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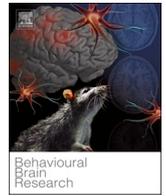
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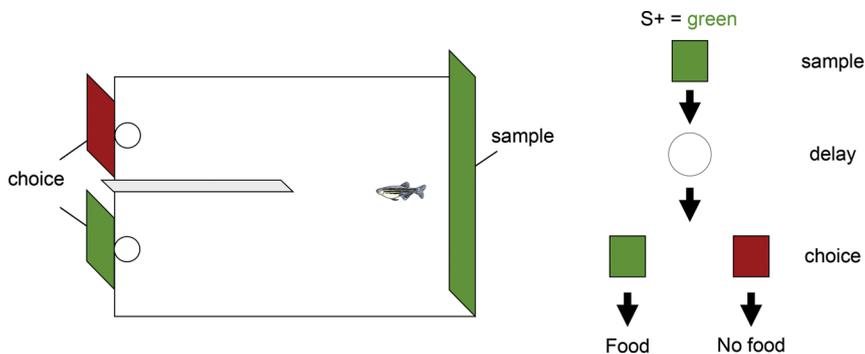
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GRAPHICAL ABSTRACT



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ABSTRACT

Operant conditioning is a powerful tool to study animal perception and cognition. Compared to mammals and birds, there are very few behavioral studies using operant conditioning paradigm in teleosts. Here we aim to establish matching-to-sample task (MTS) in adult zebrafish, using visual cues (colors) as discriminative stimuli. Unlike simple one-to-one color-reward association learning, MTS requires ability for context integration. In this study, zebrafish learned to perform the simultaneous-matching-to-sample (SMTS) within 15 sessions. After the SMTS training, working memory was tested by inserting a delay period (delayed matching-to-sample; DMTS). Zebrafish could perform the DMTS with a delay of at least 3–4 seconds. They could also learn to perform the DMTS even with a delay period from the beginning of the training session. These results strongly suggest that adult zebrafish possess working memory. However, our study also indicates limitations of zebrafish in cognitive flexibility or attention: they could perform SMTS/DMTS only in a certain set-up. The presence of working memory without the mesencephalic dopamine neurons indicates the convergent evolution of this function in amniotes and teleosts.

1. Introduction

Brain complexity is still commonly believed to increase linearly

with the phylogenetical “modernity” of vertebrate species. In this view inherited from the *scala naturae*, so-called “higher” vertebrates have increased their brain size by adding newer brain structures on the older

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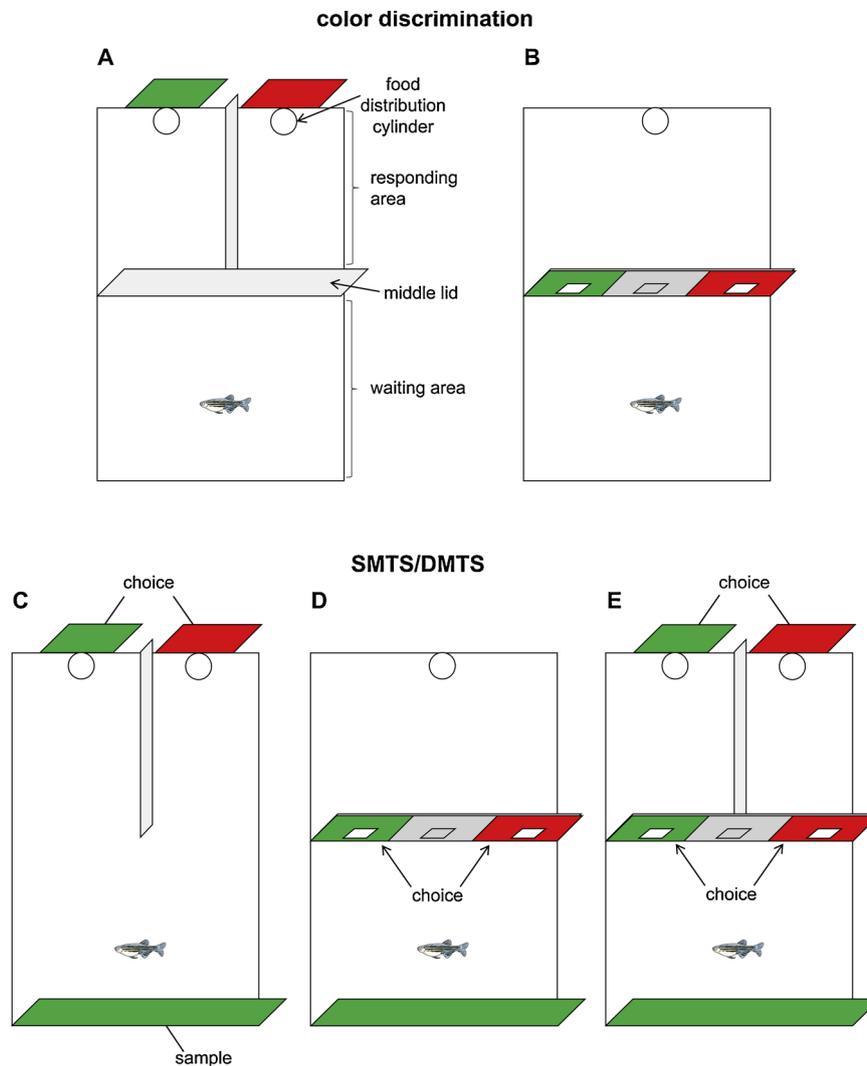


Fig. 1. Schematic drawings of operant boxes used in this study. (A and B) Set-ups used for color discrimination tasks: “Entering the hallway” set-up (A) and “Passing through the window” set-up (B). (C, D, E) Set-ups used for matching-to-sample tasks: “Entering the hallway” set-up (C), “Passing through the window” set-up with a single food distributor (D), and “Passing through the window” set-up with two food distributors (E).

ones. For instance the prefix “neo” referring to the six-layered mammalian “neocortex” reflects this view [1,2]. However, many behavioral studies suggest that birds such as corvids and parrots demonstrate cognitive capacities rivaling those of primates, such as theory of mind, tool use and manufacture, although they do not possess a mammalian-like “neocortex” [3–5]. It is now well accepted that the presence of so-called higher order cognitive functions is not specific to mammals.

In order to analyze specific cognitive components of complex behaviors, behavioral studies under controlled conditions are necessary. Operant conditioning is a powerful tool to decipher animal cognition. For example, studies using operant conditioning tasks based on visual discrimination have revealed that pigeons are capable of “categorizing objects” [6,7]. Furthermore, presence of executive functions in birds (mainly in pigeons) has been demonstrated by operant conditioning: reversal learning task to test learning flexibility [8,9], moving-dot paradigm (shell game) for selective attention [10], and delayed matching-to-sample task (DMTS) for working memory [11–13]. Executive functions are crucial for cognitive processes such as planning, cognitive flexibility, decision-making, and inhibiting inappropriate actions [14]. In mammals, these functions are performed in the prefrontal cortex (PFC). Several studies have demonstrated that the avian pallium (dorsal telencephalon) also contains an area functionally equivalent to the mammalian PFC: the nidopallium caudolaterale (NCL) [14–17].

Outside of amniotes (a group containing mammals and birds), some species of teleosts also demonstrate cognitive capacities such as transitive inference [18] and tool use [19]. Such behavioral repertoires have been observed only in some teleost species in the families of cichlids or wrasses. Interestingly, these teleost species have an enlarged pallium. Mammals and teleosts are phylogenetically much more distant than mammals and birds. Thus comparative studies including teleost species will provide considerable information on evolution of cognitive functions, notably on their convergent evolution [20].

In this study, we developed operant conditioning tasks in the adult zebrafish, a teleost species that is becoming a popular animal model in neuroscience. Although the size of the pallium and behavioral repertoires are not as remarkable as cichlids or wrasses, the accessibility for genetic tools makes it an interesting model to correlate the behavioral studies with brain anatomy and physiology. A previous study has demonstrated that zebrafish can perform reversal learning task [21], suggesting that zebrafish also have some learning flexibility. Thus zebrafish may possess a primordium of executive functions. To test this hypothesis, we examined whether zebrafish can perform matching-to-sample tasks (MTS): simultaneous matching-to-sample (SMTS) and delayed matching-to-sample (DMTS). MTS requires capacity for context integration (the logic of “if... then...”). DMTS is a MTS with a delay period, and it is often used to study working memory in non-human

animals.

We specially aimed to establish an operant conditioning paradigm directly comparable to previous studies in terrestrial animals. For this purpose, we tried two different types of set-up. In order to validate the adequacy of our set-up for zebrafish, we also performed simple color discrimination tasks in each set-up, since zebrafish is known to be able to perform color discrimination in operant paradigm.

2. Materials and methods

2.1. Subjects

90 experimentally naive adult zebrafish (*Danio rerio*; between 3–12 months) were used in this study. The zebrafish were all raised in our own colony in the animal facility (maintained at 28 °C with a 14-/10-hours light/dark cycle). The animals were maintained with their siblings in a fish tank (generally 10–25 individuals) until used for the experiment. All experimental protocols and care of laboratory animals were conducted in compliance with the official regulatory standards and approval of the French Government (reference document n°APAFIS#1286- 2015062616102603 v5, AP 2014-28 V2 mars).

2.2. Apparatus and stimuli

The subjects were tested individually in a home-made operant box. Schematic drawings of the operant boxes are shown in Fig. 1. A rectangular aquarium (32 cm × 14 cm width, approximately 7 cm height of water) was divided into two areas: the waiting area and the responding area. During the inter-trial interval (ITI), the waiting area and the responding areas were separated by a removable plastic board (middle lid). The middle lid was in opaque white so that the subject cannot see the stimuli before the lid is removed. At the end of the responding area, there were one or two food distribution cylinders (2 cm diameter). A small piece of reinforcer was provided manually after a correct response.

As a reinforcer (reward), Gemma Micro 600 (SKRETTING, Westbrook, Maine, USA) was used in the Experiment 1 (Exp 1) and Exp 3, while a small piece of dried mosquito larva (NovoFil; JBL, Neuhofen, Germany) was used for the rest of the experiments (see Discussion).

We have tried slightly different set-ups in order to find the most suitable operant box for zebrafish. The main difference is the definition of the response of the fish: one is “Entering the hallway” (Fig. 1A, C), and the other is “Passing through the window” (Fig. 1B, D, E) (see below for more details). In the “Entering the hallway” set-up, the responding area is further divided into right and left responding areas. In the “Passing through the window” set-up, a panel containing square windows (2 cm × 2 cm, 2 cm above the bottom of the box) was placed at the position of the middle lid.

Since zebrafish is known to be able to distinguish colors [21], we used colored panels in green (approximate RGB color code = R: 5, G: 170, B:110) and in red (R: 240, G: 40, B:80) as discriminative stimuli. The luminance of the two panels was almost the same (around 18 cd/m²). In the “Entering the hallway” set-up, the two color panels were placed at the end of the responding area behind each food distributor (Fig. 1A, C), and in the “Passing through the window” set-up, the colors were displayed at the position of the middle lid (Fig. 1B, D, E). In SMTS and DMTS, the “sample” (either green or red color depending on the trial) was demonstrated at one end of the box in the waiting area. In SMTS/DMTS with “Passing through the window” response, most of the data shown here were obtained with the set-up shown in Fig. 1E, in which two choice stimuli and two food distributors are placed at the end of the responding area. This is due to the fact that the fish showed a difficulty for performing the task in the set-up with a single distributor shown in Fig. 1D (see Results for details).

2.3. Experimental procedure

2.3.1. Habituation

Naive zebrafish were raised in group in a large tank in the animal facility, but during the experiment, the fish were kept individually in the same tank used for the experiment (operant box). In most cases (except Exp 2), 10 fish were used in each experiment. Each subject was used only for one experiment, and we never used the same subject in different experiments. The fish were separated in the operant box for 5–7 days, and deprived of food during 2 days before starting the pre-training. Until the completion of each experiment, the fish were kept in the same operant box.

2.3.2. Pretraining

The fish were first trained to eat food under the food cylinder. In the pretraining, the color stimuli were removed from the operant box. At the beginning of the trial, the middle lid was closed and the subject was placed in the waiting area. After the middle lid was removed, a small piece of reinforcer was dropped close to the fish and left until the fish ate it (the fish was left freely up to 2 min). When the fish consumed the food, the location of food delivery became gradually closer to the food cylinder in the following trials. The pretraining continued until the fish came to eat the food delivered through the cylinder. In the set-up with two food cylinders, the food was delivered equally often on the right and on the left side to avoid developing position preference.

In case of the “Entering the hallway” response, once the fish learnt to eat the food under the cylinder, they were reinforced only when coming close by a cylinder. The distance required to be reinforced was gradually reduced, and in the end, the food was provided only when the head of the fish came just in front of a cylinder, or tapping it.

In case of “Passing through the window” response, entering the responding area through a window was considered to be a response. Thus regardless the distance of the fish from the cylinder, the food was provided once the fish passed through the window. When the fish did not pass the window after 2 min, the experimenter pushed the window panel towards the end of the waiting area (as a consequence, the waiting area narrows) to facilitate the fish passing through the window. The advantage of the “Passing through the window” response is that it is clearer to determine whether the fish responded or not. This behavior occurred rarely for untrained fish, thus we could better validate the intention of the fish to respond (for example, it could serve to reduce the false-positives due to hyperactivity).

The middle lid was closed during the food consumption. After finishing eating, the middle lid was re-opened and the fish came back to the waiting area. When the fish did not come back by itself after 10 seconds (10 s), it was manually helped to move to the waiting area.

A session consisted of 10 trials, and one or two sessions were performed per day. That is, the subjects were fed at least 10 pieces of reinforcer every day. We could not perform more than two sessions per day because of the loss of motivation for food which occurred above 10 trials. In the end of the week, Gemma Micro 300 (SKRETTING, Westbrook, Maine, USA), which is the food normally provided in our fish facility, was fed in order to compensate the nutrition.

2.3.3. Color discrimination

After the fish established the response-reward (food consumption) association, discrimination stimuli (green/red colors) were displayed. Half of the fish were rewarded when they responded on the green side (S+ = green), while the other half were rewarded when they responded on the red side (S+ = red). The right/left position of the two colors was randomized. We have performed experiments having both colors to be S+, although the data for “S+ = green” is demonstrated in Results.

When the fish chose the S+, a small amount of reward was provided. When the fish chose the S-, reward was not provided, and the middle lid is closed and the fish was confined within the responding

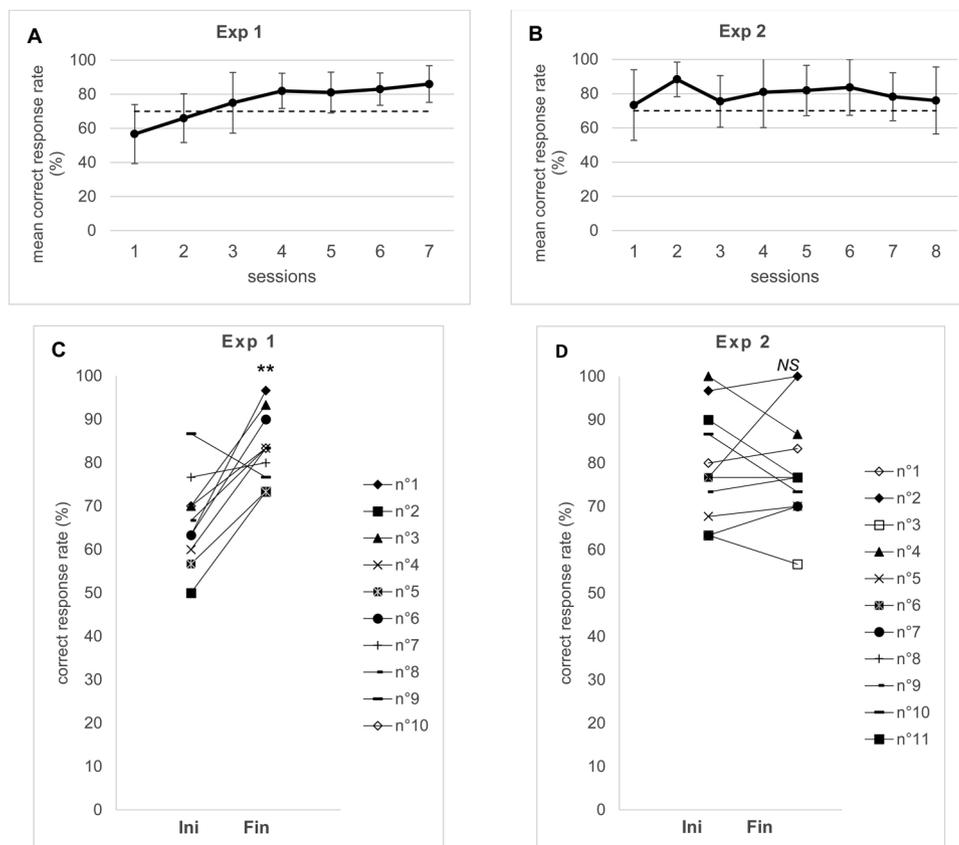


Fig. 2. Progress of the correct response rate in color discrimination tasks. (A and B) Session by session progression of mean percentage of correct responses of the group in Exp 1 (A) and in Exp 2 (B). Vertical bars show the standard deviation. The horizontal dotted line demonstrates the 70% correct response rate. (C and D) Comparison of the correct response rate of the initial phase (Ini) and the final phase (Fin). $**p < 0.01$ in Exp 1 (C), not significant (NS) in Exp 2 (D).

area for 30 s. Since zebrafish do not like to be placed in a small area, this serves as a mild punishment.

The tasks were performed using two different set-ups, one with “Entering the hallway” (Fig. 1A, Exp 1), and the other with “Passing through the window” (Fig. 1B, Exp 2). In the case of the “Entering the hallway” response (Exp 1), entering the S + hallway (responding area) and putting the head under the cylinder or poking it was considered to be a correct response. Poking the cylinder of S-, or entering the S-hallway 3 times within a trial (even without poking the wrong cylinder) was considered as an incorrect response (error). If the fish did not choose any color within 30 s, it was also recorded as “incorrect response”, thus the middle lid was closed for 30 s, and the next trial started.

Each trial was followed by an inter-trial-interval (ITI) of 10 s. One session consisted of 10 trials, and one or two sessions were performed per day. The learning criterion was 70% correct response rate or above ($\geq 70\%$) for 3 consecutive sessions.

2.3.4. Matching-to-sample (MTS)

In the matching-to-sample (MTS), subjects were trained to choose a given sample stimulus out of two subsequent choice stimuli. Choosing the choice stimulus that is the same as the sample (S+) was rewarded with food, while choosing that is different from the sample (S-) was not rewarded. The simultaneous matching-to-sample (SMTS) is a task in which the sample stimulus continues to be presented after the onset of the choice stimuli.

In the SMTS procedure, the sample stimulus (either green or red panel) was first shown alone in the end of the waiting area for 10 s. Then the middle lid opened, and choice stimuli (green and red panels) were displayed. Choosing the same color as the sample (S+) within 30 s was recorded as a correct response, and the fish was rewarded with food. When the fish chose the different color as the sample (S-), reward was not provided and the middle lid was closed for 30 s (mild punishment). If the fish did not choose any color within 30 s, the middle lid

was closed, and the next trial started after the mild punishment.

When the sample is retrieved before the appearance of the choice stimuli, the task becomes the delayed matching-to-sample (DMTS). The sample color on the wall of the waiting area was first removed, then the middle lid was removed several seconds later, and the choice stimuli were displayed. Thus the only difference between the SMTS and DMTS was the insertion of the “delay” between the removal of the “sample” and the appearance of the “choice”. In the Exp 3, different durations of the delay were tested, starting with 3 s.

Green or red “sample” was demonstrated in a random manner within a session. Appearance of each sample color was 50% in average, and the right/left position of the two choice stimuli was also randomized.

Each trial was followed by an ITI of 10 s. One session consisted of 10 trials, and one session was performed per day. The learning criterion was the same as described above: we considered that the fish mastered the task when it could retain the correct response rate $\geq 70\%$ for 3 continuous sessions.

We performed 6 experiments for SMTS and DMTS. In the Exp 3, SMTS training sessions followed by DMTS test sessions were performed with “Entering the hallway” response, and Gemma Micro 600 was used as a reinforcer. In the Exp 4, the same training was performed with a new reinforcer, using dried mosquito larva. Exp 5 was performed with “Passing through the window” response. In the Exp 6–8, the fish were divided into two groups, one was trained for SMTS while the other was training directly for DMTS with a delay of 3 s.

2.4. Data analysis

We generally started the experiments with 10–12 subjects, and often there were one or two fish that showed a freezing behavior. When the fish froze throughout the experiment, the subject was removed from the analysis. When a subject responded more than half of the trials within a session, the percentage of correct responses were calculated. Otherwise,

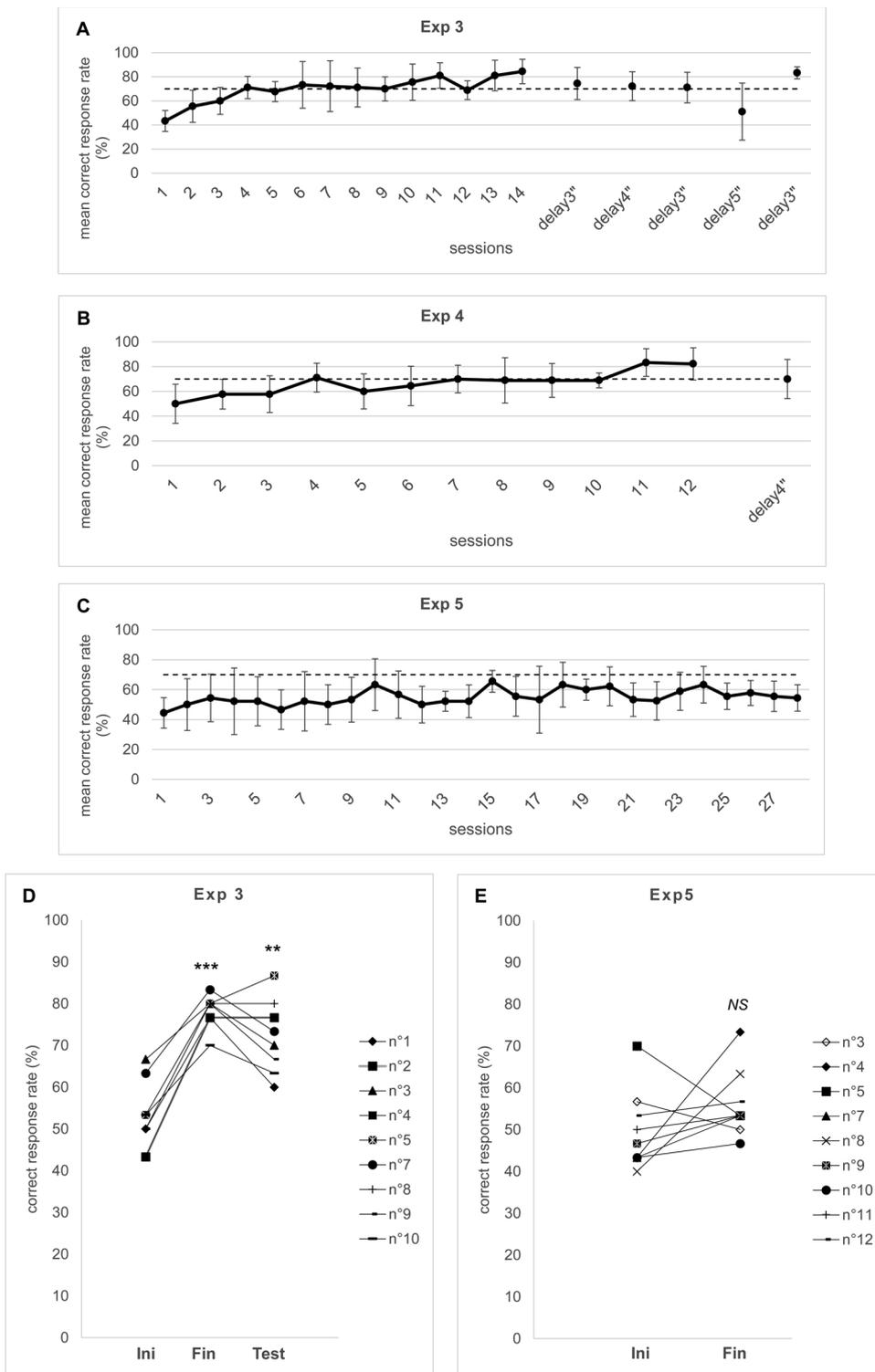


Fig. 3. Progress of the correct response rate in SMTS trainings and DMTS tests. (A–C) Session by session progression of mean percentage of correct responses of the group in Exp 3 (A), Exp 4 (B), and Exp 5 (C). Vertical bars show the standard deviation. The horizontal dotted line demonstrates the 70% correct response rate. SMTS trainings are demonstrated with lines while DMTS test sessions are demonstrated with separate dots. (D) Comparison of the correct response rate of the initial phase (Ini) and the final phase (Fin) of SMTS training sessions, as well as the first 3 sessions of the DMTS test in Exp 3. $**p < 0.01$, $***p < 10^{-4}$. (E) Comparison of the correct response rate of the initial phase (Ini) and the final phase (Fin) of the SMTS training in Exp 5. There is no significant difference (NS).

the session displayed no data (indicated “-” in the Tables).

We tested our learning criterion of “3 consecutive sessions achieved with a score $\geq 70\%$ ” using a binomial test, yielding a $p = 0.021$ for an expected probability of success for each trial at chance level ($p = 0.5$), meaning that our learning criterion is robust. “2 consecutive sessions achieved with a score $\geq 70\%$ ” using a binomial test yielded a $p = 0.058$, which we did not consider as robust enough to use as our main criterion ($p > 0.05$).

We visualized the results of each experiment in two different manners. The first demonstration is the progression of the mean correct

response rate of the group session by session, indicating the standard deviation (Fig. 2A and B, Fig. 3A-C, Fig. 4). The second demonstration is comparison of the performance between the initial phase versus final phase of the training. For the latter analysis, the average correct response rate of the last 3 sessions (30 trials of the final phase) of each subject was tested against the correct response rate of the first 3 sessions (30 trials of the initial phase) using a paired t -test (Fig. 2C and D, Fig. 3D and E, Fig. 5). Data was considered significant for $p < 0.05$.

For each dataset (initial phase or final phase of each experiment), we performed a Shapiro-Wilk test to verify the normality of our data

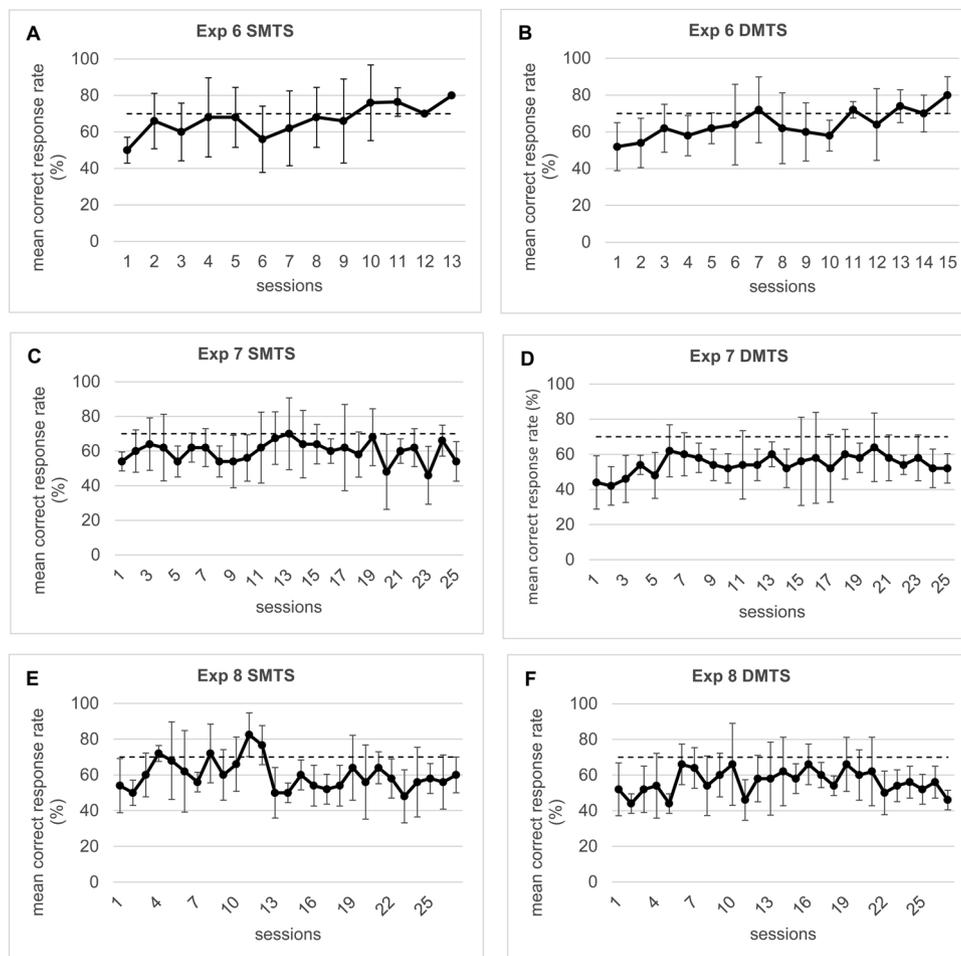


Fig. 4. Session by session progression of mean percentage of correct responses of the group in Exp 6 (A and B), Exp 7 (C and D), and Exp 8 (E and F). SMTS training groups (A, C, E) and DMTS training groups (B, D, F) are shown separately. Vertical bars show the standard deviation. The horizontal dotted line demonstrates the 70% correct response rate.

with a threshold of $p < 0.05$, which confirmed the normal distribution (H_0 accepted for this threshold), except for the final phase of Exp 5. In order to apply the paired t -test that is designed for continuous datasets, we systematically compiled 3 successive sessions (30 trials) for each subject. For this reason, some of the DMTS data could not be used for statistical analysis. For example, the DMTS test in Exp 4 is not demonstrated as the others, because there is only one test session (10 trials).

The data of Exp 6, 7, and 8 were combined for analysis, since they are the same training conditions. The performance of SMTS in the Exp 7 and 8 decreased even after reaching the learning criterion, thus it was not clear how to define the final phase. In this particular case, we performed two analyses for the subjects which had reached the learning criterion at least once. For the first analysis, the final phase was defined as the last 3 sessions of the experiments for all the subjects (Fig. 5A and B). For the second analysis, if the subject reached the learning criterion, the data of the last 3 sessions before reaching the criterion (high performance phase) were used for the statistical analysis (Fig. 5C and D). If the subject did not reach criterion, it was defined as the last 3 sessions of the experiments as in the first analysis.

3. Results

3.1. Color discrimination (Exp 1 and 2)

We first performed a two-forced choice color discrimination task. Although a previous study has already shown that zebrafish are capable

of performing color discrimination task with a set-up similar to the “Entering hallway” [21], we verified whether our set-up is well-adapted for operant conditioning in zebrafish (Exp 1). We have confirmed that adult zebrafish can learn the visual discrimination task quickly. Table 1 shows the percentage of correct responses in each session of each subject. All the fish reached the correct response rate $\geq 70\%$ within 4 sessions, and the performance was quite stable, 9 out of 10 subjects reaching the learning criterion ($\geq 70\%$ for 3 continuous sessions) within 6 sessions. The mean correct response rate of the 10 subjects in each session is shown in Fig. 2A, demonstrating that the performance of the group improved through the training sessions. We compared the correct response rate of the first 3 sessions (initial phase; Ini) and the last 3 sessions (final phase; Fin), and confirmed that the performance is significantly higher after the training ($p < 0.01$, Fig. 2C).

Zebrafish are also capable of performing the color discrimination with a “Passing through the window” set-up (Exp 2). Although individual variation seemed larger, most of the subjects reached the criteria very quickly and kept a stable performance (Table 2, Fig. 2B). As the correct response rate was already high from the initial sessions, there was no significant difference between the initial phase and final phase (Fig. 2D).

3.2. SMTS training followed by DMTS test with “Entering the hallway” response

3.2.1. SMTS training and DMTS test with a nutritious reinforcer (Exp 3)

In the beginning, we used Gemma 600 as a reinforcer. Table 3 shows

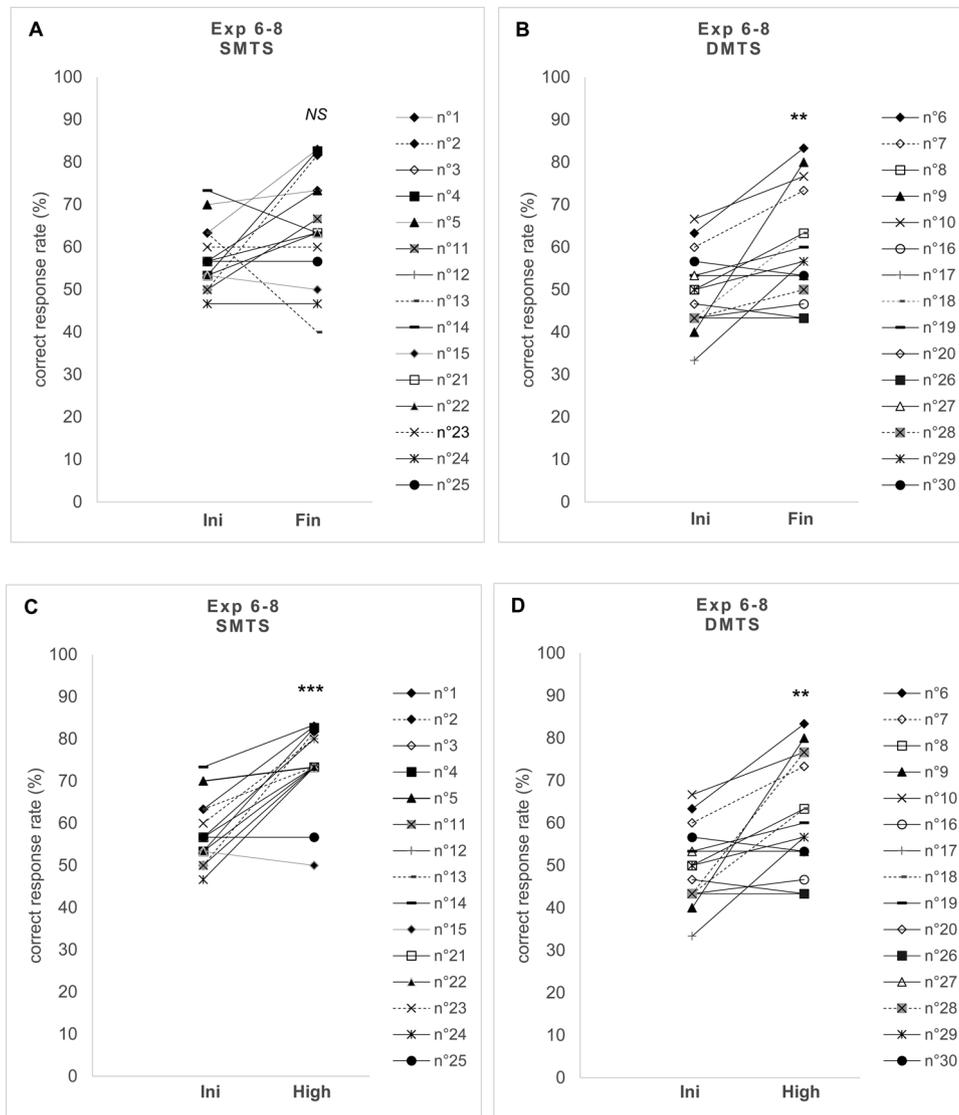


Fig. 5. (A and B) Comparison of the correct response rate of the initial phase (Ini) and the final phase (Fin) in Exp 6–8. SMTS training groups (A) and DMTS training groups (B) are shown separately. Not significant (NS) in SMTS (A), $**p < 0.01$ in DMTS (B). (C and D) Comparison of the correct response rate of the initial phase (Ini) and the last 3 sessions before reaching the criterion (high performance phase; High) in Exp 6–8. SMTS training groups (C) and DMTS training groups (D) are shown separately. $***p < 10^{-4}$ in SMTS (C), $**p < 0.01$ in DMTS (D).

Table 1

Correct response rate (%) of each animal through the color discrimination learning in Exp 1. The sessions with $\geq 70\%$ correct response rate are indicated in italic. The level of the performance retention is indicated with asterisks: “*” indicates achieving $\geq 70\%$ for 2 continuous sessions, and “***” in bold indicates $\geq 70\%$ for 3 or more continuous sessions. The “–” indicates that the subject did not respond more than half of the trials.

sessions	Percentage (%) of correct responses										mean
	n°1	n°2	n°3	n°4	n°5	n°6	n°7	n°8	n°9	n°10	
1	50	50	40	–	50	60	50	60	100	50	56.7
2	70	60	70	50	50	50	80	60	90*	80	66.0
3	70*	40	100*	60	70	80	100*	80	70**	80*	75.0*
4	100**	80	90**	70	80*	70*	80**	70*	90**	90**	82.0**
5	100**	60	90**	90*	80**	90**	80**	70**	70**	80**	81.0**
6	100**	80	90**	80**	70**	80**	70**	90**	90**	80**	83.0**
7	90**	80*	100**	80**	70**	100**	90**	90**	70**	90**	86.0**

the correct response rate in each session for each subject. The correct response of all animals was around a chance level (30–60%) in the first session, but most of them achieved the learning criterion after 14 sessions (some fish have reached the criterion during the DMTS test). The mean correct response rate also demonstrates the general improvement

of the group during the SMTS training (Fig. 3A). The paired *t*-test showed significant difference ($p < 10^{-4}$) in performance between the initial phase (Fig. 3D, Ini) and the final phase of the training (Fig. 3D, Fin).

The DMTS test was performed after the 14 sessions of SMTS

Table 2

Correct response rate (%) of each animal through the color discrimination learning in Exp 2. The sessions with $\geq 70\%$ correct response rate are indicated in italic. The level of the performance retention is indicated with asterisks: “*” indicates achieving $\geq 70\%$ for 2 continuous sessions, and “**” in bold indicates $\geq 70\%$ for 3 or more continuous sessions. The “–” indicates that the subject did not respond more than half of the trials.

sessions	Percentage (%) of correct responses											
	n°1	n°2	n°3	n°4	n°5	n°6	n°7	n°8	n°9	n°10	n°11	mean
1	80	100	60	100	60	60	40	–	70	–	90	73.3
2	70*	100*	80	100*	83	90	80	–	90*	90	100*	88.3*
3	90**	90**	50	100**	60	80*	70*	70	60	80*	80**	75.5**
4	100**	100**	40	100**	60	70**	60	80*	90	90**	100**	80.9**
5	100**	100**	90	100**	70	70**	90	80**	60	70**	70**	81.8**
6	80**	100**	50	100**	70*	80**	100*	100**	70	80**	90**	83.6**
7	90**	100**	80	80**	70**	60	60	100**	80*	70**	70**	78.2**
8	80**	100**	40	80**	70**	90	50	100**	80**	–	70**	76.0**

training. We found that the high correct response rate was retained during the DMST test sessions. The duration of the delay was 3 s for the first test. 7 out of 9 fish could achieve $\geq 70\%$ correct response. We then increased the duration of the delay, and the delay of 4 s was tested in the following session. Still 7 out of 9 fish could achieve $\geq 70\%$ correct response, thus we further tested the delay of 5 s. There were only 2 subjects that could reach $\geq 70\%$ correct response. When we reduced the delay (3 s) in the following session, all the subjects could achieve again $\geq 70\%$ correct response rate. The correct response rate obtained after SMTS training was maintained during the DMST tests ($p < 0.01$; Fig. 3D, Test). We thus conclude that zebrafish could perform the DMST, with the delay up to around 4 s.

Although all subjects could achieve 70% correct response rate, certain subjects had difficulty to retain the high performance continuously. We considered that this may be due to a lack of motivation (food saturation), thus we reduced the frequency of the experiments at later stages of the Exp 3: after the 6th session, there were at least 2 days of gaps between sessions. This improved the performance of many subjects. For this reason, the number of sessions does not correspond to the number of days. In the Tables showing MTS trainings, the numbers of days are indicated together with the number of sessions. The gap was sometimes long because of practical limitation. Interestingly, we found that zebrafish could retain the high correct response rate even after 11 days of gap between 2 sessions (between the 8th and 9th sessions, Table 3). This result suggests that zebrafish can learn SMTS regardless

Table 3

Correct response rate (%) of each animal through SMTS training sessions and DMST test sessions in Exp 3. The sessions with $\geq 70\%$ correct response rate are indicated in italic. The level of the performance retention is indicated with asterisks: “*” indicates achieving $\geq 70\%$ for 2 continuous sessions, and “**” in bold indicates $\geq 70\%$ for 3 or more continuous sessions.

sessions	days	Percentage (%) of correct responses									
		n°1	n°2	n°3	n°4	n°5	n°7	n°8	n°9	n°10	mean
1	1	40	30	50	40	40	60	40	50	40	43.3
2	2	60	40	70	30	60	70	50	60	60	55.6
3	3	50	60	80*	60	60	60	70	40	60	60.0
4	4	70	80	60	80	80	60	80*	70	60	71.1
5	5	80*	60	60	60	60	70	80**	70*	70	67.8
6	8	80**	40	70	60	80	60	70**	100**	100*	73.3
7	10	60	60	90*	70	40	90	90**	50	100**	72.2*
8	12	80	70	60	50	60	100*	70**	90	60	71.1**
9	23	60	60	80	80	80	80**	60	70*	60	70.0**
10	25	70	90	60	50	80*	70**	80	100**	80	75.6**
11	29	60	80*	90	80	70**	90**	90*	80**	90*	81.1**
12	32	80	60	70*	80*	70**	70**	60	70**	60	68.9
13	36	60	90	70**	80**	100**	90**	90	80**	70	81.1
14	39	90	80*	100**	70**	70**	90**	90*	90**	80*	84.4*
delay 3s	43	50	80**	60	90**	80**	80**	90**	70**	70**	74.4**
delay 4s	46	50	70**	70	80**	90**	70**	80**	80**	60	72.2**
delay 3s	52	80	80**	80*	60	90**	70**	70**	50	60	71.1**
delay 5s	59	30	60	70**	40	100**	60	30	30	40	51.1
delay 3s	61	90	80	90**	80	80**	80	80	90	80	83.3

of the irregular training schedule.

3.2.2. SMTS training and DMST test with a new reinforcer (Exp 4)

Since we observed the food saturation in the Exp 3, we changed the reinforcer in this experiment. Dried mosquito larvae were chopped in small pieces, and one piece was provided at a time as a reinforcer.

Among the 9 subjects, 8 of them (except fish n°10) had achieved the criterion at least once by the end of the 12th session (23 days) (Table 4). The high performance was relatively well retained: some individual (n°1) kept $\geq 70\%$ correct response for 8 continuous sessions (Table 4). The general high performance is also visible by the mean score (Fig. 3B).

After majority of the subjects reached the learning criterion, a DMST test with 4 s was tested (Table 4, Fig. 3B). 5 subjects out of 9 reached $\geq 70\%$ correct response rate.

3.3. SMTS training with “Passing through the window” response (Exp 5)

We initially tried the “Passing through the window” set-up with a SMTS training with “Passing through the window” response with a single food distributor (Fig. 1D). In the color discrimination task, the same set-up (Fig. 1B) nicely worked and fish could learn the task quickly (Table 2, Fig. 2B). However, fish had difficulty for performing SMTS using this set-up. They could not achieve the criteria, and there were many sessions in which the fish did not even respond (data not

Table 4

Correct response rate (%) of each animal through SMTS training sessions and DMTS test sessions in Exp 4. The sessions with $\geq 70\%$ correct response rate are indicated in italic. The level of the performance retention is indicated with asterisks: “*” indicates achieving $\geq 70\%$ for 2 continuous sessions, and “***” in bold indicates $\geq 70\%$ for 3 or more continuous sessions.

sessions	days	Percentage (%) of correct reponses									
		n°1	n°2	n°3	n°4	n°5	n°6	n°7	n°8	n°10	mean
1	1	20	50	50	40	60	70	40	70	50	50.0
2	2	50	40	50	80	60	70*	60	50	60	57.8
3	3	60	40	50	50	60	70**	50	90	50	57.8
4	4	60	60	80	90	70	60	80	80*	60	71.1
5	5	70	70	50	60	70*	70	70*	30	50	60.0
6	8	70*	50	60	70	80**	80*	70**	30	70	64.4
7	9	70**	70	70	80*	50	60	70**	70	90*	70.0
8	11	80**	90*	80*	70**	40	70	50	90*	50	68.9
9	12	70**	80**	60	70**	50	90*	70	80**	50	68.9
10	16	70**	70**	80	70**	60	70**	70*	70**	60	68.9
11	17	70**	70**	100*	80**	80	80**	90**	100**	80	83.3
12	23	90**	70**	90**	80**	100*	90**	70**	90**	60	82.2*
delay 4s	24	70**	50	90**	50	60	90**	80**	80**	60	70.0**

shown). We thought that close spatial association of choice and reward may be important for the fish, thus we further modified the set-up (Fig. 1E) and another experiment was performed using naive fish.

Nonetheless, even after the modification, all fish had difficulty for learning the task (Table 5, Fig. 3C). 8 out of 9 could perform $\geq 70\%$ for 2 consecutive days, but there were only 3 animals achieving for 3 consecutive days (Table 5). These animals could not retain the high performance in a stable manner. A paired *t*-test with the correct response rate of the last 3 sessions against that of the first 3 sessions did not yield a significant difference in performance (Fig. 3E).

DMTS (delay for 3s) was tested only with one animal, and the

Table 5

Correct response rate (%) of each animal through SMTS training sessions in Exp 5. The sessions with $\geq 70\%$ correct response rate are indicated in italic. The level of the performance retention is indicated with asterisks: “*” indicates achieving $\geq 70\%$ for 2 continuous sessions, and “***” in bold indicates $\geq 70\%$ for 3 or more continuous sessions. There is only one DMTS test session that is indicated with “3s)” (delay for 3 seconds).

sessions	days	Percentage (%) of correct reponses									
		n°3	n°4	n°5	n°7	n°8	n°9	n°10	n°11	n°12	mean
1	1	50	50	60	50	30	50	30	40	40	44.4
2	2	50	30	80	50	30	60	40	40	70	50.0
3	3	70	50	70*	30	60	30	60	70	50	54.4
4	5	50	50	20	40	50	90	30	80*	60	52.2
5	15	70	30	70	50	50	30	70	40	60	52.2
6	16	40	50	40	50	60	60	60	40	20	46.7
7	18	30	80	40	70	50	20	50	60	70	52.2
8	19	50	40	50	*80	40	60	40	40	50	50.0
9	22	60	50	20	60	60	60	60	70	40	53.3
10	23	60	90	60	60	90	50	40	70*	50	63.3
11	25	70	60	40	70	60	70	30	40	70	56.7
12	30	50	50	30	60	40	40	70	50	60	50.0
13	37	50	60	40	60	50	60	50	50	50	52.2
14	38	30	60	40	60	60	60	60	50	50	52.2
15	39	70	70	50	70	70	60	70	70	60	65.6
16	40	50	60	30	60	60	50	60	80*	50	55.6
17	43	10	70	50	80	50	30	70	70**	50	53.3
18	44	80	80*	60	60	60	60	70*	70**	30	63.3
19	45	50	60	70	60	70	60	60	60	50	60.0
20	47	60	70	70*	80	70*	50	40	50	70	62.2
21	51	50	40	60	40	70**	50	70	50	50	53.3
22	52	70	50	60	50	3s)60	70	40	40	50	53.3
23	53	50	70	40	70	60	80*	60	50	50	58.9
24	54	80	50	50	60	70	50	80	60	70	63.3
25	58	40	60	60	50	60	50	50	60	70*	55.6
26	59	50	70	60	50	70	60	50	50	60	57.8
27	65	50	80*	50	50	60	50	50	60	50	55.6
28	66	50	70**	50	60	60	50	40	50	60	54.4

correct response rate was 60% (Table 5, n°8 in the 22nd session). This animal went back to SMTS training in the following session, but it could not reach again the criterion.

3.4. Insertion of the delay in training sessions (Exp 6–8)

We tested whether zebrafish are capable of establishing the MTS even with a delay from the beginning of the training. Half of the subjects was trained with SMTS as the previous experiments (positive control), and the other half was trained with DMTS with the delay of 3 s. Because of the low performance using the “Passing through the window” response, we used the “Entering the hallway” response (mosquito larva as a reinforcer) for this experiment. As there are only 5 animals for each condition, we repeated the experiments 3 times (n = 15 for each group). The correct response rate of each subject in Exp 6–8 are shown in Tables 6–8, and progression of the mean score of each group is shown in Fig. 4A-F.

In contrast to the SMTS training, in which most subjects reached the criterion within 15 sessions (Tables 6–8 SMTS), there was a larger variation in the DMTS training groups. In Exp 6, 4 fish out of five reached the criterion within 15 sessions (Table 6, Fig. 4A). However, in Exp 7 and Exp 8, most of them could reach $\geq 70\%$ correct response rate for 2 consecutive sessions, but not for 3 consecutive sessions (Tables 7 and 8). Nonetheless, when we tested the difference between initial phase and the last phase, we found a significant difference ($p < 0.01$; Fig. 5B and D).

As the DMTS training groups did not reach the criterion quickly, we continued the experiments for more than 25 sessions in Exp 7 and Exp 8. Zebrafish were not capable of retaining the high performance after a certain number of sessions. Even in the group of SMTS training, there was no fish which could retain a high correct response rate until the end of the experiment, when it exceeded 20 sessions. This has important implications for the use of zebrafish in operant conditioning tasks.

This is clearly demonstrated by the statistical analysis. When we compared the SMTS performance of the final phase (the last 3 sessions in the end of the experiments) with the initial phase, there was no significant improvement (Fig. 5A), although there is a significant improvement when we use the data of 3 sessions reaching the learning criterion, instead of the final phase (Fig. 5C).

To conclude, our results show that zebrafish can perform DMTS with at least 3–4 seconds of delay period. However, they could obtain a high behavioral performance only in a certain set-up. Also, zebrafish could keep a high performance only within a limited number of sessions.

Table 6

Correct response rate (%) of SMTS training animals (left column) and DMTS training animals (right column) in Exp 6. The sessions with $\geq 70\%$ correct response rate are indicated in italic. The level of the performance retention is indicated with asterisks: “*” indicates achieving $\geq 70\%$ for 2 continuous sessions, and “***” in bold indicates $\geq 70\%$ for 3 or more continuous sessions.

sessions	days	Percentage (%) of correct responses											
		SMTS						DMTS					
		n°1	n°2	n°3	n°4	n°5	mean	n°6	n°7	n°8	n°9	n°10	mean
1	1	50	50	50	40	60	50.0	60	60	60	30	50	52.0
2	2	<i>80</i>	50	50	<i>80</i>	<i>70</i>	66.0	60	60	40	40	<i>70</i>	54.0
3	3	60	50	<i>70</i>	40	<i>80*</i>	60.0	<i>70</i>	60	50	50	<i>80*</i>	62.0
4	4	<i>70</i>	60	<i>70*</i>	40	<i>100**</i>	68.0	50	50	<i>70</i>	50	<i>70**</i>	58.0
5	7	<i>80*</i>	<i>70</i>	<i>80**</i>	<i>70</i>	40	68.0	<i>70</i>	50	60	60	<i>70**</i>	62.0
6	8	60	60	30	50	<i>80</i>	56.0	<i>90*</i>	30	<i>70</i>	60	<i>70**</i>	64.0
7	9	40	<i>70</i>	<i>80</i>	<i>80</i>	40	62.0	<i>90**</i>	50	60	<i>90</i>	<i>70**</i>	72.0
8	10	<i>90</i>	50	60	<i>80*</i>	60	68.0	60	40	<i>90</i>	50	<i>70**</i>	62.0
9	15	<i>70*</i>	<i>90</i>	60	<i>80**</i>	30	66.0	<i>80</i>	60	<i>70*</i>	40	50	60.0
10	16	<i>90**</i>	<i>80*</i>	<i>80</i>	<i>90**</i>	40	76.0	50	<i>70</i>	60	50	60	58.0
11	17	<i>89**</i>	<i>75**</i>	<i>70*</i>	<i>78**</i>	<i>70</i>	76.4*	<i>80</i>	<i>70*</i>	<i>70</i>	<i>70</i>	<i>70</i>	72.0
12	18			<i>70**</i>		<i>70*</i>		50	50	50	<i>90*</i>	<i>80*</i>	64.0
13	21					<i>80**</i>		<i>80</i>	<i>70</i>	60	<i>80**</i>	<i>80**</i>	74.0
14	22							<i>80*</i>	<i>70*</i>	60			
15	23							<i>90**</i>	<i>80**</i>	<i>70</i>			

4. Discussion

4.1. Establishment of operant conditioning tasks in adult zebrafish

Although there are some studies investigating spatial learning and memory in some teleosts including zebrafish [22–25], the presence of executive functions remains to be investigated. In this study, we developed an operant conditioning paradigm to study teleost executive functions using adult zebrafish, which allows to compare the data in teleosts with those in mammals and birds.

We demonstrate that adult zebrafish is able to perform SMTS and DMTS. It has been shown that zebrafish is capable of performing color discrimination task using operant conditioning by Parker et al. (2012) [21], and we also confirmed that zebrafish can perform two-choice color discrimination tasks. The main difference with this previous study is the food delivery system. In Parker et al. (2012), a small amount of water containing artemia (around 10 μ l) was delivered by turning a screw. In our study we delivered solid food, so that we could confirm the reward was completely consumed by the fish before starting the next trial.

Table 7

Correct response rate (%) of SMTS training animals (left column) and DMTS training animals (right column) in Exp 7. The sessions with $\geq 70\%$ correct response rate are indicated in italic. The level of the performance retention is indicated with asterisks: “*” indicates achieving $\geq 70\%$ for 2 continuous sessions, and “***” in bold indicates $\geq 70\%$ for 3 or more continuous sessions. For test sessions (with a delay period) in the SMTS training animals, the duration of the delay is indicated: 4s) = delay for 4 seconds, and 5s) = delay for 5 seconds.

sessions	days	Percentage (%) of correct responses											
		SMTS						DMTS					
		n°11	n°12	n°13	n°14	n°15	mean	n°16	n°17	n°18	n°19	n°20	mean
1	1	50	60	50	60	50	54.0	40	30	30	60	60	44.0
2	2	50	60	60	<i>80</i>	50	60.0	30	40	60	40	40	42.0
3	3	50	50	<i>80</i>	<i>80*</i>	60	64.0	60	30	40	60	40	46.0
4	4	<i>70</i>	40	50	<i>90**</i>	60	62.0	50	60	50	60	50	54.0
5	7	60	40	50	60	60	54.0	40	30	60	60	50	48.0
6	8	50	<i>70</i>	<i>70</i>	60	60	62.0	60	<i>70</i>	40	60	<i>80</i>	62.0
7	9	50	50	<i>70*</i>	<i>70</i>	<i>70</i>	62.0	60	<i>70*</i>	40	<i>70</i>	60	60.0
8	10	50	60	40	60	60	54.0	50	60	50	<i>70*</i>	60	58.0
9	11	<i>70</i>	50	40	<i>70</i>	40	54.0	<i>70</i>	50	50	50	50	54.0
10	15	<i>70*</i>	50	<i>70</i>	40	50	56.0	60	60	50	40	50	52.0
11	16	<i>80**</i>	<i>70</i>	<i>80*</i>	40	40	62.0	40	<i>80</i>	60	30	60	54.0
12	17	5s)60	60	<i>70**</i>	50	90	66.0	40	50	60	60	60	54.0
13	18	4s)60	<i>90</i>	5s)50	40	<i>80*</i>	64.0	50	<i>70</i>	60	60	60	60.0
14	21	50	<i>80*</i>	50	<i>90</i>	50	64.0	50	50	<i>70</i>	40	50	52.0
15	22	60	<i>70**</i>	<i>80</i>	50	60	64.0	<i>90</i>	60	60	20	50	56.0
16	24	60	5s)60	50	60	<i>70</i>	60.0	<i>90*</i>	50	40	<i>80</i>	30	58.0
17	25	<i>80</i>	<i>80</i>	40	<i>80</i>	30	62.0	60	<i>80</i>	30	40	50	52.0
18	28	50	50	60	50	<i>80</i>	58.0	50	<i>80*</i>	50	<i>70</i>	50	60.0
19	29	<i>80</i>	50	<i>90</i>	60	60	68.0	50	60	60	<i>70*</i>	50	58.0
20	30	60	60	20	30	<i>70</i>	48.0	50	50	<i>90</i>	50	<i>80</i>	64.0
23	31	<i>70</i>	50	60	60	60	60.0	60	50	<i>70*</i>	40	<i>70*</i>	58.0
22	32	60	60	60	50	<i>80</i>	62.0	50	60	50	50	60	54.0
23	35	60	50	20	60	40	46.0	50	60	<i>70</i>	<i>70</i>	40	58.0
24	36	<i>80</i>	<i>70</i>	60	60	60	66.0	40	50	<i>70*</i>	50	50	52.0
25	37	60	50	40	<i>70</i>	50	54.0	50	60	50	60	40	52.0

Table 8

Correct response rate (%) of SMTS training animals (left column) and DMST training animals (right column) in Exp 8. The sessions with $\geq 70\%$ correct response rate are indicated in italic. The level of the performance retention is indicated with asterisks: “**” indicates achieving $\geq 70\%$ for 2 continuous sessions, and “***” in bold indicates $\geq 70\%$ for 3 or more continuous sessions. For test sessions (with a delay period) in the SMTS training animals, the duration of the delay is indicated: 3s) = delay for 3 seconds, 4s) = delay for 4 seconds, and 5s) = delay for 5 seconds. The same indication is used in case a delay period more than 3 seconds was tested in the DMST training group.

sessions	days	Percentage (%) of correct responses											
		SMTS					DMTS						
		n°21	n°22	n°23	n°24	n°25	mean	n°26	n°27	n°28	n°29	n°30	mean
1	1	50	<i>70</i>	60	30	60	54.0	50	<i>70</i>	30	50	60	52.0
2	2	50	50	60	50	40	50.0	50	40	40	40	50	44.0
3	3	<i>70</i>	40	60	60	<i>70</i>	60.0	30	50	60	60	60	52.0
4	13	<i>70*</i>	<i>70</i>	<i>70</i>	<i>80</i>	<i>70*</i>	<i>72.0</i>	<i>70</i>	40	30	<i>70</i>	60	54.0
5	20	60	<i>80*</i>	50	<i>100*</i>	50	68.0	40	50	40	40	50	44.0
6	21	60	<i>80**</i>	<i>90</i>	40	40	62.0	<i>70</i>	60	<i>80</i>	<i>70</i>	50	66.0
7	22	60	50	60	50	60	56.0	60	<i>80</i>	60	<i>70*</i>	50	64.0
8	24	<i>80</i>	50	<i>90</i>	60	<i>80</i>	<i>72.0</i>	60	40	<i>80</i>	40	50	54.0
9	27	<i>70*</i>	50	40	<i>70</i>	<i>70*</i>	60.0	<i>70</i>	60	<i>70*</i>	40	60	60.0
10	28	<i>70**</i>	<i>70</i>	<i>80</i>	<i>70*</i>	40	66.0	50	<i>90</i>	60	40	<i>90</i>	66.0
11	29	<i>3s)70**</i>	<i>80*</i>	<i>70*</i>	<i>80**</i>	<i>100</i>	<i>80.0</i>	50	40	50	60	30	46.0
12	30	4s)60	<i>70**</i>	<i>90**</i>	<i>4s)70**</i>	<i>70*</i>	<i>72.0*</i>	<i>70</i>	40	60	<i>70</i>	50	58.0
13	31	3s)70	4s)40	<i>4s)70**</i>	<i>5s)70**</i>	50	60.0	60	40	<i>90</i>	40	60	58.0
14	34	4s)50	3s)50	4s)60	5s)60	50	54.0	<i>90</i>	50	40	<i>70</i>	60	62.0
15	35	3s)50	60	3s)50	4s)40	60	52.0	60	60	50	<i>70*</i>	50	58.0
16	41	<i>70</i>	40	50	60	50	54.0	<i>70</i>	<i>70</i>	<i>80</i>	60	50	66.0
17	42	60	40	60	50	50	52.0	60	60	<i>70*</i>	50	60	60.0
18	43	50	50	<i>70</i>	60	40	54.0	60	50	50	50	60	54.0
19	44	60	40	60	<i>70</i>	<i>90</i>	64.0	<i>80</i>	<i>70</i>	<i>70</i>	40	<i>70</i>	66.0
20	48	<i>90</i>	40	60	50	40	56.0	<i>70*</i>	50	<i>70*</i>	<i>70</i>	40	60.0
21	49	60	<i>80</i>	60	60	60	64.0	50	60	<i>5s)90**</i>	40	<i>70</i>	62.0
22	50	40	<i>70*</i>	60	60	60	58.0	60	60	50	50	30	50.0
23	51	<i>70</i>	40	30	50	50	48.0	50	60	60	60	40	54.0
24	52	50	<i>90</i>	40	50	50	56.0	60	50	50	50	<i>70</i>	56.0
25	55	<i>70</i>	60	60	50	50	58.0	40	50	50	60	60	52.0
26	56	50	<i>80</i>	60	40	50	56.0	50	<i>70</i>	50	60	50	56.0
27	57	<i>70</i>	50	60	50	<i>70</i>	60.0	40	40	50	50	50	46.0

After comparing the two different solid foods, Gemma Micro 600 (Exp 3) and dried mosquito larva (Exp 4), we decided to use the dried larva as a reinforcer, because the latter seems to maintain the motivation for food better (less nutritious). The Exp 3 required longer training (39 days) because we could not perform the experiment every day due to the saturation with the food. However, the long-lasting training experiment with a long interval between sessions have revealed an interesting point: the task performance did not decline much despite pauses lasting days. This suggests that the adult zebrafish are capable of retaining the rule at least for several days.

By contrast, our experiments using two different types of set-up (“Entering the hallway” and “Passing through the window”) revealed that zebrafish are not flexible enough to adapt their performance to different set-ups. The interesting point is that they could perform the “Passing through the window” response in the color discrimination task, but not in the MTS. It is probably because the cognitive attention required for MTS is much heavier than that for a simple visual discrimination task. Based on the behavioral observation, passing through the window by itself may require considerable attention for zebrafish. Thus they can perform correctly when the task is simple (color-food one to one association), but may be distracted when the task requires additional attention.

We also show that zebrafish may not be able to perform a task which requires long training sessions (more than 20 sessions). The decline of the performance could be because of lack of long-term attention and/or motivation, or stress from the long isolation. In any case, correct choice of the task is important for a good use of zebrafish in behavioral studies.

4.2. Working memory in zebrafish

The ability to perform DMST shows the presence of working

memory in zebrafish. Working memory is a kind of active memory, holding ongoing information of any modality online. In this aspect, it can be considered as a kind of short-term memory, but the important point is that working memory involves the ability to manipulate the information according to the contextual needs of the moment [17,26,27]. This internal maintenance of goal states is necessary for goal-directed behaviors.

In pigeons, such internal maintenance has been directly demonstrated by the presence of neurons activated during the delay period of DMST in NCL (working memory neurons; [11,28]). The authors could nicely correlate the working memory task with brain activity, since the behavioral paradigm is programmed like Russian “Matryoshka”, in which cognitive subcomponents increases in a stepwise manner [13]. The only difference between the SMTS and DMST is the presence of the delay period. Thus we can consider that cognitive components required for performing DMST consists of those required for SMTS plus working memory.

Based on behavioral observation of certain species, it is not surprising that teleosts possess working memory. For example, goal-directed behavior such as tool use observed in the sixbar wrasse [19] should require working memory. Nonetheless the presence of working memory has never been demonstrated experimentally. Our study showing that adult zebrafish can perform the DMST task strongly supports the presence of working memory in teleosts. Adult zebrafish can keep the memory at least for 3–4 seconds. This is similar to the result performed in honey bee [29]. In our experiments, it seemed difficult for zebrafish to keep the information in working memory for more than 5 s. However, our experiments were not aiming to assess the maximal timespan of zebrafish working memory and additional experiments would be needed to determine it.

In this study, we tried two types training procedures. 1) Animals are

trained with SMTS and then tested with a trial inserted a delay period. 2) Training with delay of 3 s from the start. In pigeon, it has been shown that training with delays from the start, results in rather slow acquisition [30]. In our data in zebrafish also, training with delays from the start was obviously more difficult (Exp 7 and 8).

Our results support the presence of working memory in the adult zebrafish, but zebrafish could perform DMSTs only under certain conditions. This brings a difficulty for performing behavioral experiments combining with electrophysiological or imaging set-ups, which would be required to identify working memory neurons *in vivo*. For now, imaging techniques on freely moving animals can be applied only in zebrafish larvae, but our preliminary data suggest that the executive functions are not yet developed at this stage. On the other hand, adult zebrafish is too small to perform the classical brain lesion. Indeed, we have tried lesion study in zebrafish, but could not obtain stable behavioral data after the brain lesion. Further studies and practical considerations are required to pursue the anatomical investigation of the executive areas in teleosts.

4.3. Convergent evolution of the executive functions

Working memory is one of the well-defined cognitive components of executive functions. Our current study together with a previous study showing that zebrafish are capable of performing reversal learning test, it is likely that zebrafish possess at least a primordium of the executive area. The next step would be to identify it.

In birds, based on combination of behavioral, anatomical, pharmacological, and electrophysiological studies, Güntürkün and his colleagues have proposed that NCL is a functional equivalent to the mammalian PFC (reviewed in [14,17]). Although mammalian PFC and avian NCL are functionally similar, they are not considered to be homologous as an executive pallium because of the topological location within the pallium (PFC at the anterior end while NCL at the posterior end of the pallium). Thus the similar functions are considered to have evolved independently in two lineages.

Güntürkün argues that recruitment of dopamine (DA) neurotransmission in the NCL may be a critical factor for evolving the functional properties similar to the mammalian PFC [17]. Indeed, to perform the DMSTs, DA innervation from midbrain DA cells (corresponding A10) to the executive pallium is necessary both in mammals and birds. Nonetheless, there is a significant difference between the DA systems of birds and mammals. For example, D1-family DA receptors play important roles in DA neurotransmission, but the DA receptor composition is different between mammals and birds. The avian pallium express additional DA receptor subtypes that had secondary lost in mammals (previously called D1D and newly proposed to rename as D6; reviewed in [31]). Thus it seems that DA neurotransmission is similarly important, but detailed mechanisms on how the executive pallia work may be different between mammals and birds [20].

Teleosts are phylogenetically distant from mammals, and the teleost executive area, if any, is unlikely to be homologous to the mammalian PFC. Our comparative studies of DA systems suggest that the teleost DA system is very different from the amniote DA systems, more specifically this difference is extreme when compared with mammals [20,31–33]. For example, teleosts have much more DA receptor subtypes than other vertebrates, with 14 DA receptor genes in case of zebrafish, while only 5 in mammals [31,34]. In addition, teleosts do not have DA cell in the mesencephalon. Thus it is likely that non-homologous DA cells play an equivalent role to the amniote A9/A10 cells. Finding the DA cells involved in the working memory task would help to identify the anatomical requirements playing the same role as A9/A10 cells.

Disclosure

Patent pending for the protocol and set-up of SMTS and DMSTs (French national patent n°1858985)

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