



ELSEVIER

Neuroscience Letters 356 (2004) 159–162

Neuroscience  
Letters

[www.elsevier.com/locate/neulet](http://www.elsevier.com/locate/neulet)

## Mushroom body structural change is associated with division of labor in eusocial wasp workers (*Polybia aequatorialis*, Hymenoptera: Vespidae)

Sean O'Donnell<sup>a,\*</sup>, Nicole A. Donlan<sup>b</sup>, Theresa A. Jones<sup>b</sup>

<sup>a</sup>Neurobiology and Behavior Program and Department of Psychology, University of Washington, Seattle, WA 98195, USA

<sup>b</sup>Neuroscience Institute and Department of Psychology, University of Texas, Austin, TX 78712, USA

Received 8 August 2003; received in revised form 4 November 2003; accepted 10 November 2003

### Abstract

Highly eusocial insect workers exhibit age-related division of labor. Adults begin working inside the nest, moving to the nest periphery and later to foraging. Passage through this task sequence is associated with neuroanatomical changes in the mushroom bodies (MB) of honey bee (*Apis*) and ant (*Camponotus*) workers. We asked whether eusocial wasp workers (*Polybia aequatorialis*) exhibit similar changes in adult neuroanatomy. Wasps were identified as working in-nest, on-nest, or foraging. The volumes of the somata of workers' MB intrinsic neurons (Kenyon cells), and of the neuropils containing the Kenyon cell dendritic arbors (calyces), were estimated using stereological methods. In-nest workers had significantly smaller calyx to Kenyon cell volume ratios than on-nest and foraging workers. Age-related task specializations in *Polybia* workers are associated with major neuroanatomical reorganization in the mushroom bodies.

© 2003 Elsevier Ireland Ltd. All rights reserved.

**Keywords:** Division of labor; Kenyon cells; Calyx; Structural plasticity

Eusocial insect colonies are tractable systems for analyzing how individuals coordinate to generate group behavior [16]. The properties of the nervous system that influence worker behavior are poorly understood, but the mushroom bodies (MB) have received increasing attention because of their putative roles in sensory integration, learning and memory [2,9,18]. Eusocial insects have relatively larger MB, particularly MB calyces, than related solitary species [10]. This pattern implies that the MB calyces play an important role in regulating division of labor.

Research in honey bees (*Apis mellifera*) and ants (*Camponotus floridanus*) supports this hypothesis by linking plasticity of the mushroom bodies with division of labor among workers [7,19]. Honey bee foragers have larger Kenyon cell dendritic arbors [6], and increased calycal volume relative to the Kenyon cell body region, than do nurse bees. This difference is more strongly related to individual differences in task performance than to absolute age [5,20]. These studies implicate the MB in the regulation of worker division of labor. Patterns of division of labor in *Polybia* paper wasps are convergent on those of honey bees,

but whether similar MB changes are associated with task performance in eusocial wasps is not known.

The purpose of the present investigation was to test the generality of MB structure/task associations in advanced eusocial paper wasps. We asked whether MB anatomy corresponds to task performance in *Polybia aequatorialis* (Vespidae). Like honey bees, *Polybia* wasps initiate new colonies as swarms [11], and workers exhibit strong task specialization [13]. An important component of *Polybia* worker division of labor is temporal polyethism, a task performance pattern where workers pass through a predictable sequence of task specializations during adult life. Young adults begin by working inside their enclosed nests, progressing to tasks on the nest exterior such as building, and later they leave the nest to forage [15]. Temporal polyethism is shared by *Polybia* wasps, honey bees, and many ants [11]. *Polybia* wasps are therefore a good system in which to test the generality of the brain/behavior relationships documented in *Apis* and ants.

If changes in MB structure are functionally associated with task performance differences, then *Polybia* workers performing different task sets will exhibit consistent differences in brain structure. We focused on attributes of MB anatomy that are associated with temporal polyethism

\* Corresponding author. Tel.: +1-206-543-2315; fax: +1-206-685-3157.  
E-mail address: sodonnell@u.washington.edu (S. O'Donnell).

in honey bees: the volumes of the Kenyon cell region and of the adjacent neuropil, the calyx. The Kenyon cells are the intrinsic neurons of the mushroom bodies and are believed to participate in multimodal sensory integration and learning. Their cell bodies are enclosed by, and surround, the cup-shaped neuropil of the calyces, in which their dendrites arborize and receive much of the sensory afferent projections to the MB, including synaptic input from antennal and optic lobes. Kenyon cell axonal projections travel via the peduncle to a set of lobes, where they intermingle with the dendrites of the extrinsic neurons that connect the MB with the protocerebrum [3,12,17,18]. Because the calyces contain Kenyon cell dendritic arbors and synaptic input, changes in calycal volume may reflect dendritic growth and synapse addition onto Kenyon cells.

Workers were observed and collected from four *P. aequatorialis* colonies at Monteverde, Costa Rica (10° 18'N, 84° 48'W). The colonies' nests were moved from road banks and wooded areas, and mounted on frames approximately 1 m above the ground in shelters that shielded them from direct sun and rain. Workers had unimpeded flight access outside the shelters and foraged freely. Colonies were in the shelters for at least 3 weeks prior to worker collection. We collected workers from three colonies in July 2000, and from one colony in July 2002. Workers were collected during a single 1–2 h session from each colony. All wasps were collected directly into plastic tubes containing 4% paraformaldehyde that were cooled in an ice bath. Specimens were stored and transported refrigerated until histological processing.

Behavioral data were collected by an observer (S. O'D.) seated 1 m from the colonies, facing the nest entrance hole. We carefully plugged the nest entrances with cotton, then collected workers that were observed performing tasks on the nest surface (on-nest tasks: building and receiving materials from foragers). Foragers were collected when they landed on the nest and transferred materials they were carrying to nest mates (e.g. insect prey and nectar). See ref. [14] for an ethogram of *Polybia* worker behavior. After approximately 20 workers were collected in each category, we unplugged the nest and tapped on the envelope to elicit in-nest workers to exit. After several hundred workers had moved from the interior onto the nest envelope, we collected approximately 20 in-nest workers as they exited the nest.

Workers' head capsules were dissected from the bodies and embedded in resin. Entire head capsules were sectioned on the frontal plane (Fig. 1). We made 1.5  $\mu\text{m}$  serial sections using a Leica Ultracut R microtome with a diamond knife. Sections were mounted on gelatin-coated slides and stained with toluidine blue, a Nissl stain (Fig. 1). Sections 24  $\mu\text{m}$  apart were viewed using a high resolution digital camera and a computer-interfaced light microscopic stereological work station at a final magnification of 800 $\times$ . All histological data were collected blind to the behavioral category of the wasp.

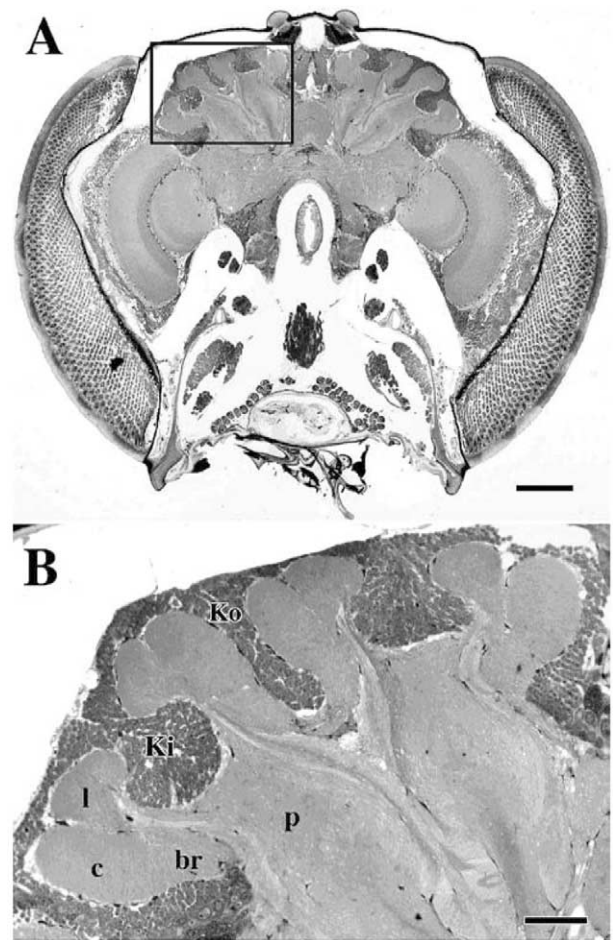


Fig. 1. Frontal section of the head capsule of a *P. aequatorialis* worker. (A) The location of the calyx of the mushroom bodies (left hemisphere inside box) can be seen in relation to the compound eyes (to the sides). Scale bar = 200  $\mu\text{m}$ . (B) The left-hemisphere calyx is shown at higher magnification. The inner Kenyon cells (Ki) are nestled within the cup created by the lip (l) and collar (c) and basal ring (br) of the calyx, the outer Kenyon cells (Ko) form a layer surrounding the calyx, and the neuropil of the peduncle (p) projects ventrally. Scale bar = 50  $\mu\text{m}$ .

We measured the volume of brain regions that were shown to be associated with task performance and/or age in honey bees: the Kenyon cell body region and the calyx neuropil. Volumes of the Kenyon cell body region and calyces of one randomly chosen hemisphere of each brain were estimated using stereological techniques [1] and NeuroLucida™ software (MicroBrightfield, Inc., Williston, VT). The Kenyon cell region contains darkly stained somata in contrast to the lightly stained neuropil of the calyces. The areas of the medial and lateral calyces per section were obtained using NeuroLucida™ contour tracing software. The calycal subdivisions of the basal ring and collar were traced together as one structure because the boundary between the two was frequently unclear, and because preliminary data indicated that group differences in neuropil to Kenyon cell volume ratios did not vary significantly between these subdivisions. The lip was traced separately because of its obvious borders, as shown in Fig. 1. Systematic point

counting was used to measure the area per section occupied by inner and outer Kenyon cells. A grid of test points was superimposed over randomly oriented images of the mushroom bodies and points falling on Kenyon cell bodies were counted. Area was determined as the product of the number of points counted and the area per point, adjusted for magnification ( $625 \mu\text{m}^2$ ). Systematic point counting was more efficient than contour tracing for estimating the volume of the Kenyon cell body region because of its irregularly shaped borders. Volumes were computed using the Cavalieri method (a highly efficient and unbiased volume estimation method; [8]), i.e. the product of the sum of the area per section of each structure and the distance between sections. These data were used to calculate the ratio of calycal neuropil to Kenyon cell region volume.

Comparisons of in-nest, on-nest, and foraging wasps from four colonies reveal dramatic task-associated differences in MB structure (Fig. 2). The calyx neuropil of in-nest wasps were smallest relative to the adjacent Kenyon cell regions (multiple ANOVA; behavior effect  $F_{2,47} = 18.5$ ,  $P < 0.0001$ ; colony and colony  $\times$  behavior interaction effects were non-significant). Although the strongest differences in MB structure were between in-nest workers and the other two categories, calyx:Kenyon cell region ratios were intermediate for on-nest workers, and therefore the ratio apparently increased as workers progressed from in-nest to foraging tasks.

As shown in Table 1, the difference in calyx:Kenyon ratios among the behavioral groups was primarily an effect of greater calyx volume in on-nest workers and foragers, although Kenyon cell region volume was slightly lower in foragers. Foragers had both the smallest Kenyon cell regions and the largest calyces (Table 1). The calyx volume in on-nest workers was intermediate between that found in

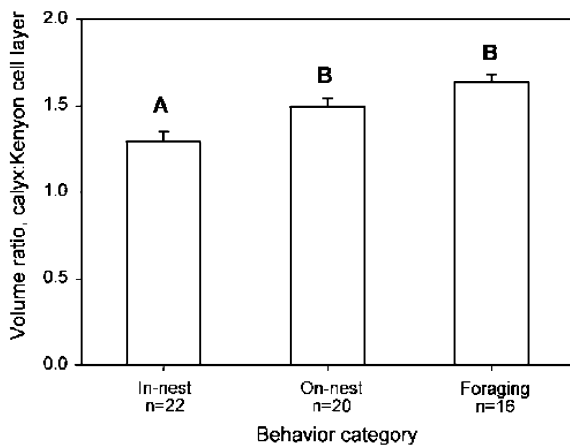


Fig. 2. Bar graph showing the association of relative volumes of MB subregions (calyx to Kenyon cell layer ratio) with task performance for *P. aequatorialis* workers from four colonies. Sample sizes (number of workers) are indicated below each behavioral category. Bar height represents overall mean  $\pm$  SE. Letters above bars indicate significantly different group means according to post-hoc comparisons (Tukey's studentized range test).

Table 1

Volumes of mushroom body subregions ( $\text{mm}^3$ ) in *P. aequatorialis* workers

Behavioral category	Kenyon	Calyx
In-nest	$13.06 \pm 1.12$	$16.23 \pm 1.19$
On-nest	$13.04 \pm 0.93$	$19.22 \pm 1.27^*$
Forager	$12.73 \pm 0.85$	$20.79 \pm 1.21^*$

Data are means  $\pm$  SE. ANOVA of behavioral category effects on MB region volumes: Calyx  $F_{2,47} = 10.8$ ,  $P < 0.005$ ; Kenyon cell layer  $F_{2,47} = 1.64$ ,  $P > 0.20$ . \* $P < 0.05$ : on-nest workers' and foragers' calyx volumes significantly different from in-nest workers, Tukey's studentized range test. All other comparisons NS.

foragers and in-nest workers. There was no evidence that volume changes were specific to a particular calycal subregion (lip versus collar plus basal ring), inner versus outer Kenyon cell regions, or medial versus lateral MB structures.

The volume ratio of MB calycal neuropil to Kenyon cell somata varied significantly with division of labor in *Polybia* workers. The greatest calyx to Kenyon volume ratio was found in foragers, consistent with the findings in honey bees and in ants [5,7,20]. Adult MB structural plasticity has also been documented in primitively eusocial paper wasps. Ehmer et al. [4] found that one MB calycal subregion, the collar, was larger in *Polistes dominulus* females that nested in social groups, compared to solitary nesting females. MB structure did not covary with *P. dominulus* females' dominance rank in the social groups. Advanced eusocial *Polybia* wasps differ from *Polistes* in several important ways. *Polybia* colonies are always initiated by swarms, which are large groups of queens and workers [11]. Therefore, *Polybia* workers are never solitary. Furthermore, *Polistes* workers do not exhibit temporal polyethism, and their open-comb nests are simpler than envelope-covered *Polybia* nests.

In *Polybia*, the strongest difference in the calyx to Kenyon ratio was associated with workers moving away from the nest interior. Because *Polybia* nests are enclosed, the visual and olfactory sensory environments that workers experience change dramatically as they move to on-nest and foraging tasks. *Polybia* workers exhibit partial ovary development when young, and the ovaries regress at around the age of transition from in-nest to on-nest tasks [14]. Taken together with the neuroanatomical data presented here, these data suggest that the transition from in-nest to on-nest work is a major milestone in the development of adult *Polybia* workers.

Our assignment of workers to task categories was based on short-term observations. *Polybia* workers exhibit strong task specialization, and there is little overlap in the location of task performance in the three worker behavioral categories [15]. Nonetheless, we may have misidentified some individuals' behavioral categories, particularly if on-nest workers or foragers were inside the nest when the entrance was sealed. Such errors of behavioral categoriz-

ation would tend to make our analysis of MB/behavior relationships more conservative.

Like honey bees, *Polybia* workers exhibit expanded MB calyces, relative to the volume of the Kenyon cell region, at more advanced stages of their temporal polyethism sequence. The mushroom bodies are considered to be important for multimodal sensory integration, associative learning, and memory [2,9,18]. These capacities are likely to be central to the transitions between working in the nest, working on the nest and leaving the nest to forage. The present results provide further support for the hypothesis that adult behavioral transitions are supported by MB plasticity, and extends these findings to eusocial wasps. Our subject species provides some advantages over honey bees, and is also important from a comparative standpoint. In *Apis*, dramatic manipulations of colony structure are often necessary to decouple worker age and task performance [6]. *Polybia* workers exhibit greater behavioral flexibility by varying in their rates of passage through the temporal polyethism task sequence. For example, first age of foraging ranged from 5 to over 40 days among simultaneously observed nest mates in unmanipulated colonies [15]. In *Polybia* wasps, worker variability in the rate of temporal polyethism is greater than in *Apis*, allowing measurements of naturally-occurring associations of behavior and brain structure variation.

Although we found evidence for consistent MB anatomy differences between different task groups, this study does not address the possibility of age effects on MB structure, nor could it detect individual differences in MB and behavioral development. Future studies could take advantage of individual differences in the rate of temporal polyethism to assess whether MB structure is associated with task performance variation, after accounting for age effects.

## Acknowledgements

This study was supported by NSF grant IBN-9904885 to S. O'D. Field research was conducted under permits (to S. O'D.) from the Costa Rican Ministry of the Environment and Energy (MINAE), obtained with the assistance of the Organization for Tropical Studies.

## References

- [1] H. Braendgaard, H.J. Gundersen, The impact of recent stereological advances on quantitative studies of the nervous system, *J. Neurosci. Methods* 18 (1986) 39–78.
- [2] J.B. Connolly, I.J.H. Roberts, J.D. Armstrong, K. Kaiser, M. Forte, T. Tully, C.J. O'Kane, Associative learning disrupted by impaired Gs signaling in *Drosophila* mushroom bodies, *Science* 274 (1996) 2104–2107.
- [3] B. Ehmer, R. Hoy, Mushroom bodies of vespidae wasps, *J. Comp. Neurol.* 416 (2000) 93–100.
- [4] B. Ehmer, H.K. Reeve, R.R. Hoy, Comparison of brain volumes between single and multiple foundresses in the paper wasp *Polistes dominulus*, *Brain Behav. Evol.* 57 (2001) 161–168.
- [5] S.E. Fahrbach, D. Moore, E.A. Capaldi, S.M. Farris, G.E. Robinson, Experience-expectant plasticity in the mushroom bodies of the honeybee, *Learn. Mem.* 5 (1998) 115–123.
- [6] S.M. Farris, G.E. Robinson, S.E. Fahrbach, Experience- and age-related outgrowth of intrinsic neurons in mushroom bodies of adult worker honeybee, *J. Neurosci.* 21 (2001) 6395–6404.
- [7] W. Gronenberg, S. Heeren, B. Hölldobler, Age-dependent and task-related morphological changes in the brain and the mushroom bodies of the ant *Camponotus floridanus*, *J. Exp. Biol.* 199 (1996) 2011–2019.
- [8] H.J.G. Gundersen, T. Bendtsen, L. Korbo, N. Marcussen, A. Møller, K. Nielsen, J.R. Nyengaard, B. Pakkenberg, F.B. Sorensen, A. Vesterby, M.J. West, Some new, simple and efficient stereological methods and their use in pathological research and diagnosis, *APMIS* 96 (1988) 379–394.
- [9] M. Heisenberg, What do the mushroom bodies do for the insect brain? An introduction, *Learn. Mem.* 5 (1998) 1–10.
- [10] P.E. Howse, Design and function of the insect brain, in: L.B. Browne (Ed.), *Experimental Analysis of Insect Behavior*, Springer, Berlin, 1974.
- [11] R.L. Jeanne, The swarm-founding Polistinae, in: K.G. Ross, R.W. Matthews (Eds.), *The Social Biology of Wasps*, Cornell University Press, Ithaca, 1991, pp. 191–231.
- [12] P.G. Mobbs, The brain of the honey bee, *Apis mellifera* L. The connections and spatial organization of the mushroom bodies, *Philos. Trans. R. Soc. Lond. B* 298 (1982) 309–354.
- [13] S. O'Donnell, Genetic effects on task performance, but not on age polyethism, in a swarm-founding eusocial wasp, *Anim. Behav.* 55 (1998) 417–426.
- [14] S. O'Donnell, Worker age, ovary development, and temporal polyethism in the swarm-founding wasp *Polybia occidentalis* (Hymenoptera: Vespidae), *J. Insect Behav.* 14 (2001) 201–213.
- [15] S. O'Donnell, R.L. Jeanne, Life-long patterns of forager behaviour in a tropical swarm-founding wasp: effects of specialization and activity level on longevity, *Anim. Behav.* 44 (1992) 1021–1027.
- [16] T.D. Seeley, Honey bee colonies are group-level adaptive units, *Am. Nat.* 150 (1997) 22–41.
- [17] N.J. Strausfeld, Organization of the honey bee mushroom body: representation of the calyx within the vertical and gamma lobes, *J. Comp. Neurol.* 450 (2002) 4–33.
- [18] N.J. Strausfeld, L. Hansen, Y.S. Li, R.S. Gomez, K. Ito, Evolution, discovery, and interpretations of arthropod mushroom bodies, *Learn. Mem.* 5 (1998) 11–37.
- [19] G.S. Withers, S.E. Fahrbach, G.E. Robinson, Selective neuroanatomical plasticity and division of labour in the honeybee, *Nature* 364 (1993) 238–240.
- [20] G.S. Withers, S.E. Fahrbach, G.E. Robinson, Effects of experience and juvenile hormone on the organization of the mushroom bodies of honey bees, *J. Neurobiol.* 26 (1995) 130–144.