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Mushroom Body Volume Is Related to Social Aggression and Ovary Development in the Paperwasp *Polistes instabilis*

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Key Words

Division of labor · Dominance · Mushroom bodies · Neural plasticity · Paperwasp · Social insects

Abstract

The mushroom bodies (MB) are a complex neuropil in insect brains that have been implicated in higher-order information processing such as sensory integration and various types of learning and memory. Eusocial insects are excellent models to test functional neural plasticity in the MB because genetically related nest mates differ in task performance, environmental experience and social interactions. Previous research on eusocial insects shows that experience-dependent changes in brain anatomy (i.e., enlarged MB calyces) are positively correlated with task performance and social interactions. In this study, we quantified relationships of task performance and social and reproductive dominance with MB volume in *Polistes instabilis*, a primitively eusocial paper wasp. We used experimental removals of dominant workers to induce changes in aggressive behavior and foraging by workers. Ovary development and social dominance were positively associated with the volume of the MB calyces relative to the region containing the Kenyon cell bodies. In contrast to highly eusocial insect workers, foraging behavior was not positively correlated with MB calycal volume. We conclude that mushroom body volume is more strongly associated with dominance rank than with foraging behavior in *Polistes instabilis*.

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Introduction

Structural variation in insect's brains is associated with individual behavioral differences, possibly reflecting the individual's demands for sensory processing and integration [Erber et al., 1980; Kaulen et al., 1984; de Belle and Heisenberg, 1994; Heisenberg et al., 1995; Heisenberg, 1998; Strausfeld et al., 1998; Gronenberg and Holl-dobler, 1999; Dubnau et al., 2001; Pascual and Preat, 2001]. The mushroom bodies (MB) are paired neuropils in arthropod forebrains that are implicated in higher order information processing [Heisenberg, 1998]. Neuroethological studies on insect model systems (*Drosophila*, roaches) implicate the MB in diverse cognitive functions [Heisenberg et al., 1995; Mizunami et al., 1998; Pascual and Preat, 2001; Waddell and Quinn, 2001; Davis, 2005]. For example, MB neurons function in several types of learning and memory, including context-dependent and spatial memory [Schildberger, 1984; Li and Strausfeld, 1997, 1999; Strausfeld et al., 1998; Waddell and Quinn 2001; Heisenberg, 2003; Farris, 2005; Fahrbach, 2006].

Comparative and neuroethological data suggest that the MB play a special functional role in eusocial insects [Strausfeld et al., 1998; Farris and Roberts, 2005; Fahrbach, 2006]. MB development is functionally related to individual differences in task performance in workers of several species (honey bees, ants, and paper wasps) [Erber et al., 1980; Mobbs, 1982; Kaulen et al., 1984; Withers et

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al., 1993; Durst et al., 1994; Gronenberg et al., 1996; Dubnau et al., 2001; Farris et al., 2001].

The MBs are mainly composed of several anatomical classes of intrinsic neurons called Kenyon cells. The Kenyon cell bodies cluster in a region that outlines cup-shaped neuropils called calyces in which the Kenyon cell dendrites arborize [Mobbs, 1982; Ehmer and Hoy, 2000; Gronenberg, 2001]. Volumes of MB regions can reflect the amount of dendritization of the MB Kenyon cells, either via growth of dendrites or increases in the numbers of dendrites, as well as number of synapses the Kenyon cells make with afferent neurons [Farris et al., 2001; Gronenberg, 2001; Ehmer and Gronenberg, 2004; Seid et al., 2005; Fahrbach, 2006].

The MB calyces receive neural input from other parts of the brain, including the antennal and optic lobes [Heisenberg, 1998; Farris, 2005]. There are three generally recognized anatomical and functionally distinct subregions of the eusocial hymenopteran MB calyx: the lip, which receives information from the antennal lobes; the collar, which receives visual input; and the basal ring, which is further subdivided into two parts that receive information either from the optic or antennal lobe [Ehmer and Hoy, 2000; Ehmer and Gronenberg, 2004; but see Gronenberg, 2001]. Relative volumes of the MB calyx and calycal subregions have been used to estimate neural development in this important neuropil. Differences in MB subregion volumes might indicate the relative contributions of chemosensory (i.e., lip) versus visual (i.e., collar) input to MB development [Gronenberg and Holldobler, 1999; Gronenberg and Liebig, 1999; Gronenberg, 2001]. For example, Hymenoptera that rely more on visual input (bees and wasps) have relatively larger MB collars than those that rely less on vision [e.g., ants; Gronenberg, 2001].

In some eusocial hymenoptera, MB calycal volume correlates with foraging behavior [Farris, 2005; Fahrbach, 2006]. Volumetric increases in the mushroom bodies have been observed when individuals shift from tasks within the nest to tasks on the surface of the nest in the highly eusocial wasp, *Polybia aequatorialis* [O'Donnell et al., 2004]. Leaving the nest to forage is correlated with increased calycal volume in honey bees (*Apis mellifera*), carpenter ants (*Camponotus floridanus*) and eusocial wasps (*Polybia aequatorialis*), possibly because of increased cognitive demands associated with flight such as object recognition and memory when collecting specific materials (e.g., nectar versus pulp), and place memory when navigating to and from the nest [Withers et al., 1993; Gronenberg et al., 1996; O'Donnell et al., 2004].

The effects of foraging on MB volume have been observed in highly eusocial species with temporal polyethism. In these species, adult workers generally progress through age-specific tasks, moving from in-nest tasks to foraging as they develop [Jeanne, 1991; Robinson, 1992; Seid et al., 2005]. However, many eusocial insect workers do not exhibit strong temporal polyethism. In these primitively eusocial species, task performance is less age-dependent, and instead varies according to an individual's social rank. Individuals with the same dominance rank (e.g., subordinate forager) might vary widely in age [Hughes and Strassmann, 1988; O'Donnell, 1996, 1998a, b; Heinze and Oberstadt, 1999]. Many if not all individuals are capable of reproduction, but ovary development in subordinate workers is suppressed via dominance interactions [Pardi, 1948; West-Eberhard, 1969; Roseler et al., 1980; Strassmann, 1981; Strambi, 1985]. Social and reproductive dominance are often negatively associated with foraging rates for primitively eusocial insect workers [Reeve, 1991; Roseler, 1991; O'Donnell and Jeanne, 1995; Foster et al., 2004]. Socially dominant individuals not only forage less frequently, but may also pressure subordinate nest mates to forage via aggressive interactions [Reeve, 1991; O'Donnell, 1998b, 2006; Bruyndonckx et al., 2006].

The goal of this study was to assess whether social interactions are associated with MB structure in primitively eusocial paper wasps. Several lines of evidence suggest that social conditions can affect insects' MB volume. For example, the calyces of *Drosophila* fruit flies reared at higher larval densities are enlarged relative to those of flies reared at lower densities [Heisenberg et al., 1995]. Ehmer et al. [2001] provide evidence for this in *Polistes* paper wasps by showing that solitary nest foundresses have smaller antennal lobes and MB collars relative to members of multiple foundress associations. Positive relationships of social dominance rank with MB volume have been documented in *Mischocyttarus mastigophorus*, another independent-founding paper wasp [O'Donnell et al., 2007].

In this study, we quantified the relationships among reproductive state, dominance interactions and MB volume in the primitively eusocial paper wasp *Polistes instabilis*. As in other *Polistes* species, dominance interactions such as biting and abdominal vibrations are positively associated with reproductive dominance in *Polistes instabilis*, but are negatively associated with foraging rates, particularly food foraging [O'Donnell, 1998b; Molina and O'Donnell, unpublished data]. Ehmer et al. [2001] found increased brain volume in *Polistes* multiple foundresses

relative to solitary foundresses, suggesting an effect of rate of social interactions on MB volume. Our study extends this work by assessing brain/behavior associations in post-emergence (worker producing) *P. instabilis* colonies. If social dominance is positively associated with MB volume, dominant individuals, which are generally nest workers in this species, should have a greater ratio of neuropil to Kenyon cell body volume than subordinate foragers [O'Donnell et al., 2007]. Alternatively, if foraging task performance is positively associated with MB volume, as has been found in several highly eusocial insect species [Withers et al., 1993; Gronenberg et al., 1996; O'Donnell et al., 2004], we would predict foragers to have a greater ratio of neuropil to Kenyon cell body volume than nest workers. We focused on the MB calyces because they are developmentally plastic and they have shown the predicted associations with behavior in other species [Withers et al., 1993; Ehmer et al., 2001; Farris et al., 2001; O'Donnell et al., 2004; O'Donnell, 2006].

To test for brain/behavior relationships in *P. instabilis*, we performed experimental worker removals. We manipulated the dominance hierarchy by removing the most dominant workers [O'Donnell, 1998b; Molina and O'Donnell, unpublished data]. We tested whether MB volume was related to changes in social aggression (dominance) and foraging (task performance) before versus after nestmate removal. Because MB volume might have changed following the worker removals, we also analyzed relationships between individuals' MB volume and their rates of behavior post-removal. We tested for predicted associations among MB volume, ovary development, and behavior, to assess whether dominance or foraging task performance was associated with MB calyx volume.

Material and Methods

Behavioral Data and Subject Collection

Behavioral data were collected from 27 June to 24 July 2005. We studied seven post-worker emergence *Polistes instabilis* colonies. The nests were observed in situ near Cañas in Guanacaste Province, Costa Rica [10°26'N; 85°07'W; O'Donnell, 1998b]. Nests were located at heights of 0.5 to 2.5 m above the ground, in vegetation or on the eaves of buildings. All adult wasps on the nests were individually marked with paint pens 1–2 days before observations began. Colonies were observed daily for 3–4 days each.

Behavioral data were collected using a portable tape cassette recorder while sitting 1 m from the nest for three hours continuously in the morning, between 08:00h–12:00 h local time, and for two hours in the afternoon, between 13:00–17:00 h. All occurrences of the following acts were noted: arrivals and departures from the nest, abdominal vibrations, chases/bites and number of

times bitten, and egg laying [See O'Donnell, 1998b for ethogram]. We also recorded the identity of all adults present by visually scanning the nest every 15 minutes. Time spent on nest was calculated from the scan data. Queens were identified based on observations of egg laying; one female per colony laid eggs during the study.

Control observations were performed for 1 to 2 days before experimental manipulation. The workers selected to be removed were non-ovipositing individuals with the greatest number of bites and chases. One individual was removed from colonies with fewer than 15 adult females, and two individuals were removed from larger colonies. Behavioral data from colonies were collected for one day following the removals, after which the removed dominant workers were returned to their nests during the night, except for Colony H. Behavioral data were recorded the day after the dominant workers were returned and colonies were subsequently collected after dark on the last day of observations.

Dissections and Neuroanatomical Measurements

Head capsules and gasters were dissected from the thorax, and all body sections were placed in paraformaldehyde based fixative immediately after collection. The wasps' gasters were fixed for at least 3 weeks and then transferred into 70% ethanol. The ovaries were dissected from each female's gaster, and the egg cells (oocytes) were photographed under a dissecting microscope at 10× magnification using a digital camera. The two largest oocytes were measured from the digital photographs using Adobe Photoshop. Length measurements were converted to mm by photographing and measuring a stage micrometer. Because oocytes are roughly elliptical, area was calculated as: $\pi * \frac{1}{2} \text{length} * \frac{1}{2} \text{width}$. We used the mean area of the two largest oocytes as an index of ovary development [Keeping, 2000; Markiewicz and O'Donnell, 2001; Keeping, 2002; Foster et al., 2004].

Body size affects dominance status in many *Polistes* species [Pardi, 1946; Turillazzi and Pardi, 1977; Tannure-Nascimento et al., 2005]. Although Hughes and Strassmann [1988] did not find body size to be an indicator of dominance in *Polistes instabilis* workers, body size could still influence and/or confound relationships between MB volume and behavior in our study [Mares et al., 2005]. We used wing length as an index of body size [O'Donnell and Jeanne, 1995; Ehmer et al., 2001; O'Donnell, 2001]. To measure wing length, we dissected the forewings away from the thorax and mounted them flat on microscope slides using transparent tape. We photographed the wings under a dissecting microscope at 10× magnification using a digital camera. We measured length along the stiff anterior edge of the wing from the base to the proximal end of the pterostigma [i.e., the costa vein; O'Donnell and Jeanne, 1995; O'Donnell, 2001]. Lengths were determined from the digital photographs using Adobe Photoshop. Length measurements were converted to mm by photographing and measuring a stage micrometer. We used the mean length of the left and right wings as an index of body size; when one wing was damaged, we used the other alone to index size.

Individuals were chosen for neuroanatomical measurements if they were queens, foragers or dominant workers. We collected neuroanatomical data for two to five marked subjects per colony (total $n = 20$, mean = 2.9 wasps/colony). The head capsules were cut in half to improve infiltration and were embedded in resin. We made 10 μm thick coronal sections using a rotary microtome and steel histology knives (fig. 1). Sections were mounted on gela-

Fig. 1. Coronal section of one lobe of the mushroom body from a female *Polistes instabilis* wasp. The insert gives the approximate delineation of the anatomical regions that we quantified. The Kenyon cell body region (kbc) is shaded light gray, and the calyx (subregions labeled lip, and c + br = collar plus basal ring) are shaded dark gray.



tin-coated slides and stained with toluidine blue. The head capsule sections were photographed through a 4× objective using a digital camera mounted on a compound light microscope. To quantify the area of targeted brain regions in the sections, we overlaid a 4.6 cm square grid atop the digital image in Adobe Photoshop, and counted the total number of intersection points that fell within the lip, collar, basal ring and Kenyon cell body region in an arbitrarily chosen hemisphere. Every other section was quantified moving caudally after quantifying the rostral-most section containing mushroom bodies [Mayhew, 1992]. Volume was calculated as a product of the sum of points counted over sections, the area per point (0.67 mm²) and the distance between section planes (20 μm).

Quantification was performed on every other section. Measurements were made blind to the individual subject's behavior. We measured volume of the following brain regions for one hemisphere per adult wasp: the Kenyon cell body region, and subregions of the calyx neuropil: lip and collar + basal ring [Mobbs, 1982; Farris, 2005; O'Donnell et al., 2007]. We investigated both relative volumes (calyx: Kenyon cell body ratio) and absolute volumes of MB subregions. The collar and basal ring were grouped because boundaries between these subdivisions were often ambiguous, whereas boundaries between the lip and collar were always distinct.

Statistical Analyses

The proportion of time spent on the nest was estimated from the number of scans in which an individual was present on the nest. If an individual, such as a forager, was not present for any scans on an observation day, they were arbitrarily assigned one scan on nest for that day. Changes in behavior were determined by the difference in rates before versus after nest mate removal. Individual rates of behaviors were calculated as frequency divided

by the product of the proportion of time an individual spent on the nest and number of observation hours, except for foraging, which was calculated as overall frequency of arrivals per observation hour. Behavioral change data met assumptions of parametric tests [i.e., normally skewed, kurtotic distributions and equal variances; Pedhazur, 1982] but rates of behavior did not and were therefore square-root transformed. We used a box plot test to insure that extreme values were not statistical outliers [Iglewicz and Hoaglin, 1993].

We used partial correlations to independently analyze the relationships among ovary development, changes in behavior and MB volume ratios as well as absolute MB volume. Hierarchical regression analyses were used to test associations between volume ratios as well as absolute volumes and square-root transformed rates of behavior post-removal. Colony identity was included as a factor in all multivariate analyses. Wing length was included as a factor in analyses of absolute brain region volume.

Results

Reproductive State, Caste, and MB Anatomy

Ovary development was positively correlated with a greater calyx: Kenyon cell body ratio (fig. 1, $r = 0.60$, $n = 19$, $p < 0.05$). Ovary development was positively associated with the collar + basal ring: Kenyon cell body ratio ($r = 0.61$, $n = 19$, $p < 0.05$), and there was a marginally non-significant positive association between ovary development and the lip: Kenyon cell body ratio ($r = 0.54$, $n = 19$, $p = 0.06$). MB subregion volume ratios were posi-

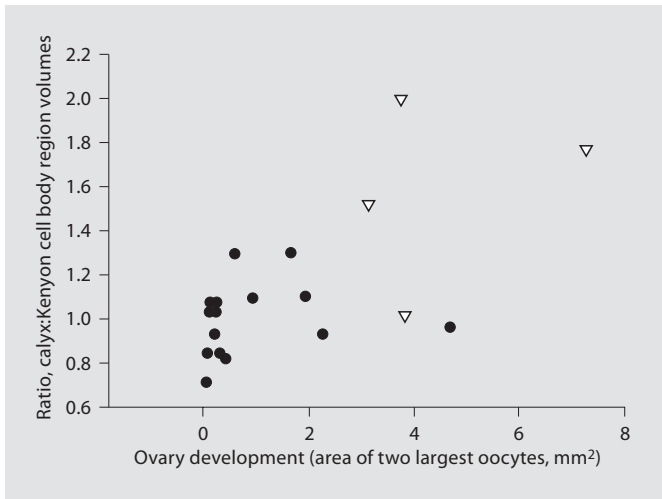


Fig. 2. Scatterplot depicting the relationship between calyx:Kenyon cell body region ratios and ovary development, as indexed by the average size of the two largest oocytes. Shaded circles indicate workers, open triangles indicate queens.

tively correlated, suggesting that there were increases in calycal volume across regions (lip: Kenyon cell body ratio vs. lip: collar + basal ring ratio: $r = 0.70$, $n = 19$, $p < 0.01$; lip: Kenyon cell body ratio vs. collar + basal ring: Kenyon cell body ratio: $r = 0.85$, $n = 19$, $p < 0.0001$).

Queens had a greater calyx: Kenyon cell body ratio than workers (fig. 2; $F_{1,11} = 19.07$, $p < 0.001$). Queens had both greater lip: Kenyon cell body ratios ($F_{1,11} = 12.10$, $p < 0.01$) and greater collar + basal ring: Kenyon cell body ratios ($F_{1,11} = 19.82$, $p < 0.001$) than workers.

Changes in Behavior and MB Volume

There was a non-significant trend toward a positive correlation between calyx: Kenyon cell body ratio and changes in biting rates ($r = 0.50$, $p = 0.08$). Changes in biting rates were positively correlated with lip: Kenyon cell body ratio ($r = 0.55$, $p < 0.05$; fig. 3a) and lip: collar + basal ring ratio ($r = 0.58$, $p < 0.05$; fig. 3b). The calyx: Kenyon cell body ratio was not correlated with changes from pre- to post-removal treatments in the number of vibrations ($r = -0.09$, $p = 0.76$), foraging trips ($r = 0.09$, $p = 0.77$), or times bitten by other nest mates ($r = 0.05$, $p = 0.88$).

Post-Removal Behavior and MB Volume

Post-removal foraging rates were negatively associated with calyx: Kenyon cell body ratio (fig. 4; $t_8 = -2.41$, $p < 0.05$). Foraging rates were specifically negatively associ-

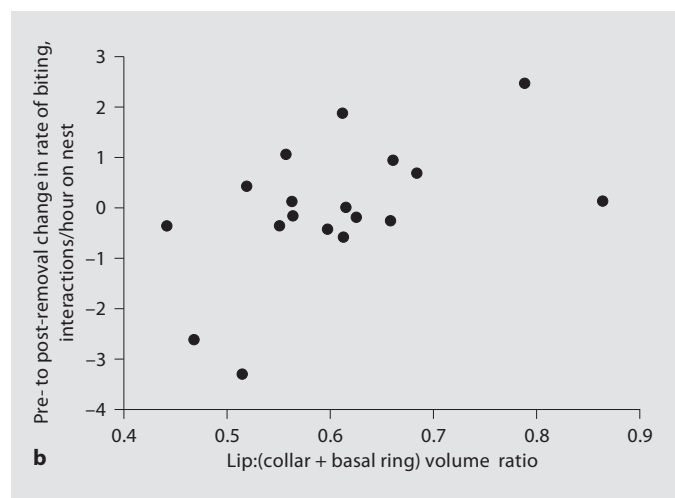
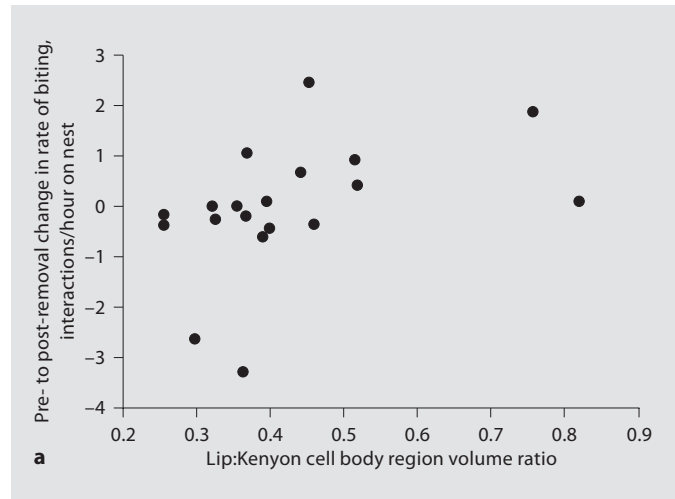


Fig. 3. Scatterplot depicting the relationships between change in biting rate and ratios of calycal subregions to the Kenyon cell body region. **a** Scatterplot depicting the relationship between change in biting rate and lip: Kenyon cell body region after accounting for colony identity. **b** Scatterplot depicting the relationship between change in biting rate and lip: collar + basal ring after accounting for colony identity.

ated with the collar + basal ring: Kenyon cell body ratio ($t_8 = -2.59$, $p < 0.05$) and there was a non-significant trend toward negative association between post-removal rates of foraging and lip: Kenyon cell body ratio ($t_8 = -2.01$, $p = 0.08$). Additionally, there was a non-significant trend toward association between post-removal rates of biting nest mates and collar + basal ring: Kenyon cell body ratio ($t_8 = 1.93$, $p = 0.09$). The calyx: Kenyon cell body ratio was not associated with the proportion of time an individual spent on the nest post-removal ($R^2 = 0.12$, $F_{1,11} = 3.20$,

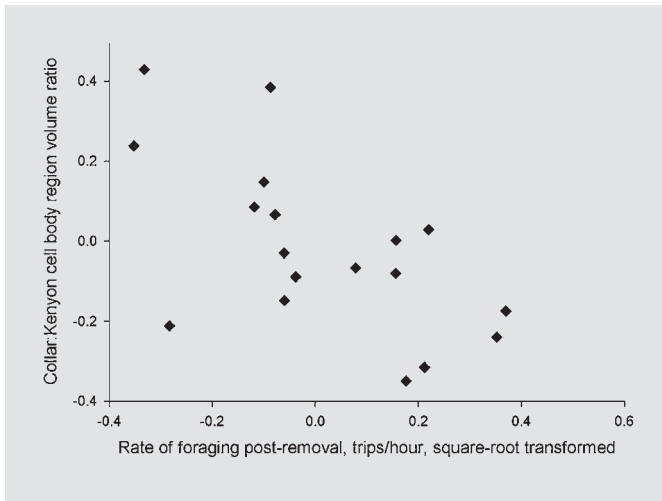


Fig. 4. Partial regression plot of collar: Kenyon cell ratio and square-root transformed rates of foraging during post-removal observations, after accounting for colony identity and square-root transformed rates of biting other nest mates, vibrating and times bitten by other nest mates.

$p = 0.10$) nor the rate of abdominal vibrations ($R^2 = 0.01$, $F_{1,11} = 0.30$, $p = 0.60$).

Body Size Effects

Body size had little effect on MB volume or behavior. Wing length was not significantly associated with either total calyxal volume ($N = 19$, $r = 0.52$, $p = 0.10$) or with the Kenyon cell body volume ($r = -0.38$, $n = 19$, $p = 0.26$). Body size was not correlated with ovary development ($r = 0.44$, $n = 19$, $p = 0.18$), and queens were not significantly different in size from workers ($F_{1,9} = 2.89$, $p = 0.12$). Additionally, body size was not significantly associated with time spent on the nest ($r = 0.04$, $n = 19$, $p = 0.91$) or post-removal rates of vibrations ($r = 0.11$, $n = 19$, $p = 0.75$), bites ($r = -0.09$, $n = 19$, $p = 0.80$), foraging trips ($r = -0.43$, $n = 19$, $p = 0.19$) or times bitten by other nest mates ($r = -0.43$, $n = 19$, $p = 0.19$).

Discussion

Reproductive physiology and MB volume were correlated in post-emergence *P. instabilis* colonies. Specifically, ovary development was positively associated with calyx: Kenyon cell body region ratio, and queens had larger calyx: Kenyon cell body region ratios than workers. We did not find that reproductively dominant individuals

were larger bodied than subordinate individuals, in agreement with previous studies on this species [Hughes and Strassmann, 1988]. Furthermore, body size had little effect on MB volumes.

Social dominance was also positively correlated with MB volume. Individuals that increased their biting rates post-removal also had greater lip: Kenyon cell body ratios and greater lip: collar + basal ring ratios. Because the lip is primarily innervated by axons from the antennal lobes [Gronenberg and Holldobler, 1999; Gronenberg, 2001], this pattern suggests that olfactory communication may be especially important in *P. instabilis* dominance interactions. However, the high correlations among MB sub-region volumes suggest that neural responses to social interactions might occur throughout the MB, and therefore depend on multiple sensory modalities. For example, there may be a visual component to this relationship, as suggested by the marginally non-significant positive associations between collar + basal ring: Kenyon cell body region and biting rate.

Previous studies have suggested that social interactions can influence MB volume. Members of *P. dominulus* multiple foundress associations had enlarged MB collars and antennal lobes relative to single foundresses [Ehmer et al., 2001]. In contrast to our study, Ehmer et al. [2001] did not find a significant relationship between MB volume and dominance rank among nest mates in multiple foundress groups. Effects of dominance may be more pronounced in our study as a function of colony size; their colony size ranged from 1 to 3 foundresses whereas our colony sizes ranged from 7 to 26 members. Our analysis might also have been able to detect subtle dominance-related differences because we focused on internal MB volume whereas they focused on MB sub-regions (i.e., collar) in relation to total brain volume.

We found that task performance was related to MB volume, again suggesting that dominant wasps, that generally do not forage, had better-developed MB. Post-removal rates of foraging were negatively associated with calyx: Kenyon cell body ratios, and specifically with the collar + basal ring: Kenyon cell body ratios. The patterns we found are contrary to previous studies on highly eusocial insects which showed that increases in MB calyx volume were associated with foraging behavior [Withers et al., 1993; Gronenberg et al., 1996; Farris et al., 2001; O'Donnell et al., 2004]. This difference may be explained in part by nest architecture. Workers in the highly eusocial species move out from dark nest interiors as they age. O'Donnell et al. [2004] found the largest MB anatomical differences between *Polybia* workers within the nest and

on-nest workers. In contrast to most highly eusocial species, *Polistes* have open, exposed nests [Reeve, 1991]. These differences in stimulus levels do not exist between *Polistes* nest workers and foragers which are all subjected to similar levels of light. Initiation of flight might also be more important to neuropil expansion than material collection and continued foraging performance per se. For example, similar increases in brain region volume have been observed in honey bee queens and drones after orientation flights [Fahrbach et al., 1995, 1997]. Experiences such as flight might have irreversible effects on brain volume. Fahrbach et al. [2003] found that honey bee foragers do not have a reduction in brain volume if foraging is stopped. Finally, task specialization is reversible and dependent on social rank instead of age in *Polistes* [Reeve, 1991]. As such, task performance's relationship to brain volume might be a function of social organization in primitively eusocial groups as subordinates often per-

form the majority of foraging [Pardi, 1948; Strambi, 1985; Reeve, 1991; O'Donnell, 1998a; but see O'Donnell, 1996]. We suggest that in some species, MB anatomy reflects dominance status and social experience more strongly than the cognitive challenges of foraging tasks.

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