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Octopaminergic system in the brain controls aggressive motivation in the ant, *Formica japonica*

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Abstract

The ants, *Formica japonica* is polyphagous and workers hunt other insects as foods. In this study, interspecific aggression was examined in the workers and queens. Behavior experiments demonstrated that interspecific aggressiveness was significantly higher in workers than queens. Workers showed predatory aggressive behavior towards crickets, on the other hand, queens elicited threat behavior but they didn't attack crickets. In order to investigate neuronal mechanisms underlying regulation of aggressive motivation, the role of biogenic amine in the brain in evoking aggressive behavior was examined by measuring biogenic amine using high-performance liquid chromatography (HPLC) with electrochemical detection (ECD). No significant difference in the octopamine (OA) level was found between workers and queens, but the level of N-acetyloctopamine (NacOA) in the brain of queens was significantly higher than that of workers. This study suggests that OAergic system in the brain must involve in controlling aggressive motivation in the ants.

Introduction

Aggressive behavior is common among most of all animals. They conflict not only among intraspecific animals but also among interspecific animals. In most case, animals show violent attacking towards the opponents. Ants elicit offensive and defensive aggressive behaviors when they encounter intraspecific hetero colonial ants or interspecific insects. Ants must provide us great experimental system for investigating the neuronal mechanisms underlying controlling aggressive motivation in animals.

The Japanese wood ant, *Formica japonica* is one of the most common ants in Japan. The colonies of this species are largely polygynous and contain thousands of workers and broods. *Formica japonica* is a polyphagous and the foragers sometime hunt other small insects and take them into their nest as foods. This study focused on the predatory aggressive behavior in the ants towards interspecific insect (cricket) to investigate the neuronal mechanisms underlying the motivation for aggressive behavior. We investigated here the role of biogenic amine in the brain in motivating aggressiveness. Biogenic amine in the brain is believed to function as neurotransmitter, neuromodulator and neurohormone and play important role in insect behavior [1, 2]. According to the similarities in the action, the octopaminergic (OAergic) system in invertebrate and noradrenergic system in vertebrate have been thought to be homologous [8]. In insect, OAergic system is closely linked to aggressive behavior [3, 4, 7, 10]. In ants, OAergic system is known to link to nestmate recognition [11]. Nestmate recognition is important for releasing intraspecific aggression. We here discuss about the role of OAergic system in the ant brain in motivating aggressive behavior.

Materials and methods

A colony of ants *Formica japonica* was collected at Hokkaido University and reared at 26 °C under L/D 12:12. It contained several hundreds of workers and pupas of reproductive ants. Workers were collected outside of the nest and queens that were hatched within 1 week were collected in the nest. Some queens were also collected after their copulation. Response of worker and queen to a mechanical tactile stimulus was observed and the responses were classified into 4 levels, i.e. level1: lie down, level 2: dart, level 3: turn toward the source of stimulus (fine brush), level 4: turn toward the brush and bite it. In order to observe predatory aggressive behavior, an ant was placed in a plastic laboratory dish (φ5cm) and an infant cricket (5mm) was installed in it. The responses of ants to a cricket were classified into 5 levels, i.e. level 1: no response, level 2: escape, level 3: threat, level 4: chase and level 5: bite and capture. Behavior of ants was recorded using a digital video recorder for later analysis.

Biogenic amines in the ant brain were measured using high-performance liquid chromatography (HPLC) with electrochemical detection (ECD). The detail of the HPLC-ECD system was described in our previous report [5]. In short, an ant was quickly frozen using liquid N_2 and the brain was dissected out in ice cold saline. A single brain was homogenized in 50 µl of ice-cold 0.1 M perchloric acid containing 5 ng of 3, 4-dihydroxybenzylamine (DHBA) as an internal standard. After centrifugation of the homogenate (0°C, 15,000rpm, 30min), the supernatant of sample was injected directly onto the HPLC column. The mobile phase containing 0.18 M chloroacetic acid and 16 µM disodium EDTA was adjusted to pH 3.6. Sodium-1-octanesulfonate at 1.85 mM as an ion-pair reagent and CH_3CN at 8.40% (v/v) as an organic modifier were added into the mobile phase solution. The chromatographs were acquired using the computer program PowerChrom (eDAQ Pty Ltd).

Results

F. japonica is believed not to have major workers and minor workers. However, workers perform different task such as nest building, guarding and foraging. Nest-builders maintain their nest and bring nest materials to the outside of the nest. Guards stay around the entrance of the nest and attack invaders hardly. Foragers go collecting foods outside. We here examined interspecific aggression in workers and queens of *F. japonica.* The responses of workers and queens to mechanical tactile stimulus to the abdomen were observed (Fig 1A). Nest builders evoked dart response (avoidance behavior) if abdomen was touched with a fine brush. Foragers and guards turn toward the brush and aggressively bite it (aggressive response). On the other hand, queen showed mainly turn response toward the source of the stimulus (threat response). Interspecific aggression towards a cricket was also observed in the ants (Fig 1 B). Foragers and guards showed similar predatory aggressive behavior toward a cricket but nest builders showed agile avoidance behavior (not shown). On the other hand, queen showed threat-behavior without predatory aggression.

In order to investigate the role of biogenic amine in aggressive motivation, the contents

of biogenic amines in the brain was measured using HPLC-ECD. OA levels in the brain of forager and virgin queen were significantly higher than that of nest builder and queen that started laying eggs (data not shown). However the ratio of OA in the brain of forager was significantly higher than both queens (Fig 2A). On the other hand, there were no significant differences in the level of 5-HT in the brain of forager and nest builder. The levels of 5-HT in the brain of queens were significantly lower than that of workers (data not shown). However, the ratio of 5-HT to Nac5-HT in the brain of foragers and virgin queen were significantly higher than queens (Fig 2B). We also examined the levels of tyramine (TA) and dopamine in the brain (DA) (data not shown). The level of TA in the brain of queens was significantly higher than that of worker and virgin queen; however there were no significant difference in the ratio of TA to NacTA between forager and nest builder, between virgin queen and queen and between workers and queens. On the other hand, DA level in the brain of queens was significantly lower than that of workers and virgin queen although there was no significant difference in the ratio of DA to NacDA between forager and nest builders, between virgin queens and queens and between workers and queens.

Discussion

This study demonstrated that the wood ant, *F. japonica* has great potential for elucidating the key to motivate aggressive behavior. Like other ants, the colony of *F. japonica* has queens and workers that perform different tasks. In general, the number of tasks performed by workers increase and change depending on their age in ants. However, there still remains plasticity in shifting between different tasks according to the demand of colony, for example, between nursing and foraging [12], foraging and nest building (our observation). However, at this stage, little is understood neuronal mechanisms how they change their task and which social demand in the colony makes workers change their task.

We here demonstrated that interspecific aggression is evoked in workers and queen and that aggressiveness in ants change depending on the task and caste. Biogenic amine in the brain is thought to be a key that regulates aggressive behavior in insects [3, 4, 10]. The wood ant *F. rufa* shows interspecific aggression and it has been demonstrated that 5-HT increases if ants evoke aggressive behavior [6]. However, it has been still remained unclear how aggressive behavior is motivated in the central nervous systems. Our study demonstrated that the OAergic system in the ant brain is closely linked to motivating aggressive behavior. Workers that have higher level of OA in the brain like foragers showed higher aggressiveness. Although minor worker *Pheidole dentata* shift the task from nursing to foraging, the OA level in the brain is demonstrated not to change with age [9]. Therefore OA might not be linked to the determination of task in the ant. On the other hand, our data demonstrated that, in the queens, even if OA level in the brain is kept high; aggressive motivation seems to be kept low to evoke just threat behavior, if activity of N-acetylation in the brain is enhanced. Our studies

also suggest that the 5-HTergic and DAergic systems are not closely linked to motivating aggressive behavior, although the 5-HTergic system might be linked to an evoked aggression as shown by Kostowski et al [6], and DAergic system in the brain, on the other hand, is demonstrated to be linked to social interaction in the ant [5]. We also observed that social interaction maintains contents of biogenic amine in the brain. Thus our study suggests that increase in OA level in the brain enhances aggressive motivation in the ants. It is now necessary to investigate which neurons are involved in motivating aggression in the ant brain.

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Figure legends

Fig. 1. Aggressive response in worker and queen. A: A gentle mechanical tactile stimulus to the abdomen evoked aggressive response in foragers but avoidance dart response in nestbuilders. The tactile stimulus evoked turn response in queens. (N=10) B: Interspecific aggression in ants. Foragers evoked predatory aggression but queen elicited threat behavior. (N=15) *: $p < 0.05$: ANOVA with Tukey's test.

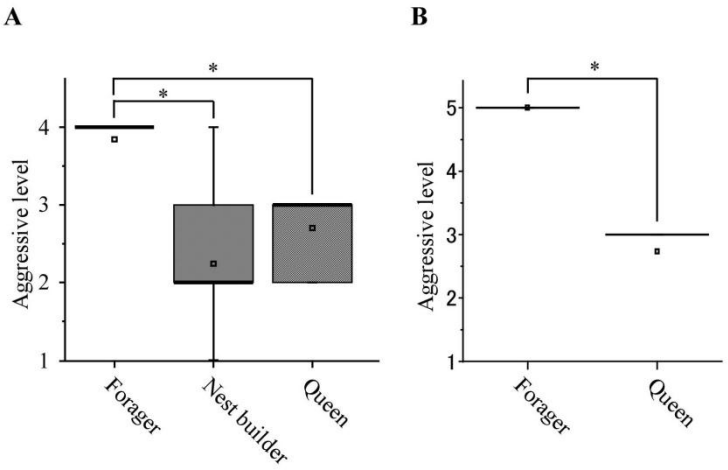

Fig. 2. Biogenic amines in the brain of ants. A: The ratio of OA to NacOA in the ant brain. The ratio of OA in the brain of foragers was significantly higher than queens. B: The ratio of 5HT to Nac5HT in the ant brain. The ratio of 5-HT in the brain of foragers and virgin queens were significantly higher than queens. (*$P < 0.05$, two-tailed Kruskal-Wallis ANOVA with Bonferroni-type multiple nonparametric comparison)