Manipulation of aggressive behavior of the cricket using a small robot

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Abstract: Male crickets *Gryllus bimaculatus* are known to exhibit intensive aggressive behavior towards other males. These crickets are often used as an experimental model in aggression researches. We establish a real-time recording system and behavior manipulation system using a small robot that can elicit aggressive behavior in a test cricket.

Keywords: insect, aggressive behavior, biogenic amine, social interaction.

1. INTRODUCTION

It is widely observed in animals that dominant hierarchy is established by agonistic behavior, through the complex interaction among physiological, motivational, and behavioral systems. Social and physical environment must be two of the most important factors to understand agonistic behavior in animals, which makes it difficult to fully understand it.

The cricket provides one of the greatest model systems to investigate neuronal mechanisms underlying aggressive behavior. Aggressive behavior in crickets is released by antennal contact detecting cuticular substances between two conspecific males [1]. When a male cricket encounters another male by chance, it exhibits intensive aggressive behavior. The battle starts out slowly and escalates into a fierce struggle [2]. Once the fighting is settled, the loser (subordinate) will refuse to fight again for a while [3]. It has been demonstrated that biogenic amine system is closely linked with agonistic behavior [5, 6]. Octopamine level of the hemolymph might mediate aggression level. Biogenic amines in the insect brain work as neurotransmitters, neuromodulators and as neurohormones. However, it remains unclear how the biogenic amine system in the central nervous system is mediated during a fight. In this study, we attempt to manipulate aggressive behavior of a male cricket using a small robot that is a kind of decoy of a conspecific male cricket so that we can record neuronal activities in the cricket brain.

2. MATERIALS AND METHODS

2.1 Animals

Cricket *Gryllus bimaculatus* used were raised in our laboratory colony. They were reared at 25-30°C under L/D 14:10 (lights on at 6:00 h) and fed a diet of insect pellets, carrots and water *ad libitum*. We used sexually mature male crickets for all experiments. To reduce the influence of prior fighting experience and to motivate fighting, each cricket was individually separated in a 100 ml glass beaker lined with filter paper for 2 days before the experiments.

2.2 Robot

The robot has dimensions of 18x18x22mm and is driven by two differential wheels. It has a size comparable to that of a typical cricket. It has no sensors except for an infrared receiver used for receiving commands encoded into pulses of infrared light. Movements can be controlled both in open or closed-loop with or without real-time feedback from insects' position [7]. In order to elicit aggressive behavior in a male cricket, another male cricket body parts that has head and thorax parts were attached on the robot or extraction of cuticular carbons were painted in the surface of the robot.

2.3 Electrophysiological recording from a free moving animal

Electromyograms (EMG) of mandible muscles were obtained using two varnish-coated copper wires (17 µm diameter) inserted proximally into the mandible muscle. Large amplitude mandible muscle potentials were recorded in free moving male crickets. The cricket recorded EMGs were placed in an experimental arena (12x12x10cm). Other male cricket or a robot was placed at the arena to observe the behavior and to record the activities of mandible muscles.

2.4 Measurement of biogenic amines using a micro-dialysis system

Animals were anaesthetized by rapid cooling on crushed ice. Microdialysis probe (0.22mm outer diameter, 2.0 mm total length) was implanted in the head. After implanted, crickets can walk around freely. Then another cricket or a robot was placed in the arena to elicit aggressive behavior to the cricket with the probe implanted. Probes were perfused at a rate of 2.0µl/min with cricket saline (85.55mM NaCl, 5.63mM KCl, 2.25mM $CaCl_2$). Probes were perfused 30min prior to the experiment. For all microdialysis experiments, samples were collected every 10 min. The samples collected were injected directly onto the HPLC column for qualitative and quantitative analyzing [8].

3. RESULTS AND DISCUSSION

3.1 Aggressive behavior of the cricket

Male crickets show intensive aggressive behavior when they encounter another male (Fig.1). The previous agonistic interaction between males had influence over the following behavior in subordinates. We focused on the subordinate behavior to understand how animals alter their behavior dependent on previous experience. Pharmacological experiments demonstrated that nitric oxide (NO) signaling and biogenic amine octopamine (OA) play a crucial role in the behavioral decision making in fighting. We found that NO has an inhibitory effect on aggressive motivation whereas OA has a facilitating effect on aggressive motivation. We hypothesize that NO and OA signals mediate internal state of the cricket to introduce aggressive behavior. We also designed dynamic behavior models and neurophysiology model to understand how cricket develop internal state for aggressive motivation. We then understand that social interactions constantly improve internal state of animals.

Fig. 1. Fighting between male crickets.

3.2 Real time analysis of the cricket internal state

In order to investigate how animals realize real time adaptation, we established a real time recording system from a free moving animal. In male cricket, aggressive behavior is elicited by cuticular pheromone from conspecific male. We have developed a behavior manipulation system using a small robot that can easily introduce aggression behavior from a male cricket (Fig. 2). Cricket showed intensive aggressive behavior toward the robot. During aggressive behavior, we succeeded in recording EMG from mandible opener muscle using a fine copper wire electrode. Cricket attacked the robot again and again although it stopped attacking another male cricket once the fighting was settled. Therefore we can elicit aggressive behavior any time in a test animal.

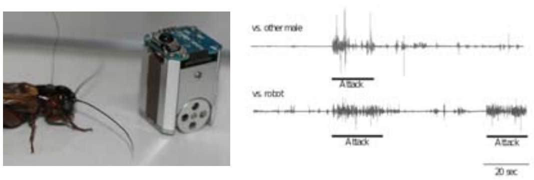

Fig. 2 Cricket and robot. EMG was recorded from a test animal that elicit attacking toward another male or the robot.

Aggressive motivation in animals is thought to be mediated by biogenic amines. In cricket, octopamine and serotonin are thought to play important role in releasing aggressive behavior [4]. Then we also established a microdialysis system to analyze dynamical change in the contents of biogenic amines in the test animals (Fig. 3). The samples of the microdialysis were collected every 10 min. The samples collected were quantitatively and qualitatively analyzed using a high-performance liquid chromatography (HPLC) with electrochemical detection (ECD) system [8]. Using this system we can evaluate our multi-feedback model that was designed to describe dynamical change of internal state of the cricket [9].

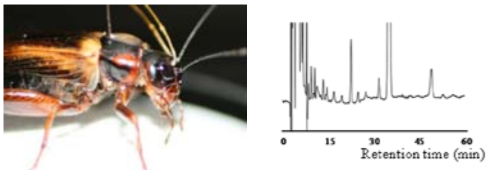

Fig. 3. Microdialysis system for a cricket.

4. CONCLUSION

We hypothesize that important mechanism underlying behavior adaptability is a multiple feedback structure that is composed of feedback loop in the nervous systems and feedback loop through the social environment [9]. In order to evaluate this hypothesis, we tried to establish a real-time recording system and behavior manipulation system using a small robot that can elicit aggressive behavior in a test cricket.

5. REFERENCES

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