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SHORT COMMUNICATION

Social isolation and brain development in the ant *Camponotus floridanus*

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Abstract Social interactions play a key role in the healthy development of social animals and are most pronounced in species with complex social networks. When developing offspring do not receive proper social interaction, they show developmental impairments. This effect is well documented in mammalian species but controversial in social insects. It has been hypothesized that the enlargement of the mushroom bodies, responsible for learning and memory, observed in social insects is needed for maintaining the large social networks and/or task allocation. This study examines the impact of social isolation on the development of mushroom bodies of the ant *Camponotus floridanus*. Ants raised in isolation were shown to exhibit impairment in the growth of the mushroom bodies as well as behavioral differences when compared to ants raised in social groups. These results indicate that social interaction is necessary for the proper development of *C. floridanus* mushroom bodies.

Keywords Ant · *Camponotus floridanus* · Social animals

Introduction

Group learning is often associated with higher-order primates in which social interactions are necessary for proper intellectual development (Castro and Toro 2004). This intellectual development is frequently accompanied with increased brain mass, and it has been shown that primates housed in larger groups have increased gray matter in major brain areas (Sallet et al. 2011). Primates, including humans, when socially isolated during early development conversely exhibit abnormal behaviors and social disabilities which are accompanied by decreased brain volumes (Chugani et al. 2001; Fromkin et al. 1974; Harlow and Suomi 1971). Although mammalian models of the social brain have shown a correlation between brain size and social complexity, insect models due to their vast diversity and ecological dominance may shed greater light on how brain plasticity and/or brain evolution and development are shaped by environmental influences (Lihoreau et al. 2012).

Eusocial insects (ants, bees, and wasps) also live in complex social groups, and these groups are marked by their highly organized collective behaviors and learning abilities (Leadbeater and Chittka 2007). Like primates, social complexity in social insects plays a role in the enlargement of their brains (Ehmer et al. 2001; Riveros et al. 2012; Smith et al. 2010) and recent reviews on the *social brain* hypothesis in insects have emphasized environmental components in brain size and/or development of specific regions of the brain (Lihoreau et al. 2012; Muscedere et al. 2014; O'Donnell et al. 2015). Furthermore, learning in social insect societies is underscored by socially relevant tasks. Wasps are able to learn faces of other colony members (Gronenberg et al. 2008; Tibbets 2002). Honeybees *teach* each other resource locations by the use of social dance (Dornhaus and Chittka 1999), while several species of ants will use tandem running (Franks and Richardson 2006) and other forms of recruitment to communicate nest and/or food sites (Goss et al. 1989; Hölldobler and Wilson 1990). Ants, in particular, are obligatory eusocial, in which individuals depend on one another for survival and socialization (Hölldobler and Wilson 1990). Communication

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within the colony often controls social behavior which leads to division of labor within the nest (Moglich and Hölldobler 1974; Seid and Traniello 2006). This division of labor often follows age-based developmental stages that correspond to task changes accompanied by increased brain volumes and complexity (Gronenberg et al. 1996; O'Donnell et al. 2004; Seid et al. 2005; Seid and Wehner 2009).

The mushroom bodies of the ant brain are of particular interest during their development because they are the centers of learning and memory and have been shown to increase in size and/or synaptic complexity during ant adult maturation (Seid et al. 2005; Kühn Bühlmann and Wehner 2006; Seid and Wehner 2009; Stieb et al. 2010). The calyces of the mushroom bodies are most often divided into the lip and collar in ants, which serve as secondary integrators for olfaction and visual modalities, respectively (Gronenberg et al. 1996), and are believed to be responsible for more complex behaviors in ants (Gronenberg and López-Riquelme 2004). These brain regions also increase in volume during development (Gronenberg et al. 1996) which may be associated with social development as ants mature (Seid et al. 2005).

This study addresses the impact of social isolation on the behavioral and brain development in the ant species *Camponotus floridanus*. Although most behavioral tasks in ants are modifications of solitary insect behaviors, i.e., foraging (Fischman et al. 2011), these behaviors are now under social control and are initiated by social interactions. This social initiation is similar to what is seen in primates (Sallet et al. 2011); thus, ants may show similar brain- and social-related changes associated with group size. In this study, we found that ants isolated and deprived of all social contact as newly eclosed adults showed retarded brain growth, specifically in the mushroom bodies, compared to ants raised in social groups. Additionally, the isolated ants showed significant behavioral delays compared to ants reared in groups. We discuss the implications of these findings below in light of social organization of social species in an effort to dissect out the effects of social isolation on brain development in these ants.

Methods and materials

Anatomical analysis procedure

A queen-right colony of *C. floridanus* ants was collected in Gainesville, Florida, USA, and housed as a colony in the laboratory. Newly eclosed ants, identified by their light pigmentation (presumably 1 to 2 days post-eclosion), were removed from the colonies and placed into two groups. In the first, the isolated group (IG), ants were housed alone without social contact in a small test tube (1 cm diameter, 100 cm long) filled part way with water and a cotton plug. In the second group, single newly eclosed ants were placed in a social group (SG), a queenless sub-colony of six older ants, housed in similar tubes to the IG ants. Ant tubes were placed in individual petri dishes (2 cm high × 150 cm diameter), and ants fed approximately 1 mL of 1.0 M sucrose solution ad libitum, three times a week on a 1-cm^2 piece of Parafilm placed in the center of the petri dish. They were housed in a 12-h L/12-h D light-controlled room in the laboratory at 24–25 °C. Ant brains were dissected and the day of dissection was recorded. Although we tried to maintain an equal number of individuals for a given age, ants varied so much in time of eclosions that exact match aging was impossible. Still, we were able to sample ants at relatively equal age intervals between the two groups.

Brain staining

The ant brains were dissected with fine tip forceps and prepared for a standard immunoreactive staining method (Seid et al. 2008). The ants' brains were dissected out in 0.1 M phosphate-buffered solution (PBS), and then stored at 4 °C overnight in 4 % paraformaldehyde in PBS. Next, they were rinsed three times in PBS and treated with collagenase activated with $CaCl_2$ for 10 min. The brains were then rinsed three times in 3 % Triton X-100 in PBS for 20 min each. Brains were stored overnight in a 10 % normal goat serum and PBS with Triton X-100 (blocking solution). After rinsing in the blocking solution, brains were incubated for 2 days in Alexa Fluor 488 phalloidin (Invitrogen) to stain for F-actin and mouse anti-synapsin antibody (DSHB 1:1000) to stain neuropils. Brains were then washed three times in PBS with Triton X-100 for 10 min each, followed by incubation in goat α-mouse Alexa Fluor 594 (Invitrogen) to fluoresce the synapsin staining. Finally, the brains were washed three times in PBS, dehydrated through an ethanol series, and mounted in glycerol on glass slides for viewing.

Brain tracing

Stained brains were imaged with an Olympus FluoView FV1000© laser confocal microscope. Images were optically sectioned at 5 μm until the entire brain was imaged in series (Fig. 1a). The PC-based software Reconstruct was utilized to obtain a 3D image and volumes for the individual brain structures by tracing and then compiling each image (Fiala 2005) (Fig. 1b). The calyces of the mushroom bodies, containing the lip and the body regions, and the total brains were traced for each image so as to obtain volumetric measurements for each (Gronenberg et al. 1996).

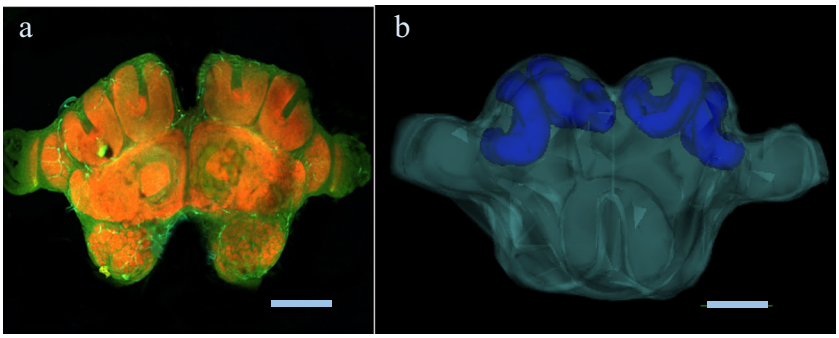

Fig. 1 **a** Laser confocal image of an ant brain. Red staining is synapsin antibody staining of neuropils within the ant brain. **b** Three-dimensional reconstruction of an ant brain. *Blue* indicates calyces of the mushroom bodies. *Clear outline* indicates the total brain. *Error bars* = 100 μm

Behavioral assay

An established behavioral assay used to evaluate aggression in several *Camponotus* species was modified to avoid endangering our subject ants through aggressive interaction with a live ant (Carlin and Hölldobler 1986). Ten ants were placed under IG conditions and ten ants under SG conditions for 30 days. Each ant was placed in an empty, 100 × 30 mm, Fluon-lined petri dish and allowed to acclimate for 1 min. A worker ant from the subject's source colony was crushed and then placed in the center of the petri dish. The subject ant was observed for 60 s and given a behavioral score once every 5 s. After 60 s, the experimental ant was removed from the petri dish and the total behavioral score was calculated by summing the total observational score across the 60 s. Behavioral scores were assessed blind to ant condition on a 0–3 scale that went from ignore/avoid, turning towards without approaching, approaching and finished with physical interaction/contact with the crushed ant, respectively. The crushed ant was disposed of while the petri dish was wiped down with 100 % ethanol.

Statistical analysis

Statistical analysis was performed with the computer program *R* (www.R-project.org). We calculated the ratio of the average mushroom body volume (AMBV) to the total brain volume, which was compared across the IG and SG ants using a regression analysis. For the behavioral test, scores were summed across the entire sample period and a Mann-Whitney *U* test was used to identify statistical differences between the social group and the isolated ants.

Results

Total brain volume and mushroom body growth

Brain volume did not increase significantly in the SG nor in the IG with ant age (regression analysis: $F_{1,9} = 2.41$, $p = 0.155$, $R^2 = 0.211$; regression analysis: $F_{1,12} = 0.065$, $p = 0.8031$, $R^2 = 0.0053$, respectively) (Fig. 2a). However, the SG ants did show significant increases in the relative enlargement of the mushroom bodies (brain-to-mushroom body volume ratio) with age (regression analysis: $F_{1,12} = 9.87$, $p < 0.01$, $R^2 = 0.451$) (Fig. 2b), while IG ants did not show a significant mushroom body increase with age (regression analysis: $F_{1,9} = 0.379$, $p = 0.553$, $R^2 = 0.041$) (Fig. 2b).

Behavior

In the behavioral test, SG ants were significantly more likely to react aggressively to the stimulus than IG ants, as noted by totaling their scores over the minute time trial (Mann-Whitney

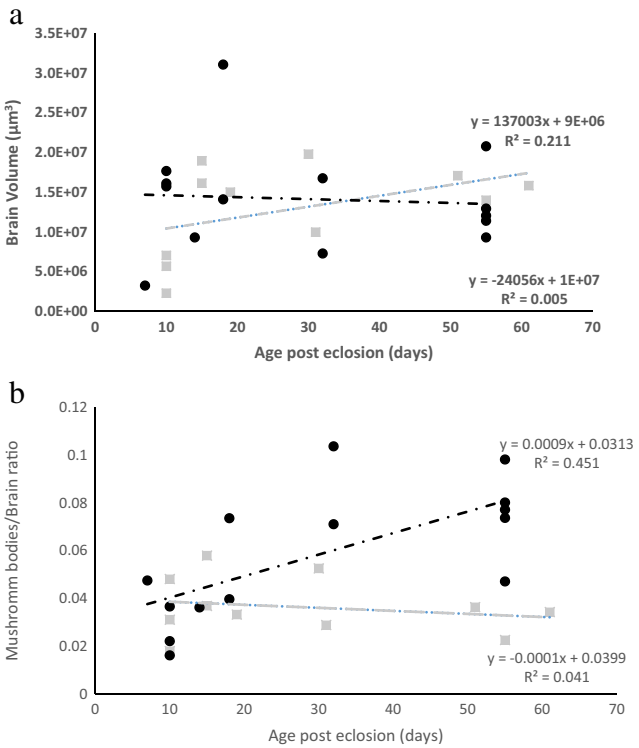

Fig. 2 **a** The total brain volumes of SG and IG ants comparing post-eclosion age. The relationship is slightly negative for SG ants and positive for isolated ants but neither are statistically significant ($F_{1,12} = 0.065$, $p = 0.8031$, $R^2 = 0.005$; $F_{1,9} = 2.41$, $p = 0.155$, $R^2 = 0.211$, respectively). *Gray square* = IG, *black circle* = SG. **b** The ratio of mushroom body to total brain volume shows a statistically significant increase vs time in SG ants (regression analysis: $F_{1,12} = 9.87$, $p < 0.01$, $R^2 = 0.451$) but not in IG ants (regression analysis: $F_{1,9} = 0.379$, $p = 0.553$, $R^2 = 0.041$). *Gray square* = IG, *black circle* = SG

U test, $n = 10$, $W = 8.5$, $p < 0.01$) (Fig. 3a). Ants in the SG group were over ten times more aggressive according to their total aggression scores. The average score for the SG ants at each time point also shows a trend of an increasing score in the first half of the test followed by a decrease in the second (Fig. 3b). The IG ants maintained a steady score, averaging below 0.5, with more receiving a score of 0 at each time point than not, throughout the testing. In fact, almost all of the IG ants remained in a small area after adjusting to the new environment and made little effort to explore, even after the dead ant was introduced. None of the IG ants physically touched the crushed ant (a score of 3), while all but one of the SG ants touched the crushed ant in an aggressive encounter. Similarly, only three of the IG ants progressed to a behavioral score of 2, approaching the crushed ant. Most IG ants either ignored the crushed ant or quickly retreated after a short investigation.

Discussion

Our isolated ants exhibited brain structural differences when compared to ants reared in a group as well as differences in their aggressive interactions in a new environment. The mushroom body volumes of SG ants increased in size relative to the total brain volume as they maturated, while IG ants did not show a change in relative mushroom body size. Furthermore, IG ants exhibited markedly different behaviors when introduced to a novel social situation and failed to physically engage a conspecific. Our results parallel those found in macaque monkeys that were isolated during the first 6 months of life (Harlow and Suomi 1971) in which these primates fail to engage in social interactions after being raised in isolation. Also similar to our findings, monkeys living in larger social networks had an increase in brain areas responsible for learning (Sallet et al. 2011), analogous to our relative mushroom body size increases.

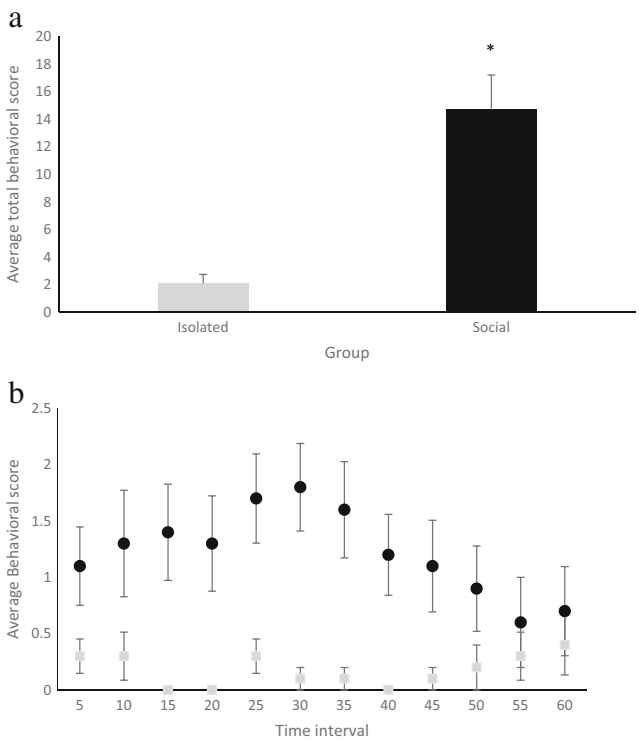

Fig. 3 **a** Average aggression scores summed across the 1-min trial. SG ants were significantly more aggressive than IG ants (Mann-Whitney *U* test, $n = 10$, $W = 8.5$, $p < 0.01$). *Asterisk* indicates significantly greater value. **b** The average behavior (±SE) score at each time point across the 1-min test for the SG and IG ants. *Gray square* = IG, *black circle* = SG

Our results support the social brain hypothesis that brain size is shaped by group size/social interactions (Jolly 1966; Dunbar 1992) and show how the environment (Lihoreau et al. 2012), in our data the social environment, plays a critical role in brain development. We show that a lack of social interactions inhibits the brain growth of learning and memory centers (i.e., mushroom bodies), leading to decreased relative brain volumes as found in other social organisms. Social interactions appear necessary for the proper brain development in *C. floridanus*; thus, the development of the social brain may be an across-taxa phenomenon and the construction of the social brain may be governed by similar processes from social insects to mammals. Although our study does not directly test how the evolution of the brain is influenced by environment (see review by Muscedere et al. 2014; O'Donnell et al. 2015), our data suggests that the developmental processes that shape social species are modified by the environment to elicit different phenotypes and these phenotypes may be under selection for social species.

Although our observed growth of the mushroom bodies of *C. floridanus* during development could be attributed to the ants' behavioral progression correlated to an increase in brain volume (Gronenberg et al. 1996), the increase seen in the mushroom bodies of the SG ants cannot solely be attributed to starting foraging because the isolated ants also had to forage and care for themselves. Solitary insects have to do a larger number of tasks themselves than social insects because they cannot rely on other colony members to help them in division of labor (Wilson 1971). In fact, our IG ants seemed to perform all the necessary tasks for survival in the isolated state, i.e., foraging. Although some brain development should be expected, we believe that a critical period of social interaction plays a role in social ant brain development. In *C. floridanus* as well as other ants, mushroom bodies enlarge in the first months after eclosion into an adult (Gronenberg et al. 1996; Seid et al. 2005). We found this enlargement is at least partially due to social interactions during the first 30 days post-eclosion, and we suggest that during this time, a critical phase for social development is occurring for proper ant brain growth and social maturation. When older ants are isolated, they exhibit an increased need for social interactions (Boulay and Lenoir 2001), and this is the opposite of what we found in

our young ants and suggests that adult ants reared within the colony may be resistant to the negative effects of isolation compared to our IG ants. This suggests that a critical period for social interaction is necessary for proper social development in ants and that isolation is particularly damaging when the ant brain is developing. Similar critical periods are known in primates (Scott 1962) and may exist in social hymenoptera as well.

The differences in brain morphology and behavior we found could also be related to stress involved in isolating an obligatory social species. In other ants, isolation has been found to cause stress and increase mortality (Boulay et al. 1999, 2000). Furthermore, isolation has also been shown to have a neurochemical effect on ants, specifically influencing the dopaminergic and octopaminergic systems (Boulay et al. 2000; Wada-Katsumata et al. 2011). Interestingly, these systems are also correlated with brain development in some social insects (Schulz and Robinson 1999; Fahrbach 2006) and may be in part related to both the behavioral and morphological differences we observed.

Future studies looking at using the genome of *C. floridanus* (Bonasio et al. 2010) could be used to elucidate the genetic underpinnings of the social brain as well as examining the specific proteins involved in enlarging specific regions of the brain. Social isolation in ants could serve as a new model for developmental disability affecting behavioral and neuronal development, and since our study suggests that the processes governing social maturation are similar across taxa, our ants may yield findings across many social species.

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