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Food-rewarded conditioning and neurophysiological analysis of cheliped gripping behavior in crayfish

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ABSTRACT

Animals act on their environment to intentionally manipulate it with a defined purpose. This behavior generally needs a special organ suited for the purpose and a highly complex neural mechanism to perform voluntary motor control. Crustaceans with a pair of chelipeds show various manipulative behavior for dietary, exploratory, and reproductive purposes, but the neuronal mechanism underlying the cheliped manipulative behavior has not been clarified yet. In the present study, we trained crayfish *Procambarus clarkii* to perform a cheliped manipulative task by a newly developed operant paradigm in which animals gripped a specific object for food reward when a visual cue was presented. Animals were then tethered in an operant chamber during the task to enable reliable physiological recordings from the central nervous system. Neural activities descending from the brain were recorded extracellularly from the connective nerves between the brain and the subesophageal ganglion in the trained animals. We found those units showing spike activities that were significantly correlated with cheliped muscle activities, but not with strict timing of visual cue presentation. Although we could not test if those descending activities were necessary or sufficient for initiating the cheliped action by their selective stimulation, the present findings suggest that neural activities for controlling operant gripping behavior are formulated in the brain rather than in the subesophageal ganglion where cheliped motoneurons are present and visual information is transmitted through the brain.

1. Introduction

Animals have an ability to act on the environment to accomplish a defined purpose such as getting food or defending themselves using a special motor organ suited for its purpose [1]. Butterflies, for example, suck nectar from flowers using their flexible proboscis, and racoons wash food using their forelimbs [1,2]. A range of these actions are referred to as manipulation, distinguished from forms of reflexes and locomotor behavior. Manipulation needs highly complex motor control mechanisms because it is composed of complex sequence of heterogeneous events unlike reflexive and locomotor behavior that emerge as an episode or repetition of a simple action. In addition, manipulative behavior generally depends on voluntary control involving the intention of animals to act on the environment. Volition participates in the highest level of motor hierarchy, a framework of motor behavior in which neural activities flow from the higher levels making a general decision to the lower levels controlling specific muscle fibers [1,3].

Forelimbs of mammals are one of the most sophisticated organs performing various manipulative tasks. The neural mechanisms underlying forelimb manipulative behavior has been studied for a long time using animals trained to accomplish a food-reward task with their forelimbs (reviews [4,5], monkey [4–6], cat [7,8], and rodent [9,10]). Interestingly, cortical recordings in awake and behaving monkeys revealed neural activities characteristic of voluntary control (reviews [4,11]) such as reward representation [12–14] and motor preparation [15–19].

Chelipeds of crustaceans, although cumbersome in morphology, is a versatile organ to enrich their behavioral repertoire by performing, for example, defense/threat action [20], righting reaction [21] and food manipulation [22]. Moreover, in some decapod crustaceans, cheliped-motor learning ability was reported by classical [23] or operant conditioning procedure [24–26]. American lobster, in particular, is capable of discriminating the intensity of sensory cues [26], suggesting voluntary control of chelipeds. Thus, the crustacean brain is capable of executing complex motor control to accomplish cheliped manipulation although it is much simpler and smaller than vertebrate brains [26,27]. However, neural activities related to cheliped manipulative behavior have not yet been recorded from the crustacean central nervous system.

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We chose crayfish *Procambarus clarkii* Girard in this study as the subject of our experiments to record and analyze neural activities related to manipulative behavior. Crayfish show cheliped action to a small fish as prey in natural condition [28] and has the ability to accomplish a motor task to pull out food by passing a cheliped through a small access point [23]. We first developed an operant conditioning paradigm and an experimental chamber for tethered crayfish to perform a cheliped manipulative task, that is, to grip a specific object for food reward when a visual cue was presented. Training procedures were performed in two conditions: the underwater condition to confirm an operant learning ability of crayfish and the midair condition to make easier extracellular recording from the circumesophageal commissure of the trained animals while they are carrying out the cheliped manipulative task. We demonstrated for the first time the descending spike activities from the brain representing the motor command for cheliped manipulative behavior.

2. Materials and methods

We used a total of 87 crayfishes *P. clarkii* of both sexes (6.0 to 8.5 cm in body length from rostrum to telson): 59 animals for conditioning experiments and 28 animals for physiological recording experiments. Animals were obtained from retail stores, kept in a group in large tanks under a 12-light/dark cycle, and fed dried crayfish pellets twice a week. We used R programming software (version 2.15.1–3.3.1) [29] to perform statistical tests. All results were considered to be significant when $P < 0.05$.

2.1. Operant conditioning

2.1.1. Animals and apparatus

Seven days before experiments, animals were transferred into small tanks separately and starved to increase the motivation for food. We cut animals' second antennae at 1–2 mm from the base to prevent their mechanical stimulation that would cause reflexive gripping during training. A nut was glued to the carapace with a quick-drying adhesive (Aron Alpha, Toagosei, Tokyo, Japan) to tether animals to a holder during training (Fig. 1A).

Training was performed in a white acrylic chamber (20 × 18 × 7 cm, Fig. 1A and B) filled with tap water. Animals were tethered to a holder above the chamber at the level that the whole body was immersed under the water with no appendage touching the floor. After each training session that typically took 10–20 min, animals were released from the holder and returned to the small tanks filled with water. Some training sessions were performed in the midair condition in which the chamber was emptied of water completely: animals were picked out of the tank water into the air, repositioned to the empty experimental chamber, and tethered to the holder. After training that also took about 10–20 min in the air, the animals were brought back to small tanks and kept there under water until next training session to be carried out after 24 h.

A polyethylene tube (2.0 mm in outside diameter, Fig. 1A) was used to give animals food reward for cheliped gripping. The tube was set under tethered animals along the anteroposterior axis. One end of the tube was touching animals' mouth and the other was connected to a syringe outside the chamber. The tube and the syringe were filled with water so that fish sausage pellets in the tube came out to animals' mouth by manually pushing the syringe.

Animals were trained to grip a black acrylic bar (5.0 cm in length and 1.0 cm in diameter, Fig. 1A and B) with the left cheliped. The bar was originally placed above the chamber out of animals' reach and lowered just outside of the left cheliped (Fig. 1B) to the level of merocarpopodite joint but it never touched the cheliped (Fig. 1A). The bar was presented still for 10 s and then lifted back to the initial position. Its velocity of up-and-down movement was kept at 0.4 cm per second by a linear motor (EMP 400 series, Oriental motor, Tokyo, Japan) under the control of a personal computer through serial communication (Fig. 1A). The bar position was monitored by the control signals fed to the linear motor.

2.1.2. Conditioning paradigm

We performed two experiments that were different in the

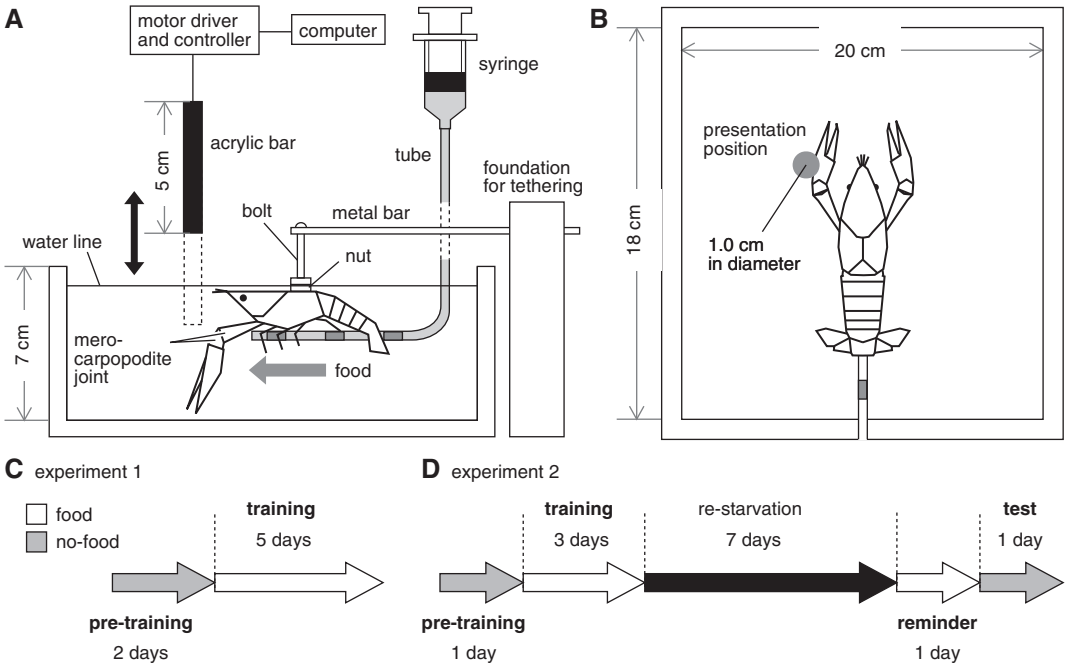

Fig. 1. Operant conditioning for cheliped gripping behavior in crayfish. A, B, Schematic drawing of the present experimental setup viewed from its side (A) and above (B). The chamber was filled with water and the animal was placed well below the surface in the underwater condition. C, D, Experimental schedule for the experiment 1 (C) and the experiment 2 (D). White arrows represent a procedure in which animals were provided with food reward while gray ones represent another procedure without reward. A black arrow represents a re-starvation period.