condition, which indicates the fish is shortening its IDIs and gathering information about its surroundings. An RI over 1 can also be indicative of a response to the stimulus, such as “freezing,” in which the fish is startled by the stimulus and emits fewer EODs (Moller, 1995, p. 122). The RIs for each fish were analyzed for change over time using the Pearson’s correlation coefficient. Fish 10 was the only fish with a significant correlation between trial number and RI, and the correlation was significant for rewarded trials, $r(21) = 0.556, p < .01$, and for punished trials, $r(18) = 0.557, p < 0.01$. Fish 10’s RI increased over time for rewarded trials and decreased for punished trials (see figures 6 & 7). Fish 10 was the only fish whose performance never improved between days, performing at 73% correct on Day 1, 53% correct on Day 2, 47% correct on Days 3 and 4, and 40% correct on Day 5. All the other fish had at least one day on which they performed better than the previous day.

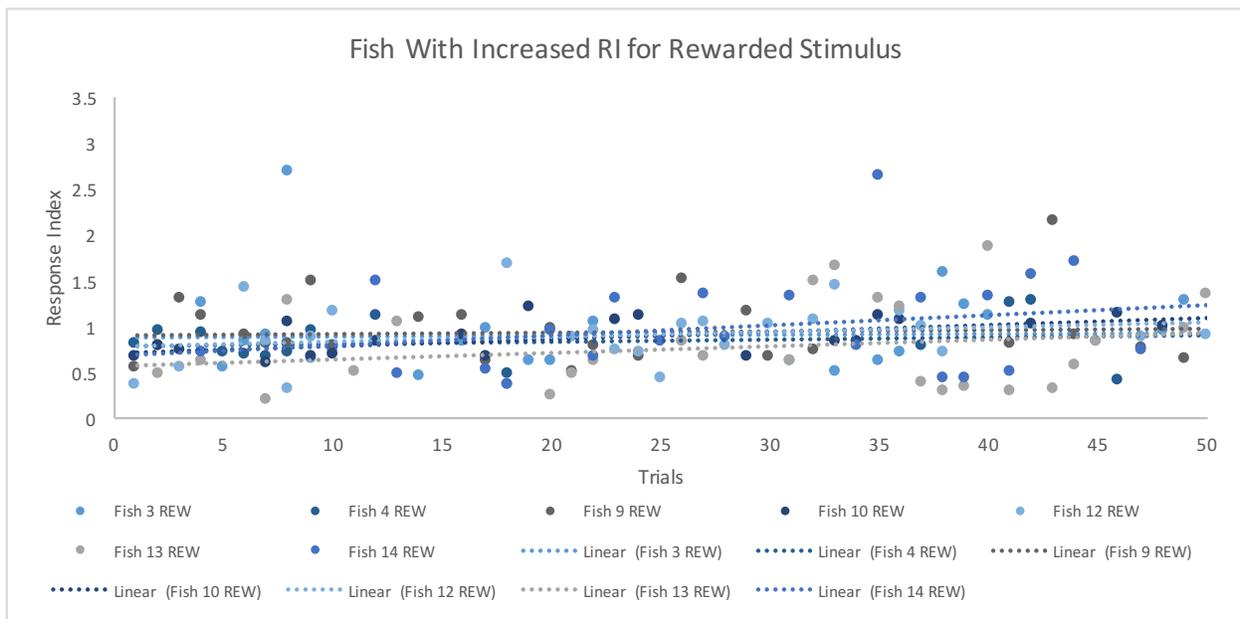


Figure 6. Fish 3, 4, 9, 10, 12, 13, and 14 had response indices that increased over time when they chose the rewarded stimulus. Trials 1-10 occur

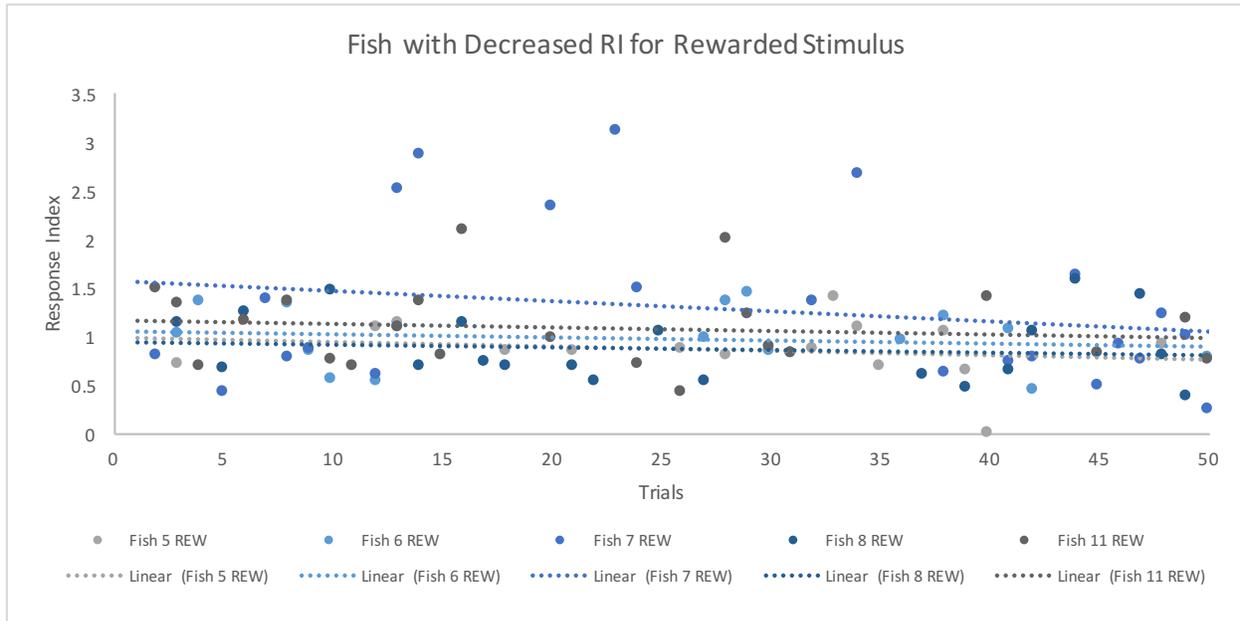


Figure 7. Fish 5, 6, 7, 8, and 11 had response indices that decreased over time when they chose the rewarded stimulus.

RIs were also analyzed for significance by finding the mean RI and standard deviation for each fish; an RI was significant for a response to stimuli if it was at least one standard deviation above or below 1. We have introduced this index as a way to quantitatively evaluate the changes in the fishes' IDI duration. Significant RIs varied greatly by fish and by day (see figure 8), and they were compared to choice by trial, but there was no apparent correlation. It is worth mentioning that fish 7, who was the only one to pass the 70% threshold on testing day, had a high mean RI and high variability, especially for rewarded trials (see figure 9). The mean RI over all rewarded trials for fish 7 was the second-highest of all the subjects and the standard deviation was the highest, $m = 1.281$, $SD = 0.82$; the mean RI over all punished trials for fish 7 was almost identical, $m = 1.285$, $SD = 0.58$. Only fish 13 had a higher variability for punished trials, $m = 0.79$, $SD = 0.69$. Fish 12 had the highest mean RI for rewarded trials, $m = 1.83$, $SD = 0.32$ and punished trials, $m = 1.81$, $SD = 0.32$. Interestingly, fish 12 chose the rewarded object on less than 30% of the trials on testing day (4 out of 15 trials). If we are to consider 70% as the threshold above which the results are not due to chance, then 30% must be considered as the

threshold below which the results are not due to chance. Fish 3 was the only other fish to perform below 30% (4 out of 15 trials), but the mean RI over all rewarded trials was close to 1, $m = 0.913$, $SD = 0.47$, the mean RI for punished trials was similar, $m = 0.907$, $SD = 0.37$.

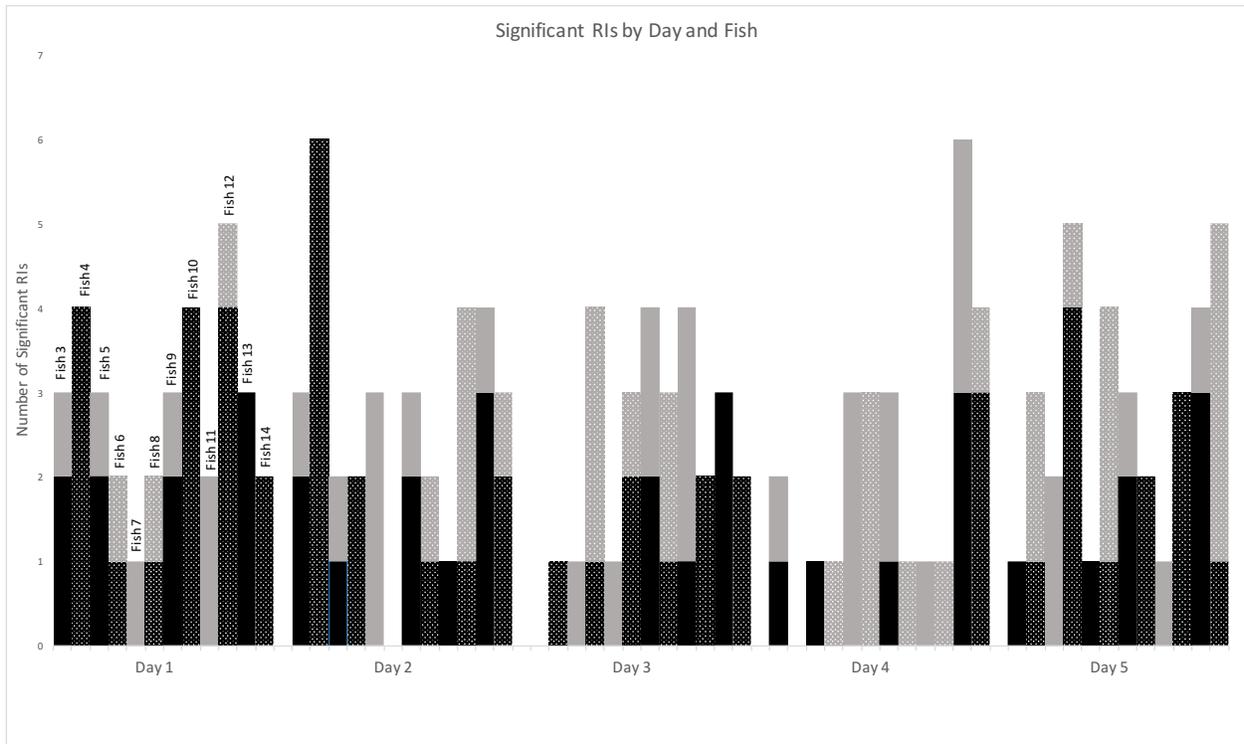


Figure 8. Total number of significant RIs for each fish on each day, separated into RIs lower than 1 (darker columns) and RIs higher than 1 (lighter columns). Each day follows the order of fish on Day 1. Gaps within a given day indicate a fish did not have any significant RIs. Only 26% of trials overall elicited a significant response, and there was no correlation between significant RIs and performance.

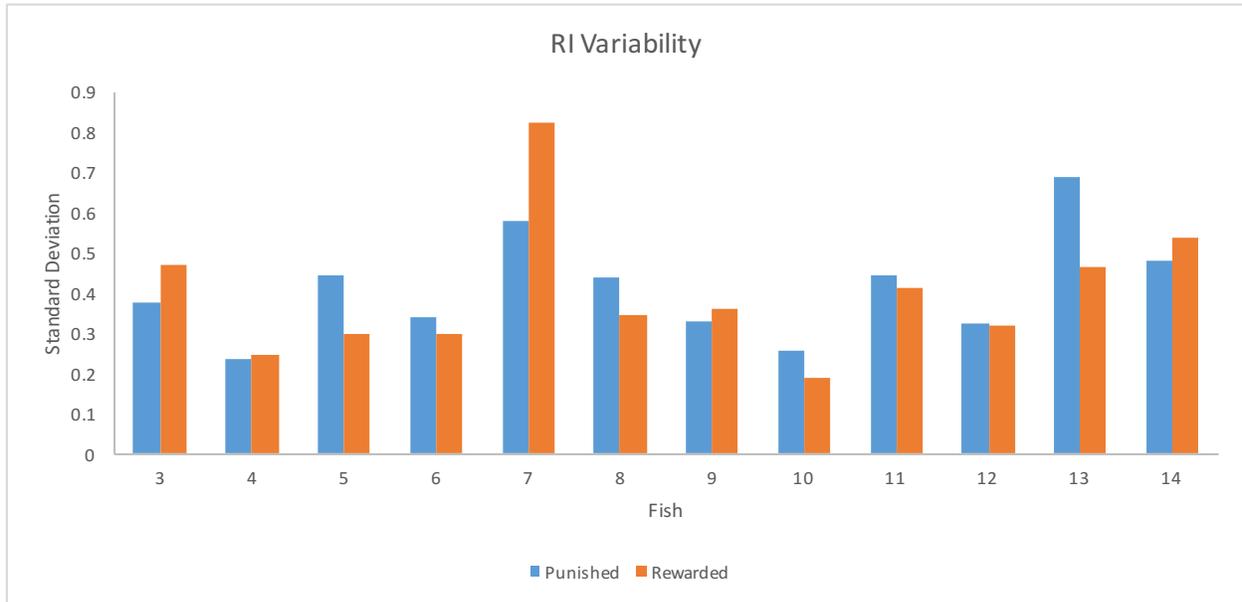


Figure 9. Variability in total RIs, as measured by standard deviation from individual mean RI by fish. Variability did not have any correlation with performance.

Discussion

The aim of this experiment was to determine whether *Gnathonemus petersii* could be operantly conditioned using darkness as positive reinforcement. With the possible exception of fish 7, the fish did not learn to discriminate between objects using darkness as a positive reinforcement and bright light as a positive punishment. Fish 7's results are somewhat inconclusive; while it did perform above the 70% accuracy threshold on testing day, the RI suggests that it may not have been learning. Additionally, the RI data for fish 10 show significant changes over time, but fish 10 did not perform better than chance levels, nor did any of the fish with significant RIs (aside from fish 7). The RI data indicate fish were attending to stimuli and changes in their environment other than the object discrimination training. It is important to note that change in IDI does not only occur in response to electric stimuli; rather, any sensory stimuli that a fish perceives may cause a change in EOD activity (Post & von der Emde, 1999). A number of stimuli could have caused the fish to shorten or lengthen their IDIs, such as perceived changes in the conductivity of near-by objects or the visual aspect of the

porcelain cylinders. The majority of trials were not met with a significant response, which may mean the fish were not attending to any stimulus in these trials. There appear to be inter- and intra-individual differences in response to stimuli, as evidenced by the inconsistent nature of the RI data, which supports the use of the response index to evaluate EOD activity this type of experiment. It is possible light and darkness were not salient enough consequences to effectively train the fish. However, there were a number of potential confounding factors, leaving light and dark as potentially viable means of training behaviors in *G. petersii* and other nocturnal animals. This is supported by previous work using darkness and bright light as a component of training with *G. petersii* (Cain et al., 1994; Walton & Moller, 2010).

The first and most obvious explanation for the lack of successful training is the experimental design. Each fish had 60 training trials over four days before the 15 testing trials on the fifth day. Previous experiments with object discrimination have subjected fish to 40 or more trials in one training session, with training sessions occurring over several weeks (Schwarz & von der Emde, 2001; von der Emde et al., 1998; von der Emde & Fetz, 2007). Such rigorous training was avoided in this experiment in an attempt to minimize stress and fatigue on the subjects, but this may have compromised the effectiveness of the training.

Another component of the experimental design that raises concerns is the punishment light. This punishment was intended to bolster the reinforcing nature of the darkness used as positive reinforcement. Daily illuminance readings on this light ranged from 500 to 2900 lx. Illuminance was measured under the water with a Samsung Galaxy S7 phone running the Lux Meter application (Angstrom Metrology, LLC, 2016), always kept in the same position. Fish are rendered functionally blind at an illuminance of 540 lx (Teyssevre & Moller, 1982), which the punishment likely did, given that the low end of the range of readings was 500 lx. Vision in

weakly *G. petersii* is currently an active area of study, and there is much yet to be understood. One component that has not been fully explored is the length of time it takes an individual's eyes to adjust between bright and dark conditions. Landsberger et al. (2008) studied the morphology of the mormyrid retina and found that the photoreceptors are organized differently in light-adapted eyes versus dark-adapted eyes. It is possible that the brightness of the punishment light affected the subjects' retinæ for a long enough period of time as to render them unable to perceive the darkness in subsequent trials during which they chose the rewarded object.

Even though care was taken to mitigate stress on the subjects, their inability to be successfully trained may have been a result of the stress imposed by the experimental tank and procedure. Stress has been shown to affect learning in mormyrids (Miller, 2015), though the effects may vary by fish. Raoult, Trompf, Williamson, and Brown (2017) found that stress led to decreased activity, and therefore a slower rate of learning in mulloway (*Argyrosomus japonicus*). This decreased activity may be reflected in the subjects of this experiment: all but two fish had to be coaxed to the door with a net at least once on two or more days, and four fish had to be coaxed at least once on all five days. The hesitance to enter the experimental side of the tank may be due to stress, though it could also simply be due to a lack of "interest" or "understanding" of the procedure. However, several subjects, including the ones that swam through the door without any assistance, attempted to jump out of the tank at least once between trials, suggesting a stronger physiological reaction to the environment than "apathy". It may have been beneficial to provide the fish with an experimental tank that promoted stress reduction. For example, Schuster & Amtsfeld (2001) used virtually the same experimental setup, but they provided their subjects with a shelter abutting the door, which likely provided a less stressful condition for the fish as they acclimated to the environment, as well as between trials.

Von der Emde and Fetz (2007) used their subjects' home tanks (complete with shelters) as training tanks, so the fish had access to shelter and did not experience handling as part of the experimental procedure. Caipang, Fatira, Lazado, and Pavlidis (2014) found that short-term handling increased stress response indices in blood plasma of Atlantic cod (*Gadus morhua*) for 24 hours. In *G. petersii*, it can take up to one hour for EODs to return to baseline after a stress-induced increase in discharge activity (Miller, 2015). In the case of the present experiment, this may explain why the fish did not vary significantly from a response index of 1. If the fish are experiencing stress by virtue of being in the experimental tank, then it is unsurprising that the experimental EODs did not vary significantly from the control EODs.

It is also possible that inter-individual personality difference played a role in learning as in the findings of Kareklas et al. (2017), that *G. petersii* with bold personalities learned a spatial task faster than those who were not bold. However, it is unlikely that only one fish out of 12 would have a bold personality, so this is not the most plausible explanation for the results of this experiment.

Another variable worth considering is the possibility that the fish did not accurately detect the differences between the objects through active electrolocation. Almost 75% of all trials that were analyzed did not have a significant corresponding RI, suggesting that every fish had several trials in which they were likely not attending to any stimulus. While electrosense is believed to be the primary sense utilized in spatial orientation and investigation by *G. petersii* (Pusch et al., 2013), these fish have highly developed retinæ and are capable of visually identifying and discriminating between objects (Landsberger et al., 2008; Teyssevre & Moller, 1982, Schuster & Amtsfeld, 2002; Kareklas et al., 2017). While the retinæ of *G. petersii* appear to be particularly well adapted to dim light (Landsberger et al., 2008), the optomotor response

functions up to illumination of 540 lx (Teyssedre & Moller, 1982). The fish in the present experiment were held behind Plexiglas before the start of each session and between trials. Through the Plexiglas in neutral light, the fish were able to see the identical porcelain cylinders without being able to perceive them electrically. By the time the door was opened for the first trial, it is possible that the fish had gathered enough visual information to obviate the need for active electrolocation. Further support for this theory is the fact that the porcelain cylinders could have been potential sources of shelter, but none of the fish swam close to the water surface to investigate an entry point, suggesting that the fish did not perceive the cylinders to be hollow. This would explain the consistency between control EODs and experimental EODs, as well as the dearth of significant RIs and the fishes' poor performance on overall object discrimination. In prior object discrimination experiments, fish either had visual access to the objects they were electrolocating or were tested in complete darkness, forcing the use of electrolocation (Schwarz & von der Emde, 2001; von der Emde, 2004; von der Emde et al, 1998). Schwarz and von der Emde (2001) controlled for vision in trials performed in dim light by testing in complete darkness, but it is possible that the fish who had visual access to the stimuli may have used vision in concert with electrosense to initially discriminate between the stimuli. Arguments have been made that *G. petersii* need lower illuminance than those in the present experiment in order to attend to visual cues. Schuster and Amtsfeld (2002) concluded that *G. petersii* needed light conditions below 10 lx to discriminate visual cues, but those results were somewhat ambiguous, as one of their three subjects was able to be trained in illuminance of 43 lx, and it showed a tendency toward correct choices (59%) at an illuminance of 990 lx. Von der Emde and Bleckmann (1998) found that some *G. petersii* individuals may actually use vision over electrolocation as the primary sense in foraging in the presence of neutral light. Furthermore,

Teyssedre and Moller (1982) present compelling evidence of the optomotor response in illuminance of up to 540 lx. The conflicting evidence as to what *G. petersii* is capable of detecting visually under neutral light conditions is an area worthy of rigorous future investigation, as this may play a key role in object discrimination. It is also possible that fish, with the exception of fish 7, were unable to detect the objects inside the porous, electrically transparent porcelain cylinders because of insufficient stimulus strength. The self-generated electric field distortion caused by the presence of the object could have been below electrosensory threshold. In prior, successful experiments though, similar cylinders were filled with conductive liquid or nonconductive paraffin wax (Lissmann, 1963; Moller, 1995, p.122).

The results of this experiment, while generally not supportive of the hypothesis, offer some intriguing questions for future study. The potential conflict between vision and electrosense has not been thoroughly examined, and future experiments may be illuminating. It is also worth further investigating the effects of stress on learning in *G. petersii*, as well as what conditions may provoke stress responses. Additionally, it may be that experiments of this type simply need to be performed over a larger number of trials and longer period of time, though consideration should be made with regard to potential stressors. There are a number of modifications that could be made to this experiment to re-test the hypothesis. A higher threshold could be set to truly declare the fish successful in the training task, as in Schwarz and von der Emde (2001). Using darkness as a positive reinforcer should be tested without using brightness as a punishment. The use of positive punishment is likely unnecessary and may have hindered the subjects from registering the positive reinforcer altogether. The influence of stress could be mitigated by allowing the fish to live in a compartment of the experimental tank to eliminate handling before training and testing. Handling between trials can also be eliminated by creating

an experimental tank that is symmetrical and moving the cylinders in which the objects are placed from one side to the other between trials, rather than moving the fish. This set-up may allow for more trials per day without undue stress or fatigue to the subjects. Potential stress can further be reduced by providing shelter for the fish in the holding area of the experimental tank. The tank could also be modified by using two doors, one directly in front of each object, to make the choice between the objects more distinct, as has been done in prior experiments (von der Emde et al., 1998; von der Emde, 2004; von der Emde et al., 2010). Additionally, the wall and doors separating the holding area from the experimental area should be made out of a plastic mesh, which would allow the fish to perceive the objects electrically between trials. By addressing these confounding factors, a more robust experiment can truly test whether darkness is an effective positive reinforcer in *G. petersii*. This study is a preliminary foray into the world of sensory reinforcement, and it will hopefully inspire future inquisition into alternative methods of positive reinforcement in weakly electric fish.

References

- Agrillo, C., Dadda, M., Serena, G., & Bisazza, A. (2009). Use of number by fish. *PloS One*, 4(3), e4786. doi: <https://doi.org/10.1371/journal.pone.0004786>
- Agrillo, C., Miletto Petrazzini, M. E., Piffer, L., Dadda, M., & Bisazza, A. (2012). A new training procedure for studying discrimination learning in fish. *Behavioural Brain Research*, 230(2), 343-348. doi:10.1016/j.bbr.2012.02.025
- Al-Imari, & Gerlai. (2008). Sight of conspecifics as reward in associative learning in zebrafish (*Danio rerio*). *Behavioural Brain Research*, 189(1), 216-219. doi: <https://doi.org/10.1016/j.bbr.2007.12.007>
- Angstrom Metrology, LLC. (2016). Lux Meter [Mobile Application Software]. Retrieved from <http://www.angstrommetrology.com>
- Baldwin, B. A., & Start, I. B. (1981). Sensory reinforcement and illumination preference in sheep and calves. *Proceedings of the Royal Society of London Series B, Biological Sciences*, 211(1185), 513-526.
- Cain, P., Gerin, W., & Moller, P. (1994). Short-range navigation of the weakly electric fish, *Gnathonemus petersii* G. (Mormyridae, teleostei), in novel and familiar environments. *Ethology*, 96(1), 33-45. doi: <https://doi.org/10.1111/j.1439-0310.1994.tb00879.x>
- Caipang, C. M. A., Fatira, E., Lazado, C. C., & Pavlidis, M. (2014). Short-term handling stress affects the humoral immune responses of juvenile Atlantic cod, *Gadus morhua*. *Aquaculture International*, 22(4), 1283-1293. Retrieved from <https://link.springer.com/article/10.1007/s10499-013-9746-2>
- Campbell, B. A., & Pickleman, J. R. (1961). The imprinting object as a reinforcing

- stimulus. *Journal of Comparative and Physiological Psychology*, 54(5), 592. doi:
<http://dx.doi.org/10.1037/h0045435>
- Castro, L., & Wasserman, E. A. (2010). Animal learning. *Wiley Interdisciplinary Reviews: Cognitive Science*, 1(1), 89-98. doi:10.1002/wcs.18
- Ciali, S., Gordon, J., Moller, P. (1997). Spectral sensitivity of the weakly discharging electric fish *Gnathonemus petersii* using its electric organ discharges as the response measure. *Journal of Fish Biology*, 50, 1074-1087.
- Cooper, J. J., & Mason, G. J. (2001). The use of operant technology to measure behavioral priorities in captive animals. *Behavioral Research Methods, Instruments, and Computers*, 33(3), 427-434. Doi: 10.3758/BF03195397
- Dawkins, M. S., & Beardsley, T. (1986). Reinforcing properties of access to litter in hens. *Applied Animal Behaviour Science*, 15(4), 351-364. doi:
[https://doi.org/10.1016/0168-1591\(86\)90127-9](https://doi.org/10.1016/0168-1591(86)90127-9)
- Gilbertson, D. W. (1975). Courtship as a reinforcement for key pecking in the pigeon, *Columba livia*. *Animal Behaviour*, 23, 735-744. doi: [https://doi.org/10.1016/0003-3472\(75\)90101-3](https://doi.org/10.1016/0003-3472(75)90101-3)
- Hogan, J. A., & Roper, T. J. (1978). A comparison of the properties of different reinforcers. In *Advances in the Study of Behavior* (Vol. 8, pp. 155-255). Academic Press. doi:
[https://doi.org/10.1016/S0065-3454\(08\)60134-5](https://doi.org/10.1016/S0065-3454(08)60134-5)
- Kareklas, K., Elwood, R. W., Holland, R. A., & Tregenza, T. (2017). Personality effects on spatial learning: comparisons between visual conditions in a weakly electric fish. *Ethology*, 123(8), 551. doi:10.1111/eth.12629
- Koistinen, T., Ahola, L., & Mononen, J. (2007). Blue foxes' motivation for access to an earth floor measured by operant conditioning. *Applied Animal Behaviour Science*, 107, 328-

341. doi: <https://doi.org/10.1016/j.applanim.2006.09.024>
- Landsberger, M., von der Emde, G., Haverkate, D., Schuster, S., Gentsch, J., Ulbricht, E., Reichenbach, A., Makarov, F., Wagner, H.-J. (2008). Dim light vision – Morphological and functional adaptations of the eye of the mormyrid fish, *Gnathonemus petersii*. *Journal of Physiology - Paris*, 102(4), 291-303. doi:10.1016/j.jphysparis.2008.10.015
- Lee, J., Floyd, T., Erb, H., & Houpt, K. (2011). Preference and demand for exercise in stabled horses. *Applied Animal Behaviour Science*, 130(3), 91-100. doi: <https://doi.org/10.1016/j.applanim.2011.01.001>
- Lissmann, H. (1963). Electric location by fishes. *Scientific American*, 208(3), 50-59. Retrieved from <http://www.jstor.org/stable/24936498>
- Lissmann, H. W., & Machin, K. E. (1958). The mechanism of object location in *Gymnarchus niloticus* and similar fish. *Journal of Experimental Biology*, 35(2), 451-486. Retrieved from <http://jeb.biologists.org/content/35/2/451>
- Lott, G. K. (2007). *Hybridizing cellular and behavioral neurobiology with modern engineering tools: microelectronics, microfabricated devices, and software solutions for physiology* (Doctoral dissertation). Retrieved from <https://ecommons.cornell.edu/handle/1813/7530>
- Moller, P. (1995). *Electric fishes: History and Behavior* (1st ed., Fish and fisheries series; 17). London; New York: Chapman & Hall.
- Moller, P. (2002). Multimodal sensory integration in weakly electric fish: a behavioral account. *Journal of Physiology - Paris*, 96(5), 547-556. doi:10.1016/S0928-4257(03)00010-X
- Parker, C. (1966). Total darkness as an aversive stimulus condition for the squirrel monkey. *Psychonomic Science*, 6(3), 111-112. doi:10.3758/BF03327982
- Perret, A., Henry, L., Coulon, M., Caudal, J.-P., Richard, J.-P., Cousillas, H., Hausberger, M.,

- George, I. (2015). Social visual contact, a primary “drive” for social animals? *Animal Cognition*, 18(3), 657-666. doi:10.1007/s10071-015-0834-8
- Post, N., & Von der Emde, G. (1999). The “novelty response” in an electric fish: response properties and habituation. *Physiology & Behavior*, 68(1), 115-128. doi:10.1016/S0031-9384(99)00153-5
- Pusch, R., Kassing, V., Riemer, U., Wagner, H.-J., Von Der Emde, G., & Engelmann, J. (2013). A grouped retina provides high temporal resolution in the weakly electric fish *Gnathonemus petersii*. *Journal of Physiology, Paris*, 107(1-2), 84. doi:10.1016/j.jphysparis.2012.06.002
- Ragir, S., & Singh, S. (2014). GriffinVC [Computer software]. Hunter College CUNY, New York.
- Raoult, V., Trompf, L., Williamson, J. E., & Brown, C. (2017). Stress profile influences learning approach in a marine fish. *PeerJ*, 5, e3445. doi:10.7717/peerj.3445
- Rojas, R., & Moller, P. (2002). Multisensory contributions to the shelter-seeking behavior of a mormyrid fish, *Gnathonemus petersii* Günther (mormyridae, teleostei): The role of vision, and the passive and active electrosenses. *Brain, Behavior and Evolution*, 59(4), 211-221. doi:10.1159/000064908
- Roper, T. J. (1975). Nest material and food as reinforcers for fixed-ratio responding in mice. *Learning and Motivation*, 6(3), 327-343. doi: https://doi.org/10.1016/0023-9690(75)90013-2
- Schuster, S., & Amtsfeld, S. (2002). Template-matching describes visual pattern-recognition tasks in the weakly electric fish *Gnathonemus petersii*. *The Journal of Experimental Biology*, 205(4), 549. Retrieved from <http://jeb.biologists.org/content/205/4/549>

- Schwarz, S., & von der Emde, G. (2001). Distance discrimination during active electrolocation in the weakly electric fish *Gnathonemus petersii*. *Journal of Comparative Physiology A*, *186*(12), 1185-1197. doi:10.1007/s003590000170
- Sovrano, V. A., Bisazza, A., & Vallortigara, G. (2002). Modularity and spatial reorientation in a simple mind: Encoding of geometric and nongeometric properties of a spatial environment by fish. *Cognition*, *85*(2), B51-B59. doi: [https://doi.org/10.1016/S0010-0277\(02\)00110-5](https://doi.org/10.1016/S0010-0277(02)00110-5)
- Teyssedre, C. and Moller, P. (1982), The optomotor response in weak-electric mormyrid fish: Can they see? *Zeitschrift für Tierpsychologie*, *60*, 306-312. doi:10.1111/j.1439-0310.1982.tb01088.x
- Urbaniak, G. C., & Plous, S. (2013). Research Randomizer (Version 4.0) [Computer software]. Retrieved from <http://www.randomizer.org/>
- von der Emde, G. (2004). Distance and shape: perception of the 3-dimensional world by weakly electric fish. *Journal of Physiology - Paris*, *98*(1), 67-80. doi:10.1016/j.jphysparis.2004.03.013
- von der Emde, G. (2006). Non-visual environmental imaging and object detection through active electrolocation in weakly electric fish. *Journal of Comparative Physiology A*, *192*(6), 601-612. doi: 10.1007/s00359-006-0096-7
- von der Emde, G., Amey, M., Engelmann, J., Fetz, S., Folde, C., Hollmann, M., Metzen, M., Pusch, R. (2008). Active electrolocation in *Gnathonemus petersii*: Behaviour, sensory performance, and receptor systems. *Journal of Physiology - Paris*, *102*(4), 279-290. doi:10.1016/j.jphysparis.2008.10.017
- von der Emde, G., Behr, K., Bouton, B., Engelmann, J., Fetz, S., & Folde, C. (2010). 3-

- Dimensional scene perception during active electrolocation in a weakly electric pulse fish. *Frontiers in Behavioral Neuroscience*, 4(26), 1-13. doi:
<https://doi.org/10.3389/fnbeh.2010.00026>
- von der Emde, G., & Bleckmann, H. (1998). Finding food: senses involved in foraging for insect larvae in the electric fish *Gnathonemus petersii*. *Journal of Experimental Biology*, 201(7), 969-980. Retrieved from <http://jeb.biologists.org/content/201/7/969>
- von der Emde, G., & Fetz, S. (2007). Distance, shape and more: recognition of object features during active electrolocation in a weakly electric fish. *Journal of Experimental Biology*, 210(17), 3082. doi:10.1242/jeb.005694
- von der Emde, G., & Schwarz, S. (2002). Imaging of Objects through active electrolocation in *Gnathonemus petersii*. *Journal of Physiology - Paris*, 96(5), 431-444.
doi:10.1016/S0928-4257(03)00021-4
- von der Emde, G., Schwarz, S., Gomez, L., Budelli, R., & Grant, K. (1998). Electric fish measure distance in the dark. *Nature*, 395(6705), 890. doi: 10.1038/27655
- Walton, A. G., & Moller, P. (2010). Maze learning and recall in a weakly electric fish, *Mormyrus rume proboscirostris* Boulenger (Mormyridae, teleostei) 1. *Ethology*, 116(10), 904-919.
doi:10.1111/j.1439-0310.2010.01807.x