Detection of Water Flow by Japanese Eel (*Anguilla japonica*): Behavioral and Pharmacological Analyses

Shigeru Watanabe

Department of Psychology, Keio University

Email: swat@flet.keio.ac.jp

Abstract – Eels migrate long distances and their spatial learning ability based on extra-maze visual cues has been examined in the laboratory. Here, I examine the discriminative properties of water flow in their spatial cognition. Individuals of the Japanese eel (*Anguilla japonica*) were trained in a circular pool containing four pipes. One pipe was open where the eels could enter, and the other pipes were closed. A small motor-driven water screw was placed beside each pipe. The water screw close to the open pipe was active to allow water flow, whereas the other screws were inactive. The position of the open pipe with the active screw was randomly changed, and the eels could learn the position of the open pipe after approximately 15 trials. Overall, five tests were conducted. Test 1: A generalization test showed a generalization gradient along the distance from the open pipe and the active screw. Test 2: A test using a screw with no blades (i.e., no water flow but possible vibration or sound by a motor is present) resulted in chance level performance, suggesting that the eels needed water flow to find the open pipe. Test 3: A test with a water pump that produced water flow by a mechanism different from that of the original motor-driven screw showed that the eels maintained the detection of water flow under this condition. Test 4: The eels also located the open pipe in a dark room test; therefore, visual cues were not used for the detection of the open pipe. To confirm that the water flow was detected by the lateral organ, streptomycin sulfate was dissolved in a home tank to impair the hair cells in the lateral line organ in Test 5, and the eels were not able to detect the open pipe under these conditions. This observation suggests that the detection of water flow depends on the lateral-line organ. In summary, the data show that eels can detect water flow to find hiding locations.

Keywords – Fish, Spatial learning, Lateral line organ, Streptomycin

_____

Fish display remarkable orientation and navigation abilities during migration (Dodson, 1988), and eels, in particular, possess strong migratory capacities. Hatched larvae of the Japanese eel (*Anguilla japonica*) in the West Mariana region migrate to Japan, and adult eels swim 2000 km back to the West Mariana (Tsukamoto et al., 2011). However, their home range after upstream trips in rivers is generally small (0.05 km^2), although transport-release experiments have demonstrated their ability to return to their original area (Itakura et al., 2017).

The relationship between large-scale spatial behavior such as migration and small-scale spatial learning in the laboratory is not well known; however, spatial learning of fish has been studied using a variety of experimental settings. The jumping goby (*Bathygobius soporator*) swims over a tidal area during high tide and learns the topography of the region. Aronson (1971) constructed artificial tide pools and reported that these fish learned the configuration of the environment during high tide and used this information to jump to safe tide pools during artificial low tide. The spatial learning of fish has been examined using the T or Y-maze (Benhaım et al., 2013; Spence et al., 2011; White & Brown, 2015a; b;

Zerboloio & Wickstra, 1980), plus maze (Rodríguez et al., 1994; Sison & Gerlai, 2010), diamond-shaped enclosure (López et al., 1999), radial arm maze (Roitblat et al., 1982), and Morris maze-like pool (Durán et al., 2008; Saito & Watanabe 2004; 2005).

However, no experimental studies have been conducted on the spatial learning of eels except for our two reports. Recently, we examined the spatial learning of the Japanese eel using the Morris maze-like apparatus (Watanabe & Shinozuka, 2019). Eels prefer to hide in pipes, and this behavior determines the reward for spatial learning. Four pipes were arranged in a circular water pool, one of which was open for the eels to enter, whereas the other pipes were closed. The position of the open pipe was fixed, and there were several extra-maze cues, such as furniture and posters, around the pool. The eels learned the task and maintained discrimination when the pool was rotated to remove possible intra-maze cues, such as small scratches on the wall, but did not maintain discrimination in the dark room condition where they could not use the visual extra-maze cues. Watanabe (2020) conducted a similar experiment with eels; however, it included both extra-and intra-maze cues. The intra-maze cue was a triangular block that was fixed to an open pipe. While some of the subjects displayed discrimination based on the position cues (extra-maze cue), others used the intra-maze cue. The Japanese eel is thus able to orient the position of an open pipe based on either type of cue.

Researchers have examined the sensory modalities of eels and reported their olfaction (Barbin et al., 1998; Westin, 1990), vision (Byzov et al., 1998; Omura et al., 1997), and magnetic sensing (Cresci et al., 2017; Naisbett-Jones et al., 2017). The retina of Japanese eel larvae contains cones (Omura et al., 1997), and Byzov et al. (1998) reported yellow-sensitive and green-sensitive cones in European eels (*Anguilla anguilla*), indicating that these animals have color vision.

It is possible that fish use multiple sensory modalities for spatial behavior in natural settings. Eels may also use information from water flow for spatial orientation. Under high water velocity gradients, eels rapidly escaped back upstream, whereas exploratory search behavior was common when water acceleration was low (Piper et al., 2015). Orientation using a lateral line system for migration in fish has been suggested (Montgomery et al., 1995). Thus, eels may also be using this mechanism for spatial orientation.

The lateral line is a mechanosensory organ in fish that detects hydrodynamic stimuli (Coombs et al., 1989; Janssen, 2004; Mogdans et al., 2004; Montgomery et al., 1997; Webb, 2014). This organ consists of two types of structures: superficial neuromasts (SNs), which are distributed on the surface of the skin, and canal neuromasts (CNs) embedded in the subepidermal canals. The SN is sensitive to water velocity or the DC component of vibration, and the CN detects the pressure gradient between the canal pores or the AC component (Kanter & Coombs, 2003). Electrophysiological responses to hydrodynamic stimuli have been recorded from the lateral line organ (mottled sculpin: Coombs & Janssen, 1990; cichlid fish (*Sarotherodon niloticus)* (Münz, 1985). By recording the lateral line afferent nerves of New Zealand long-fin eels (*Anguilla dieffenbochii)*, it was revealed that more than 80% of the nerves were water flow-sensitive (Voigt et al., 2000). Parts of the torus semicircularis of catfish (*Ancistrus spp.*) that received afferents from the lateral line organ showed selective activation by particular moving objects (Muller, 1996), and these observations suggest a complex distant sensation of the lateral line system. The lateral line system detects water surface waves (Bleckmann et al., 1989), moving objects (Vogel & Bleckmann, 1997), prey (Montgomery & Hamilton, 1997; Pohlmann et al., 2004), predators (Blaxter & Fuiman, 1989), and conspecifics (Anneser et al., 2020). This system contributes to maintaining schooling (Partrige & Picher, 1980) and spatial maps (de Perera, 2004).

There are variations in the distribution patterns of lateral line organs in different species (Cernuda-Cernuda & Garcfa-Fernnade, 1996; Wellenreuther et al., 2010). Recently, Nakae et al. (2021) reported the details of the morphology of the lateral line organ in the Japanese eel. They observed 13 well-developed groups of SNs, seven groups of CNs, and specific commissures connecting the left and right canals. Variability in the lateral line organs depends on the lifestyle or environment of the fish. Nonvisual cues are important for nocturnal predators (Montgomery & Hamilton, 1997); therefore, the lateral line system is useful for these eels.

The lateral line organ receptors are hair cells; therefore, antibiotics such as streptomycin impair the functioning of this organ (Baker & Montgomery, 1999; Kaus, 1987; Mogdans & Nauroth, 2011).

Because the streptomycin impairs protein synthesis in mycobacterium tuberculosis, it is used for treatment of tuberculosis, but it also damages hair cells in the inner ear and causes so-called streptomycin deafness in human patients. Thus, the streptomycin has been used to damage hair cells in the lateral line organ (for example, Kaus, 1987; Mogdans & Nauroth, 2011). Morphologically, cell death in the lateral line organ after gentamicin treatment has been observed (Brown et al., 2011).

There have been no known laboratory studies on the discrimination of water flow in eels, and here, I trained Japanese eel individuals to detect water flow using an apparatus invented for spatial learning of eels (Watanabe & Shinozuka, 2019). In the present experiment, the water flow cue was produced by a small motor-driven water screw, and the eels were then tested in dark conditions with a bladeless screw and a water pump to examine how the water flow controls the spatial behavior of the eel. They were also tested at different distances from the screw to the open pipe. Finally, I used streptomycin sulfate to confirm that inactivation of the lateral line organ impairs the water flow detection.

Methods

Ethical Approval

Ethical review and approval were not required for the animal study because the fish were not the target of our animal experiment committee. All animals were treated in accordance with the guidelines of the Japanese Society for Animal Psychology. No evidence of deprivation or physical invasion was found.

Subjects

Eleven Japanese eel individuals were obtained from the NPO Safty Life River (Miyazaki, Japan). The eels were cultured before arrival at the laboratory. They were all in the yellow stage (not ready for reproduction), but their exact ages were unknown. Their total body lengths ranged from 22.5 cm to 38.2 cm. The eels were housed individually in a home tank ($39.8 \times 25.4 \times 28$ cm) with an air pump. The depth of water was 24 cm. Sand was placed on the floor of each tank, and a gray vinyl polychloride tube (inner diameter: 25 mm; length: 240 mm) was placed in each tank. The experiments were initiated one week after the arrival of the eels in the laboratory. A 13 L:11 D artificial illumination cycle was used. However, the racks for the tanks were covered by a gray vinyl curtain so that the animals received only indirect, low-intensity illumination. Earthworms were provided once a week, although some eels did not consume them.

Apparatus

The experimental apparatus consisted of a white polypropylene circular pool with a diameter of 110 cm and a height of 38 cm (Figure 1d). The water level was maintained at 10 cm from the bottom of the pool. The water temperature was maintained at 22°C, and the water was changed every five days. The experimental room was illuminated by a fluorescent lamp. The illuminance of the water surface was 368 lx. The pool contained four gray vinyl polychloride pipes (inner diameter: 25 mm; length: 340 mm), and each pipe had four lead weights ($20 \times 20 \times 20$ mm) to affix it to the floor. The distance between the outer edges of the pipes was 50 cm. A transparent acrylic cylinder (length: 20 mm) was inserted into each end of the three closed pipes and fixed by acryl screws such that the eels could not enter the tube (Figure 1c). For the open pipe, acrylic cylinders were placed but not fixed with screws. Thus, the eels could push the cylinders and enter the pipe.

Small motor-driven water screws designed for toy submarines (Figure 1a. Submarine motor NINI, Tamiya Inc., Shizuoka, Japan) that were activated by a 1.5 V battery were used to produce the water flow. Each screw was 8.0 cm in length, had two blades, and was fixed on a copper cylinder (diameter: 4.0 cm; height: 1.0 cm). The screws were placed on the right side of the pipe at a distance of

5.0 cm. One of the motor screws was turned on and the pipe beside it was open. The screws faced the pool wall; thus, the water flow was directed toward the wall, and the distance between the blades and the wall was 40 mm. The other three water screws close to the closed pipes were turned off, thereby preventing water flow. The battery was changed every third day to maintain a constant power. The surface speed of the water flow was measured at 5[remove .0], 10, and 20 cm from the screw using a float (diameter: 5 mm) (Figure 1e). During measurements, no pipes were placed in the pool, and the direction of the flow was toward the open space as opposed to toward the wall. Mini water aquarium pumps (Figure 1b; Qiilu, China) with heights of 4.0 cm and diameters of 2.0 cm were used for the test 3 described later. A nozzle (inner diameter of 5 mm) was connected to the bottom to produce water flow. A DC power controller supplied electricity to the pump to produce approximately the same water flow to the screw. Dechlorinated tap water was used in both test pool and home tanks.

Eel behavior was monitored using a charge-coupled device (CCD) camera (G100, NEC Avio, Tokyo, Japan) fixed above the pool. A night scope (Super Night Compact 1000 NDX; Kenko Tokina Co., Ltd., Tokyo, Japan) was used to observe the eels in the dark room test.

Figure 1

Experimental Setting
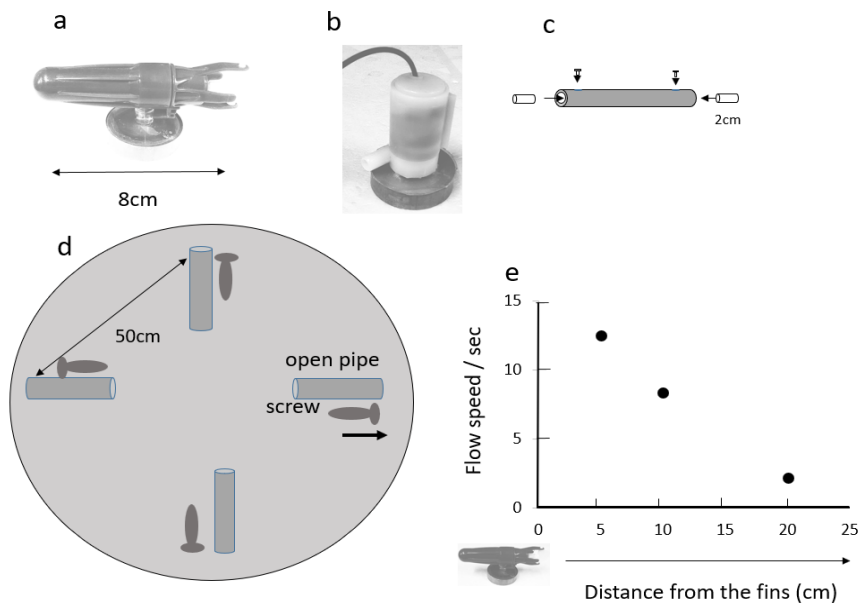
Note. a: Water screw designed for a toy submarine. It has two blades at the left end and a battery is inserted into the right part. b: Water pump designed for an aquarium. It produces water flow from a small nozzle at the bottom. Electricity is provided by a wire. c: Closed pipe with a small acryl fixed by a screw at each end. The screws are not fixed for an open pipe. d: Pool for training (the diameter 110 cm). It contains four pipes and four water screws. Only the screw closed to the open pipe produces water flow toward the wall. e: Surface speed of water flow at different distances. Horizontal axis is distance from the blades and vertical axis speed of surface water flow.

Procedure

Habituation to the Apparatus

Each eel was individually removed from the home tank into a carrying bucket using a nylon net and subsequently gently released into the pool from the bucket. During the habituation phase, all pipes were opened, and the eels were allowed to move around the pool for 10 min. Screws were not placed in the pool. Generally, eels entered one pipe and remained there. After 10 min, the pipe was removed, the eel slid back

into the net, and was returned to the home tank. All pipes were cleaned with a brush, and the water was gently stirred to remove possible olfactory cues. Subsequently, the next eel underwent habituation training. This procedure was repeated for two days.

Discriminative Training

The subjects were divided into an experimental group (n = 7) and a control group (n = 4). The experimental group underwent discriminative training that was identical to that in the habituation phase, except that the water screws were placed beside the pipes, and only one pipe with an active screw was open. An active screw refers to the activation of its motor by a battery to produce water flow. The remaining three pipes were closed. An eel could visit the closed pipes as it searched for the open pipe. Upon entering the open pipe, it could remain there for 10 min. If an eel did not enter the pipe, it was retrieved after 10 min. The eels underwent one training trial per day and the position of the open pipe was randomly changed. A visit or choice was defined as the insertion of the head into the pipe. The first choice of pipes was used as the data for the day. When the eel directly entered the open pipe without visiting other pipes, it was counted as the correct response. The number of incorrect visits to the closed pipes and the time taken to reach the goal (open pipe) were also recorded. Three correct responses in four successive trials was selected as the discrimination criterion. The eels underwent at least ten training trials.

For the four eels in the control group, the positions of the open pipe and the active screw were randomly changed independently every day; thus, the water flow did not provide any information about the position of the open pipe. However, the subjects might have learned through rapid scans of the four pipes or avoided revisiting the closed pipes that they had already visited. The subjects underwent 15 such trials.

Tests

The experimental group underwent five tests after achieving the discrimination criterion. Each test consisted of four trials. The first test was a water flow generalization test, consisting of three subtests, each containing four trials. The distance between the open pipe and the screw was 5 cm at the subset one, 10 cm at the subset two, and 20 cm at the subset three. The procedure was identical to that used for the ordinal training. The second test, the dark room test, was identical to ordinal training, except that the room light was not turned on. In the third test, the no-blade test used an active water screw without blades to produce no water flow but vibration of the motor in the toy screw. In the fourth test, a mini water pump was used instead of a screw. The pump was placed beside each pipe and only the pump near the open pipe was active. Finally, in the fifth test, the eels were treated with streptomycin. Two grams of streptomycin sulfate (Fuji film Wako Pure Chemical Corporation, Osaka, Japan) was dissolved in 10 L of water in the home tank (0.2 g/L). The eels lived in a tank with streptomycin during the four days of testing.

In addition to the number of correct responses, the numbers of incorrect visits to closed pipes and excursions to the correct pipes were recorded.

The tests were performed in the same order as described above. Two trials of regular training were performed between different tests. When the subject showed an incorrect response during this period, more training was provided to maintain the discrimination level.

Statistical Analyses

Detection was defined as the number of correct responses (i.e., entering the open pipe). To analyze performance in the tests, the number of correct responses divided by the number of trials (four) was compared with the chance level (.25) using single-sample t-tests (significance level .05). One-way analysis of variance (ANOVA) was used to analyze the generalization test. The factor was the distance between the screw and the open pipe, and the analysis was applied to the number of correct responses, number of errors, and time taken to reach the open pipe.

Results

Discrimination

Figure 2a shows the average forward learning curve of the experimental group, expressed as the cumulative number of correct responses. The number of eels decreased in later trials because the eels that met the discrimination criterion did not receive further discriminative training. The fastest eel met the criterion in 10 trials and the slowest in 19 trials (average: 14.1 trials). The solid black line indicates the average, the broken line indicates the expected cumulative number by chance, and the gray lines indicate the highest and lowest numbers in each trial. All the eels achieved the discrimination criterion. Figure 2b shows the results for the control group. The cumulative correct responses were lower than the expected values for the 15 trials. None of the eels met the discrimination criterion within the 15 trials.

Figure 2

Learning Curves
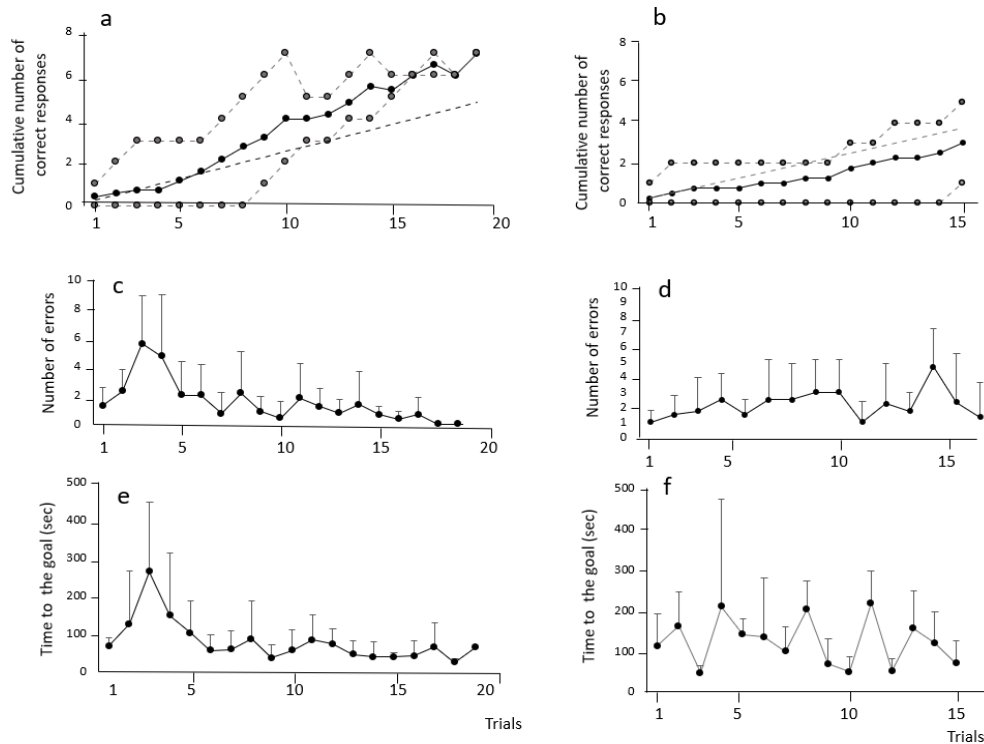
Note. Forward discriminative learning curves of the experimental group (left) and control group (right). a and b: Cumulative number of correct responses. Black line indicates average, and broken lines with circles denote the best and worst number of cumulative correct responses. The straight broken line indicates expected cumulative responses. c and d: The number of incorrect visits. e and f: time taken to reach the goal (open pipe). Vertical axis in each graph is averaged value. Small vertical bars indicate standard deviations.

Figures 2c and d show the number of incorrect visits to the closed pipes before the eels reached the open pipe. The eels often revisited the pipes that they had previously visited. Clearly, the experimental group showed a reduction in the number of incorrect visits, whereas no such reduction was observed in the control group.

Figures 2e and f present time taken to reach the goal during the trainings. The experimental group showed a decrease in time taken to reach the goal. The mean time taken to reach the goal for the first five trials was 141.2 sec (SD = 119 sec) and 52 secs (SD = 38.3 sec) for the last five trials, and these were

significantly different ($t(4) = 2.74$, $p = .05$, 95% CI [103.4, 179 for the first five and 32.7, 71.3 for the last five trials], Cohen's $d = 1.01$). In the control group, the mean time taken to reach the goal in the first five trials was 120.1 sec (SD = 40 sec) and 123.7 sec (SD = 43.1 sec) in the last five trials, and no significant difference was shown (confidence interval of the first and last five trials were 120.1 ± 110.5 and 123.7 ± 27.8 respectively, $t(4) = 0.08$, $p = .94$, 95% CI [96, 230.6 for the first five and 95.9, 151.5 for the last five trials], Cohen's $d = 0.09$).

Tests

Figures 3a–c reveal generalization along the distance from the open pipe to the screw. The eels showed a reduced number of correct responses depending on the distance (Figure 3a). The correct responses differed significantly from the chance level at 5 cm ($t(6) = 12.02$, $p < .001$, 95% CI [.73, .97], Cohen's $d = 43.1$) and 10 cm ($t(6) = 7.78$, $p < .001$, 95% CI [.52, .76], Cohen's $d = 27.6$), but not at 20 cm ($t(6) = .79$, $p = .45$, 95% CI [.1, .54], Cohen's $d = 4.95$). The eels could not find the correct pipe when the water screw was placed 20 cm away. One-way ANOVA of the correct responses/trial showed a significant effect of the distance ($F (2/20) = 16.55$, $p = .001$, $\eta^2 = 0.65$). Thus, the systematic decrement of water flow detection by distance was clearly observed. In the case of the number of incorrect visits (Figure 3b), one-way ANOVA revealed a significant effect of distance ($F (2/20) = 9.16$, $p = .002$, $\eta^2 = 0.50$), whereas no effect was shown for the times to the goal ($F (2/20) = 0.06$, $p = .94$, $\eta^2 = 0.01$) (Figure 3c).

Figure 3

Results of the Tests
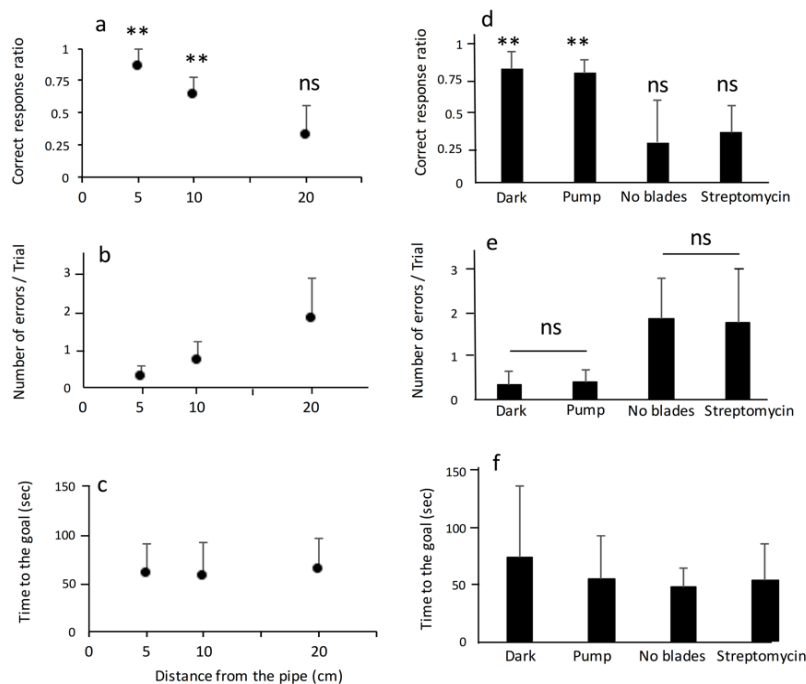
Note. Results of the tests in the experimental group. Left: generalization test. a: mean number of correct responses divided by trials. '**' indicates significant difference from the chance level ($p < .05$). b: mean number of incorrect visits divided by trials. ANOVA results indicated a significant effect of the distance. c: mean time reach to the goal (open pipe). ANOVA results did not indicate a significant effect of the distance. Small vertical bars indicate standard deviations. Right: four other tests. d: mean number of correct responses divided by trials. e: mean number of incorrect visits to the closed pipes divided by trials. f: mean times to reach the goal. Vertical axis in each graph is averaged value. Small vertical bars indicate standard deviations. '**' indicates a significant difference from the chance level in t-test (p < .05).

Figure 3d shows the correct responses in the other four tests. The correct responses were significantly higher than those by chance in the dark room test ($t(6) = 12.40$, $p < .001$, 95% CI [.71, .93], Cohen's $d = 6.72$). The test with the pump produced significantly greater correct responses than those by chance ($t(6)=15.0$, $p < .001$, 95% CI [.70, .88], Cohen's $d = 8.49$). The test with no blades resulted in chance level performance ($t(6) = 0.31$, $p = .76$, 95% CI [0, .56], Cohen's $d = 0.19$). The performance in the streptomycin test was no greater than that by chance level (confidence interval $= 0.39 \pm 0.11$, $t(6) = 1.93$, $p = .10$, 95% CI [.28, .50], Cohen's $d = 0.82$). None of the animals displayed three or more correct responses during the test. There was no significant difference between the dark test and the test with the pump (confidence interval of the dark test and test with the pump were 0.82 ± 0.11 and 0.79 ± 0.09 respectively, $t(7) = 0.55$, $p = .60$, 95% CI [071, .93 for the dark and .70, .58 for the pump tests], Cohen's $d = 0.28$) or between the test with no blades and that with streptomycin ($t(7) = 0.60$, $p = .57$, 95% CI [.01, .57 for the no blades and .11, .57 for the streptomycin tests], Cohen's $d = 0.28$).

Figure 3e shows the number of incorrect visits. There was no significant difference between the dark test and that with the pump ($t(7) = 0.37$, $p = .73$, 95% CI [0, .64 for the dark and .13, .65 for the pump tests], Cohen's $d = 0.22$) or between the test with no blades and that with streptomycin ($t(7) = 0.42$, $p = .69$, 95% CI [1.03, 2.69 for the no blades and .44, 2.92 for the streptomycin tests], Cohen's $d = 0.18$). There was no significant difference. Figure 3f shows the time required to achieve this goal. A one-way ANOVA showed no significant effects of the tests ($F (3,/27) = 0.56$, $p = .65$, $\eta^2 = 0.07$).

Discussion

The present experiments demonstrated the detection of water flow by the Japanese eel. Clearly, the experimental group showed an increment in the correct response and a reduction in the number of incorrect visits, whereas no such reduction was observed in the control group. The reduction in the correct response along the distance between the open pipe and the screw shows the control of the eels' behavior by the water flow. The dark room test demonstrated that visual cues from the active blades possibly did not provide sufficient information for detection. The test with the screw without blades showed that the water flow produced by the blades was crucial for the detection. Contrasting this, the eels maintained their discriminative behavior in the test with the water pump, indicating that the water flow produced by a mechanism different from the original screw controlled the behavior of the eels. Streptomycin was expected to impair hair cells in the lateral organs and disturb the detection of water flow.

No significant difference between the test with no blades and that with streptomycin suggested that the effects of streptomycin mimicked the absence of water flow, and no significant difference between the dark test and that with the pump suggested that the pump mimicked the water flow produced by the water screw.

The detection criterion was based on the number of correct responses, but I also analyzed the number of incorrect visits to the closed pipes and the time taken to reach the goal. The correct responses and number of incorrect visits displayed a similar pattern in the five tests, but the time taken to reach the goal did not differ among the tests. The time taken to reach the goal had a large variability owing to the spatial relationship between the release points and the goal. This large variability should result in no significant differences in the statistical analyses.

Previously, we used a maze similar to the present study to demonstrate spatial learning in the Japanese eel (Watanabe & Shinozuka, 2019). On average, the eels learned the maze task in 16.4 trials (n = 12). There was no significant difference in the number of trials required to reach the criterion between that study and the present experiment (unpaired two-tailed t-test, $t(17) = 1.04$, $p = .31$, 95% CI [13.22, 19.6 for the previous and 10.73, 17.53 for the present results], Cohen's $d = 2.80$). In another experiment, eels were trained in a pool with extra- and intra-maze cues (Watanabe, 2020). In this case, the animals learned the task in 14.3 trials on average (n = 9). There was also no significant difference in the number of trials required in that study compared to the present experiment (unpaired two-tailed t-test, $t(14) = 0.12$, $p = .90$, 95% CI [12.34, 16.32 for the previous and 10.73 , 17.53 for the present results], Cohen's $d = 0.06$). Thus, the Japanese eel learned spatial discrimination based on visual or water flow cues with approximately the same

amount of training. Goldfish demonstrated spatial learning in a Morris-type maze with a food reward in 35.2 trials on average without intra-maze cues, and in 22.4 trials with intra-maze cues (Saito & Watanabe, 2004).

Studies on spatial learning based on non-visual cues, such as that of C57/BL6 mice, have shown successful spatial discrimination in a dry-type Morris maze based on auditory cues (Watanabe & Yoshida, 2007) in an average of 12 trials (in terms of reduction of reaction time to reach the goal). The mice also learned the maze based on airflow cues (Bouchekioua et al., 2015) in approximately 12 trials. These observations suggest successful spatial learning based on non-visual cues in two distant species: eels and mice.

There have been several studies on damage to the lateral line organ due to antibiotics. Kaus (1987) trained striped panchax (*Aplocheilus lineatus*) to react to waves using a food reward, and then tested the effect of streptomycin. He added streptomycin to the home tank and found that 5.5 mg–44 mg/L streptomycin disturbed the detection of the wave. Mogdans and Nauroth (2011) trained oskars (*Astronotus ocellatus*) to discriminate a 100 Hz sinusoidal vibration from other frequencies using a food reward, and found a reduction in detection following streptomycin treatment (1 g/L); however, the detection recovered after two–four weeks. In the present experiment, 0.2 g/L streptomycin was added to the home tank, and disturbance of the water flow detection was observed. The testing was repeated for four days, but an increment in impairment over time did not occur. Most previous studies with streptomycin placed animals in a tank with the drug for 3 h and then transferred them to a pool with fresh water, while the subjects in the present experiment were kept in the tank with the drug. Because the eels remained in a tank with the drug for 24 h, it is most likely that the hair cells of the lateral line organs were already strongly damaged. Thus, an increment in impairment over the four days was not observed. The present results suggest that the eels detected the water flow through the lateral line organ but did not show an increase in the time taken to reach the correct pipe, suggesting that the drug did not disrupt their swimming ability. As described in the introduction section, the lateral line organ has two subsystems, the SN and CN, and damage to the hair cells by streptomycin is non-selective; therefore, functional separation of the SN and CN in water flow discrimination is undetermined in the present experiment.

There is wide diversity in the distribution of the lateral line organ in different fish, and this diversity is probably related to their ecology (Jansen, 2004). The eel adapts to different environments during its life cycle, namely sea water or fresh water, in different stages such as migration and staying stages. Nakae et al. (2021) suggested that morphological changes occur in lateral lines depending on the developmental phase. The eels in the present experiment were cultivated, that is, they were in the yellow phase (staying phase). Those in the silver phase (migrating phase) may display faster learning, because they require finer water flow discrimination for migration. Thus, experimental analysis of spatial learning based on hydrodynamic perception in eels at different developmental stages will provide an ecological understanding of the functions of the lateral line organ and its function in migration.

Conclusions

The present study demonstrated successful spatial learning using water-flow cues in the Japanese eel. Several tests clarified that the learning was based on water flow and not on other possible cues. In particular, the gradient of the water flow detection along the change in the water flow demonstrated the control of detection by the water flow. The streptomycin test confirmed that the lateral line organ was crucial for water flow discrimination.

Acknowledgements

References

Anneser, L., Alcantara, I. C., Gemmer, A., Mirkes, K., Ryu, S., & Schuman, E. M. (2020). The neuropeptide Pth2 dynamically senses others via mechanosensation. *Nature, 588*(7839), 653–657. https://doi.org/10.1038/s41586-020-2988-z

Aronson, L. R. (1971). Orientation and jumping behavior in the bogie fish, *Bathygobius soporator*, *American Museum Novitiates, 188*, 14861–14822. http://hdl.handle.net/2246/3993

Baker, C. F., & Montgomery, J. C. (1999). Lateral line mediated rheotaxis in the Antarctic fish (*Pagothenia borchgrevinki*). *Polar Biology, 21*(5), 309. https://doi.org/10.1007/s003000050366

Barbin, G. P., Parker, S. J., & McCleave, J. D. (1998). Olfactory clues play a critical role in the estuarine migration of silver-phase American eels. *Environmental Biology of Fishes, 53*, 283–291. https://doi.org/10.1023/A:1007469232698

Benhaım, D., Bégout, M. L., Lucas, G., & Chatain, B. (2013). First insight into exploration and cognition in wild caught and domesticated sea bass (*Dicentrarchus labrax*) in a maze. *PLoS ONE 8*(6), e65872. https://doi.org/10.1371/journal.pone.0065872

Blaxter, J. S., & Fuiman, L. A. (1989). Function of the free neuromasts of marine teleost larvae. In: Coombs, S., Görner, P., Münz, H. (eds). *The Mechanosensory Lateral line: Neurobiology and Evolution.* p. 481–499. Springer-Verlag.

Bleckmann, H., Tittel, G., & Blubaum-Gronau, E. (1989). The lateral line system of surface feeding fish: anatomy, physiology and behaviour. In: Coombs, S., Gorner, P., & Munz, H. (eds). p. 501–526. *The Mechanosensory Lateral Line: Neurobiology and Evolution*. Springer-Verlag.

Bouchekioua, Y., Minura, M., & Watanabe, S. (2015). Air flow cued spatial learning in mice. *Animal Cognition, 18*, 339–404. https://doi.org/10.1007/s10071-014-0809-1

Brown, A. D., Mussen, T. D., Sisneros, J. A., & Coffin, A. B. (2011). Reevaluating the use of aminoglycoside antibiotics in behavioral studies of the lateral line. *Hearing Research, 272*(1),1–4. https://doi.org/10.1016/j.heares.2010.10.014

Byzov, A. L., Damjanović, I., Utina, I.A., Mićković, B., Gačić, Z., & Andjus, R.K. (1998). Electrophysiological and spectral properties of second-order retinal neurons in the eel. *Comparative Biochemistry & Physiology Part A, 121*(2), 197–208. https://doi.org/10.1016/S1095-6433(98)10122-8

Cernuda-Cernuda, R., & Garcfa-Fernnade, J. M. (1996). Structural diversity of the ordinary and specialized lateral line organs. *Microscopy Research & Technique, 34*(4), 302–312. https://doi.org/10.1002/(SICI)1097-0029(19960701)34:4<302::AID-JEMT3>3.0.CO;2-Q

Coombs, S., & Janssen, J. (1990). Behavioural and neurophysiological assessment of lateral line sensitivity in the mottled sculpin, *Cottus bairdi. Journal of Comparative Physiology A, 167*(4), 557–567. https://doi.org/10.1007/BF00190827

Coombs, S., Gorner, P., & Munz, H. (eds) (1989). *The mechanosensory lateral line: Neurobiology and evolution.* Springer.

Cresci, A., Paris, C. B., Durif, C. M. F., Shema, S., Bjelland,R.M., Skiftesvik,A.B., & Browman,H.I.(2017). Glass eels (*Anguilla anguilla*) have a magnetic compass linked to the tidal cycle. *Science Advances, 3*(6), e1602007. https://doi.org/10.1126/sciadv.1602007

de Perera, B.T. (2004). Fish can encode order in their spatial map. *Proceedings of Royal Society of Science London, 271*(1553), 2131–2134. https://doi.org/10.1098/rspb.2004.2867

Dodson, J. J. (1988). The nature and role of learning in the orientation and migratory behavior of fishes. *Environmental Biology of Fish, 23*(3), 161–182. https://doi.org/10.1007/BF00004908

Durán, E., Ocańa, F. M., Gómez, A., Jimenez-Moya,F., Broglio,C., Rodoriguez,F., & Sala,C. (2008). Telencephalon ablation impairs goldfish allocentric spatial learning in a 'hole board' task. *Acta Neurobiologiae Experimentalis (Wars), 68*(4), 519–525.

Itakura, H., & Mitake, Y. (2017). Site fidelity, diel and seasonal activities of yellow-phase Japanese eels (*Anguilla japonica*) in a freshwater habitat as inferred from acoustic telemetry. *Ecology of Freshwater Fish, 27*(3), 1–15. https://doi.org/10.1111/eff.12389

Janssen, J. (2004). Lateral line sensory ecology. Von der Emide, Magdans, J., Kapoor, B.G. (eds) *The Senses of Fish*. Springer-Science+Business Media, p231–-264.

Kanter, M. J., & Coombs, S. (2003). Rheotaxis and prey detection in uniform currents by Lake Michigan mottled sculpin (*Cottus bairdi*). *Journal of Experimental Biology, 206*(1), 59–70. https://doi.org/10.1242/jeb.00056

Kaus, S. (1987). The effect of aminoglycoside antibiotics on the lateral line organ of *Aplocheilus lineatus (Cyprinodontidae)*. Acta Otolaryngol (Stockh), *103*(3), 291–298.

López, J. C., Broglio, C., Rodrıguez, F., Thinus-Blanc, C., & Salas, C. (1999). Multiple spatial learning strategies in goldfish (*Carassius auratus*). *Animal Cognition, 2*, 109–120. https://doi.org/10.1007/s100710050031

Mogdans, J., & Nauroth, I. E. (2011). The oscar, *Astronotus ocellatus*, detects and discriminates dipole stimuli with the lateral line system. *Journal of Comparative Physiology A, 197*, 959–968. https://doi.org/10.1007/s00359-011-0656-3

Mogdans, J., Krother, S., & Engelman, J. (2004). Neurobiology of the fish lateral line: adaptations for the detection of hydrodynamic stimuli in running water. In: Von der Emide, M.J., & Kapoor, B.G. (eds) *The Senses of Fish*. pp. 265-287. Springer Science + Business Media.

Montgomery, J. C., & Hamilton, A. R. (1997). Sensory contributions to nocturnal prey capture in the dwarf scorpion fish (*Scorpaena papillosus*). *Marine and Freshwater Behaviour & Physiology, 30*, 209–223. https://doi.org/10.1080/10236249709379026

Montgomery, J. C., Baker, C. F., & Carton, A. G. (1997). The lateral line can mediate rheotaxis in fish. *Nature, 389*(4), 960–963. https://doi.org/10.1038/40135

Montgomery, J., Coombs, S., & Halstead, M. (1995). Biology of the mechanosensory lateral line in fishes. *Review of Fish Biology and Fisheries 5*, 399–416. https://doi.org/10.1007/BF01103813

Muller, H. (1996). Indications for feature detection with the lateral line organ in fish. *Comparative Biochemistry & Physiology, 114*(3), 257–263.

Münz, H. (1985). Single unit activity in the peripheral lateral line system of the cichlid fish *Sarotherodon niloticus* L. *Journal of Comparative Physiology A, 157*, 555–568. https://doi.org/10.1007/BF01351350

Naisbett-Jones, L. C., Putman, N. F., Stephenson, J. F., Ladak, S., & Young, K. A. (2017). A magnetic map leads juvenile European eels to the gulf stream. *Current Biology, 27*(8), 1236–1240. https://doi.org/10.1016/j.cub.2017.03.015

Nakae, M., Kuroki, M., Sato, M., & Sasaki, K. (2021). The lateral line system and its innervation in the Japanese eel *Anguilla japonica* (*Teleostei: Elopomorpha: Anguillidae*). *Journal of Morphology, 282*(6), 863–873. https://doi.org/10.1002/jmor.21353

Omura, Y., Uematsu, K., Tachiki, H., Furukawa, K., & Satoh, H. (1997). Cone cells appear also in the retina of eel larvae. *Fishery Sciences, 63*(6), 1052–1053. https://doi.org/10.2331/fishsci.63.1052

Partridge, B. L., & Pitcher, T.J. (1980). The sensory basis of fish schools; relative roles of lateral line and vision. *Journal of Comparative Physiology A, 135*, 315–325. https://doi.org/10.1007/BF00657647

Piper, A. T., Manes, C., Siniscalchi, F., Marion, A., Wright, R. M., & Kemp, P. S. (2015). Response of seaward-migrating European eel (*Anguilla anguilla*) to manipulated flow fields. *Proceedings of Biological Sciences, 282*(1811), 20151098. http://dx.doi.org/10.1098/rspb.2015.1098

Pohlmann, K., Atema, J., & Breithaupt, T. (2004). The importance of the lateral line in nocturnal predation of piscivorous catfish. *Journal of Experimental Biology, 207*(17), 2971–2978. https://doi.org/10.1242/jeb.01129

Rodríguez, F., Durán, E., Vargas, J. P., Torres, B., & Salas, C. (1994). Performance of goldfish trained in allocentric and egocentric maze procedures suggests the presence of a cognitive mapping system in fishes. *Animal Learning & Behavior, 22*(4), 409–420. https://doi.org/10.3758/BF03209160

Roitblat, H. L., Tham, W., & Golub, L. (1982). Performance of *Betta splendens* in a radial arm maze. *Animal Learning & Behavior, 10(1)*, 108–114. https://doi.org/10.3758/BF03212055

Saito, K., & Watanabe, S. (2004). Spatial learning deficits after the development of dorsomedial telencephalon lesions in goldfish. *Neuroreport, 15*(18), 2695–2699.

Saito, K., & Watanabe, S. (2005). Experimental analysis of spatial learning in goldfish. *Psychological Records, 55*(4), 647–662. https://doi.org/10.1007/BF03395532

Sison, M., & Gerlai, R. (2010). Associative learning in zebrafish (*Danio rerio*) in the plus maze. *Behavioural Brain Research, 207*(11), 99–104.

Spence, R., Magurran, A. E., & Smith, C. (2011). Spatial cognition in zebrafish: the role of strain and rearing Environment. *Animal Cognition, 14*, 607–612. https://doi.org/10.1007/s10071-011-0391-8

Tsukamoto, K., Chow, S., Otake, T., Kurogi, H., Mochioka, N., Miller, M. J., Aoyama, J., Kimura, S., Watanabe, S., Yoshinaga, Y., Shinoda, A., Kuroki, M., Oya, M., Watanabe, T., Hata, K., Ijiri, S., Kazeto, Y., Nomura, K., & Tanaka, H. (2011). Oceanic spawning ecology of freshwater eels in the western North Pacific. *Nature Communications, 2*, 179. https://doi.org/10.1038/ncomms1174

Vogel, D., & Bleckmann, H. (1997). Water wave discrimination in the surface-feeding fish *Aplocheilus lineatus*
. *Journal of Comparative Physiology A 180*, 671–681. https://doi.org/10.1007/s003590050082

Voigt, R., Carton, A. G., & Montogomery, J. C. (2000). Responses of anterior lateral line afferent neurons to water flow. J*ournal of Experimental Biology, 203*, 2495–2502.

Watanabe, S. (2020). Spatial learning in Japanese eels using extra- and intra-maze cues. *Frontiers in Psychology, 11*, 1350. https://doi.org/10.3389/fpsyg.2020.01350

Watanabe, S., & Shinozuka, K. (2019). Spatial learning in Japanese eels (*Anguilla japonica*). *Animal Cognition, 23*, 233–236. https://doi.org/10.1007/s10071-019-01320-y

Watanabe, S., & Yoshida, M. (2007). Auditory cued spatial learning in mice. *Physiology & Behavior, 92*(5), 906–910. https://doi.org/10.1016/j.physbeh.2007.06.019

Webb, J. F. (2014). Morphological diversity, evolution and development of the mechanosensory lateral line system. In: Coombs, S., Bleckmann, H., Fay, R.R., Popper, A.N. (eds), *The Lateral Line System.* p 17–72. Springer, New York, https://doi.org/10.1007/2506_2013_12

Wellenreuther, M., Brock, M., Montgomery, J., & Clements, K.D. (2010). Comparative morphology of the mechanosensory lateral line system in a clade of New Zealand triplefin fishes. *Brain, Behavior & Evolution, 75*(4), 292–308. https://doi.org/10.1159/000317061

Westin, L. (1990). Orientation mechanisms in migrating European silver eel (*Anguilla anguilla*): temperature and olfaction. *Marine Biology, 106*, 175–179. https://doi.org/10.1007/BF01314798.

White, G. E., & Brown, C. (2015a). Cue choice and spatial learning ability are affected by habitat complexity in intertidal gobies. *Behavioral Ecology, 26*(1), 178–184. https://doi.org/10.1093/beheco/aru178

White, G. E., & Brown, C. (2015b). Microhabitat use affects goby (*Gobiidae*) cue choice in spatial learning task. *Journal of Fish Biology, 86*(4), 1305–1318. https://doi.org/10.1111/jfb.12638

Zerboloio, D., & Wickstra, L. L. (1980). Instrumental avoidance acquisition by goldfish in a Y-maze using explicit and response-contingent cues. *Bulletin of Psychonomic Society, 15*(2), 128–130. https://doi.org/10.3758/BF03199610