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SHORT COMMUNICATION

Queen regulates biogenic amine level and nestmate recognition in workers of the fire ant, *Solenopsis invicta*

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Abstract Nestmate recognition is a critical element in social insect organization, providing a means to maintain territoriality and close the colony to parasites and predators. Ants detect the colony chemical label via their antennae and respond to the label mismatch of an intruder with aggressive behavior. In the fire ant, *Solenopsis invicta*, worker ability to recognize conspecific nonnestmates decreases if the colony queen is removed, such that they do not recognize conspecific nonnestmates as different. Here, we tested the hypothesis that the presence of the colony queen influences the concentration of octopamine, a neuromodulator, in worker ants, which in turn has an effect on nestmate recognition acuity in workers. We demonstrate that queenless workers exhibit reduced brain octopamine levels and reduced discriminatory acuteness; however, feeding queenless workers octopamine restored both. Dopamine levels are influenced by honeybee queen pheromones; however, levels of this biogenic amine were unchanged in our experiments. This is the first demonstration of a link between the presence of the colony queen, a worker biogenic amine, and conspecific nestmate recognition, a powerful expression of colony cohesion and territoriality.

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Introduction

The ability of colony members to discriminate between nestmates and nonnestmates is a critical element in social insect organization, providing a means to exclude harmful individuals such as predators, parasites, and conspecific competitors (Wilson 1971). Ants detect the colony chemical label via their antennae (Ozaki et al. 2005) and, through grooming and trophallaxis, they contribute to and experience odors associated with their nest (Boulay et al. 2004). Upon contact with an intruder, the resident compares the cuticular chemistry of the intruder with the experience-based neural template of its colony odor (Vander Meer and Morel 1998) and in case of mismatch aggression occurs. Fire ants show an almost stereotypic response of high aggressiveness towards allospecific ants, presumably because such intruders are easily recognized by qualitatively different cues. The intraspecific response is not stereotypic. Between individuals, aggressiveness varies even in response to identical stimuli. This is generally supported by the graded recognition–aggressive behaviors used in the quantification of nestmate recognition bioassays (Vander Meer and Morel 1998).

The plasticity of individual recognition acuity is clearly illustrated by the powerful influence of the *Solenopsis invicta* queen on the conspecific recognition capabilities of workers. Removing the queen or if a new queen is adopted degenerates or restores, respectively, worker intraspecific recognition. We attributed this powerful queen effect to a putative recognition primer pheromone that increases the sensitivity of workers to subtle quantitative differences in conspecific nestmate recognition cues (Vander Meer and Alonso 2002). Interestingly, octopamine (OA), an important neurochemical in invertebrates, acts variously as a neurohormone, neurotransmitter, and neuromodulator (Roeder 2005) and has been reported to increase the sensitivity of individuals to pheromones (Pophof 2002). We report here efforts to decipher the physiological changes that occur as a consequence of fire ant queen presence or absence and that may influence worker sensitivity to subtle differences in conspecific recognition cues.

Materials and methods

Colony collection and separation

Queenright monogyne (QR; fully functional colony: single queen, workers, and brood) colonies were collected from a known monogyne colony field site (RKVM) located in Gainesville, FL, USA. Soil containing the ant colonies were placed in a Fluon®-lined tray containing Petri dishes with moistened Castone® on the bottom. As the soil dried out, the queen, workers, and brood moved from the soil into the Petri dish.

Preparation of experimental units

Queenright *S. invicta* monogyne (single queen) colonies (ten replicates were set up, but only seven survived to the end of the experiment) were each divided into three experimental units with approximately the same amount of workers and brood. Two units were queenless (QL) and the third was QR. All colony fragments were fed aqueous sucrose solution (10%) and crickets (*Acheta domestica*); however, one QL unit from each replicate was also fed OA (racemic, Aldrich, Milwaukee, WI, USA; 0.2%, W/V), dissolved in the aqueous sucrose solution.

Aggression bioassays

The nestmate recognition capabilities of QR colonies and two QL subunits from each colony were assessed by an established nestmate recognition bioassay that measured aggression (Obin and Vander Meer 1988). All QR and QL colony units were challenged once a week with a non-nestmate intruder worker from each of five laboratory-maintained QR monogyne colonies, not otherwise used in this experiment. The most aggressive response (scale 1 to 9 of increasing aggressive behavior) elicited by the first ten interactions of the intruder with unique resident ants was recorded. The mean response of the five introductions was used as the measure of aggression for each of the seven QR and QL colony units.

Brain dissection and biogenic amine analysis

Aggression bioassay results for the QL units reached levels 1–3 (investigative level) whereas QR- and OA-fed QL units reached aggression levels that were not significantly different by week 10. Therefore, worker ant brains were dissected and prepared for OA and dopamine (DA, Aldrich, Milwaukee, WI, USA) analysis by gas chromatography–mass spectrometry (GC–MS) from all experimental units. Worker ants subjected to brain dissection were initially cooled in a refrigerator and then quickly frozen in a vial using a Dry-Ice®–acetone bath. The ant specimen to be dissected was removed from the vial and the brain dissected in ice-cooled Ringers solution and transferred to a silanized glass vial insert (100 μl) containing 20 μl methanol. The brain was macerated with a probe in the methanol. Replicates consisted of ten pooled worker brains. Internal standard (4-hydroxy-3-methoxybenzylamine hydrochloride, Aldrich, Milwaukee, WI, USA, 4.0 ng per sample) was added to each vial insert and biogenic amines were then derivatized and analyzed by GC–MS as previously described (Barron et al. 2007a). Brain extracts were analyzed from QR-, QL-, and QL-fed OA colony units from each of the seven replicates.

Statistical analyses

Between-group differences in brain biogenic amines (OA and DA) were examined using nonparametric repeated-measures analysis of variance (ANOVA). Between-group differences in aggression over time were compared using nonparametric repeated-measures ANOVA (two factors: treatments and time after the start of the experiment). All statistical analyses were conducted using GraphPad Prism 4, version 4.0c. Data are presented as median±interquartile range and box-and-whisker plots in the figures.

Results

Effect of fed OA on levels of biogenic amines in worker brains

Brain OA and DA levels in workers from QR, QL, and QL + OA units at the tenth week of the experiment are shown in Fig. 1. One-way, nonparametric, repeated-measures ANOVA of the OA data showed significant differences (Friedman test, $F=12.29$, $P=0.0003$). The results of Dunn's Multiple Comparison posttests are summarized in Fig. 1. Octopamine-fed QL units had worker brain OA levels significantly higher than QL units but not when compared to the QR units (Fig. 1, Rank sum difference=−13.00, $P<0.001$ and rank sum difference=−5.00, $P>0.05$, respectively). The OA

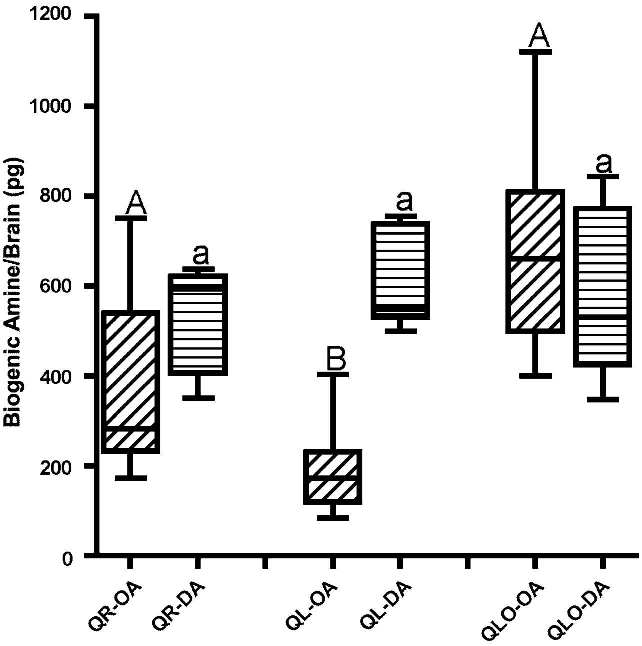

Fig. 1 The effect of queen presence or absence and dietary octopamine on octopamine and dopamine levels in fire ant worker brains. Amounts of octopamine and dopamine at week 10 in *S. invicta* worker brains are presented for the following experimental units: *QR* = queenright experimental units; *QL* = queenless experimental units; *QLO* = QL experimental units fed octopamine. The biogenic amine analyzed is indicated by *OA* = octopamine or *DA* = dopamine. QL ants experience a significant reduction in octopamine levels that is redeemed by dietary octopamine. Results are shown as *box-and-whisker* plots, N=7**.** Treatments with different *letters above* the *box* plots are statistically different (*upper case* = octopamine; *lower case* = dopamine)

levels in QL worker units dropped to significantly lower levels than the QR levels (Fig. 1, rank sum difference=8.00, $P<0.05$). This is not the case if QL workers are fed OA. One-way, nonparametric, repeated-measures ANOVA of brain concentrations of DA data showed no significant differences among QR, QL, and QL + OA units (Friedman test, F=0.2857, P=0.9640).

Effect of OA on QL worker aggression

The aggression levels of QL units fed sucrose decreased with increasing time such that, between 7 and 10 weeks posttreatment, mean aggression levels were only investigative (antennation; Fig. 2). Two-way repeated-measures ANOVA indicated that time (F=13.76, DFn=3, DFd=48, $P<0.0001$) and treatment (F=91.29, DFn=2, DFd=48, $P<0.0001$) significantly contributed to variation. Time vs. treatment interaction was not significant (F=1.80, DFn=6, DFd=48, P=0.1185). The results of Dunn's Multiple Comparison posttests are summarized in Fig. 2. Specific results for week 10 are: QR vs. QL (rank sum difference=13.5, $P<0.001$); QR vs. QL + OA (rank sum difference=6.00, $P>0.05$); QL vs. QL + OA (rank sum difference=−7.500, $P<0.05$).

Discussion

In invertebrates, OA and tyramine are functional homologs to the vertebrate adrenergic neurotransmitters, noradrenaline and adrenaline (Roeder 2005), and therefore have a wide range of physiological activities. In most insects, OA acts as a neurotransmitter and/or neuromodulator, and many sensory inputs have been reported to be modulated by OA, including olfactory inputs (Roeder 2005). Interestingly, OA increases pheromone acuity in insects by lowering the threshold of response, e.g., male moths are more sensitive to the female sex pheromone in the presence of OA (Linn et al. 1996). In addition, the lower threshold was found to be for pheromone-specific neurons, rather than neurons for general odors (Pophof 2002). These reports and our previous postulation that a lowered threshold of fire ant worker response to weakly differing nestmate recognition cues was correlated with the presence of the queen (Vander Meer and Alonso 2002) led to the octopamine manipulation studies presented here.

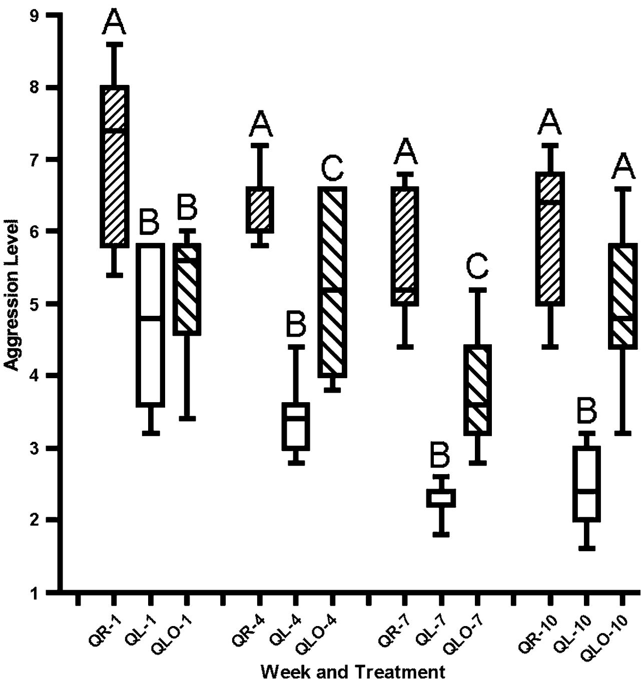

Fig. 2 The effect of dietary octopamine on the nestmate recognition capabilities of queenless *S. invicta* workers over time. The treatments are *QR* = queenright experimental units; *QL* = queenless experimental units; *QLO* = QL experimental units fed octopamine. Results are shown as *box-and-whisker* plots, N=7, for weeks 1, 4, 7, and 10. Treatments with different *letters* within each time period *above* the *box* plots are statistically different

Manipulation of OA levels in workers by feeding OA in sugar water is a noninvasive method to elevate brain levels of OA, as opposed to topical application or injection (Barron et al. 2007a). However, we do not know if the OA taken up in the diet of the QL workers is the same OA represented by the increased brain concentration shown in Fig. 1. It is possible that dietary OA is broken down but in some way influences the biosynthesis of OA in the brain. The net result, however, was the significant elevation of OA levels in QL worker treatments. Since the addition of OA to the sugar water was the only rearing difference between the paired QL experimental conditions, we conclude that the higher brain levels of neuromodulator, OA, restored the ability of QL workers to discriminate between subtle conspecific nestmate recognition cue differences.

In social insects, OA has been shown to be involved in honeybee associative learning (Menzel and Müller 1996) and honeybee dance behavior (Barron et al. 2007b) and, importantly, OA was indirectly implicated in nestmate recognition in honeybees when treatment with OA agonists improved discrimination of nestmates from nonnestmates (Robinson et al. 1999). Honeybee queen pheromone has been shown to influence worker DA levels, which influences worker behavior (Beggs et al. 2007). Our study clearly demonstrates a nestmate recognition role for OA in another social insect. However, DA was unchanged in our treatments–controls, indicating potential physiological roles other than nestmate recognition. The only other report linking OA and behavior in ants is that OA inhibited isolation-induced trophallaxis (exchange of fluids between colony members through regurgitation) in *Camponotus fellah* workers (Boulay et al. 2000). However, it is clear from the literature that there are numerous potential roles for biogenic amines in ants, including the fire ant (Roeder 2005).

The *modus operandi* of social insect primer pheromones has been reported to date only for the queen honeybee mandibular gland pheromone. It was demonstrated to regulate brain gene expression (Grozinger et al. 2003) as well as modulate brain DA levels (Beggs et al. 2007). A putative primer pheromone, which potentially regulates worker OA levels, has yet to be identified in the fire ant queen nor any other ant. However, the link between OA levels, the presence or absence of the queen, and the aggression levels may give us the tools to isolate the active compound(s). The research presented here extends the role of biogenic amines in regulating complex social insect interactions and we report for the first time the regulatory effect of an ant colony queen on a biogenic amine in workers. This is also the first direct demonstration of a role for biogenic amines in conspecific nestmate recognition, which is one of the key features of insect sociality.

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