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SHORT COMMUNICATION

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Age-related changes in biogenic amines in individual brains of the ant *Pheidole dentata*

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Abstract The behavioral development of minor workers of the ant *Pheidole dentata* involves a progression of tasks beginning with brood care and culminating in foraging as individuals age. To understand the role of brain neurochemistry in age-related division of labor, we measured the levels of serotonin, dopamine and octopamine in individual brains of minor workers of different age. Serotonin and dopamine levels were significantly correlated with worker age: both increased as minor workers matured, and serotonin rose significantly in the oldest ants. In addition, the serotonin:dopamine ratio was significantly higher in the oldest workers. Octopamine levels did not change with age, although the ratios of octopamine:serotonin and octopamine:dopamine were significantly higher in the youngest workers. These age-associated changes in biogenic amine levels suggest an involvement of neuromodulators in minor worker behavioral ontogeny and temporal polyethism in *P. dentata*.

Introduction

Understanding the regulation of the division of labor is one of the great challenges in the study of insect societies. Although a relationship between age and task performance has been described in many ant species (Hölldobler and Wilson 1990), the neurobiology of the behavior of individual workers and its relationship to age is poorly understood (Gronenberg et al. 1996; Page and Erber 2002). The ant genus *Pheidole* and *P. dentata* in particular has served as a paradigm of colony sociogenesis (Wilson 1976; 1985) and a model for studies of socioecology and caste plasticity (Brown and Traniello 1998; Calabi and Traniello 1989a, b; Wilson 1984). *P. dentata* is a completely dimorphic ant having a minor worker caste responsible for brood care, nest maintenance and foraging, and a major worker caste that is morphologically and behaviorally specialized for colony defense (Wilson 1976). Wilson (1976) described that *P. dentata* minor workers shift from brood care to foraging over the course of the first 20 days of their adult life, forming a discretized temporal caste system in which labor is divided among age cohorts with little or no overlap in tasks. This pattern of division of labor makes this species an excellent candidate for exploring neurochemical changes that may accompany behavioral development and underscore temporal polyethism.

In this paper we examine age-related changes in biogenic amines in minor workers of *P. dentata* by measuring the levels of serotonin, dopamine and octopamine in individual ant brains. We targeted these three amines for analysis because they have been identified in the brains of some ants (Punzo and Williams 1994) and are known to influence behavior and polyethism in honey bees (Wagener-Hulme et al. 1999; Schulz and Robinson 1999, 2001).

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Materials and methods

Laboratory rearing

Queenright colonies of *P. dentata* collected in Gainesville, Florida were cultured in the laboratory in test tubes partially filled with water and fitted with a tight cotton plug. Colonies were placed in Fluon-lined plastic boxes (35×22×11 cm), fed sugar water and mealworms and were maintained under a 12 h light:12 h dark cycle at 28°C and 80% relative humidity. One colony that showed vigorous activity and growth served as the source of workers for amine analysis.

Table 1 Cuticular pigmentation, age and behavior in *Pheidole dentata*. Modified from Wilson (1976) and Seid et al. 2005. *AC* Age class

Age cohort	Pigmentation	Age (days after eclosion)	Tasks
AC1	Uniformly light yellow	1–3	Groom queen, eggs and microlarvae
AC2	Gaster and head gray; thorax yellow	4–8	Groom queen and brood, move larvae and assist in adult eclosion
AC3	Gaster and head darken; thorax dark yellow	9–19	Roll and carry brood, groom and feed larvae. Carry nest material
AC4	Uniformly dark brown to black	20+	Forage and defend nest/food sources. Carry live or dead nestmates

Estimation of minor worker age and sampling

Minor workers were divided into four age classes (AC1–AC4) according to post-eclosion cuticular pigmentation (Table 1; Wilson 1976). Ten ants of each age class in one colony were selected for neurochemical study according to their pigmentation and the tasks they were observed performing. Younger ants (AC1–AC3) engaged in inner-nest tasks (i.e., brood care) and older individuals (AC4) that were foraging were collected for amine measurement. The colony from which workers were selected for analysis contained approximately 900 individuals (2.2% AC1, 3.5% AC2, 10.8% AC3, 71.7% AC4 and 12.8% majors).

Preparation of samples for HPLC

Workers brains were dissected in ice-cold saline (160 mM NaCl, 3 mM, 12 mM HEPES). Each brain (volume 0.01 mm^3) was removed from the head capsule in <1 min and homogenized in 30 µl of 0.3 M perchloric acid. Care was taken to avoid the inclusion of cuticle or other tissue in the sample. The homogenate was centrifuged for 4 min at 4°C at 11,000 r.p.m. in a Costar Spin X tube filter (0.22 µm) for HPLC. To verify that amine levels did not vary as a consequence of age-related changes in brain size, volumetric measurements were made by serial sectioning the brains of minor workers; no age-related increase in size was found: AC1 and AC4 minor worker brain volumes were 1.5×10^7 μm^3 and 1.4×10^7 μm^3, respectively (Seid et al. 2005).

Measurement of biogenic amine levels

Brain amine levels were analyzed with a Coulochem II module with two 5011 analytical cells, a model 580 pump, a MD-150 column (ESA, Chelmsford, Mass.), a Ryodine injector with a 20-µl loop, using DEMO mobile phase (phosphate buffer, citric acid, 1-octanesulfonic acid, acetonitrile) (ESA). Two 5011 analytical cells, each with two electrodes ($E1$, −175 mV; $E2$, 175 mV; $E3$, 350 mV; $E4$ 650 mV), were used to quantify amines. Internal standards run with every sample and external standards run before and after each sampling period verified the measurement of the three amines. Data were collected and analyzed with an A/D converter using LoggerPro software (Vernier, Beaverton, Ore.). Ratios of amines in single individuals were analyzed to identify potential interactions (Kravitz 2000; Djokaj et al. 2001).

Statistical analysis

ANOVA and Tukey-Kramer pairwise comparison (TKPC) were used to test the significance of differences in the study (JMPin, SAS Institute). The TKPC provides a conservative estimate of the significance of differences by taking into account the inherent error rates that accompany multiple comparisons (Ott 1994). Differences were considered significant at $\alpha=0.05$.

Results

Minor worker age and biogenic amines

Levels of serotonin and dopamine increased significantly with minor worker age (ANOVA, $F=9.95$, $df=3$, $P>0.001$, $F=10.40$, $df=3$, $P>0.001$, respectively). AC4 had significantly higher levels of serotonin than all other age classes (TKPC, $P<0.05$; Fig. 1a). Dopamine rose steadily with age: AC3 had significantly higher levels than AC1 (but not AC2) and AC4 had significantly higher levels than both AC1 and AC2 (but not AC3; TKPC, $P<0.05$; Fig. 1b). Octopamine levels did not change significantly as minor workers matured (ANOVA, $F=2.65$, $df=3$, $P=0.06$; Fig. 1c).

Analysis of the relative concentrations of amines revealed that the octopamine:serotonin and octopamine:dopamine ratios were significantly different among age classes (ANOVA, $F=3.46$, $df=3$, $P=0.026$, $F=3.20$, $df=3$, $P=0.035$, respectively). The octopamine:serotonin ratio was significantly higher only for AC1 in comparison to AC4 (TKPC, $P<0.05$; Fig. 2a) and the octopamine:dopamine ratio for AC1 was only significantly higher than for AC2 (TKPC, $P<0.05$; Fig. 2b). The serotonin:dopamine ratio contrasted sharply with that of octopamine:serotonin: minor workers in AC1 had the lowest ratio and AC4 had a significantly higher ratio compared to AC1–AC3. No significant differences were found in the serotonin:dopamine ratio between AC1, AC2 and AC3 (ANOVA, $F=5.20$, $df=3$, $P=0.004$; TKPC, $P>0.05$; Fig. 2c).

Discussion

We measured biogenic amine levels in individual *P. dentata* minor workers in spite of the minute volume of the brain (0.01 mm^3) and found significant changes in

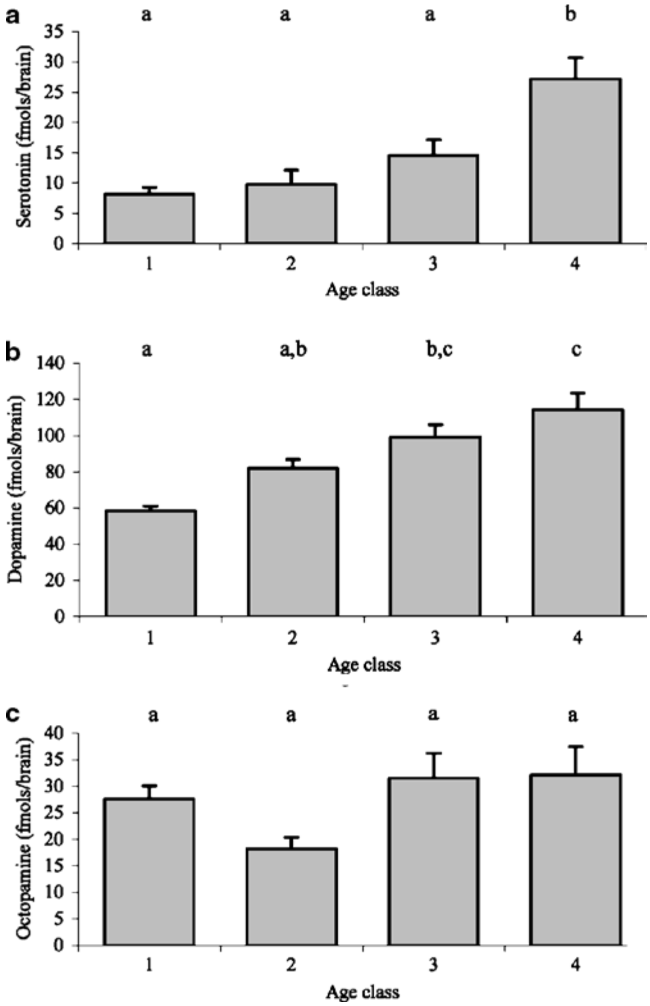

Fig. 1a–c Mean levels (+SE) of biogenic amines in each age class of the minor worker caste of *Pheidole dentata*. *Different letters* indicate significant differences between groups [Tukey-Kramer pairwise comparison (TKPC), $P<0.05$]. $n=10$ ants per age class

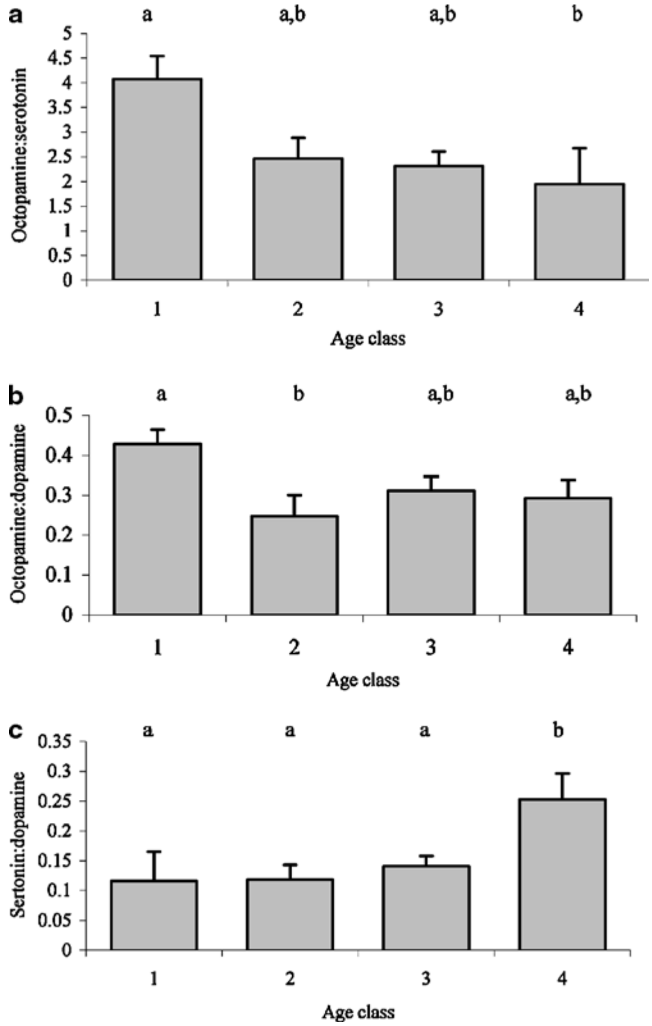

Fig. 2a–c Mean ratios (+SE) of biogenic amines in each age class of the minor worker caste of *P. dentata*. *Different letters* indicate significant differences between groups (TKPC, $P<0.05$). $n=10$ ants per age class

neurochemistry that correlated with the age and behavior of workers. Although we sampled individuals from one colony, we believe that our results are valid and generally applicable in *P. dentata* because of the low variance in amine levels among workers in each age class and the colony's health and behavior. Furthermore, amine levels measured in the brains of ants selected from additional colonies were consistent with the results presented here.

Serotonin increased roughly fourfold in AC4, the age class in which the transition from intranidal to extranidal tasks occurs. Serotonin is known to affect the coupling of endogenous circadian rhythms and diurnal cycles (Tomioka 1999). The increase we recorded is associated with the transition from inner-nest tasks that do not require tuning to photoperiod, to foraging, which must be synchronized with environmental cues. Serotonin is also generally associated with aggression (Kravitz 2000) and has been shown to be involved in agonistic behavior in ants (Kostowski and Tarchalska 1975). The age-related increase in this amine in *P. dentata* may underscore prey capture and the defense of food sources and/or territory shown by older minor workers. The association between increased serotonin and the onset of foraging is thus consistent with the general behavioral effects of this amine.

Dopamine levels increased with minor worker age, a pattern that may be related to the processing of olfactory stimuli. Dopaminergic processes are widely distributed within the insect brain and dopamine has been found to be important in antennal lobe development in bees (Kirchhof et al. 1999). It is possible that dopamine and other amines modulate olfaction as the brain develops and thus allow minor workers to discriminate among chemical cues associated with task performance in *P. dentata*.

Although octopamine did not change significantly with age, the ratios of octopamine to serotonin and dopamine were relatively high in AC1 workers. Boulay et al. (2000) found that octopamine reduced oral exchanges in *Camponotus fellah* and hypothesized that this amine may thus mediate colony cohesion. If this is correct, then the relatively high ratio of octopamine to other amines we recorded in AC1 suggests that newly eclosed ants have a reduced need for trophallaxis to reinforce social connections with nestmates. In *P. dentata*, AC1 minor workers can be easily transferred and adopted by conspecific colonies without aggression (personal observation), perhaps due to the high ratio of octopamine to other amines. Boulay and Lenoir (2001) hypothesized that octopamine mediates colony recognition, but since absolute levels are stable with age in *P. dentata*, it may be the ratio or "cocktail" of amines (Kravitz 2000; Djokaj et al. 2001) that modulates nestmate discrimination in this species.

Schulz et al. (1999) found that division of labor in honey bees was related to changes in levels of amines in specific neuropils. Because of their small size, we measured amines in whole brains and therefore do not know if levels vary in certain regions or the entire brain. Furthermore, we do not know if changes in amine levels activated the performance of tasks or were task-induced. However, AC1 minor workers in *P. dentata* colonies fed tryptophan, a precursor of serotonin, show increased responsiveness to trail pheromone, suggesting a causal relationship between serotonin and foraging (Ament et al., unpublished data).

Although Wilson (1976) described a discretized temporal caste system in *P. dentata*, our studies show that repertoire size increases with minor worker age, with new tasks augmenting task sets performed at younger ages (M. A. Seid and J. F. A. Traniello, unpublished data). Our data suggest that the shift from inside-nest to outside-nest tasks and the increase in repertoire size that we found characterizes temporal polyethism in *P. dentata* are correlated with changes in neurochemistry. Specifically, a rising dopamine level is associated with an increasing repertoire size and a pulse of serotonin occurs as ants initiate foraging. Additionally, ultrastructural studies of the lip region of the mushroom bodies of young and old ants show that synapse number on axonal terminals increases with age (Seid et al. 2005). Our results thus indicate that significant neurochemical changes and neuroanatomical remodeling occur in minor workers during behavior development, supporting a brain-based model of temporal polyethism.

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