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RESEARCH ARTICLE

Social interactions influence dopamine and octopamine homeostasis in the brain of the ant *Formica japonica*

Ayako Wada-Katsumata[1,2], Ryohei Yamaoka[3] and Hitoshi Aonuma[1,*]

[1]Research Institute for Electronic Science, Hokkaido University, Sapporo, Hokkaido, 060-0812, Japan, [2]Department of Entomology, North Carolina State University, Raleigh, NC 27695, USA and [3]Department of Applied Biology, Graduate school of Science and Technology, Kyoto Institute of Technology, Kyoto, Kyoto-fu, 606-8585, Japan

*Author for correspondence (aon@es.hokudai.ac.jp)

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SUMMARY

In ants, including *Formica japonica*, trophallaxis and grooming are typical social behaviors shared among nestmates. After depriving ants of either food or nestmates and then providing them with either food or nestmates, a behavioral change in type and frequency of social interactions was observed. We hypothesized that starvation and isolation affected levels of brain biogenic amines including dopamine (DA) and octopamine (OA) – neuromediators modifying various insect behaviors – and tested the relationship between brain biogenic amines and social behaviors of stressed ants. Ants starved for 7 days contained lower brain DA levels and they did not perform trophallaxis toward nestmates. Feeding starved ants sucrose solution re-established trophallaxis but not brain DA levels. The performance of trophallaxis induced recovery of brain DA content to the level of untreated ants. Ants that were isolated for 2 days displayed markedly increased OA levels, which following nestmate interactions, returned to levels similar to those of control (non-isolated) ants and ants isolated for 1 h. We conclude that: (1) starvation reduced brain DA level but had no significant effect on brain OA (trophallaxis recovered the brain DA levels), and (2) isolation increased brain OA level but had no effect on brain DA (trophallaxis and grooming events recovered the brain OA levels). We suggest that social interactions with nestmates influenced brain biogenic amine homeostasis in stressed *F. japonica*.

Key words: brain biogenic amine, social insect, social behavior, starvation stress, isolation stress, trophallaxis, grooming.

INTRODUCTION

Ants and other social insects perform trophallaxis and allogrooming to transfer food among nestmates (Wheeler, 1918; Wilson, 1971; Hölldobler and Wilson, 1999), eliminate pathogens and parasites for colonial sanitation (Oi and Pereira, 1993; Currie et al., 1999; Fernández-Marín et al., 2009) and exchange cuticular hydrocarbons (CHCs) to maintain nestmate recognition (Hölldobler and Wilson, 1999; Greene, 2010; Liebig, 2010; Van Zweden and d'Ettore, 2010). Nestmate recognition is a cognitive process whereby a worker accepts or rejects the encountered conspecifics after detecting CHCs on the body surface by antennal sensory organs (Ozaki et al., 2005; Ozaki and Wada-Katsumata, 2010). Nestmates achieve a uniform colony-specific odor that depends on mixing individuals' CHCs *via* trophallaxis and grooming behavior, and they discriminate differences in CHC profiles between nestmates and non-nestmates (Ozaki and Wada-Katsumata, 2010; Van Zweden and d'Ettore, 2010). Social isolation of individual ants can alter their CHC profiles (Boulay et al., 2000a). For example, *Camponotus fellah* workers that were isolated for an extended period (20–40 days) and then reintroduced into the mother colony did not perform social behaviors with the resident ants and were not accepted by the former nestmates, probably because their CHC profiles did not match those of the residents (Boulay et al., 2000a; Boulay et al., 2004). In contrast, ants isolated for a shorter duration (3–10 days) performed trophallaxis and allogrooming, which presumably allowed reacceptance into the mother colony.

Formica japonica is one of the most common ant species in Japan. Colonies are largely polygynous and contain thousands of workers and brood (Kondoh, 1968; Masuko et al., 1998). Like *C. fellah*, *F. japonica* also uses CHCs as nestmate recognition cues (Akino et al., 2004). Trophallaxis and grooming are considered important social behaviors for exchanging both food and CHCs (Akino et al., 2004). In our preliminary observations, trophallaxis was rarely performed by *F. japonica* workers in a satiated colony. However, when ants in a starved colony were given sugar solution they performed trophallaxis, transferring sugar solution to starved nestmates. Also, workers experimentally isolated for several hours and then reunited with nestmates performed both trophallaxis and allogrooming (Fig. 1). It thus seemed that starved or isolated ants displayed social behaviors more frequently than untreated ants. We hypothesized that starvation and isolation acted as stressors, causing physiological and behavioral changes.

Biogenic amines play a number of roles in insect behavior (Baumann et al., 2003). High concentrations of monoamines such as dopamine (DA), octopamine (OA), serotonin (5-HT), tyramine (TA) and histamine (HA) exist in the nervous system and function as neuromediators, i.e. neurotransmitters, neuromodulators or neurohormones (Evans, 1980; Brown and Nestler, 1985; Roeder, 1994; Manastirioti, 1999; Scheiner et al., 2006). OA has been linked to nestmate recognition in social insects such as honeybees (Robinson et al., 1999) and ants (Vander Meer et al., 2008). In *Solenopsis invicta*, worker recognition of conspecific non-nestmates decreased following queen removal (Vander Meer et al., 2008). These queenless workers

Fig. 1. Trophallaxis, allogrooming and self-grooming in *Formica japonica* foragers observed in assay tubes after several hours of social isolation. Left, two workers are performing trophallaxis; middle, a worker is grooming the abdomen of another worker; right, a worker is grooming her own foreleg.

had reduced brain OA and reduced discriminatory acuteness; however, treatment with exogenous OA restored brain OA and the ability to recognize conspecific non-nestmates in queenless workers. Also, administration of OA changed the duration of trophallaxis in socially deprived *C. fellah* workers (Boulay et al., 2000a; Boulay et al., 2000b). Additionally, it is well known that stress, resulting from temperature, mechanical or chemical stimuli, population density and starvation, modulates biogenic amine levels in the insect brain (Chen et al., 2008; Gruntenko et al., 2004; Rauschenbach et al., 1993; Woodring et al., 1988; Neckameyer and Weinstein, 2005). However, the relationship between brain biogenic amines and social behaviors in ants remains unclear.

In this study, we examined which brain biogenic amines were affected by stressors such as starvation and isolation, and whether social behaviors such as trophallaxis and grooming are related to changes in brain biogenic amine levels under stress conditions. The impact of stress on the relationship between social behaviors and brain biogenic amines is discussed.

MATERIALS AND METHODS

Ants

Formica japonica Motschoulsky 1866 were collected in April at Hokkaido University. One colony was separated into two (colonies A and B). Each colony contained two queens and approximately 800 workers. The two colonies were installed in artificial plaster nests and kept at 26°C under a 12 h:12 h light:dark photoperiod. Fresh water and food (20% sucrose solution and dead crickets) were provided. We assumed that there were no specific differences in genetic background and rearing conditions between colonies A and B. After separation, we carried out all behavioral tests within 1 month. Additionally, temporal changes of brain biogenic amines of starved ants after sucrose feeding were tested in November. Forager ants that were outside the artificial nest were used in the experiments. Behavior of tested ants was recorded using a digital video recorder (NV-GS500-S, Panasonic, Osaka, Japan) for later analysis.

Behavioral observations of starved ants

Starved ants were obtained by depriving colony A of food for 7 days. Five minutes before the test, one starved ant was introduced into an assay tube (5 cm length, 8 mm internal diameter, Tygon R-3603, Saint-Gobain K. K., Hara-Mura, Nagano prefecture, Japan). A pair of starved nestmates was introduced into another assay tube, and the single starved ant was introduced into the assay tube containing two starved nestmates. Test ants were divided into three treatment groups: S, SS and SST. In treatment S, a single starved ant was allowed encounters with two nestmates. Trophallaxis and grooming events of the single ant were observed for 3 min after it made antennal contact with either nestmate. After observation, the single ant was used for brain biogenic amine analysis. In treatment SS, a single starved ant was provided 20% sucrose solution and allowed to encounter the two starved nestmates. Trophallaxis and grooming events were observed after the single ant made antennal contact with either nestmate. The single ant was collected just before performing trophallaxis and used for brain biogenic amine analysis. In treatment SST, a single starved ant was provided 20% sucrose solution and allowed to perform trophallaxis with either starved nestmate. The ant was observed until finishing one bout of trophallaxis, then collected for biogenic amine analysis. As a control group, untreated ants (non-isolated and non-starved) were obtained from colony B. A single ant was introduced to an assay tube containing two non-starved nestmates. We observed trophallaxis and grooming for 3 min after antennal contact with either nestmate and then used the single ant for brain biogenic amine analysis. The first antennal contact occurred within 10 s in the SS, SST and control groups after ants were introduced into the assay tube containing nestmates. Forty replicates were performed in each test group. The brains of eight ants in each test group were homogenized together as one sample. Five samples were prepared for each treatment for biogenic amine analysis.

Additionally, after the experiments described above, temporal changes in the brain biogenic amines of starved ants after sucrose feeding were tested. Ants from colony B (reared for 18 months in the laboratory) were deprived of food for 7 days and divided into three treatment groups: S0, SS3 and SS5. In treatment S0, one single starved ant was introduced into an assay tube, observed for 3 min and immediately used for brain biogenic amine analysis. In treatment SS3, one single starved ant was provided 20% sucrose solution, observed for 3 min and used for brain biogenic amine analysis. In treatment SS5, one single starved ant was provided 20% sucrose solution, observed for 5 min and used for brain biogenic amine analysis. Forty replicates were performed for each test group. For the brain biogenic analysis, four brains were homogenized together as one sample, and 10 samples were prepared.

Behavioral observations of isolated ants

Ants from colony B were introduced individually into the assay tubes and isolated from the mother colony for either 1 h or 2 days in order to test the effect of social isolation on behavior and biogenic amine titer. From preliminary tests, the 1 h isolation period was considered representative of a normal foraging period under natural conditions whereas 2 days of forager isolation was considered abnormal. Each isolated ant was provided 20% sucrose solution and held within the assay tube with a plug of cotton. Following isolation, each ant was introduced into a new assay tube. There were two treatment groups: I and IC. In treatment I, the isolated ant without nestmates was observed for 3 min after introduction into a new assay tube. In treatment IC, a single isolated ant was allowed encounters with two non-isolated nestmates in a new assay tube. Trophallaxis and grooming behaviors were observed and recorded for 3 min after the single ant made antennal contact with either nestmate; their first contact occurred within 10 s. Forty replicates were performed in each test group. Eight brains were pooled for each sample. Therefore, five samples were prepared for biogenic amine analysis.

Measurement of brain biogenic amines

Each ant from the treatment and the control groups was quickly frozen using liquid N_2. Frozen ant brains were dissected out in cold saline (140 mmol l^{-1} NaCl, 10 mmol l^{-1} KCl, 6 mmol l^{-1} $CaCl_2$, 2 mmol l^{-1} $MgCl_2$, 44 mmol l^{-1} glucose, 2 mmol l^{-1} TES, pH 7.2). The optic lobes were cut off from the brain to prevent contamination with retinal pigments. Ant brains were collected into a micro glass homogenizer and homogenized in 50 µl of ice-cold 0.1 mol l^{-1} perchloric acid containing 5 ng of 3,4-dihydroxybenzylamine (DHBA; Sigma, St Louis, MO, USA) as an internal standard. After centrifugation of the homogenate (4°C, 15000 ***g***, 30 min), 35 µl of supernatant was collected. Biogenic amines in the brain were measured using high-performance liquid chromatography (HPLC) with electrochemical detection (ECD). The HPLC-ECD system was composed of a pump (EP-300, EICOM Co., Kyoto, Japan), an auto-sample injector (M-504, EICOM Co., Kyoto, Japan) and a C18 reversed-phase column (250 mm×4.6 mm internal diameter, 5 µm average particle size; CAPCELL PAK C18MG, Shiseido, Tokyo, Japan) heated to 30°C in the column oven. A glass carbon electrode (WE-GC, EICOM Co.) was used for electrochemical detection (ECD-100, EICOM Co.). The detector potential was set at 950 mV *versus* an Ag/AgCl reference electrode, which was also maintained at 30°C in a column oven. The mobile phase containing 0.18 mol l^{-1} chloroacetic acid and 16 µmol l^{-1} disodium EDTA was adjusted to pH 3.6 with NaOH. Sodium-1-octanesulfonate at 1.85 mmol l^{-1} 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 flow rate was kept at 0.7 ml min^{-1}. The chromatographs were acquired using the computer program PowerChrom (eDAQ Pty Ltd, Denistone East, NSW, Australia). The supernatants of samples were injected directly onto the HPLC column. After the acquisition, they were processed to obtain the level of biogenic amines in the same sample by the ratio of the peak area of substances to the internal standard DHBA. We used a standard mixture for quantitative determination that contained amines, precursors and metabolites (20 compounds at 100 ng ml^{-1} each): DL-3,4-dihydroxymandelic acid (DOMA), L-β-3,4-dihydroxyphenylalanine (DOPA), L-tyrosin (Tyr), N-acetyloctopamine (Nac-OA), (–)-noradrenaline (NA), 5-hydroxy-L-tryptophan (5-HTP), (–)-adrenaline (A), DL-octopamine (OA), 3,4-dihydroxybenzylamine (DHBA, as an internal standard), 3,4-dihydroxy phenylacetic acid (DOPAC), *N*-acetyldopamine (Nac-DA), 3,4-dihydroxyphenethylamine (DA), 5-hydroxyindole-3-acetic acid (5-HIAA), *N*-acetyltyramine (Nac-TA), *N*-acetyl-5-hydroxytryptamine (Nac-5HT), tyramine (TA), L-tryptophan (Trp), 3-methoxytyramine (3-MTA), 5-hydroxytryptamine (5-HT) and 6-hydroxymelatonin (6-HM). Nac-OA and Nac-TA were gifts from Dr Nagao [Kanazawa Institute of Technology, Hakusan, Ishikawa prefecture, Japan, see Sasaki et al. (Sasaki et al., 2007)]. All other substances were purchased from Sigma.

Statistical analysis

The results of behavioral observations were tested by Pearson's χ^2 test (P<0.05) and the Mann–Whitney U-test (P<0.05). Differences in the levels of biogenic mines were tested using ANOVA with Tukey's test and Dunnett's test (P<0.05).

RESULTS

Behavior of starved ants and brain biogenic amine levels

The number of starved ants that performed social behaviors under different conditions is shown in Table 1. We classified grooming behavior into three categories: receiving grooming, giving grooming and self-grooming. Grooming behavior was not observed in any of the treatment groups. In the control group obtained from colony B (one non-starved ant allowed encounters with non-starved nestmates), none of the ants performed trophallaxis, received grooming or gave grooming. Ten ants from the control group self-groomed. None of the ants within the S group (one starved ant allowed encounters with starved nestmates) performed trophallaxis. All starved ants provided 20% sucrose solution in the SS and SST groups solicited trophallaxis. We observed abdominal distention in recipient SST ants, indicating that trophallaxis occurred. A trophallaxis event took 12.2±1.9 s (mean ± s.e.m.) in SST ants (N=40).

Table 1. Number of starved ants that performed social behaviors under different conditions

	Control (N=40)	S (N=40)	SS (N=40)	SST (N=40)
7 days starvation	–	+	+	+
Sucrose	+	–	+	+
Nestmates	+	+	+*	+
Trophallaxis	0	0	n.d.	40
Receiving grooming	0	0	0	0
Giving grooming	0	0	0	0
Self grooming	10	0	0	0

Control, untreated ants; S, starved ants; SS, starved ants provided sucrose and allowed to interact with starved nestmates; SST, starved ants provided sucrose and allowed to perform one bout of trophallaxis with starved nestmates.
+ and – indicate treatment types; +*, SS group ants were collected just before they performed trophallaxis, and used for brain biogenic amine analysis.
n.d., no data.

We focused our attention on DA and OA levels because we could not find pronounced changes in the levels of other brain biogenic amines. The DA level in the brains of the S group ants was markedly lower than in the control group (ANOVA, $F_{3,13}$=6.36, P=0.007; Fig. 2A). The DA level in the SS group was slightly higher than in the S group, but significantly lower than that of the control group. The DA level of the SST group was similar to that of the control group. The difference in DA levels between SS and SST groups could be due to either interaction with nestmates or the fact that SST ants were withdrawn from the assay later than SS (sucrose-fed) ants (before *vs* after trophallaxis). To control for temporal changes in brain DA of the sucrose-fed ants (Fig. 3A), the DA levels of the S0 (one starved ant), SS3 (one starved ant kept for 3 min after sucrose feeding) and SS5 ants (one starved ant kept for 5 min after sucrose feeding) were compared. Ants in all groups were not allowed to interact with nestmates. The brain DA levels in both SS3 and SS5 treatments were significantly lower than those of the S0 group (ANOVA, $F_{2,27}$=8.88, P=0.001; Fig. 3A). The observed values of amine levels of Fig. 3 were not compared with the result of Fig. 2 because the tests were carried out in different seasons. The DA levels of SS3 and SS5 ants were approximately one-third of the DA level of S0 ants. These results suggest that the DA level of starved ants increases during interaction with nestmates but not shortly after sucrose feeding. However, because sucrose feeding caused the ants to perform trophallaxis, higher DA levels in the SST ants than in the SS treatment (Fig. 2A) could be accounted for by social interaction and trophallaxis.

There were no differences in OA levels between the treatment and control groups. However, the OA levels tended to decrease after starvation and increased after the ant ingested sucrose solution and performed trophallaxis with nestmates (ANOVA, $F_{3,13}$=3.21,

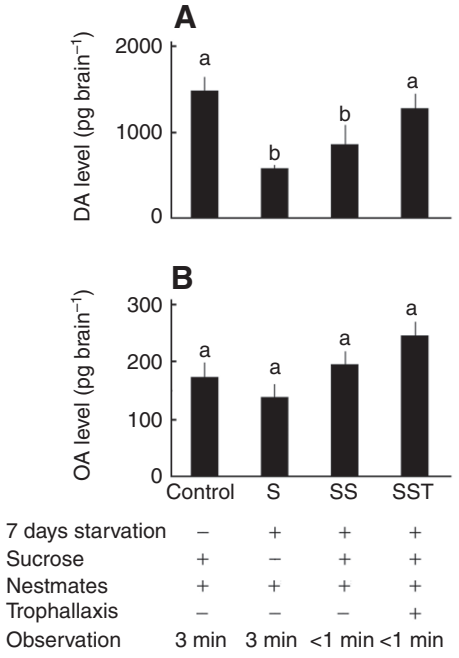

Fig. 2. Dopamine (DA) and octopamine (OA) levels in the brains of starved ants interacting with nestmates. (A) DA levels per brain of sucrose-fed ants. (B) OA levels per brain of sucrose-fed ants. Ants in all treatment groups were allowed to interact with nestmates. + and – indicate treatment types. S group ants were observed for 3 min, and used for brain biogenic amine analysis. SS group ants were collected just before they performed trophallaxis, and used for brain biogenic amine analysis. SST ants were collected just after they performed trophallaxis, and used for brain biogenic amine analysis. Observations of these treatment groups were completed within 1 min. Groups marked by the same letter did not differ at the 5% significance level (ANOVA, Dunnett's test). All columns were exclusively compared with the control. The level of DA was significantly decreased by starvation. After ingesting sucrose solution and then performing trophallaxis, the DA level recovered. The level of OA was not affected by starvation, sucrose feeding or interaction with nestmates. Data are means ± s.e.m.

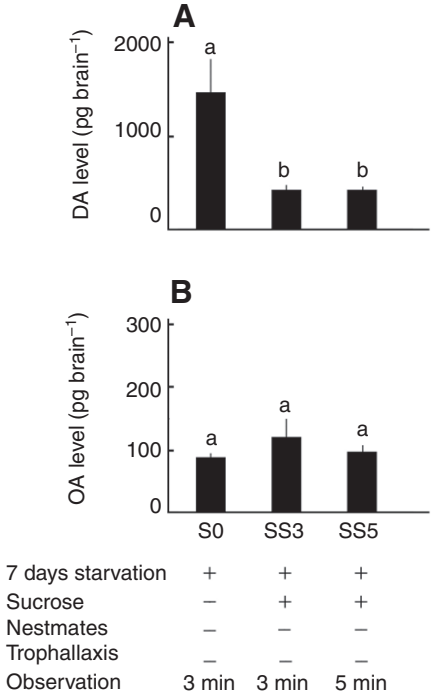

Fig. 3. DA and OA levels in the brains of starved ants with non-nestmates. (A) DA levels per brain of sucrose-fed ants. (B) OA levels per brain of sucrose-fed ants. Ants in all groups were not allowed to interact with nestmates. S0, one single starved ant was observed for 3 min; SS3, one single starved ant was provided sucrose solution, observed for 3 min; SS5, one single starved ant was provided sucrose solution, observed for 5 min. + and – indicate treatment types. Groups marked by the same letter did not differ at the 5% significance level (ANOVA, Tukey's test). DA levels in sucrose-fed ants did not recover even 5 min after sucrose feeding. The level of OA was not affected by sucrose feeding. Data are means ± s.e.m.

P=0.059; Fig. 2B). There were no differences in OA level between the S0 group and the other treatment groups (Fig. 3B).

Behavior of isolated ants and brain biogenic amine levels

In the IC group (one isolated ant allowed encounters with non-isolated nestmates), 75% of the 1-h-isolated ants performed trophallaxis with their nestmates (Table 2). The number of 1-h-isolated ants showing trophallaxis and grooming (Table 2) was higher than that of control ants (Table 1) (trophallaxis, χ^2=48.0, d.f.=1, P<0.001; receiving grooming, χ^2=40.7, d.f.=1, P<0.001; giving grooming, χ^2=30.3, d.f.=1, P<0.001; self-grooming, χ^2=48.0, d.f.=1, P<0.001). The 2-day-isolated ants attempted to engage their nestmates and solicit trophallaxis but were ignored. Fewer ants that were isolated for 2 days (68.5%) performed trophallaxis compared with ants isolated for only 1 h (25%) (χ^2=22.1, d.f.=1, P<0.001). More 1-h-isolated ants received grooming than 2-day-isolated ants (χ^2=9.8, d.f.=1, P=0.002). There was no difference in the number of ants giving grooming between the 1 h and 2 days isolation periods. All isolated ants performed self-grooming. Aggressive behavior was not observed in either treatment group.

Trophallaxis events and receiving grooming events were higher in the 1-h-isolated ants than in the 2-day-isolated ants (Mann–Whitney U-test: trophallaxis, U=37.5, P=0.001; receiving grooming, U=104, P=0.039; Fig. 4A). However, self-grooming occurred more frequently in 2-day-isolated ants than in 1-h-isolated ants (Mann–Whitney U-test: U=501, P=0.002; Fig. 4A). There was no difference in the number of giving grooming events between the 1-h- and 2-day-isolated ants. After 2 days of isolation, ants extended the durations of trophallaxis bouts, giving grooming and self-grooming more than the 1-h-isolated ants (Mann–Whitney U-test: trophallaxis, U=40, P=0.001; giving grooming, U=26, P<0.001; self-grooming, U=42, P<0.001; Fig. 4B). There was no difference in the duration of receiving grooming between ants isolated for 1 h and those isolated for 2 days. By comparing the proportion of time spent on each of the four behavioral acts, we found that the 1-h-isolated ants performed trophallaxis and received grooming more than ants

Table 2. Number of isolated ants that performed social behaviors after encountering nestmates

Behavior type	1 h isolation (N=40)	2 days isolation (N=40)
Trophallaxis	30	9*
Receiving grooming	27	13*
Giving grooming	22	27
Self grooming	40	40

Asterisks indicate significant difference between 1-h and 2-day-isolated ants (χ^2 test, P<0.05).

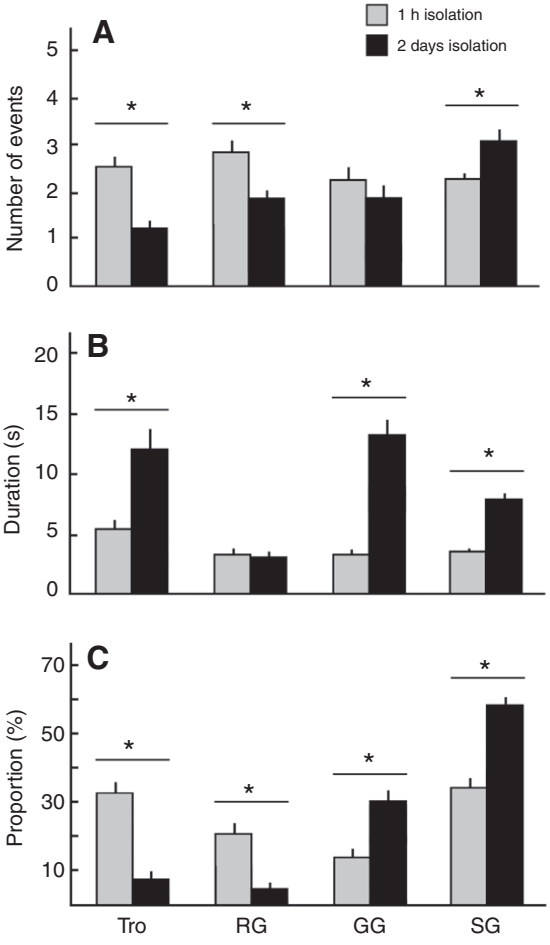

Fig. 4. Behavioral acts performed by the isolated ants during the first 3 min of an encounter with nestmates. (A) Frequency of behavioral acts. (B) Duration of behavioral acts. (C) Proportion of time spent in behavioral acts. The four behaviors sum to 100%. GG, giving grooming; RG, receiving grooming; SG, self-grooming; Tro, trophallaxis. Asterisks indicate significant differences between 1-h and 2-day isolation treatments (Mann–Whitney *U*-test, $P<0.05$). Data are means ± s.e.m.

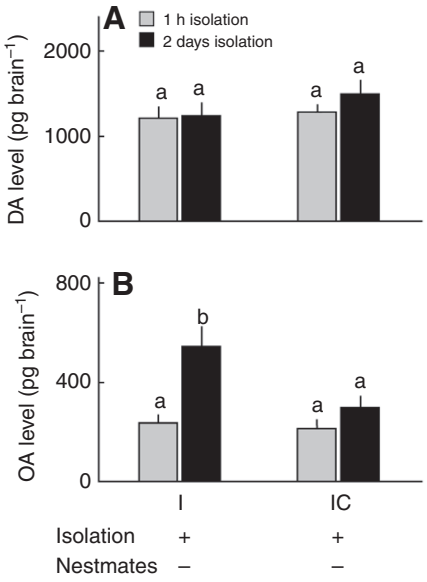

Fig. 5. DA and OA levels in the brain of isolated ants. (A) DA levels per brain. (B) OA levels per brain. I, the isolated ant without nestmates; IC, a single isolated ant allowed encounters with two non-isolated nestmates. + and – indicate treatment types. Letters above columns summarize the results of ANOVA. Groups marked by different letters differ at the 5% significance level. Isolation for 1 h did not significantly affect the DA and OA levels. OA levels increased during isolation. After encountering nestmates, OA levels decreased. Data are means ± s.e.m.

isolated for 2 days (Mann–Whitney *U*-test: trophallaxis, $U=329$, $P<0.001$; receiving grooming, $U=361$, $P<0.001$; Fig. 4C). Ants isolated for 2 days spent a greater proportion of the time grooming nestmates and self-grooming than ants isolated for 1 h (Mann–Whitney *U*-test: giving grooming, $U=437$, $P<0.001$; self-grooming, $U=196$, $P<0.001$; Fig. 4C).

In order to determine the effect of social interactions on brain biogenic amines of isolated ants, the DA and OA levels of ants with different isolation periods were compared. There was no difference in DA levels between ants isolated for 1 h and those isolated for 2 days (Fig. 5A); both groups were not significantly different from control untreated ants (Fig. 2A). There was also no difference in OA levels between 1-h-isolated ants in the I (one isolated ant without nestmates) and IC groups (one isolated ant allowed encounters with non-isolated nestmates) (Fig. 5B). However, ants in the I group isolated for 2 days contained a remarkably high level of OA (ANOVA: $F_{3,4}=12.47$, $P<0.001$; Fig. 5B). There were no differences between the IC group of the 2-day-isolated ants (Fig. 5B) and the control group (Fig. 2A). The results indicate that isolation for 2 days resulted in elevation of the OA level and that the higher OA level decreased after social interactions.

DISCUSSION

The relationship between biogenic amines and social behaviors induced in stressed social insects remains unclear. To determine whether the biogenic amines were affected by stress conditions and related to social behavior, we starved or isolated *F. japonica* and analyzed brain biogenic amines.

We found that in *F. japonica*, DA levels in foraging worker brains decreased under starvation, as previously shown for 5-day-old-female *Drosophila melanogaster* (Neckameyer and Weinstein, 2005). In mice, the dopaminergic circuitry is involved in feeding, and disrupting dopamine signaling decreased motivation for eating and drinking (Narayanan et al., 2010). Interestingly, our results indicate that sucrose-feeding dramatically reduced brain DA levels of starved ants (Fig. 3A) and that one feeding event did not lead to recovery of brain DA levels within a few minutes. Additionally, DA level in the brain did not recover after interaction with only starved nestmates (Fig. 2A, Table 1; although the ants were in the same assay tube, they did not show social behaviors). By contrast, the DA level in the brain increased, and approached the level of the control group, after ingestion of sucrose solution followed by performance of trophallaxis. Unfortunately, the observed values of amine levels shown in Figs 2 and 3 could not be compared because the assays were conducted in different seasons. It is known that the levels of brain biogenic amines are affected by various environment factors such as season and availability of food (Harris and Woodring, 1992; Božič and Woodring, 1998). Nevertheless, our results indicate that both starving and sucrose feeding reduced brain DA to levels that could not be recovered by sugar feeding within a few minutes,

and social interaction resulted in rapid elevation of DA levels. Conversely, starvation stress did not affect OA levels and there was no relationship between OA and trophallaxis in the starved ants. Yet, it is well known that various stressors such as high/low temperature and high population density affect OA level in insects (Woodring et al., 1988; Rauschenbach et al., 1993; Gruntenko et al., 2004; Neckameyer and Weinstein, 2005; Chen et al., 2008). We demonstrated a slight effect of starvation on OA level and thus there may be a role for OA in starvation stress and social behavior in *F. japonica*.

Isolation for 1 h did not affect DA and OA levels in the brain of *F. japonica* but it induced social behavior such as trophallaxis, receiving grooming, giving grooming and self-grooming. In contrast, isolation for 2 days increased the duration of trophallaxis, giving grooming and self-grooming as well as OA levels, though many ants failed to perform trophallaxis or receive grooming. After long trophallaxis and grooming behavior, OA levels decreased to control levels. Likewise, isolation of *C. fellah* workers resulted in the failure of many to reunite with their nestmates, but those that did extended the duration and increased the frequency of trophallaxis, thus maintaining colony-specific CHC profiles (Boulay et al., 2000a; Boulay et al., 2000b; Boulay et al., 2004). *Formica japonica* also uses CHCs for nestmate recognition (Akino et al., 2004). Ants isolated for 2 days may require an exchange of individual CHCs *via* social behaviors to maintain social cohesion. In honeybees and ants, OA enhanced nestmate recognition information processing (Robinson et al., 1999; Vander Meer et al., 2008) and affected the duration of trophallaxis in *C. fellah* (Boulay et al., 2000b). Thus we suggest that 2 days of isolation increased OA levels in the brain of *F. japonica*, which affected nestmate recognition, although it was unclear whether higher OA levels directly triggered trophallaxis and grooming behavior or might have modified social behaviors such as extending the duration of trophallaxis and giving grooming. Furthermore, OA levels in 2-day-isolated ants declined to the same levels as those of control ants following encounters with nestmates. We suggest that social interactions, including physical contact with nestmates, decreased OA levels. Brain DA levels in 2-day isolated ants were different from those of 1-h-isolated ants and thus DA was not involved in isolation stress.

Biogenic amines, including DA and OA, control endocrine and exocrine secretion, contraction properties of muscles, activity of neurons and generation of motor patterns in animals (Evans, 1980; Brown and Nestler, 1985; Roeder, 1994; Monastirioti, 1999; Weisel-Eichler et al., 1999; Baumann et al., 2003; Scheiner et al., 2006; Sasaki et al., 2007). Certain biogenic amines are involved in learning and memory (Menzel and Müller, 1996; Mizunami et al., 2009). Multiple dynamic equilibrium adjustment and regulatory mechanisms of biogenic amines are essential for proper body function. We found that starvation decreased brain DA levels and isolation increased brain OA levels in *F. japonica* workers. We could not discern from these experiments whether the changes in DA and OA levels influenced the mechanisms that evoke social behaviors, such as trophallaxis and grooming. The starved ants, which had lower brain DA levels, did not perform social behaviors without sucrose feeding. The 1-h-isolated ants performed social behaviors even though there were no differences in DA and OA levels between them and the untreated group. So, varying DA and OA levels were likely not direct triggers for evoking social behaviors. However, the converse appeared to be true – that social interactions including physical contact, trophallaxis and grooming brought about a recovery to normal biogenic amine levels. Thus social interactions with nestmates have important roles in biogenic amine homeostasis in the brain of stressed ants and can buffer the physiological effects of stress.

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