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**Birds Do It, Bees Do It, And Even Electric Fish Do It: Cultural
Transmission of Maze Learning in the African Weakly Electric
Fish, *Gnathonemus petersii* (Mormyridae, Teleostei)**

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Birds Do It, Bees Do It, And Even Electric Fish Do It:
Cultural Transmission of Maze Learning in the African Weakly Electric Fish,

Gnathonemus petersii (Mormyridae, Teleostei)

By

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Date

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Abstract

In this study, the ability of a maze-experienced, weakly electric fish to transmit spatial information to an inexperienced conspecific through exposure via paired training in a maze is evaluated using maze trial latency, electric signals discharged within the maze and the subsequent effect of training on molecular markers of memory.

Key words

Navigation; memory; maze learning; communication; electric fish; PKM ζ ; EOD; social behavior

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Introduction

Communication is a fundamental, complex aspect of an animal's relationship with its conspecifics and environment. Characterized as a system involving two or more individuals, transmission of information occurs between a sender and one or more receivers. While the functional significance of communication is consistent across species, the sensory modalities, signal channels, and environmental factors, however, vary widely. Organisms may depend on one or more sensory modalities such as visual, chemical, thermal, mechanical, acoustic, or electric cues. Sensory modalities evolved in environments for optimal signal transfer. Thus, animals communicate having adapted to living underground, in air, in water, or in different lighting conditions.

This diversity is intriguing and invites an exploration of the transfer of information between or among animals in social groups. Members of social groups have developed means of communication in order to coordinate tasks, manage risks, and provide information to offspring. Individuals in a group can provide information about places to seek out or avoid, location of food resources, foraging methods, and tool use. For example, primates teach their young foraging methods by demonstrations of tool use (Boesch, 1991), and meerkat pups both observe and are taught predator avoidance and foraging, skills that would be costly to develop individually, by adults in the group (Thornton & Clutton-Brock, 2011). Similarly, fish learn by observation how to engage in aggregation behavior to avoid predators and attain foraging advantages. Using visual, mechanical, and/or electric cues (Moller, 1976) to sense one another, fish transmit information about their movement allowing them to move together. In birds, social learning plays a vital role not only in acquiring foraging techniques and predator recognition, but also in song learning (Soha & Marler, 2000), mate choice (Slagsvold *et al.*, 2002) and migration

(Slagsvold & Wiebe, 2011). Migration patterns can be learned socially from older birds, as seen in captive-bred whooping cranes reintroduced to the wild (Mueller *et al.*, 2013). After an initial period of artificial migration flight training, subsequent flights that were flown with more proficient conspecifics show less deviation from a straight line migratory path and a 38% improvement in accuracy (Mueller *et al.*, 2013).

But a most remarkable example of social communication is the honeybee “dance language” discovered by Karl von Frisch (1968). The honeybee, *Apis mellifera*, has developed a unique “waggle dance” to communicate a coded message regarding the distance and direction of reliable food sources. Foragers returning from a successful resource gathering expedition display this unique locomotor behavior in the hive. While the forager dances by moving its abdomen and wings back and forth, naïve bees follow very closely, detecting the vibrations generated, through which information is purportedly transferred (Ai, 2010). Depending upon the distance of the food source, the bees either dance in a round (up to 50 meters away) or a figure-eight (exceeding 50 meters) configuration. Once other bees are recruited, sometimes requiring multiple iterations of the dance, they decode the message and set off on search flights, utilizing additional external cues such as visual or scent markers when in the vicinity of the food source (Riley *et al.*, 2005). This is a remarkable example of animal communication because we can physically observe a direct transfer of information as a result of the particular physiological and molecular mechanisms involved (Brockmann & Robinson, 2007).

Similarly remarkable is the behavior of weakly electric mormyrid fish, *Gnathonemus petersii*. This fish is endemic to African lakes and rivers and is found in large social groups, having adapted to the dark, murky, foliage-rich freshwater environments. These fish are nocturnal and have evolved a unique communication system to meet the challenges of their

environment. Besides acoustic, visual and mechanical senses, these fish are endowed with an electric organ. In an adult mormyrid fish this organ is located in the caudal peduncle and comprised of flattened, electric discharge-generating electrocytes, which are cells modified from muscle tissue (Bennett, 1971; Szabo, 1960). The rate of electric organ discharge (EOD) activity is controlled by command signals sent via spinal electromotor neurons from the medullary command nucleus, or pacemaker, to the electric organ causing the depolarization of the electrocyte membranes and resulting in the generation of a single EOD (Bennett, 1971).

Mormyrids not only generate EODs, but through an array of electroreceptors, they also detect changes in their self-generated electric field and the EODs of conspecifics (Zakon, 1986). There are two types of electroreceptors, and in concert with their electric organ discharge, they allow the fish to form an image of the environment via distortions of their electric field (Von der Emde, 1999). Weakly electric fish detect impedance or resistance in their environment using tuberous receptors, known as mormyromasts. A second class of tuberous organs, knollenorgans are sensitive to high frequencies and function in intraspecific communication (Bell, 1989). A second receptor, the ampullary receptor, is more sensitive to lower (Direct Current) frequencies and is involved in location of food and navigation (Hopkins *et al.*, 1997).

Foraging in *G. petersii* requires displacement from their day-time shelters in the evening, and returning to those shelters at day break (Moller *et al.*, 1976); fish employ their unique electro-senses for this navigational task. Communication in conjunction with navigation and movement is important since the nocturnal *G. petersii* are social and exhibit aggregation behavior. The electrosenses, working synergistically with visual and mechanical senses, help to maintain group cohesion and survival (Khait *et al.*, 2009).

Maze learning is a standard method of testing spatial navigation in a wide range of experimental animals. In previous studies, Walton & Moller (2010) have shown that mormyrid fish, predominantly using their electrosenses, can learn to navigate their way through a meander maze to a sheltered goal). In an earlier, related study, Cain, Gerin, & Moller (1994) demonstrated the importance of active electro-location in learning spatial navigation, but discovered that by rendering the electric organ surgically inoperative and eliminating the active electrosense trained fish switch to using other cues available to them to achieve a task.

The lateral pallium of the fish is a homologous structure to the mammalian hippocampus, which functions in spatial navigation and memory as well as consolidation of information from short to long-term memory (Rodriguez *et al.*, 2002). Thus, the lateral pallium is thought to play a role in spatial cognition in fish, with evidence suggesting that trauma to the dorsolateral telecephalon (or, lateral pallium) results in spatial learning deficits (Walton & Moller, 2010). Comparable to rodent learning, the spatial memory acquisition and consolidation of mormyrid fish (*G. petersii* and *Mormyrus rume proboscirostris*) subjected to a maze learning task is associated with an increase in density of the memory molecule PKM ζ in the fish's telencephalic lateral pallium. During training, the increase of PKM ζ in the post-synaptic fraction helps to strengthen long-term spatial memory (Moller *et al.*, 2010). Bliss and Lomo (1973) describe long-term potentiation (LTP) as a model for learning and memory, which involves various protein kinase enzymes, including PKM ζ , within the neuron. Serrano *et al.* (in prep.) demonstrated that as fish become familiar with the maze, molecular changes occur within the lateral telencephalon, resulting in an increase in PKM ζ levels with subsequent enhancement of synaptic transmission and facilitation of LTP.

Being familiar with the social organization and sensory systems of *G. petersii*, it is likely that in nature a naïve fish may join an experienced fish on a foraging excursion. Fish can learn and remember distance and trajectory, using path integration or, conditions permitting, electric landmarks to negotiate a maze (Walton & Moller, 2010). This, however, begs the question whether *G. petersii*, like honey bees or cranes, can transfer spatial information, direction and distance, to naïve fish?

This study will use the maze learning paradigm to test the hypothesis that inexperienced fish will acquire spatial information through exposure to an experienced fish's EOD patterns supported by concurrent increase of telencephalic PKM ζ .

Methods

Subjects

Twenty-four 8-10 month old *Gnathonemus petersii* were utilized in this project. They were obtained from a fish importer (Zorina Khan, South Richmond Hill, NY) and housed in tanks equipped with standard heating and filtration devices. Fish were fed *ad libitum* with blackworms (*Lumbriculus* sp.), and kept under controlled L12:D12 (lights on 800h) and water conditions (22-27° C, 150-300 μ S/cm and pH 6.0-7.5). Experimental animals were housed together in a large aquarium tank (122 cm L x 46 cm W x 61 cm H) that was filtered and aerated constantly and enriched with shelters and rocks. All experimental procedures complied with federal and local regulations and were approved by the Hunter College IACUC (PM teaching ac/14-01).

Materials

Experiments were performed in a circular (1-m diameter) maze embedded in a Plexiglas, electrically shielded tank (Fig. 1). Before each session, water temperature, conductivity and pH was monitored (Hanna Combo waterproof pH/EC/TDS meter) to ensure similar conditions to the home tank. A medium standard aquarium net was used to transport the subject from its home tank to the maze's starting box. A stopwatch was used to measure run time of each trial and recorded in a laboratory notebook. An overhead-mounted video camera (Sony DV) was used to record an aerial view of the tank for each trial, so that all maze runs and subsequent behaviors witnessed within the maze tank were recorded if needed for further investigation. The fishes' electric organ discharges were monitored with a pair of stainless electrodes arranged in kitty-corner position and fed into the audio channel of the video camera. Subject ID, trial IDs, and time and date of experiment were noted on a whiteboard and recorded previous to the fish's maze trial.

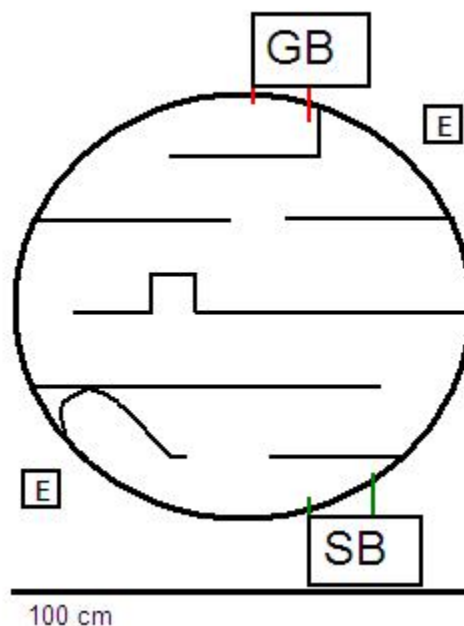


Figure 1. Maze apparatus for *G. petersii* navigation trials. Start box (SB), goal box (GB), and recording electrodes (E), 100 cm x 100cm.

Procedures

In order to differentiate the subjects, small fin clips were applied to either the anal fin, dorsal fin or both fins under a light anesthesia (tricainemethanesulfonate, MS 222; 5 mg/L. Fish were allowed a recovering time of one day before starting experimental trials. Subjects were gently removed from their home tank by net and placed in the start box (SB) of the maze; the door to the start box was lifted for the fish to enter the maze. Timing of the trial was started once the fish entered the maze, then the door was closed to prevent re-entry to the start box. Fish were trained to find the darkened goal box (GB); they were tested twice daily for ten days, or until reaching the criterion of showing no further improvement in learning (asymptote of trial latency). After the fish completed the maze twice (with a maximum end point time of ten minutes per run), the fish was gently removed and returned to its home tank. On day 11, a naïve, untrained fish (student) was introduced to the start box with the previously trained fish (teacher). An acclimation period of 2-3 minutes was allowed, and the subjects were released together into the maze. The pair was run on five consecutive trials, during which only the student fish was timed. On day 12, experiments were continued with the student alone until the performance criterion was met. Training trials were continued in the manner described above, on a daily basis, until performance latency matched that of the teacher after initial training or the end point of 10 individual training days was reached (see Table 1). The electric organ discharges (EODs) of both the teachers and students were also measured during the trials from electrodes strategically placed in the maze tank. When both teacher and students were observed simultaneously, EODs

were recorded simultaneously and so individual EODs needed to be distinguished by amplitude comparison. The dependent variables included: time to complete the maze (performance latency), and inter-discharge intervals (IDIs). EOD data were compiled and analyzed using GPrime (Lott, 2007) and Microsoft Excel (Microsoft, 2013) software.

Following the last trial, student fish were returned to their home tank for 1 hour to allow for memory consolidation before euthanasia and dissection. The fish were deeply anesthetized in a solution of buffered MS-222 (500 mg/L) until cessation of gill movements was observed. Fish were decapitated and selected brain tissues (telencephalon, optic tectum, and the remainder of the brain) were immediately dissected and stored at - 80° C. For each fish the following demographics were taken: standard length, body weight, total brain weight, sex, developmental status, and length and weight of gonads.

After completion of the maze trials, teacher fish were anesthetized (MS-222, 5 mg/L) and their electric organ then surgically rendered permanently inoperative (“silent”) by spinal cord transection anterior to the location of the electric organ (for details, see Rojas & Moller, 2002). Since tail movement in *G. petersii* is controlled by tendons, the procedure does not affect the fish’s locomotor behavior. Fish were allowed a recovery period of one day before tested again in the maze to ensure retention of their spatial memory.

When silent fish had shown retention of maze memory, a group of naïve, intact student fish were housed together with the silenced teachers and allowed to acclimate for 5-7 days. After acclimation, these students were first run in paired trials with teachers, and subsequently alone, as per previous procedure (see Table 1). Following a consolidation period of one hour, fish were euthanized and brain tissue collected.

Table 1.

Group	Group size (N)	# of trials/day	Days run	Time endpoint
Naïve/Teacher Student	9	2	7-10	600 s
	13	5 on training day 2 subsequent alone	8-11	600 s
Silent-trained student	3	5 on training day 2 subsequent alone	4	600 s

Table 1. Experimental conditions

Molecular:

Molecular data was gathered by fractionating the telencephalon brain samples into cytosolic and synaptic portions and running western blot to determine the density of the “memory” molecules, PKM ζ and PKC ζ . The collected telencephalon was thawed from -80° C storage and homogenized in 200 μ l of pre-made TEE buffer (Tris 50 mM, EDTA 1 mM, EGTA 1 mM) with a motorized pestle (20 pumps at 40 rpm). Homogenates were placed in a 1.5 mL Eppendorf tube and centrifuged at 3,000 g at 4° C for 5 minutes. The resulting supernatant was transferred to a clean centrifuge tube and the remaining pellet (nuclear fraction) disposed of. The supernatant was centrifuged in an ultracentrifuge at 100,000 x g (acceleration of gravity) at 4° C for 30 minutes. The new supernatant (the cytosolic fraction) was removed and stored safely. The remaining pellet was resuspended in 100 μ L of TEE buffer with added .001% Triton X-100, incubated on ice for 1 hour and then centrifuged at 100,000 g at 4° C for 1 hour. The resulting supernatant (the membrane fraction) was removed and the remaining pellet (the post-synaptic density fraction) resuspended in 50 μ L of TEE buffer. Total protein concentration was then determined using a bicinchoninic acid assay (BCA). The BCA solution (Pierce, Thermo Scientific) was added and samples covered and incubated in a water bath at 60° C for 30 minutes. After incubation, 10 μ L of each sample were loaded onto a 96-well plate and read in a plate reader (Bio-imaging Center, Hunter College) at a wavelength of 562 nm. Samples were reduced

with 4X Laemmli sample buffer (1/4 of total volume) and boiled in a hot water bath at 85° C for 2 minutes before frozen for storage at -80°.

Western blots:

Experimental samples, as well as 3 lanes of loading controls (all brain sample, or ABS) (20mg), were loaded into a Tris/Gly 4-20% midi gel (Novex Nu-PAGE SDS-PAGE, Life Technologies) for resolution of GluA2(102 kDa) GAPDH (37 kDa), PKM ζ (55 kDa) and PKC ζ (70 kDa) (Braren, et.al, 2014) and transferred to nitrocellulose membranes using the iBlot Dry Blotting System gel transfer device (Life Technologies) for nine minutes (Zanca, 2015). The membrane was incubated in a blocking solution of 5% sucrose in Tris buffered saline and Tween-20, for 30 minutes at room temperature. After, the membrane was incubated in primary antibodies PKM ζ (1:2000), PKC ζ (1:2000), GluA2 (1:2000) and GAPDH (1:2000) overnight. The membranes were then rinsed with TBST buffer for 20 minutes and probed with Horseradish peroxidase (HRP) conjugated secondary antibodies. The membranes were then incubated with Enhanced Chemiluminescence (ECL) substrate and exposed on CL-X Posure Film (Thermo Scientific). Resulting films were scanned and quantified using an imaging software (NIH Image J, Rasband 2014) (Zanca et.al, 2015).

Statistical analysis

The independent variable was the type of maze training, including self-taught, intact-conspecific-taught, and silent conspecific-taught. The dependent variables included trial latency (maze performance time), the electric organ inter-discharge interval (IDI), and the density of

PKM ζ . Descriptive statistics listed the number of subjects in each trial with average trial latencies, median latency, standard deviations in averages, and standard error of mean. Independent t-tests were used to determine significance of differences between mean maze trial latency of the subject groups on particular training days, as well as the differences between slopes of regression of the subject groups' performance over time (or, "learning curves"). The significance of best-fit linear regression of EOD frequency time between teacher and student were evaluated using significance of a correlation coefficient. Independent t-tests were also used to determine significance of relative levels of PKM ζ and PKC ζ in two fractions between student and naïve fish subjects groups. Statistics were run using a combination of programs, including SPSS (IBM Inc., 2013), Vassarstats (Lowry, 1998) and Microsoft Excel.

Adjustments/missing data

Due to unfortunate and unforeseeable events, various samples and data points were removed from this study so as not to skew the results. The data removed fell into three categories: latency (time), EOD and molecular data points. Firstly, a number of experimental animals perished over the course of experimentation and these unexpected deaths led to the inability to continue maze trials and gather subsequent latency data, as well as the loss of brain tissue samples due to autolysis. Other data loss, namely the EOD data, resulted from instances of high noise obscuring the analysis of electric pulse discharges on the video recorded maze trials.

Results

Latency

On the first day of training, the teacher fish ($N=9$) spent more time navigating from the start box to the goal box (Day 1, $M= 259.47$, $SEM= 48.90$) than the student fish (intact-trained) ($N=13$) on their first solo training day (Day 9, $M=116.69$, $SEM= 20.08$). The student fish paired with the silent teacher (silent-trained student) for maze training ($N=3$) spent more time in the maze than either of the other groups on their initial day of solo trials (Day 17, $M = 261.1$, $SEM= 136.39$).

However, by the end of the training period, the performance latency of the teachers (Day 7, $M= 177.54$, $SEM=55.34$) was reduced, as was that of both the intact-trained (Day 15, $M = 96.83$, $SEM= 31.52$) and silent-trained groups (Day 20, $M= 206.28$, $SEM = 101.7$). The difference in performance of the two groups, teachers versus intact-trained students, on the first day of independent trials was significant ($t(20) = 2.83$, $p = 0.01$). The average of all teacher-student paired training trials (Day 8) was significantly lower than the average of the first day of the teacher trials (Day 1) ($t(11) = 2.04$, $p = 0.03$). The students performed significantly better after being guided through the maze by a conspecific than a naïve fish introduced to the maze with no prior training, indicating possible transfer and acquisition of spatial information. The difference of performance between these groups on the last day of training was no longer significant ($t(20) = 1.37$, $p = 0.185$), which was to be expected.

The difference in performance between the teachers and the silent-trained students on their first day of solo maze trial was not significant ($t(10)= -0.01$, $p = 0.496$), with the silent-trained fish having average latencies as if no training session had occurred. There was a significant difference in the performance of the intact-trained students compared to the silent-trained students, in that the silent-trained students performed significantly worse than the intact-trained students on their first day alone in the maze ($t(14) = -1.84$, $p = 0.04$). The silent-trained

students also performed significantly worse on average during the training trials paired with the teacher fish as compared to the intact-trained fish ($t(14)=-3.44$, $p = 0.002$). These results were expected, given that the electric organ was impaired in the teacher for the silent-trained subject group, thus impairing communication and impacting transmission of spatial information to those conspecifics.

The learning curves of the teacher and both the intact-trained and silent-trained student illustrate the differences between both the initial trial day and final trial day (see Figure 2). Intact-trained students' started at a faster run time, with a shallow curve before asymptoting to the same level as that of the maze-experienced (learned) teachers, whereas both the teachers and silent-trained students start at much higher latencies and have steeper learning curves (Teacher slope $m = -12.95$, student $m = -6.68$, silent student $m = -27.41$). The linear best-fit trendlines over maze trials show no significant differences in slope between teacher and intact-trained student trials ($t(10)= 2.23$, $p = 0.16$) (see Figure 2a). There was, however, a significant difference in the best-fit linear trend lines of the intact-trained students and the silent-trained students (intact-trained $m= -6.68$, silent-trained $m= -27.41$), where the intact-trained fish had much shallower trend lines ($t(7)= -1.89$, $p = 0.003$). The silent-trained students started at a much higher average trial latency, similar to that of the teachers, indicating little or no conspecific learning or transmission of the spatial map of the maze after a training period. Data was cut off at day 3 and further training progress of the silent trained students was not evaluated due to illness of subjects.

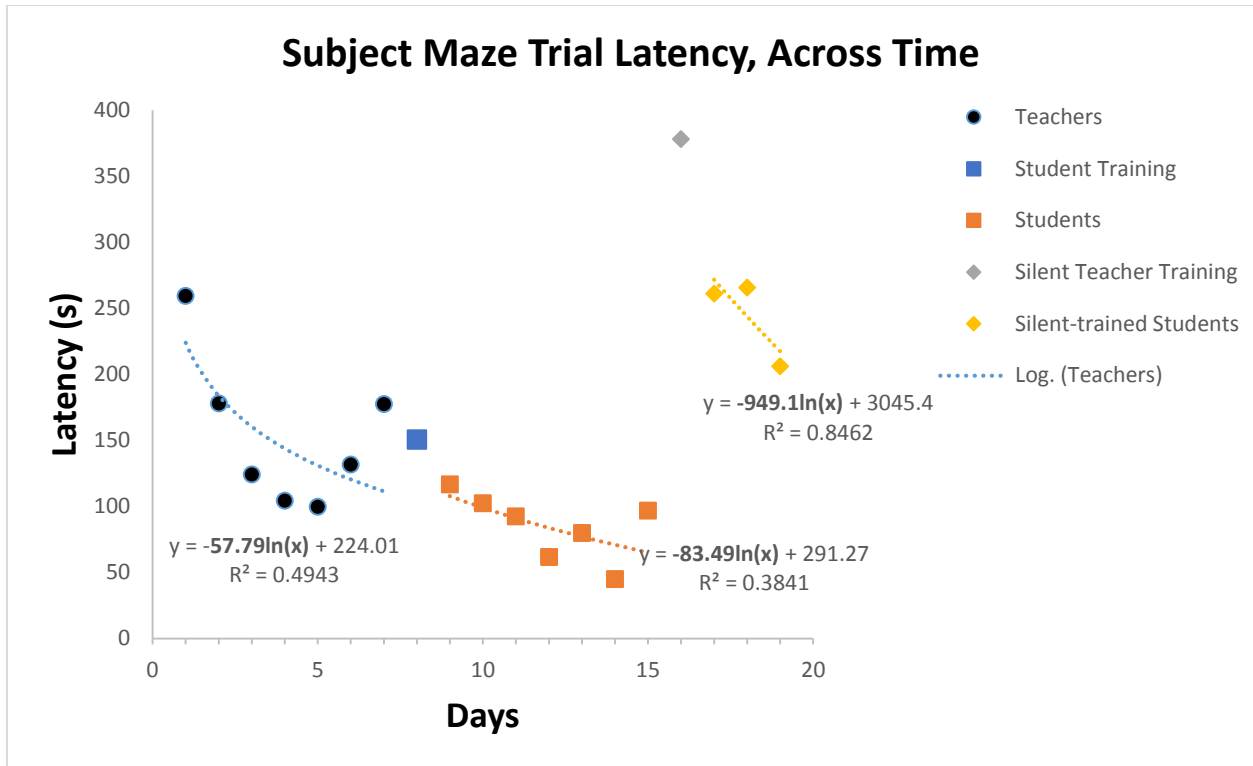


Figure 2. Performance latencies on respective intact-trained student and silent-trained student training days (Day 8 and 16) and respective teacher, intact-trained student and silent-trained test days (Days 1-7, 9-15, 17-19). Dashed lines indicate curvilinear trend lines (slopes are in bold).

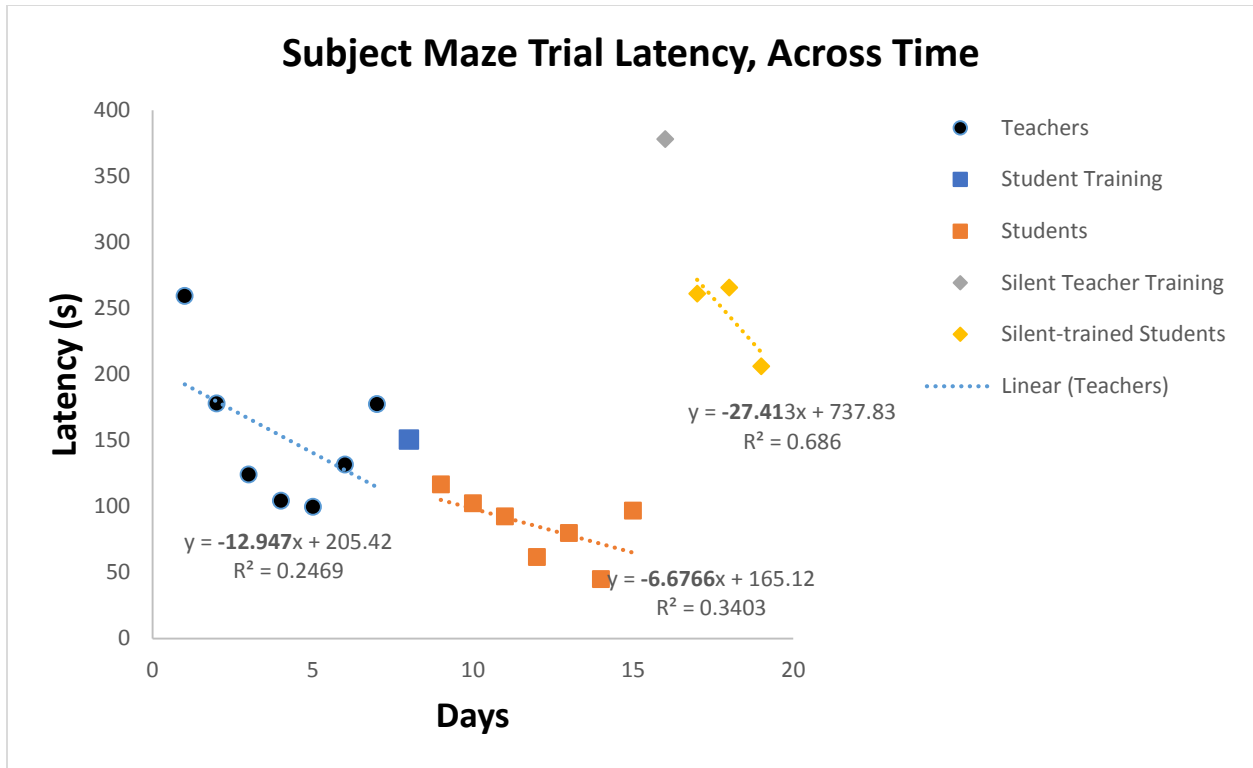
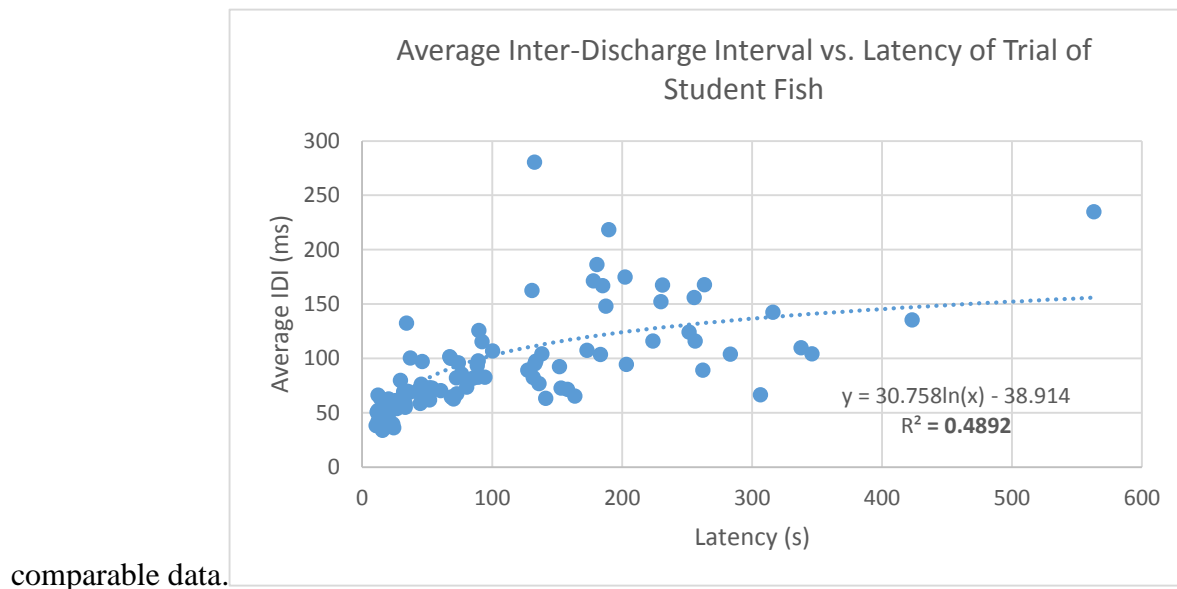


Figure 2a. Performance latencies on respective intact-trained student and silent-trained student training days (Day 8 and 16) and respective teacher, intact-trained student and silent-trained test days (Days 1-7, 9-15, 17-19). Dashed lines indicate best-fit trend lines (slopes are in bold).

Electric organ discharges

The electric organ discharge (EOD), in concert with electroreceptors, is employed by *G. petersii* as the major modality to interact with the environment. As the individual negotiates its surroundings, the interval duration between EODs (inter-discharge interval, IDI), can fluctuate, indicating levels of involvement. A shorter interval indicates a higher discharge rate and could be interpreted as more interest in, and investigation of, objects within the fish’s emitted electrical field.

The inter-discharge interval was significantly positively correlated with performance latency for student subjects ($t(5)=2.188$, $p = 0.04$) (see Figure 3). The slower the subject navigated the maze, the longer the IDIs, i.e. the lower the EOD frequency. Evaluating the data by training day and subject (see Figure 4), IDIs averaged by subject group per day, showed slightly upward slope within the teacher subject group ($m= 0.48$). However, within the intact-trained student group, a steeper trend downwards is seen ($m= -2.66$), with a decrease of IDI (increase in EOD frequency) as the training days progress, but neither slope correlations are significant. There is a significant difference ($t(10)= 2.23$, $p = 0.04$) between the slopes of the teacher and intact-trained student, perhaps indicating a higher-quality learning as a result of a previous transfer of spatial information via the EOD from the students' teacher. The IDI information for the silent teacher trained student subject group is not evaluated here due to lack of sufficient



comparable data.

Figure 3. Average inter-discharge intervals of individual student subjects over time indicating a positive correlation; as latency increases, IDI increases.

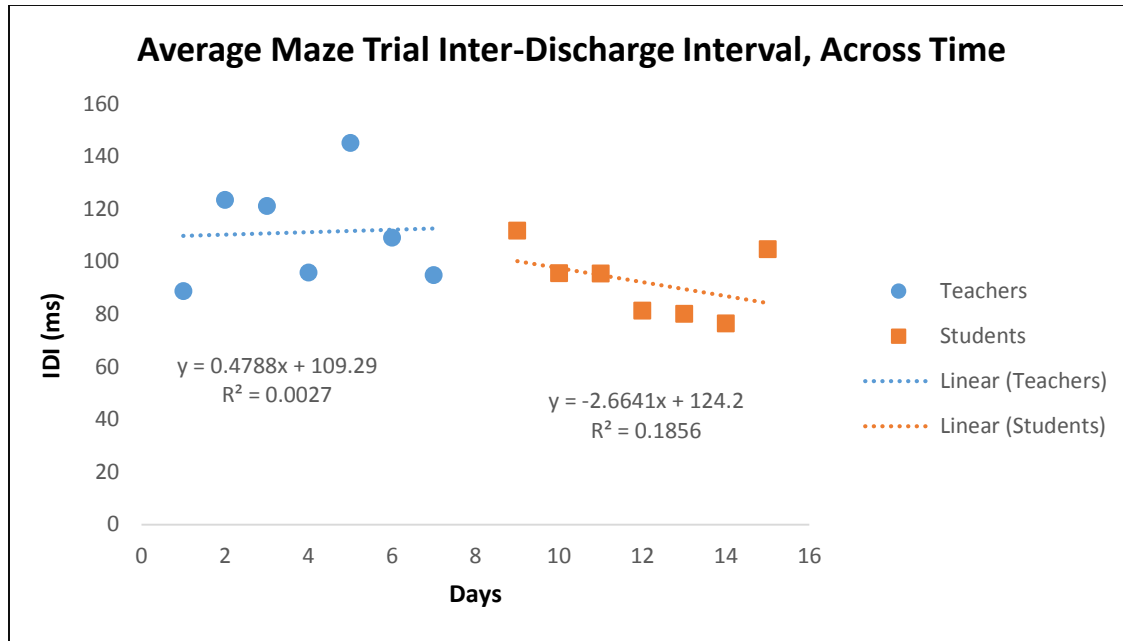


Figure 4. Inter-discharge interval over respective teacher and student training days (Days 1-7, 9-15), with dashed lines indicating linear best-fit trend lines over time.

Molecular

We examined the GAPDH corrected, relative chemiluminescence as an indicator of levels of PKC ζ and PKM ζ in cytosolic and post-synaptic density fractions, in the lateral telencephalon of the conspecific-trained student fish. As compared to a group of naïve fish, the students had significantly higher levels of PKM ζ in the cytosolic fraction in the lateral telencephalon ($t(13)=2.83, p = 0.0074$) and no significant difference in levels of PKC ζ between the two groups ($t(5)=0.58, p = 0.29$) (see Figure 5).

The post-synaptic density fraction contained no significant difference between levels of PKM ζ in the lateral fraction of the naïve and trained fish ($t(7)= -0.11, p = 0.46$) (see Figure 6). There was, however a significantly higher level of PKC ζ in the lateral telencephalon of the trained student as compared to the control group ($t(14)= 1.85, p = 0.04$). PKC ζ is an isoform of

PKM ζ and higher levels of this isoform may still indicate long-term memory induction and potentiation.

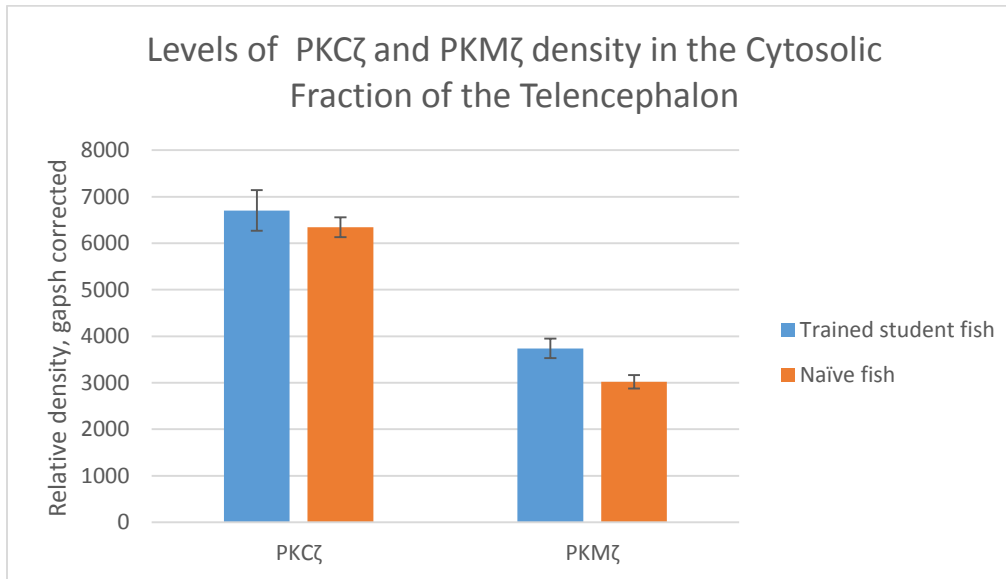


Figure 5. Relative differences in levels of PKC ζ and PKM ζ between trained student fish and untrained naïve fish in the cytosolic fraction of the lateral telencephalon.

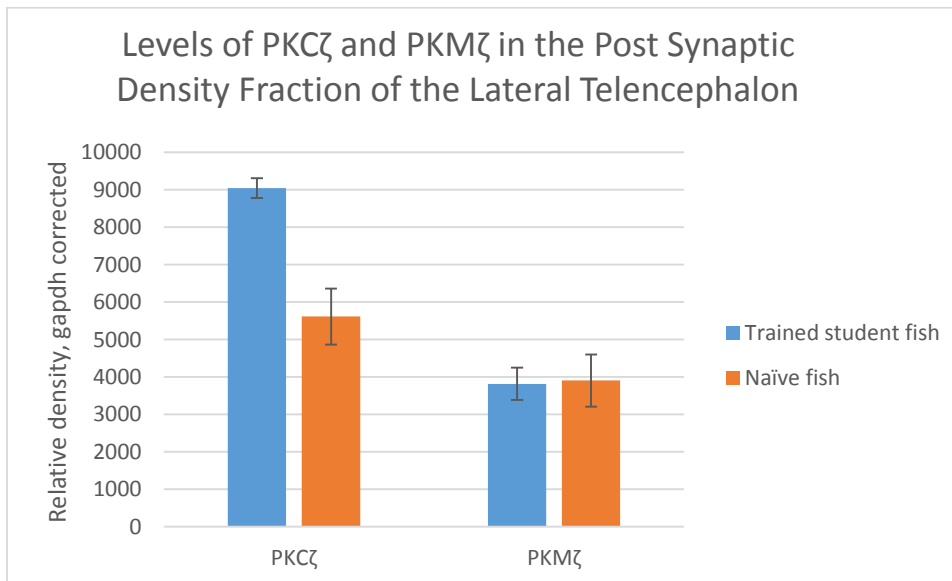


Figure 6. Relative differences in levels of PKC ζ and PKM ζ between trained student fish and untrained naïve fish in the post-synaptic fraction of the lateral telencephalon.

Discussion

The present study investigated the ability of *G. petersii* to transmit spatial information and path acquisition to an inexperienced conspecific, and the impact of the electric organ on that ability, by analyzing maze trial latency times, frequency of electric organ discharge and the levels of PKM ζ in the telencephalon across each experimental group.

Maze trial latency as an indicator of learning

In this study we compared the learning curves of maze acquisition of a naïve fish to those of a naïve “student” fish after initial paired training trials with a trained “teacher” fish. We then continued on to investigate how an electrically silenced, trained teacher fish would impact the learning of more naïve student fish. The average time of maze trials of each teacher per day were plotted and a trend line was extrapolated through the daily averages of all fish to determine the best-fit learning curves. The performance latencies of the independently run trials of the students and silent-teacher trained students were treated in the same manner. Students started performing much faster and took fewer days to asymptote in their maze performance than did the teachers, indicating possible social facilitation in the faster acquisition of a cognitive map. Silent-trained students started performing at a much slower time than even that of the naïve teachers, and subsequently began showing a steep learning curve that was similar to that of the teachers’ maze acquisition. Silent-trained student trials were suspended early due to subjects’ illness.

Independent sample t-tests statistically compared the average trial latencies of teacher and student fish, as well as student and silent-trained student fish. The latencies were significantly different on the initial day, indicating a faster initial acquisition of a cognitive map

for the student fish. The initial paired training day of experienced teachers and naïve students showed a significantly lower average latency than the initial teacher training day, showing that conspecific-aided maze navigation result in faster trials. The final day showed no significant difference between the groups, indicating an asymptoting of both learning curves to a comparable level. Indeed, the best-fit linear trend lines, or “learning curves”, of these two subject groups were not significantly different from each other. These results support the initial hypothesis of this study in that, after a training period with a conspecific, the ability to navigate a maze in an independent trial by a student immediately improved compared to the teacher, indicating some transmission of spatial information, leading to a shallower learning curve.

The naïve fish that were paired with an electrically silent teacher fish for training in maze navigation showed higher latencies during their initial trials, and steeper learning curves. The first day of solo training in the maze resulted in run times that were not significantly different from those of the initial training of the naïve teacher fish. However, both the trial latency and best-fit trend line were significantly greater than those of the intact-trained fish, indicating an advantage for the intact-trained fish. This result also indicates that the student does not simply gain an advantage from exposure to the maze during the paired training trials. The silent-trained student, after the same amount of initial exposure to the maze during training, still runs with latencies, as well as a steeper learning curve, comparable to those of a naïve fish on its first day alone in the maze, whereas the intact-trained fish has significantly shorter run times, so therefore the intact teacher must impart some benefit.

The abnormally poor initial performance and ensuing steep learning curve of the silent-trained students may be explained by the function of the electric organ discharge in conspecific communication. If this means of communication is impaired, the teacher may not be able to

effectively transfer information regarding the environment to its student. In fact, one could argue that the distraction from a fish with reduced sensory functions could result in an overall worse performance by the silent-trained student in the experimental maze, compromising its learning. Beyond that, deficits in active electrosense, on the part of the teacher, could diminish the ability to detect the presence of a conspecific (via distortion of its self-generated electric field) (Khait et al., 2009). This disrupts natural aggregating behaviors as well as any communication that would take place within that dynamic. Lack of close proximity in training trials could therefore negatively impact successful transmission of spatial information. Without electrical output of spatial information, the behaviors of the teacher may become more salient to its conspecific, and the student may resort to input via other sensory modalities, such as somatosensory or mechanosensory, to navigate the maze (confusing learning). Once alone in the maze, the fish can rely on its own electrical output and feedback to navigate to the goal box.

Role of the electric organ discharge in communication and learning

The electrical organ discharges generated by the *petersii*, as well as their electroreceptive capabilities, are employed in communication, and specific conspecific interactions produce observed changes to the EOD (Terleph & Moller, 2003). The frequency of the electric organ discharge, as well as the discharge itself, is an indicator of the behavioral state of the sender, in that the patterns of interval lengths between, and duration of, the EODs are indicative of specific behavioral situations and are important in communication (Carlson, 2002). Therefore, the inter-discharge interval (IDI), the length of time between organ discharges, can give insight into the interaction between the fish and the environment. Previous research shows longer discharges, with longer intervals between are produced by a fish at rest, and shorter discharge lengths, with

shorter intervals, are produced during times of active electrolocation, signaling a conspecific or increased presence of environmental stimuli (Carlson, 2002). In the present study, a fish swims faster through the maze as it learns how to navigate the course, and, in this excited state, produce more frequent EODs, thus IDI decreases.

In this study, the average IDI per day for the duration of training in the teachers and students, neither slopes have significant correlations. However, the best-fit linear trend line slopes of average IDI per day are significantly different between the teacher and the student. The difference in average IDI trends between the two subject groups indicates a complicated relationship between learning and behavior.

The EODs of the silent-trained students were not evaluated in this manner, because we did not get clean electrical readings from the trials and therefore did not have corresponding data to match the other experimental groups.

Role of PKM ζ in learning and memory

To understand learning and memory we look at long-term potentiation (LTP), a physiological model of memory. LTP generation requires different protein kinase enzymes (PKCs) for both induction and maintenance, with one specific PKC isoform, protein kinase M zeta (PKM ζ), being the only known molecule responsible for LTP maintenance (Bliss & Lomo, 1973); (Sacktor, 2008). PKC ζ is the only specific isoform of PKC that is expressed as stable PKM ζ in hippocampus (Sacktor, 2008; Serrano, 1993). Since the lateral pallium of the fish has been described as functionally homologous to the mammalian hippocampus, the same proteins involved in LTP in the mammalian brain would be active in the lateral pallium during learning and memory induction and maintenance.

Previous research has established that, during the induction phase of LTP, PKC is translocated to the particulate phase (including the post-synaptic density or, PSD) but increased levels do not persist into the maintenance phase. During the maintenance phase of LTP, there is an increase in PKM ζ in the cytosol (Sacktor *et al.*, 2003). PKM ζ production is a result of transcription and translation from a unique, PKM ζ specific mRNA that arises from a promoter within the PKC ζ gene (Sacktor, 2008). As these proteins are activated, they are transported to the membrane and increase AMPA receptors, which in turn enhances synaptic transmission (Sacktor, 2008). While it has already been documented in rats, recent research shows, for the first time, that while fish learn, there is an increase in the amount of PKM ζ in the lateral pallium of the telencephalon in teleost fish (Moller *et al.*, 2010).

In this study, a group of intact conspecific-taught student fish show a significantly greater average density of PKM ζ in the cytosolic fraction of the lateral telencephalon when compared to a group of naïve fish. The data also indicated, that while there was no significant increase of PKM ζ in the PSD of the lateral telencephalon, the student group had a significantly higher average density of PKC ζ in in this fraction as compared to the naïve fish. The PKC ζ levels in the PSD indicate possible induction of LTP, without yet achieving production and elevation of PKM ζ levels, indicating maintenance of LTP. However, increased levels of PKM ζ in the cytosolic fraction indicate possible maintenance of LTP.

Limitations

In the present study, there were a number of unforeseeable or insurmountable limitations that perhaps effected the results and did not allow the full and accurate completion of the intended study. Firstly, small sample sizes did not allow for any loss of subjects without severely

impacting the study. Secondly, upon reviewing the electrical data recorded, background noise that was unable to be filtered out impeded the accurate interpretation of electric signals from the fish in the maze and therefore, unreliable data was omitted from the study. Similarly, when two fish were in the experimental set-up together, the electric organ discharge emitted was too close to be able to parse out using the technology currently available to us. Since it was impossible to distinguish teacher fish from student fish, paired inter-discharge interval (IDI) data was omitted from this study.

This study spanned two laboratories over the course of three years, and during that time changes to protocols and experimental paradigms were made out of necessity. For example, to deal with fish that began to refuse to leave the start box, the protocols needed to be altered to allow light prodding of these subjects in such circumstances. Also, a new, improved maze system was built when the laboratory it was housed in was renovated. Further studies could build upon the experience and knowledge gained through these experimental iterations, and using a more refined and stable experimental design, help to better elucidate the methods of spatial transmission and communication, as well as the function of different sensory modalities, in navigation and memory retention of the weakly electric fish.

As previously mentioned, small sample sizes could significantly impact the results and skew data and anecdotal evidence of this was witnessed during the course of the study on the level of an individual fish. This fish was added to the home tank with the entire group of experimental animals a day before its training as a replacement for an initial subject that was found deceased. All other fish had been housed together through the acclimation phase and into the teacher training phases of the study. Fish that were housed together from the study's onset anecdotally showed novel behaviors of the teachers returning to the start box to retrieve lagging

students, as well as nudging them along in the correct direction of the maze path. The only fish to not be treated in this manner was the new student fish, added to the home tank late in the study. The teacher fish proceeded immediately to the goal box, where it remained, never returning to retrieve, or appear to communicate with, the new fish at all. This phenomenon is, perhaps, due to the social nature of *G. petersii*, and that they are only willing to teach familiar conspecifics; those that live in their social group. Nevertheless, the particular fish that was not aided, consistently had a longer maze run time than the other student fish for the duration of the study; potentially skewing the latency data, as well as the biochemistry data as a result of poor learning and memory consolidation. This behavioral phenomenon questions the impetus of an animal to teach its conspecific and anecdotally supports the basis the hypotheses in this study rely upon, which is that, as a social animal, there is an intrinsic motivation to transmit helpful information, spatial or otherwise, to your conspecific.

Molecular limitations

The sample size of fish used in the western blot analysis was affected by the ability to collect and microdissect the sample of telencephalon from each fish, as well as complete the subsequent fractionation of that sample. Subjects whose brains were not collected within an hour of their expiring were not eligible for this study, as changes due to autolysis could affect the results. Furthermore, the process of microdissection to retrieve lateral and medial sections from the telencephalon was not able to be performed on each sample collected, and so those samples were excluded as well. Finally, the process of fractionating the medial and lateral samples into the post-synaptic density and cytosolic fractions, relied on aged equipment that, during the process of extraction, malfunctioned and caused potential damage to a subset of the samples,

thereby excluding them from the data analysis as well. Lacking adequate samples of naïve or teacher fish to analyze via western blot, medial and lateral telencephalic samples from naïve fish unrelated to this study were substituted in as a comparison to the trained student fish used. Limitations on collection and extraction of the brain samples left us with a limited sample size, some of which not all conditions were controlled for, to gather molecular data from and therefore may not accurately reflect how learning and training affect densities of PKM ζ in the brain regions we examined in this study.

If we had, however, had adequate samples of maze-trained teacher fish and examined the molecular composition, we would expect to find non-significant differences in the levels of PKM ζ between the teachers and the trained students, since the maze latencies plateaued to similar times by the time of sacrifice and dissection. Similarly, we did not have adequate samples of the silent trained fish, and in this instance, we would expect to see lower levels of PKM ζ than in the teacher and intact-trained fish, since we saw no indication of learning in the silent trained fish.

Future studies

Firstly, trials with larger numbers of experimental animals need to be performed, to better understand the trends that began emerging from the present study and reduce the probability of error. Particularly of interest is the question of these “silent-trained students”, and how an impaired sensory modality impacts learning. Continuing a deeper, more systematic study of this effect would be necessary to better understand the complete narrative surrounding these questions. Secondly, better, more sensitive technology could be employed to record electric discharges and reduce the amount of noise in data. More sophisticated software can be obtained

and used to analyze these recordings and help parse out those discharges of two individuals within the same space so we can further study what happens in the maze within a teacher-student pair group. Furthermore, experiments can be designed, building off of those performed in this study, to more deeply explore the socio-behavioral impact on communication and learning in this social species. By comparing conspecifics housed together versus those housed alone or with other animals not part of the study, we can investigate altruism in the teaching/learning dynamic of unfamiliar individuals.

Since the electric sense is a specially adapted sensory system that aids in communication and navigation, it would seem to play a critical role in conspecific interactions, experience and learning. However, lack of one sensory modality may increase other methods of spatial navigation as well as effective communication and teaching. Like the honeybee, the electric fish predominantly relies on its preferred sensory modality, but can engage multiple modalities in a synergistic fashion to achieve its goals. If there becomes a deficit in that modality, it can resort to the others in a hierarchical order (von Frisch, 1967; Walton & Moller, 2010). Information gathered from these studies could help understand the importance of other sensory modalities in learning and communication, and how and why these modalities arise.

To better understand the role of PKM ζ , it is necessary to gather more complete baseline data regarding the density of this molecule within the telencephalon. Comparing PKM ζ levels as a response to acquisition of spatial information, across different conditions and time points, including the initially naïve “teacher” condition, comparing to the trained students and the silent-trained students. By comparing performance in the maze after longer periods away from the initial training event versus the amount of PKM ζ present in the lateral telencephalon at those time points, we can also look at long-term potentiation, or how long memory is retained. As in

the honeybee, the sensory system and social organization of this species allows for a unique opportunity to observe a direct transfer of information as a result of physiological and molecular processes taking place. Although what that information is, and how it conveys distance and trajectory remains a mystery, but speculation could indicate that specific patterns of EODs produced, physical proximity of animals in a space, and perhaps the activation of place cells in the lateral pallium, producing an internalized cognitive map, are involved. Studies of social learning and memory in the context of a hippocampal homologue is useful in better understanding the role of conspecific communication and specially adapted sensory systems in the animal kingdom, as well as spatial navigation, learning and memory in humans.

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