Social experience dependent behavior selection in the cricket
– from neuroethological approaches to modeling -

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Abstract— Insects provide good model systems to investigate neuronal mechanism underlying adaptive behaviors in social population. Insects use species specific pheromone as a communication signals. Understanding the neuronal mechanism of pheromone behaviors helps us to elucidate the neuronal mechanisms of adaptive behaviors. The cuticular substances (pheromone) elicited aggressive behavior in male crickets. We here focused on cricket agonistic behavior to understand the neuronal mechanism how they changed behavior depending on previous experiences. Experienced memory of agonistic interactions between male crickets had influence over the following behavior selection in subordinate males. After dominant hierarchy was established, the dominant cricket showed aggressive behavior when it encountered other males whereas subordinate crickets showed avoidance. NO/cGMP signaling would regulate biogenic amine system in the brain, which in turn mediates agonistic behavior among male crickets. Based on results from biological experiments, we constructed behavior model and neuronal model to reveal the neuronal mechanisms of adaptive behavior in a social population.

Keywords: crickets, social interaction, adaptive behavior, multi-agent robot systems

1. INTRODUCTION

One of the common goals of biologists and engineering researchers must be to understand how nervous systems adapt animal behaviors to the dynamic environments. It is necessary for us to perform systematic analysis to elucidate the biological mechanisms of animal behaviors for applications.

Animals have evolved nervous systems as an adaptive mechanism through long history of the life. They perceive several kinds of signals from environment to adjust behavior. They do not always respond same way to the same external stimuli. The state of central nervous system must be dependent on their experiences as well as internal and/or external conditions. Insects have rather simple and identical nervous systems than brains mammalians that have about 10^{12} neurons. Insect brain has about 10^{6} neurons and so it is called "micro-brain". It allows us to access each neuron easily, which accelerate us to investigate how animals elicit adaptive behavior from cellular level to behavior level analysis. Insects perceive information from dynamic environment and the signals perceived are processed in the micro-brain to show adaptive behavior. The behavior of insects has been understood that internal factor such as internal timer, experiences and external environments drastically mediate threshold of releasing a behavior or behavioral pattern. In particular, previous social behavior such as mating behavior and agonistic behavior modulate following behaviors.

In this study, we have focused on insect communication behavior using male crickets to understand how animals establish social organization and adapt the society. Most of pheromone induced behaviors have been thought to be hard-wired: a behavior that could be turn on and off but with no plasticity. However, some of pheromone behaviors are revealed to be modified by the previous experiences. Cricket aggressive behavior is an example of such pheromone induced behaviors. The response of males to the pheromone can be modified by the previous fighting experiences [1, 2]. The goal of our project is to unravel the mechanism of adaptive behavior in a society to understand mobiligence of social adaptation, and we have investigated 1) how animals show socially adaptive behavior in the changing circumstance, 2) how they recognize and distinguish each other, 3) how they divide labor and share knowledge. We have investigated the mechanisms for formation of social hierarchy and adaptation mechanisms for individuals to exist in a society, which are both, emerged from individual interactions. We combine neuronethological approaches and engineering approaches by constructing dynamic model to understand how animals form social communities, how they learn and retain previous experience and how they change their behavior depending on the situation.

2. BIOLOGICAL APPROACHES

2-1. Fighting behavior of male crickets

Adult male crickets *Gryllus bimaculatus* 1-2 weeks after the imaginable molt were used. Five days before the start of experiments, male crickets were kept isolated individually to increase motivation of fighting. Two of male crickets were placed into an experimental arena to observe behavioral response to the cuticular substances that are on the surface of cricket's body. The main components of the cuticular substances are hydrocarbons. The male and female cuticular pheromones introduce different behaviors in male crickets. When male crickets come across the female and perceive female pheromone, they start courtship behavior for mating. When they, on the other hand, come across conspecific male, they start agonistic behavior (Fig. 1). The interaction usually escalated to hard fighting. After the fighting, the dominant (winner) starts aggressive song and chased after the loser (subordinate) to let it go away. The subordinate crickets wouldn't fight again against the dominant male crickets.

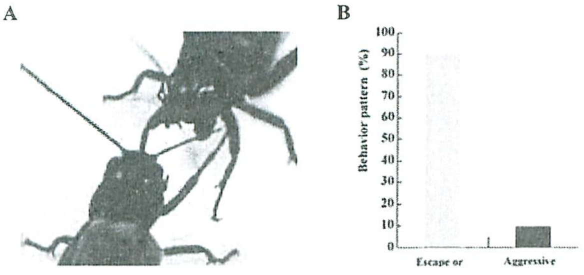

Fig. 1. Agonistic behavior of male crickets. **A:** Crickets bit each other until one of retreat from opponent. **B:** Behavioral response of the subordinate crickets to the dominants during reengagement. Escape or avoidance behaviors were evoked in

2-2. Memory of previous fighting

It is known that many insects learn odor, color, place etc. Crickets have been demonstrated that they learn odor source associated with water reward and salt water punishment[3]. They retain odor memory more than 1 week. This indicates that insect behavior can be modulated by their experiences. Pheromone behaviors in insects have been thought to be hard-wired. For example, male moths respond with a highly stereotyped response when they detect a pheromone plume released by the conspecific female. Only a few works demonstrated that pheromone behavior could be modulated by experience[4]. After the agonistic behavior in males, subordinate male change their behaviors from aggression to avoidance. Subordinate crickets must learn to associate cuticular substances of opponents to the dominant cricket during the fighting. If the inter-training interval of twice sequential fighting was more than 1hr, the subordinate crickets showed the aggressive behaviors again, but most subordinate crickets would not fight again if the inter-training interval was within 30-60min. The beaten memory was reinforced if subordinate reengaged 3 times after 15min inter-training intervals. This indicates that the aversion to the fighting in the male cricket is short-term memory and the memory can be reinforced by repetitive experiences. These results strongly support that pheromone behaviors of insects can be modulated by their experiences.

How do subordinate animals retain the previous experiences and change their behaviors after they lose fighting? As the next step, we examined the effects of NO on the agonistic behavior to investigate the neuronal mechanisms of the memory of previous experience in subordinate.

2-3. Effect of NO on avoidance behavior of the subordinate crickets

Gaseous molecule NO diffuses three dimensionally at about 100μm/sec in the tissue and it plays important roles in the peripheral and central nervous systems to regulate synaptic transmissions in invertebrate animals[5,6]. The components of NO/cGMP cascade in insects have been known as an important factor in the formation of memories. However, little is known of the role of NO signaling during the formation of dominant hierarchy.

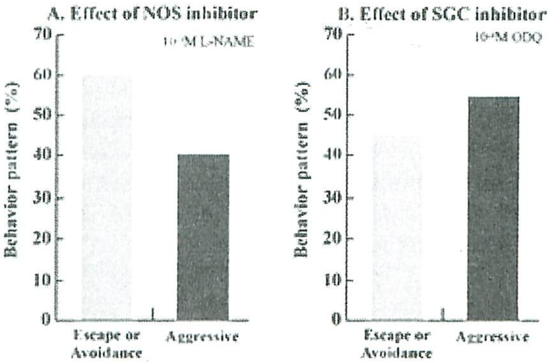

Fig. 2. Effects of NOS inhibitor L-NAME and SGC inhibitor ODQ. **A:** Head-injection of L-NAME at 1mM increased aggressive behavior in the subordinate males at the second engagement with the dominant male crickets. **B:** Head-injection of 100μM ODQ also increased aggressive behavior in the subordinate males at the second engagement.

Behavioral and pharmacological experiments were performed to investigate the effects of NO signaling on the agonistic behavior of subordinate crickets. The agents that inhibit NO/cGMP signaling pathway were applied into the brain with a volume of 1 μl using a Hamilton micro syringe. To inhibit NOS activity, the L-NAME at 1mM was head-injected. Fifteen minutes after injecting L-NAME, two of males were gently placed in a same experimental arena observe agonistic behavior. Even if they were head-injected L-NANE, the behaviors at the first contact were almost same as untreated crickets, i.e. they suddenly started fighting. After dominant hierarchy was established at the initial fight, both of them were kept isolated for 15min to have a rest. They were then gently moved again into the arena for reengagement.

The behavior of subordinates were changed significantly ($p<0.05$, χ^2-test) at the reengagement fighting. At the second engagement, only 10% of the subordinates elicited aggressive behavior if they were not treated with NOS inhibitor (Fig. 2). However, subordinates injected

L-NAME did not elicit avoidance behaviors but aggressive behaviors (Fig. 2). These results suggest that NO generation in the cricket brain would play important role on the formation of dominant hierarchy.

Nitric oxide activates soluble guanylate cyclase (SGC) in the target cells in nervous systems[7]. We examined the effects of NO/cGMP signaling on the agonistic behavior of subordinate crickets using SGC inhibitor ODQ. The behavior of both males injected ODQ were similar to control animals at the initial contact. At the second engagement, however, they elicited aggression again (Fig. 2). These results indicate that NO/cGMP signaling regulates formation of social hierarchy.

2-4. Effect of NO on biogenic amine levels in the brain

Biogenic amines are thought to regulate aggressiveness of insects[8]. However, it has little known how these biogenic amines are regulated by the external stimuli. Here we hypothesize that NO system might regulate biogenic amines in the brain to meditate agonistic behavior of the crickets. As the first step, biogenic amine levels were measured after dominant hierarchy was established using high-performance liquid chromatography (HPLC) with electrochemical detection (ECD). Accumulated levels of some biogenic amines in the cricket brain decreased after agonistic behavior was settled (Fig. 3A). The levels of OA and DA in the subordinate cricket brain, in particular, were significantly decreased. However OA and DA levels in the brain of dominants did not change.

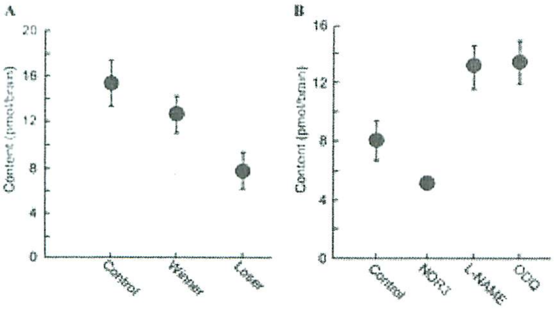

Fig. 3. A: Decrease in OA level after agonistic behavior. **B:** Head-injection of NOR3 decreased OA level in the brain, but L-NAME and ODQ increased.

Then we investigate how NO mediates biogenic amine system in the brain. Inhibition of NO/cGMP signaling in the brain increased aggression of subordinate males. NO/cGMP signaling must play important role to establish dominance hierarchy among male crickets. On the other hand, conspecific interaction between male crickets emerged decrease in biogenic amines such like OA, DA in the brain. We performed head-injection of NO-donor NOR3 to elevate NO level in the brain and then measured biogenic amine level in the brain (Fig. 3B). A NOR3 significantly decreased OA and 5-HT level of the brain although DA level in the brain was not changed. A NOS inhibitor L-NAME had opposite effect of NOR3. A head-injection of L-NAME increased OA and 5-HT levels. The effect of an ODQ on the biogenic amine levels in the brain was similar to that of L-NAME. The OA level was significantly higher than control. These result suggested that NO system regulated biogenic amine system in the brain, which mediate aggressiveness of the crickets.

3. MODELING AND SIMULATION

3-1. Behavior modeling of artificial crickets

We have constructed models of cricket agonistic behavior based on biological analysis. First of all, observation ability and motion ability of artificial cricket are assumed based on real crickets. We defined a personal field of a cricket. Then cricket behaviors were simplified to three major primitive patterns that were wandering, avoiding and fighting. We simulate our model by changing the density of the population. The rules of artificial cricket behaviors are as follows. a) Wandering: cricket walks around randomly with going straight, turning and stopping or staying. b) Avoiding obstacle: when artificial cricket sense an obstacle wall by touching one of antennae, it will turn opposite side. In case, both antennae touch an obstacle at the same time, it will turn left or right randomly. When it arrives at a corner to touch obstacle using both antennae, it turns also left or right randomly. c) Fighting: if other cricket comes into the personal field, cricket turns to the alien to start fighting. The fighting terminates when opponent treat from the personal field.

Subordinate turns and escapes from dominant opponent. The subordinate will avoid opponent for a while. On the other hand, dominant recognize its win if opponent cricket retreats from its personal field. Crickets change their behavior based on their experiences. Hence, we need at least one internal state variable for the cricket model. The probability (*P*) of losing at cricket fighting depends on a parameter α that runs from 0 through 1. The parameter α describes an internal state of the cricket. We determined this parameter from the behavior experiments of crickets. The value of α gradually decrease depending on time. Losing at fight increases the value of fight but decreases while winning at the fight.

$$P = \alpha (0 \le \alpha \le 1) \qquad (1)$$

The value of α is revised with the following equation.

$$\alpha_{n+1} = (1-\omega)\alpha_n + \varepsilon_{lose}\eta_{lose} - \varepsilon_{win}\eta_{win} \qquad (2)$$

Here,

$$\eta_{lose} = \begin{cases} 1 & \text{if lose} \\ 0 & \text{else} \end{cases}, \eta_{win} = \begin{cases} 1 & \text{if win} \\ 0 & \text{else} \end{cases}$$

We simulate how crickets change their behavior depending on the density of animal population. Three kinds of fields (128×128(pix), 256×256(pix) and 512×512(pix)) are utilized for simulations (Fig. 4). The number of crickets was fixed to four. In the case of higher density, the value of α converged to rather high and most of all avoided each other. In the case of middle density, one of the crickets increased its α value to be dominant. In the case of low density, α did not converge. The aggressiveness of each cricket was similar and α value is not so low. The behavior of the artificial crickets was described by using a probability that is defined by the parameter α. Optimization of the simulation model suggests that the cricket behaviors among males were mainly influenced by the previous fighting experience in the particular previous losses. The simulation results are similar to that of behavior observation results of crickets, suggesting that the parameter α would contain internal model that must be neuronal modulation system in animals.

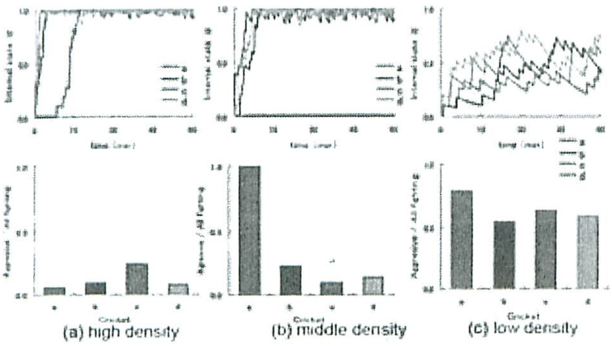
(a) high density (b) middle density (c) low density

Fig. 4. Simulation of results of the artificial cricket behavior.

3-2. Neuronal circuit modeling of the cricket's brain - from sensory input to behavior selection –

In order to understand dynamic activities of the cricket's brain, we also constructed a neuronal circuit model based on biological experiments. We have demonstrated that NO system and biogenic amine system mediate aggressiveness of the cricket. We consider neuromodulators in the neuronal model that consists of a diffusion equation for NO level, differential equations for cGMP and OA levels and a threshold model for behavior selection utilizing OA level. These components are connected in series. Since the hypothesis which OA level will affect behavior, we assume that fighting behavior is selected when OA is over the threshold (0.5) and avoidance behavior is selected when OA is under that. By the computer simulations, we observe that a rising trend in cGMP level by the increase in NO level and a consumption in OA level by a rising trend in cGMP level. As memory mechanism of defeat experience in fighting, it assumes that OA level of the winner increases by a certain value and one of the losers reduces by another certain value in proportion to fighting period.

Figure 5 shows the response of internal state of the winner and the loser in a time series. The winner continually takes the aggressive state because OA level is over the threshold. The loser takes the negative state (under the threshold) for a short time. These results indicate that proposed model is not to contradict to biological data and is an explicable one for an internal structure of the cricket's brain. As future work, we consider applying the effect of other factors in the brain to proposed neuronal circuit model and also examine to construct an explicable model both of internal physiological state and swarm behavior based on the interaction among individuals.

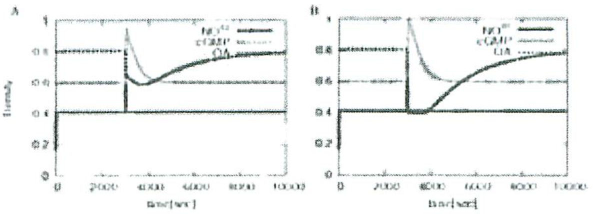

Fig.5. Simulated internal state. A: Internal state of a winner. B: Internal state of loser.

4. CONCLUSION

Cricket agonistic behavior was focused to investigate neuronal mechanism underlying adaptive behaviors in social population. They recognize conspecific animals using cuticular substances called cuticular pheromones. Understanding the neuronal mechanism of the cricket agonistic behavior as a model system helps us to elucidate the mechanisms of social adaptation. Experienced memory of agonistic interactions between male crickets had influence over the following behavior selection in subordinate males. NO/cGMP signaling was suggested here to regulate biogenic amine system in the brain, which in turn mediates agonistic behavior among male crickets. Based on results from biological experiments, we constructed behavior model and neuronal models. Further investigation performing neuronethological analysis and engineering modeling will reveal neuronal mechanisms of adaptive behavior in a social population.

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