

論文の内容の要旨

論文題目 **The neural substrate for internal-directional information exploitation revealed by dissection of the higher-order cognitive function in zebrafish**

(ゼブラフィッシュの高次脳機能の解析によって解き明された身体方向情報の活用のための神経基盤)

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Abstract

The quantitative observation of behavior as an observable outcome of brain function in organism level has been one of the most powerful tools of modern neuroscience to understand cognitive functions. Zebrafish has been drawing attention in behavioral neuroscience. Anatomical and lesion studies have evolutionarily and functionally revealed the homologous brain regions to the mammalian counterparts. Several behavior paradigms of adaptive learning, e.g. active avoidance, revealed that zebrafish has the conserved cognitive function in learning and memory. However, the higher-order cognitive functions of it are poorly known. In this research, I aimed at addressing the question of whether the different aspects of higher-order cognitive function are conserved in zebrafish and tried to provide some new insights into the mechanism by the newly designed behavioral paradigms with high-throughput behavioral analysis.

Many studies have shown that zebrafish can be trained by aversive reinforcement learning by using noxious stimuli as a reinforcer. However, aversive stimuli may transfer the state of the animal into fear or anxiety, and concomitantly induce defensive behavior such as freezing or agitation. Consequently, they could interfere with behavioral measurement or even cause the failure of subsequent behavioral training. Therefore, a reinforcement behavior paradigm with positive reward would be more favorable for studying the higher-order cognitive function. To achieve this goal, I devised an automated system to train zebrafish through appetitive reinforcement training by precise control of reward dispensing on a millisecond scale in aquatic environment. The system was applied to the operant conditioning paradigm by associating the positive valence (reward) with the visual information from the environment. The system automatically trained the fish without artificial bias at high training frequency by dispensing the rewards only when fish choose a correct assigned color cue. The results showed that zebrafish is capable of associating the reward with the assigned visual cue. The learner fish reduced error of color selection within several training sessions with declined latency of choice response and achieved around 80%-plateau correct response within a week by repeated training. Inspired by the result, I then modified the system for addressing the question in higher-order brain function.

Working memory is one fundamental function among higher-order brain functions, defined as the function to hold the information for a second-scale period and keep its amenability to further processing.

To test the working memory, I modified the hardware with the extended designs for establishing delayed nonmatching-to-sample (DNMTS) task. The DNMTS task is designed here specifically for visual-spatial working memory examination with a reference stimulus and a set of answer stimuli. By comparing the answer stimuli color with the reference color, fish needs to reach the arm that displays the different color from the reference to earn the food reward. As the fish needs to hold the information for comparison, the span of working memory can be detected by the different delay periods. The delay was set to 0 s in introductive sessions for teaching the rule, and gradually increased to measure the maintenance ability of visual-spatial working memory. The results demonstrated that zebrafish was almost unable to complete the task even for the introductive session. The mean success rate was around 0.5 to 0.6 which was not significantly different from a chance level with the binomial test. Consequently, fish showed no statistical significance in learning among the trials with further delay durations in the test period. Although I failed to train fish in DNMTS, the results revealed that this type of task, which demands fish to use information by reasoning with the implicit rule, may have surpassed the upper limit of the cognitive capacity of zebrafish.

The attention control is essential as a prior step to decision making and is highly related to behavioral flexibility. The brain is a resource-limited processor. Therefore, attention control is important to save the calculation power for the right purpose by selectively extracting useful information alone. To test if zebrafish hold the function in attention control, the rule-shift task (RST) was designed to examine the capacity of zebrafish to attend to specific information among two intermingled rules. I selected the navigation behavior, to conceptually separate the information of the internal source (idiothetic) or external source (allothetic) in information exploitation by behavioral paradigm design. It is crucial for animals to adapt to the variable environment by using both these two classes of the information and keep the flexibility to switch between them. Therefore, in Rule 1, fish was requested to follow internal-directional (idiothetic) information, e.g. right turning. In contrast, in Rule 2, the fish needed to ignore the internal information and to attend to the visual (allothetic) environmental cues, e.g. red color at the goal. The correct response was rewarded to reinforce the behavior. Once fish achieved the learner criteria in one rule, another rule was imposed on the task without notification. The fish needed to recognize the change of the hidden rule by the error feedback from the result of choice. Our data showed that fish could reach the learner criteria in both rules, the latency of choice decreased as the number of training trials increased. Furthermore, fish adapted more quickly to the altered rule when it experienced the rule changes repeatedly, indicating the capacity of retrieving the rule memory to prioritize information selection.

The Habenular-interpeduncular nucleus (Hb-IPN) circuit integrates information from multiple sensory inputs, basal ganglion, and forebrain. With the characteristic left-right asymmetric axonal projection pattern and asymmetrical representation in sensation, the Hb-IPN circuit is a great candidate as the neural substrate in directional information exploitation and attention control between internal-external information. I, thereby, specifically perturbed the neural activities in the subregion of the dorsal habenula (lateral and medial subregions) which are specifically connected with the dorsal/intermediate and intermediate/ventral interpeduncular nucleus pathway (dHbL-d/iIPN, dHbm-i/vIPN) respectively to test the possible involvement of the circuit for attention regulation. Surprisingly, I found that the zebrafish with

silenced dHbL-d/iPN showed specific impairment in learning by internal-directional information exploitation but kept intact capacity of learning by external-cued associated information. Control analyses showed that the dHbL-d/iPN silenced fish were indistinguishable from the control group in locomotion activity, behavioral flexibility, and body coordination, showing that the dHbL-d/iPN pathway is indispensable in self-directional sensation. The two parallel pathways from the dHb to the IPN are also well known for regulating the winner/loser conflict outcome. Therefore, the dHbL and dHbM could play together as a switchboard for the synergistic control of both social behaviors (dominant or submissive) and attentional orientation (internally or externally directed).