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OCTOPAMINE REVERSES THE ISOLATION-INDUCED INCREASE IN TROPHALLAXIS IN THE CARPENTER ANT *CAMPONOTUS FELLAH*

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Summary

Social deprivation is an unusual situation for ants that normally maintain continuous contact with their nestmates. When a worker was experimentally isolated for 5 days and then reunited with a nestmate, she engaged in prolonged trophallaxis. It is suggested that trophallaxis allows her to restore a social bond with her nestmates and to re-integrate into the colony, particularly *via* the exchange of colony-specific hydrocarbons. Octopamine reduced trophallaxis in these workers as well as hydrocarbon transfer between nestmates, but not hydrocarbon biosynthesis. Administration of serotonin to such 5-day-isolated ants had no effect on the percentage of trophallaxis. Administration of phentolamine alone, an octopamine antagonist, had no effect, but when co-administrated with octopamine it reduced the effect of octopamine alone and restored trophallaxis to control levels. Moreover, the observed effect of octopamine was not due to a non-specific effect on locomotor activity. Therefore, we hypothesise that octopamine mediates behaviour patterns linked to social bonding, such as trophallaxis. On the basis of an analogy with the role of norepinephrine in vertebrates, we suggest that the levels of octopamine in the brain of socially deprived ants may decrease, together with a concomitant increase in their urge to perform trophallaxis and to experience social contacts. Octopamine administration may reduce this social deprivation effect, and octopamine could therefore be regarded as being partly responsible for the social cohesion between nestmates in ant colonies.

Key words: octopamine, trophallaxis, social deprivation, colony odour, hydrocarbon, carpenter ant, *Camponotus fellah*.

Introduction

The manner in which animals adapt their behaviour to environmental changes results from functional modifications in their central nervous system and depends on the release of various neurotransmitters and neurohormones. Examples from vertebrates indicate that the construction of inter-individual bonds, which requires both motivation for social interactions and a recognition process, is mediated by neurochemicals in the brain (Kraemer, 1992; Nelson and Panksepp, 1998). Many correlative studies suggest that mother–infant attachment in most primates depends on central levels of norepinephrine (for a review, see Kraemer, 1992). For example, social separation in rhesus monkeys induces both behavioural stress and a decrease in the norepinephrine concentration in the cerebrospinal fluid (Kraemer et al., 1989). In sheep, the development of 'maternal olfactory imprinting' between the ewe and her new-born lamb involves norepinephric projections in the olfactory bulb (Pissonier et al., 1985; Kendrick et al., 1992; Levy et al., 1990, 1993). Olfactory learning of the characteristics of kin and mating partners in mice also appears to be norepinephrine-dependent (Rosser and Keverne, 1985; Sullivan et al., 1989). Moreover, in rats, an elevated titre of neural norepinephrine induces the consolidation of mother–pup bonding (Moffat et al., 1993).

High levels of social bonding also exist in eusocial insects (termites, ants and some bees and wasps) that are usually unable to live in complete isolation (Grassé and Chauvin, 1944; Franks and Partridge, 1994; Boulay et al., 1999b). Although nestmateship is now extensively documented from an evolutionary point of view (Lenoir et al., 1999), very little is yet known of the proximate mechanisms involved. In most ants, the social bond that links individuals living in the same colony allows them to direct altruistic patterns of behaviour towards nestmates. Experiments conducted with myrmicine and formicine ants indicate that these biased behaviour patterns are facilitated by learning of colony-specific recognition cues (Jaisson, 1975; Carlin and Hölldobler, 1986; Morel et al., 1988; Errard, 1994a,b). It has also been demonstrated, at least in the ant *Cataglyphis niger*, that these cues consist mainly of

cuticular hydrocarbons (Lahav et al., 1999). As established for other insects (Lockey, 1988), hydrocarbons are probably synthesised internally by the oenocytes and transported to the epicuticle through the haemolymph by a lipophorin. Using *C. niger* as a model system, it was shown that individually produced hydrocarbons accumulate in the postpharyngeal gland, which opens to the buccal cavity, both by internal sequestration and during self-grooming. This exchange explains the congruency generally found between the cuticular and postpharyngeal gland hydrocarbon profiles. In addition, a continuous exchange of hydrocarbons between individuals occurs, mostly by trophallaxis but also by allogrooming, facilitating the formation of a uniform and unique colony odour (Soroker et al., 1994, 1995). Trophallaxis, which was assumed for many years to be mostly a means of exchanging food (Wilson, 1971), may therefore have been the evolutionary step that facilitated the establishment of large ant colonies while maintaining an efficient recognition system. Since colony odour is dynamic (i.e. it changes over time), it is necessary for each worker to maintain a social bond as well as to exchange hydrocarbons with her nestmates to maintain integration into the colony. Indeed, when a worker is experimentally isolated for a few days and then reunited with a nestmate, she solicits for multiple bouts of trophallaxis (Boulay et al., 1999b; Cybulska et al., 1999), which presumably allows her to be reaccepted into the colony (R. Boulay, A. Hefetz, V. Soroker and A. Lenoir, in preparation).

We investigated potential neurobiological mechanisms that could underlie social bonding in the ant *Camponotus fellah* through the analysis of trophallaxis and of the flow of hydrocarbons. Since norepinephrine has not been systematically identified in the nervous system of the insect, we focused on its phenolic analogue and functional equivalent in invertebrates, octopamine. In most insects, octopamine acts as a neurohormone, a neurotransmitter and a neuromodulator (Evans, 1980; Orchard, 1982). In addition to its stimulatory effect on the general arousal of an insect (Livingstone et al., 1980; Arnesen and Olivo, 1988; Adamo et al., 1995; Bacon et al., 1995), octopamine also induces more specific behavioural modifications such as anorexia in the American cockroach *Periplaneta americana* (Ismail and Matsumura, 1991) and in lepidopteran larvae (Ikemoto et al., 1995; Adamo et al., 1997) or associative learning in honeybees *Apis mellifera* (Mercer and Menzel, 1981, 1983; Hammer and Menzel, 1994, 1998; Menzel and Müller, 1996). In a recent report, Robinson et al. (1999) showed that octopamine agonists facilitate nestmate recognition by young honeybee workers. In ants, octopamine was found in large quantities in the brain of *Camponotus floridanus* adult workers (Punzo and Williams, 1994), but its role remained elusive.

Various studies indicate that octopamine also controls aspects of lipid metabolism in insects. It induces increases in levels of lipids in the haemolymph of *Locusta migratoria* (Orchard et al., 1981; Orchard and Lange, 1984) as well as stimulating lipid release from the fat body *in vitro* in this species (Orchard, 1982) and in the cricket *Acheta domesticus* (Fields and Woodring, 1991). In the red flour beetle *Tribolium freemani*, octopamine induces the biosynthesis of ecdysteroids (Hirashima et al., 1998), which are thought to control hydrocarbon biosynthesis in insects (Blomquist and Jackson, 1979; Blomquist and Dillwith, 1985).

By analogy with the situation observed in vertebrates, we hypothesised that octopamine mediates social bonding in ants. As a consequence, we expected that octopamine might reverse the behavioural modifications induced by social deprivation. Experiment 1 studied the effects of octopamine in trophallaxis and allogrooming in individual *C. fellah* isolated for 5 days. In a second experiment, we compared the effect of octopamine with those of serotonin and of the octopamine antagonist phentolamine. In a third experiment, we investigated the effects of octopamine on the locomotor behaviour of the ants to examine whether the behavioural effects observed in the previous experiments were specific or were due to a non-specific modification of the activity level of the ants. Since trophallaxis is linked to hydrocarbon exchange, in a fourth experiment, we studied the effects of octopamine on both the biosynthesis and transfer of hydrocarbons in this species.

Materials and methods

Animals

Experiments were conducted with *Camponotus fellah* Della Torre workers from eight queenright colonies each composed of approximately 200 individuals. The foundresses of the colonies were collected near Tel Aviv and Beer Sheva (Israel) in March 1997. The ants were reared in the laboratory under controlled conditions (12 h:12 h L:D, 26±2 °C, 25±5 % relative humidity). The artificial plaster nests consisted of six chambers (each 6 cm×6 cm×1 cm), each covered with a separate glass plate. We could therefore collect ants from any chamber without the need to sedate all the individuals, including the queen. Each nest was connected *via* a plastic tube to a large enclosure (30 cm×3 cm×10 cm) that served as a foraging area where the ants had *ad libitum* access to food (mealworms and nectar for humming birds, i.e. honey supplemented with amino acids and vitamins, supplied three times a week). Workers for each experiment were taken from all colonies, as available.

Drug administration

Ants were sedated by cooling on ice for 5 min and injected (0.5 μl) between the sixth and seventh abdominal tergites using a microcapillary. Octopamine and serotonin hydrochlorides and phentolamine methanosulphonate salt were purchased from Sigma and Fluka. They were dissolved either in saline (experiment 1) or in saline plus 3 % ethanol (experiment 2). In all experiments, we chose workers of medium size that showed little degree of gaster distension and were engaged in intranidal tasks (soldiers were not used since the effects of social deprivation on this sub-caste are more variable). The mean body mass of these ants was 15.4±2.4 mg (mean ± S.E.M., N=30). Doses used were 0.75, 7.5, 75, 750 and 7500 ng per ant

for octopamine, and 750 ng per ant for phentolamine and serotonin.

Experiment 1: behavioural effects of octopamine

Behavioural experiments were conducted during the first hours of the scotophase (under red light) in accordance with the nocturnal habits of *C. fellah*. Workers were isolated in test tubes (1.8 cm×18 cm) for 5 days with or without food, but supplied with water that was held in the tube by means of a tightly fitting cotton plug. After the isolation period, the ants were injected with the octopamine or saline and were replaced in their respective test tubes until the behavioural tests began. Preliminary results showed that, following a saline injection, normal behaviour of the ants was restored after 1 h (R. Boulay, unpublished data), and that during this period the quantity of octopamine in the brain reached its maximal value (Boulay et al., 1999a). We therefore started the experiment 1 h post-injection. Additional control groups of 5-day-isolated ants and of non-isolated ants received no injection and were either fed or food-deprived during the isolation period.

Behavioural tests (lasting 15 min) consisted of dyadic (one-to-one) encounters between ants submitted to the same treatment and isolated under the same feeding conditions. At the beginning of each encounter, the open ends of the tubes housing the isolated ants were placed in contact. We then recorded the total duration of trophallaxis between the two ants and the total duration of allogrooming. Using the same protocol, we also recorded the duration of trophallaxis in non-isolated pairs. Workers were placed in the test tubes for 20 min to acclimate before being tested.

At the end of each encounter, the two workers were kept together for an additional 24 h to ensure that the behavioural manifestations were not due to some non-specific failure in the treated ants. Only cases in which both ants survived for 24 h after the behavioural test were taken into account.

Experiment 2: behavioural effects of serotonin and phentolamine

This experiment was conducted as in experiment 1, but with observations being shortened to 5 min. The effect of each treatment was estimated as the percentage of the total interaction time spent in trophallaxis. Drug administration included serotonin (750 ng), phentolamine (750 ng), octopamine (750 ng and 7500 ng) and octopamine+phentolamine (7500 and 750 ng, respectively). Two controls were included: non-injected and saline-injected (+3 % ethanol) ants.

Experiment 3: the effects of octopamine on locomotor activity

To test the effects of octopamine on locomotor activity, 30 pairs of workers were injected with octopamine (7500 ng) and 30 pairs with saline without ethanol. Immediately following injection, the two workers from the same treatment group were placed together in a circular corridor (internal cross section 0.8 cm; internal diameter 3 cm) and the locomotor activity of the two nestmates was recorded using a photoelectric cell connected to an automatic counter. For each pair, the cumulative number of passages was recorded after 5, 30, 60 and 120 min.

Experiment 4: hydrocarbon biosynthesis and exchange

De novo *biosynthesis of hydrocarbons*

Workers of medium size were injected with [1-^{14}C]sodium acetate either alone (56 mCi mmol^{-1}; NEN Boston, USA) or supplemented with octopamine (7500 ng) into the haemolymph through the intersegmental membrane of the abdomen. They were then incubated for 24 h (28 °C), frozen and monitored for labelled hydrocarbons in the postpharyngeal gland, crop, epicuticle and in the internal pool.

For extraction, the postpharyngeal gland and crop were immersed in 100 µl of pentane for at least 24 h. To separate epicuticular from internal hydrocarbons, thoraces (including the legs) were first washed by immersion in 400 µl of pentane for 5 min and subsequently extracted for longer periods (24 h or more) together with the abdomens (from which the Dufour's gland had been removed to eliminate the possibility of sample contamination by the hydrocarbon content of this gland). The extracts were subjected to silica gel (polygram Sil G) thin-layer chromatography (TLC). The radioactivity of various TLC lipids fractions was monitored using an IP autoradiography system (Fuji BAS 100 analyser) (Lahav et al., 1999).

Hydrocarbon transfer between nestmates during dyadic encounters

Workers, classified as donors, were injected with 0.5 µl of medium containing 1 µCi of [1-^{14}C]sodium acetate, as described above. They were incubated individually for 24 h (28 °C), and each was then presented with a single nestmate of a similar size, classified as a recipient.

Donors were supplied to satiation with food dyed with a blue colour, while recipients were starved for 24 h. Approximately 1 h before the encounter, recipients (which were non-isolated ants) were injected with 0.5 µl of octopamine (7500 ng) in saline or with 0.5 µl of saline. Experiments were stopped 24 h later by freezing. Food transfer by trophallaxis was monitored by determining the presence of a blue colour in the crop of the recipient. Both donor and recipient were monitored for the presence of labelled hydrocarbons in the postpharyngeal gland, in the crop and on the cuticular surface of the thorax, as described above. The amount of hydrocarbon transferred was determined as described by Soroker et al. (1995). The behaviour of the ants was observed over the first 6 h of each encounter, and the incidents of trophallaxis, allogrooming, self-grooming and body contact were recorded every 5 min.

Statistical analyses

All statistical analyses were performed using Statistica software for Microsoft Windows. In experiment 1, we analysed the relative effects of satiation and treatments on trophallaxis and allogrooming using two-way analysis of variance (ANOVA), followed by the *post-hoc* least significant difference (LSD) test for inter-group comparisons. Non-

isolated workers were considered as a separate control group. In experiment 2, we compared the percentage of the total interaction time time spent in trophallaxis in the seven experimental groups using a one-way ANOVA and a *post-hoc* LSD test. In experiment 3, we compared the cumulative number of passages in front of the photoelectric cell using one-way ANOVA for repeated measures. In experiment 4, the results of hydrocarbon biosynthesis, labelled hydrocarbon transfer and behavioural recordings obtained for control and octopamine-treated individuals were compared using the Mann–Whitney *U*-test (significance was taken as $P<0.05$).

Results

Experiment 1: behavioural effects of octopamine administration

Once reunited, workers deprived of social contact for 5 days and receiving no injection engaged in extensive trophallaxis (347.3±50.2 s for starved ants and 302.1±48.4 s for satiated ants compared with 42.4±9.0 s for non-isolated ants). The nutritional state of the isolated ants significantly affected the duration of trophallaxis (two-way ANOVA, $F=5.18$, $P=0.023$), which was lower for satiated ants (234.4±19.6 s) than for food-deprived ants (271.4±15.7 s). Treatment also had a highly significant effect on the total duration of trophallaxis (two-way ANOVA, $F=7.14$, $P<0.001$). However, there was no significant interaction between these two factors (alimentary state *versus* treatment $F=0.30$, $P=0.934$). Therefore, we pooled the results of satiated and food-deprived ants for further analysis using the *post-hoc* LSD test to compare differences between treatments (Fig. 1). An injection with saline 1 h before the behavioural test did not significantly affect the total duration of trophallaxis (*post-hoc* LSD test, $P=0.374$). In contrast, administration of octopamine induced a decrease in total trophallaxis duration in a dose-dependent manner. The duration of trophallaxis in ants that received 750 ng or 7500 ng of octopamine was significantly shorter than in ants administered lower doses of octopamine, saline or no injection (*post-hoc* LSD test, $P<0.05$). For instance, the maximal octopamine dose resulted in a decrease of 65 % in the duration of trophallaxis compared with ants treated with saline (*post-hoc* LSD test, $P<0.001$). Moreover, the duration of trophallaxis expressed by isolated ants receiving the maximum dose of octopamine (103.1±18.28 s) was not significantly different from that of non-isolated ants (42.2±9.0 s, planed comparison, $F=2.436$, $P=0.119$).

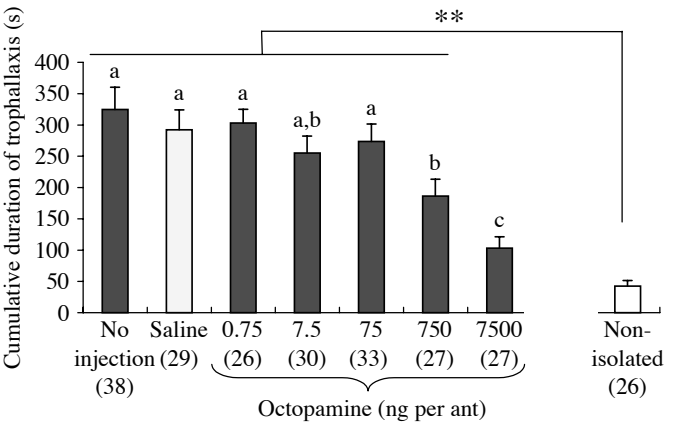

Fig. 1. Cumulative time (means + S.E.M.) spent in trophallaxis by 5-day-isolated *Camponotus fellah* workers in dyadic encounters lasting 900 s. The ants received either no injection, or were injected with saline or octopamine. Data for isolated workers with and without access to food were pooled. The right-hand column represents non-isolated, non-treated workers. The number of dyads studied in each case is given in parentheses. Different letters denote significant differences between treatments, and asterisks denote significant differences between non-isolated workers and isolated workers under the different treatments (two-way ANOVA, $P<0.05$).

The duration of allogrooming was extremely variable in all groups tested. There was no significant effect either of the state of satiety or of treatment (two-way ANOVA; $F=1.01$, $P=0.420$ and $F=1.86$, $P=0.097$, respectively; Table 1).

Experiment 2: behavioural effects of serotonin and phentolamine

The results presented in Fig. 2 show significant differences between treatments (ANOVA, $F=6.69$, $P<0.01$). Non-treated ants engaged in trophallaxis for 27.4±3.7 % and saline-injected ants for 29.7±5.2 % of the total interaction time. Similarly, confirming the results of experiment 1, administration of octopamine (750 and 7500 ng) significantly inhibited the expression of trophallactic behaviour (to 12.4±0.9 % and 1.7±2.3 %, respectively). However, after concurrent administration of both phentolamine (750 ng) and octopamine (7500 ng), the percentage of time spent in trophallaxis

Table 1. *Total duration of allogrooming expressed by non-fed workers reunited after 5 days of isolation*

			Octopamine dose (ng per ant)				
	No injection	Saline	0.75	7.5	75	750	7500
Food-deprived	102.1±50.9	123.5±94.1	114.3±115.5	67.1±53.1	68.6±58.5	93.4±79.6	69.4±34.4
Satiated	46.1±13.3	84.7±45.2	95.9±47.0	118.0±95.4	35.0±9.4	97.4±93.5	67.6.4±36.1

Values are the duration of allogrooming (s) and are presented as means ± S.E.M.
Behavioural tests lasted for 900 s.
There are no significant differences between treatments (one-way ANOVA, $P>0.05$).
Numbers of dyads are given in Fig. 1.

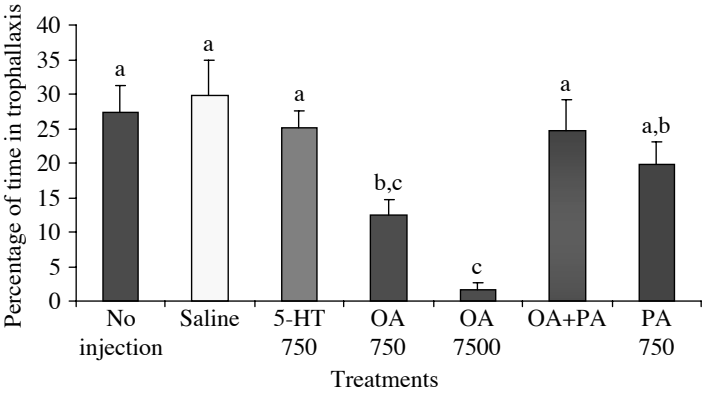

Fig. 2. Effects of octopamine (OA; 750 and 7500 ng per ant), its main pharmacological antagonist phentolamine (PA; 750 ng per ant) and serotonin (5-HT; 750 ng per ant) on the percentage of time spent in trophallaxis between workers isolated for 5 days (N=10 dyadic encounters). Tests lasted 300 s. Values are means + S.E.M. Different letters indicate significant differences between treatments (one-way ANOVA, P<0.05). OA+PA, 7500 ng of octopamine plus 750 ng of phentolamine.

(24.6±4.5 %) was not significantly different from that of either non-injected or saline-treated ants (*post-hoc* LSD test, P=0.57, P=0.34, respectively), but was statistically different from those of ants given the two highest octopamine doses (*post-hoc* LSD test, P=0.32 for 750 ng, P<0.01 for 7500 ng). Neither the administration of phentolamine (750 ng) alone nor of serotonin (750 ng) changed the duration of trophallaxis (*post-hoc* LSD test, P=0.07 for phentolamine and P=0.39 for serotonin) compared with the saline-injected group.

Experiment 3: effects of octopamine on locomotor activity

The effects of octopamine on general locomotor activity was tested by counting the cumulative number of times that a worker passed in front of the photoelectric cell. The number of passages increased linearly with time (ANOVA, F=55.9, P<0.001), but there were no statistical differences between the activities of ants treated with saline or with octopamine (7500 ng) (Fig. 3, ANOVA, F=0.2, P=0.65).

Experiment 4: hydrocarbon biosynthesis and exchange

De novo *biosynthesis of hydrocarbons*

There was appreciable lipid biosynthesis in workers during the 24 h of incubation, but there were no differences in the amount of newly synthesised hydrocarbons between octopamine- and saline-injected ants (17 852± 2759 disints min^{-1} ant^{-1} for control ants compared with 19 385±3263 disints min^{-1} ant^{-1} for octopamine-injected ants). The highest quantity of labelled hydrocarbons was found in the postpharyngeal gland (61±7.1 % of total newly synthesised hydrocarbons), while only 5.0±1.6 % was found in the crop. The distribution of hydrocarbons between the postpharyngeal gland and the thorax was also similar for octopamine- and saline-injected ants (4.0±0.7 % in control ants compared with 3.1±0.5 % in octopamine-treated ants) as was the distribution between the postpharyngeal gland and the crop (30.9±9.5 % in control ants compared with 38.1±12.4 % in octopamine-treated ants).

Hydrocarbon transfer between nestmates during dyadic encounters

The behaviour of the ants during the first 6 h of dyadic encounter is presented in Table 2. Although not statistically significant, there was an almost twofold decrease in the tendency to perform trophallaxis and allogrooming following

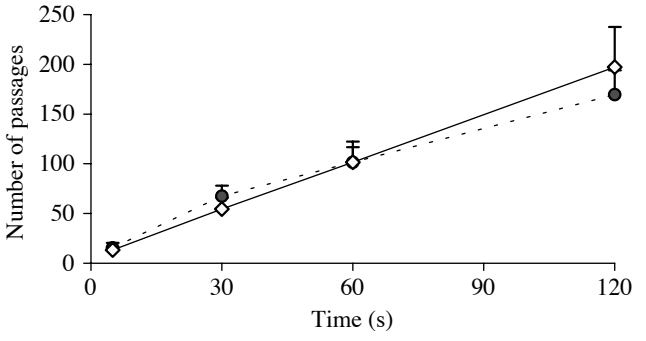

Fig. 3. Effects of octopamine on locomotor activity of non-isolated workers. Points are mean number of passages (means ± S.E.M.) in front of the photoelectric cell. Values increase significantly with time (ANOVA, P<10^{-5}) but are not different between groups (ANOVA, P=0.65). N=30 dyads for octopamine (filled symbols) and saline-injected (open symbols) ants.

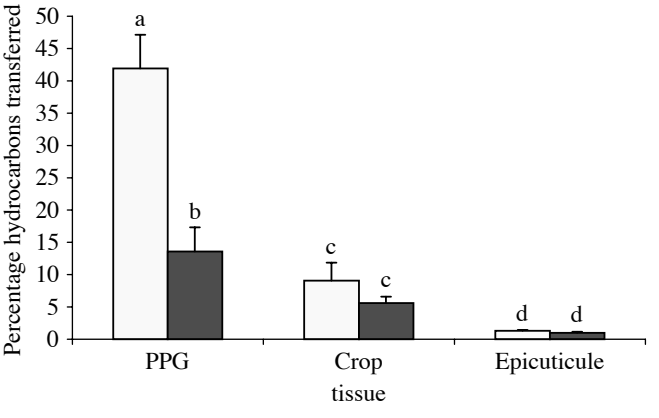

Fig. 4. Effect of octopamine (7500 ng) on the transfer of hydrocarbons (means + S.E.M.) between two nestmates of *Camponotus fellah*. Different letters indicate significant differences between control (open columns) and octopamine-injected (filled columns) ants (Mann–Whitney U-test, P<0.05). PPG, postpharyngeal gland.

Table 2. *Frequency of behavioural acts performed by recipients and donors during the first 6 h of a dyadic encounter*

	Recipient			Donor		
Behaviour	Control	Octopamine	*P*	Control	Octopamine	*P*
Trophallaxis	4.0±0.9	2.3±0.7	0.22	4.0±0.9	2.3±0.7	0.22
Allogrooming	1.1±0.4	0.4±0.2	0.19	2.0±1.0	2.5±0.7	0.40
Self-grooming	5.3±0.8	8.4±1.1	0.05	4.8±0.9	5.4±1.0	0.77
Contact	76.4±4.4	68.9±6.2	0.30	75.7±4.6	68.9±6.5	0.40
Ignore	13.2±3.6	20.0±6.0	0.30	13.3±3.7	21±6.1	0.32

Frequencies were calculated as a percentage of a total of 82 observations.
Results are presented as means ± S.E.M. Significant differences were assessed using the Mann–Whitney *U*-test.

an octopamine injection. In contrast, levels of self-grooming increased in recipient ants following injection with octopamine.

The degree of hydrocarbon transfer between nestmates in the control and octopamine-treated groups is shown in Fig. 4. A significantly smaller amount of hydrocarbon was transferred (more than twofold) to the postpharyngeal gland in the octopamine-treated group (41.9±5.21 % in control ants compared with 13.6±3.72 % in octopamine-treated ants; P=0.001, Mann–Whitney U-test). Transfer to the crop and the epicuticle followed the same trend, but the differences were not statistically significant.

Discussion

Social cooperation in animals must be continually reinforced by social bonding. Under natural conditions, ant workers maintain frequent or even continuous contact with their nestmates. Specific social interactions such as trophallaxis and allogrooming permit them to homogenise their hydrocarbons and to carry a uniform epicuticular mixture. This hydrocarbon mixture serves as a colony recognition cue and is necessary to maintain colony integrity. Consequently, an absence of stimulation by the social environment may lead to impairment in the recognition process and probably also causes a profound increase in the so-called 'social appetising' described by Wheeler (1926).

In *C. fellah*, social deprivation for 5 days was found to increase trophallaxis in both satiated and starved workers, although it was less marked in fed ones (Boulay et al., 1999b). An enhancing effect of social deprivation on readiness to engage in trophallaxis was also recently reported in satiated workers of *Camponotus acvapimensis* (Cybulska et al., 1999). A similar effect of alimentary conditions on trophallaxis and, consequently, hydrocarbon transfer was found in *C. niger* (Soroker et al., 1994). It is possible that, in isolated ants, the effects of food deprivation on the readiness to display trophallaxis were entirely masked by the much stronger effect of social deprivation.

The results of the present study confirmed the above phenomenon, but also showed that an administration of octopamine 1 h before reunion could block the effects of isolation and restore trophallaxis to a level not significantly different from that of non-isolated ants (Fig. 1). Although octopamine levels have been quantified in the brain of *C. floridanus*, no octopamine receptor has been identified in any ant species. However, several similarities exist between octopamine receptors of other insect species and vertebrate α-adrenergic receptors (Roeder et al., 1995). The fact that co-administration of octopamine with the α-adrenergic antagonist phentolamine partially blocked or at least reduced the effect of octopamine alone (Fig. 2) suggests that the observed effect is specific to an octopamine receptor rather than reflecting non-specific binding of the high level of octopamine to other biogenic amine receptors. This was also confirmed by the lack of effect of serotonin administration on 5-day-isolated ants. Whether octopamine and serotonin have opposite effects in trophallaxis, as demonstrated for other invertebrate behaviour patterns (Livingstone et al., 1980), should now be tested in a non-isolated situation.

In many insect species, octopamine is known to be associated with motor excitation (Evans and O'Shea, 1978; Bailey et al., 1983; Davenport and Evans, 1984; Woodring et al., 1989). However, the higher doses of octopamine employed had no effect on locomotor activity for at least 2 h after administration (Fig. 3). This implies that the reduction in trophallaxis in isolated workers is not due to a non-specific effect on the general activity of the ants.

The absence of an effect of octopamine on hydrocarbon biosynthesis and distribution in the body of the ants is surprising in view of the multiple reports regarding the effects of octopamine on lipid metabolism (Gole and Downer, 1979; Orchard et al., 1981; Orchard, 1982; Fields and Woodring, 1991). It is possible that octopamine induces only the production of specific lipid species that are used as fuel by muscles. This hypothesis is supported by the results of Goosey and Candy (1980, 1982) and Orchard et al. (1981), which indicated that the increase in the haemolymph octopamine level parallels changes in energy requirements.

Administration of octopamine significantly reduced the exchange of radiolabelled hydrocarbons between ants, probably as a result of the decrease in trophallaxis and allogrooming. Although the reduction in levels of these latter behaviour patterns was not significant, it may have been sufficient for the observed significant reduction in the transfer of label. The octopamine-induced reduction of trophallaxis was

not significant when the donors were isolated for only 24 h and the recipients consisted of non-isolated ants, conditions that normally do not induce augmented trophallaxis (Boulay et al., 1999b).

In view of our results, we hypothesise that social contacts between individuals may stimulate some octopaminergic neural projections, which could further stimulate the individual to search for such specific nestmate interactions. Octopaminergic neuronal projections occur between the antennal lobes and more integrative brain areas in the honeybee and are involved in the mediation of olfactory reward conditioning (Hammer and Menzel, 1994, 1998). It is therefore possible that octopamine may also partially mediate the hypothetical 'social reward' implicated in the control of social interactions between nestmates in insect societies. Social deprivation, according to the present hypothesis, would lead to a decrease in octopamine levels and to an increase in the motivation for social interaction. The augmented trophallaxis demonstrated here expresses such a motivation, and the fact that it could be decreased by octopamine lends credence to this hypothesis. We suggest that administration of octopamine mimicked the possible octopamine-mediated rewarding effects of trophallactic interactions with nestmates. Moreover, nestmate recognition is based on the comparison of an encountered phenotype with a template (Holmes and Sherman, 1983; Sherman et al., 1997). The learning of the template from the individual's own characteristics or from its social environment could also be mediated by similar octopaminergic projections. This may explain why the administration of octopamine agonists improved the recognition capacity of young honeybees (Robinson et al., 1999). It remains to be tested whether the trophallactic behaviour shown by workers of *C. fellah* when reunited with their nestmates is accompanied by an increase in endogenous octopamine levels.

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