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Age-Related Changes in the Number and Structure of Synapses in the Lip Region of the Mushroom Bodies in the Ant *Pheidole dentata*

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ABSTRACT

Behavioral development in the worker caste of many adult ants follows a pattern of task transitions that contribute to the division of labor within colonies. In the ant *Pheidole dentata*, the number of tasks that minor workers attend to increases as they progress from brood-care activities within the nest to acts outside the nest such as foraging and defense. In this study we investigated synapse maturation in the lip region of mushroom bodies in young and old minor workers because of its potentially crucial role in behavioral development, task performance, and repertoire expansion. As minor workers aged, individual presynaptic boutons enlarged and acquired more synapses and vesicles, but the total number of synapses in the lip region did not change significantly. Glial cell processes occupied less of the synaptic neuropil as ants matured. These findings indicate an expansion and enhancement of efficacy at specific sets of synaptic connections between the projection interneurons and Kenyon cell dendrites and a commensurate loss of other connections as minor workers age and expand their behavioral repertoire. J. Comp. Neurol. 488:269–277, 2005.

Indexing terms: synaptic plasticity; synapse; insect brain; Formicidae, division of labor; bouton

Both experience- and age-related neural changes are known to accompany behavioral development in vertebrates and invertebrates (Kolb and Whishaw, 1998; Farris et al., 2001). In ants (Gronenberg, 1996) and honey bees (Durst et al., 1994; Farris et al., 2001; Fahrbach et al., 1998; Withers et al., 1993) experience and age-related development are accompanied by elaboration of the neuropil and dendritic outgrowth, but little is known about the role of synapses in behavioral maturation in these social species (Gronenberg, 1996; Farris et al., 2001). In ants, the behavioral repertoire of workers is readily observed, and their brain structures are accessible, thereby providing the opportunity for a socioneuroethological analysis of synaptic and behavioral maturation.

Division of labor in ants is based on the evolution of castes, which are groups of individuals that specialize in different tasks in the colony (Hölldobler and Wilson, 1990). The neural mechanisms associated with task specialization are not known. The ant genus *Pheidole* is a useful model system for delineating size- and age-related patterns of division of labor (Wilson, 1976; Calabi and Traniello, 1989a,b; Brown and Traniello, 1998; Seid, 2004). In particular, *P. dentata* has been described as having a discrete system of age-related division of labor (temporal polyethism) in which newly eclosed adult workers care for brood within the nest and progress, without task overlap, to foraging and other acts outside the nest (Wilson, 1976). Recent work has shown that *P. dentata* minor workers expand their behavioral repertoire as they age without the loss of inner-nest behaviors (Seid, 2004).

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Dedicated to Peter M. Black, M.D. Ph.D. in appreciation for his expertise with brains larger than those of ants.

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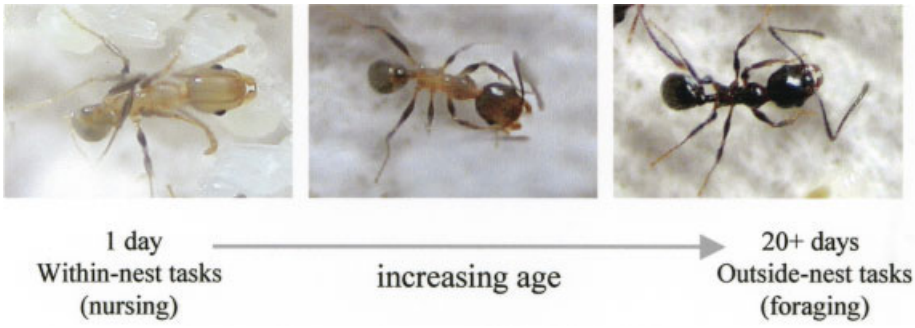

Fig. 1. Age-related pigmentation and behavior in *P. dentata* minor workers.

The mushroom bodies of insect brains play a crucial role in the control of information processing, complex behaviors, and task transitions (Strausfeld et al., 1998; Ganeshina and Menzel, 2001; Zars, 2002; Heisenberg, 2003). Ants have relatively large mushroom bodies compared with other insects, comprising as much as 40% of an ant's brain (Gronenberg, 1996; Gronenberg et al., 1996). The mushroom bodies are multimodal sensory integrators, and their role in integration, especially in regard to olfactory input, may underscore temporal polyethism in ants (Gronenberg, 1996; Hölldobler, 1999; Gronenberg and Hölldobler, 1999).

Mushroom bodies have a complex network of both excitatory and inhibitory connections (Ganeshina and Menzel, 2001; Yusuyama et al., 2002), yet the processes by which synapse number and synaptic efficacy are associated with task performance and behavioral development in social insects are virtually unexplored (Ganeshina and Menzel, 2001; Farris et al., 2001). We hypothesized that the age-related repertoire expansion of *P. dentata* involves synaptic remodeling in the calyces of the mushroom bodies where projections from olfactory (antennal) interneurons are concentrated (Gronenberg, 2001; Ganeshina and Menzel, 2001). The calyces of the mushroom bodies can be divided into three major regions, the lip, the collar, and the basal ring (Mizunmi et al., 1998; Strausfeld et al., 1998; Strausfeld, 2002), with the lip being the largest region in most ants (Gronenberg and Hölldobler, 1999). Although inputs to the lip are primarily from the projection interneurons of the antennal lobes, it also receives some input from other sensory regions (Gronenberg, 2001). The axonal boutons of the projection interneurons are the most prominent structures in the calyces and are likely to be cholinergic (Ganeshina and Menzel, 2001; Yusuyama et al., 2002).

In many systems, behavioral maturation is accompanied by important changes in synapse number and structure (Harris, 1999; Acebes and Ferrus, 2001; Yusuyama et al., 2002). Task performance in social insects has also been associated with neuroanatomical changes. For example, in response to specific tasks, dendritic processes in the mushroom bodies of honey bees undergo dramatic changes in the number of dendritic spines, where most of the synapses are thought to occur (Coss et al., 1980; Farhbach et al., 1998; Farris et al., 2001). In this paper we explore synaptic development in minor workers of the ant *P. dentata* and describe changes in synapse number, axon volume, vesicle number, and glia that may subserve temporal polyethism.

MATERIALS AND METHODS

Study species

Queenright colonies of *P. dentata* were collected in Gainesville, Florida and cultured in the laboratory in test tubes partially filled with water and fitted with a tight cotton plug. Colonies were placed in Fluon® (Woonsocket, RI)-lined plastic nest boxes (35 × 22 × 11 cm) and fed sugar water and meal worms ad libitum. All colonies were maintained in a Harris (Andover, MA) environmental chamber under a 12-hour L/12-hour D light cycle at 28°C and 80% relative humidity.

P. dentata is a completely dimorphic ant species with a major and a minor worker caste. The major worker caste is specialized for colony defense, whereas the minor workers care for brood, maintain the nest, and forage. Minor workers show an age-related behavioral progression of tasks (temporal polyethism). *P. dentata* minor workers also show a progressive change in cuticle coloration that can be used as a reliable marker of age (Wilson, 1976). Worker pigmentation changes gradually from light yellow at 1–3 days of age to dark brown/black at, 20+ days (Fig. 1). Two groups of adult ants thought to have the greatest degree of behavioral difference were studied: callow minor workers (1–3 days old) and older (20+ days) minor workers. We hypothesized that these two age groups would have the greatest neurological difference because young workers remain in the nest whereas older workers tend to perform tasks outside the nest. The behavior and neural organization of three ants from each age group were analyzed. The youngest ants were collected while tending brood, and older ants were selected while foraging in the nest arena.

Electron microscopy and sample preparation

Minor worker brains were quickly removed from the head capsule in physiological Ringer's solution (160 mM NaCl, 3 mM MgCl, 12 mM HEPES) and then fixed in 2% paraformaldehyde, 6% glutaraldehyde, 2 mM $CaCl_2$ and 4 mM $MgCl_2$ in 0.1 M cacodylic buffer. Fixation of the brains was accelerated by using microwave irradiation for 1–3 minutes (modified from Jensen and Harris, 1989). The brains remained in fixative for 24 hours and were then rinsed in 1% cacodylic buffer five times in preparation for routine processing for electron microscopy (EM). Brains were soaked in 1% OsO_4 (aq), 1.5% KFeCN (aq) in 0.1 M cacodylic buffer while being cooled to 15°C in an ice bath and irradiated using a Pelco 3451 Laboratory Microwave

Processor (Ted Pella, Redding, CA) under vacuum for 2 minutes at 37°C. After several brief rinses, the brains were placed in 1% OsO_4 (aq) in 0.1 M cacodylic buffer, cooled, microwave irradiated for an additional 2 minutes, rinsed five times with 0.1 M cacodylic buffer and twice with water, and placed in 1% aqueous uranyl acetate while being cooled on ice. They were then microwaved for an additional 2.5 minutes.

After two more brief water rinses, the brains were dehydrated in an acetone series (50, 70, 90, and 100%) for 40 seconds each in the microwave oven. Infiltration of epoxy resins was then accomplished first by placing the brains in a 1:1 epoxy resin and acetone mixture and microwave irradiating the brains at 45°C for 15 minutes; then they were placed in a 4:1 epoxy resin and acetone mixture and microwave irradiated at 45°C for 15 minutes. The process was completed with two infiltrations of 100% epoxy resin and microwave irradiation at 45°C for 15 minutes each. Brains were then placed in coffin molds or beem capsules with 100% fresh Epon and cured for 48 hours at 60°C.

Semithin and ultrathin sections were cut by using a Leica (Deerfield, IL) Ultracut T. Semithin sections were stained with 1% toluidine blue, and these sections were used to locate and orientate the lip region of the mushroom bodies under a light microscope. Ultrathin sections (45–50 nm) were then cut in series, mounted on Pioloform-coated (SPI Supplies, West Chester, PA) slot grids (Synaptek, Ted Pella), and stained with lead citrate and then 1% uranyl acetate. Series of 100–200 ultrathin sections were cut from three young worker brains and three older worker brains. Only the superior innermost part of the calyx (the upper part of the lip) was cut in the ultrathin series. Semithin sections were used to confirm that the serial sectioning was performed in the lip region (Fig. 2). The thin sections were visualized and photographed by using a JEOL (Peabody, MA) 2010 electron microscope at 10,000× magnification. Serial EM negatives were scanned and aligned digitally by using PC-based sEM Align software (Fiala and Harris, 2001a; http://synapses.bu.edu/tools), and the magnification was calibrated by using a calibration grid (Fiala and Harris, 2001b). Measurements and three-dimensional (3D) reconstructions were made by using PC-based IGL Trace software (Fiala and Harris, 2001a; http://synapses.bu.edu/tools) to outline neuronal ultrastructure on individual sections. Axonal boutons and their synapses in the brains of ants from the two age groups were quantified for comparison.

Synapse and bouton reconstructions

Synapses in the serial sections were identified by first locating electron-dense staining (putative synaptic densities) between boutons and their adjacent dendrites (Fig. 3). Synapses were further identified by the presence of vesicles in close vicinity to the densities. Synaptic densities and vesicle clusters cut in cross section extended over multiple sections, whereas synapses sectioned en face occurred on one or a few sections with vesicles clustered on the adjacent section beneath the density. The density was traced on sections (e.g., Fig. 4) to create the 3D reconstructions and make volumetric measurements. Each synaptic density was counted as one synapse, although more than one postsynaptic partner may have been involved.

Boutons were identified in serial sections as the largest objects containing numerous vesicles with synaptic densities located on their outer membrane (Fig. 3). The boutons were also traced (e.g., Fig. 4) to create the 3D reconstructions and make volumetric measurements. Vesicles within the boutons were quantified by counting all vesicles in three sections (at each quartile) within the bouton and extrapolating the number to the entire bouton volume.

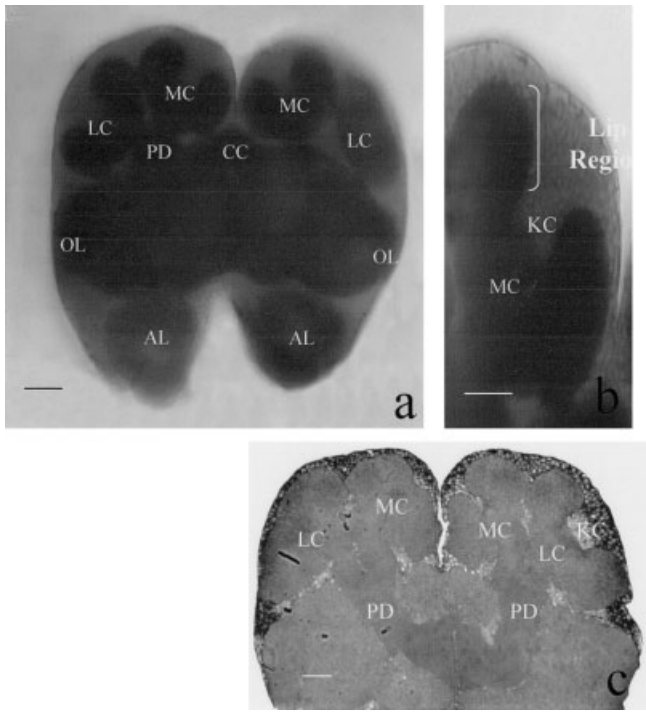

Fig. 2. Overview of the brain of a minor worker of *P. dentata* illustrating the mushroom bodies and calyces. AL, antennal lobes; CC, central complex; KC, Kenyon cells; LC, lateral calyx); MC, medial calyx; OL, optic lobe; PD, penduncle. **a:** A 60-μm-thick section of the brain showing the orientation of the mushroom bodies. **b:** Close-up of the sample area of the lip region. **c:** Semithin 1 μm thick section of an ant brain showing the details of the mushroom bodies. Scale bars = 20 μm in a,c; 10 μm in b.

Estimating total volume and synapse number in the lip region

Semithin serial sections of the entire brain of a young worker and an older worker were cut and analyzed to estimate the volume of lip region of the calyces of the mushroom bodies. All four calyces of each brain were measured to estimate the volume of the lip region. The whole calyx was measured because of the difficulty in distinguishing accurately the borders of the other two regions (collar, basal ring) in *P. dentata* and the predominance of the lip region (Gronenberg and Hölldobler, 1999; Gronenberg, 2001). Because the same measurements were obtained for both ages, it provides a useful comparison of total synapse number.

Semithin sections (0.5 μm) were viewed and photographed by using a Nikon Coolpix® 4500 digital camera attached to an Olympus BX40 microscope. Every fifth section was photographed to provide reconstructions based on 2.5-μm section separation. Digital photographs were aligned and analyzed by using the IGL align and trace programs. The size of an average lip was determined for each age worker and standardized by using the brain

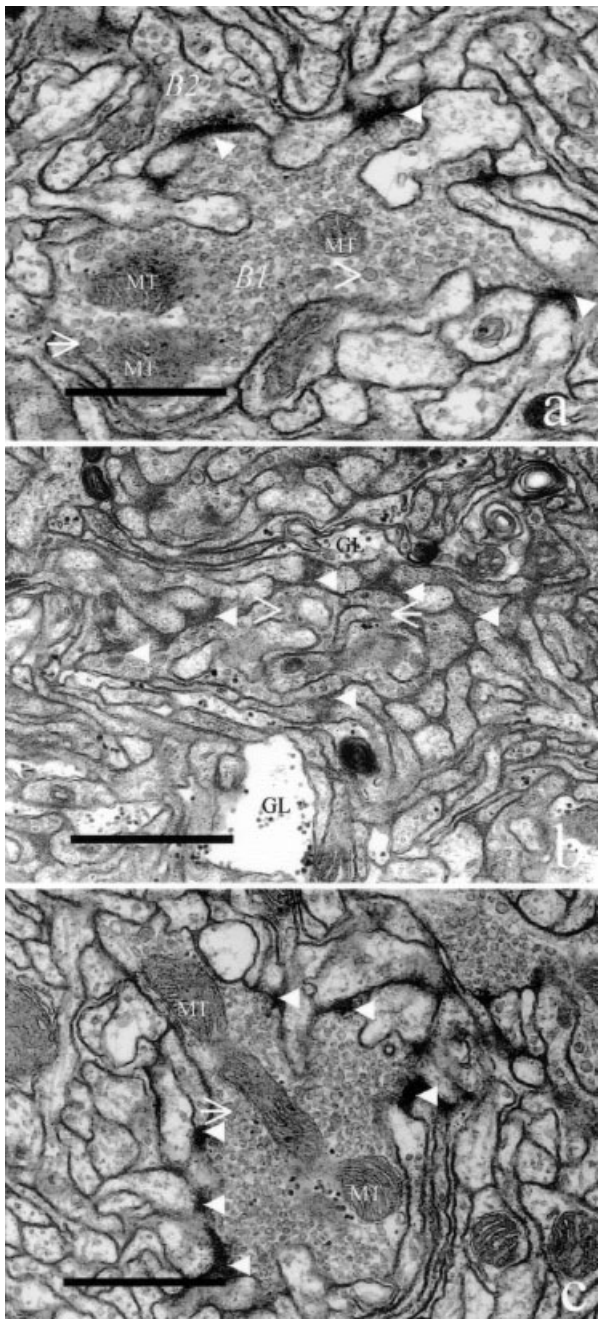

Fig. 3. Ultrastructure of axonal boutons. **a:** Bouton of an old worker with monosynaptic connections with two boutons (B1 and B2). **b:** Bouton of a young worker possessing fewer vesicles than the old worker and putative synapses with six postsynaptic dendrites. **c:** Bouton of old worker with several synaptic connections and bouton packed with vesicles. Closed arrowheads, putative synapses; open arrowheads, dense-core vesicles. MT, mitochondria. GL denote glia processes. Scale bars = 1 μm in a–c.

volumes of the young and old ant to obtain a normalized lip volume. These normalized volumes were used to extrapolate the total number of synapses, boutons, and vesicles within the lip region of the mushroom bodies. The lip volume was 4.10×10^5 μm^3 for the young worker and 4.74×10^5 μm^3 for the older worker, respectively (Fig. 2). The bouton number for the entire lip was estimated for three ants of each age with an "unbiased brick" sample technique developed by Fiala and Harris (2001b) in which every bouton found within a 1.25 μm^3 "brick" (a 25-μm^2 area summed across 20 sections) was counted providing that no portion of the bouton touched the last section or the north or east sides of the "brick." Total synapse and vesicle numbers were extrapolated by multiplying their averages per bouton by the total bouton number in the lip of both age ants.

RESULTS

The most prominent ultrastructural features in the lip region of the mushroom bodies of both young and older worker ants were the boutons of the projection interneurons, as has been described in other insects (Ganeshina and Menzel, 2001; Yusuyama, 2002). These boutons measured 1–3 μm in diameter and were full of both clear and dense-core vesicles (Fig. 3). The boutons each contained at least one mitochondrion. Several putative synapses with electron-dense staining in the extracellular space were located along the membranes of the boutons.

In young workers, the second most prominent features were astrocytic-like glial processes (Fig. 3). The cytoplasm of these processes was relatively clear and contained an abundance of dark granules, similar to the glycogen granules in vertebrate astrocytes. The glial processes surrounded and invaginated the axonal boutons. Older workers, on the other hand, lacked prominent glial processes; where they were present, they were thin and inconspicuous (Fig. 4). The remaining space within the lip was filled with densely packed dendrites that most likely originated from the Kenyon cells (Ganeshina and Menzel, 2001; Farris et al., 2001). In the lip region, most of the dendrites ranged in diameter from 0.1 to 0.3 μm and rarely exceeded 0.5 μm.

Boutons

Axonal boutons in the mushroom bodies were readily identified by their irregular shape and abundance of vesicles (Fig. 3). Four to eight boutons occurred in 100 μm^3 of the lip neuropil. The boutons in the older workers were more than twice as large as those in the young workers (Figs. 5a, 6, Table 1, column 1; t-test = 12.1, df = 60, $P < 0.0001$). However, young workers had more boutons in the lip region of the mushroom bodies than older workers (Fig. 5b, Table 2, column 2; t-test = 4.1, df = 4, $P < 0.02$). Hence, the total volume of the axonal boutons in the total estimated lip volume did not differ significantly with age (Fig. 5c, Table 2, column 3, t-test = 1.6, df = 4, $P = 0.2$).

Synapses

Synaptic densities along a single axonal bouton usually had multiple postsynaptic partners, with two to four connections being common (Figs. 3b,c, 4). Most of the multisynaptic connections occurred between boutons and dendrites in workers of both ages. Only a few synapses had a single postsynaptic partner, and in most cases these were bouton-to-bouton connections (Fig. 3a). The synapses with a single postsynaptic partner may be inhibitory, as described in honey bees (Ganeshina and Menzel, 2001).

Old workers had more synapses (i.e., postsynaptic densities) per bouton than young workers (Fig. 7, Table 1, column 3; t-test = 6.4, df = 60, $P < 0.0001$). Bouton

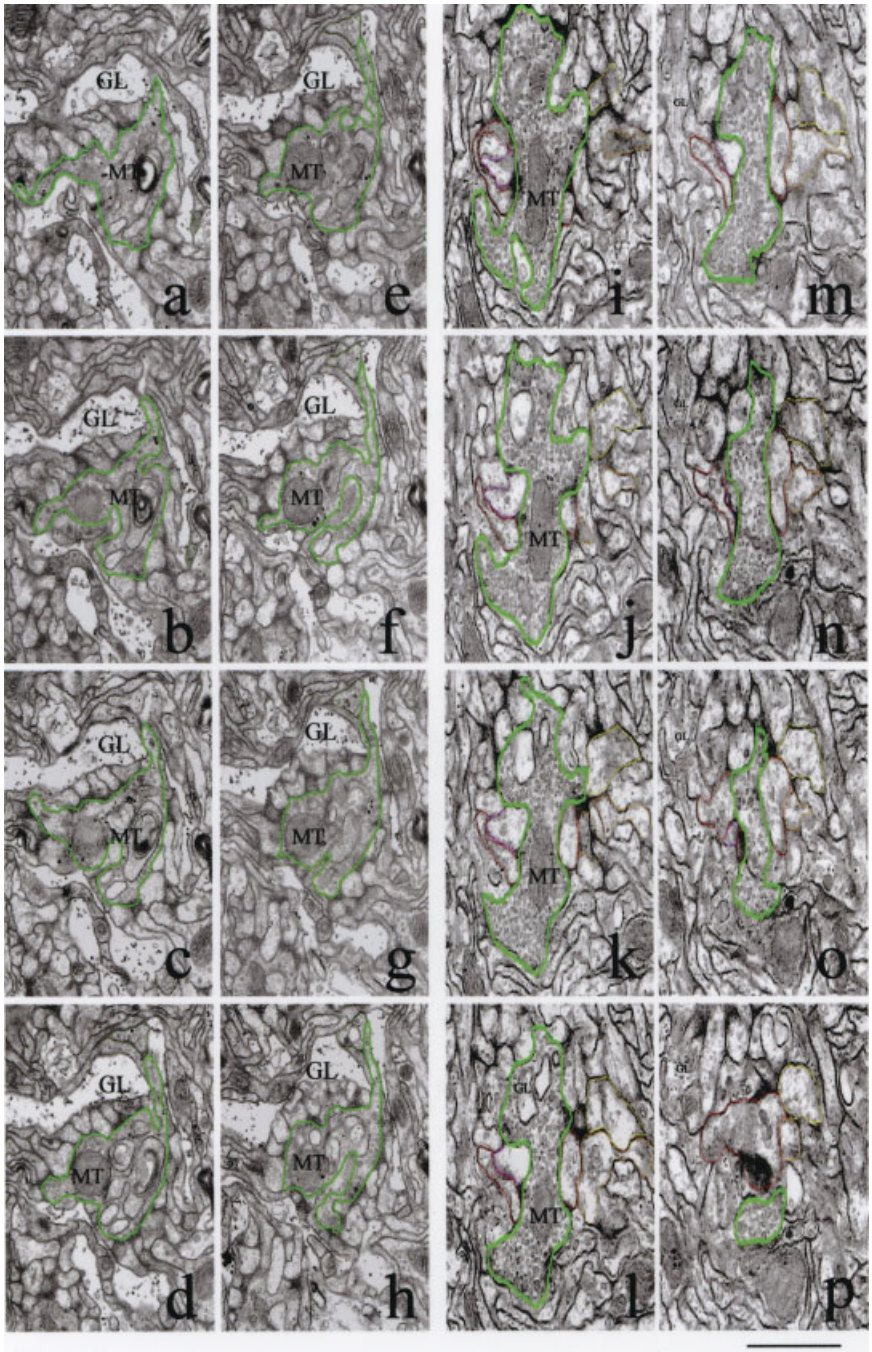

Fig. 4. Serial sections through the boutons of a young and an old worker. **a–h:** Young worker bouton in close contact with glia processes (GL). **i–p:** Old worker bouton tightly packed with vesicles. Tracings (in green) illustrate one bouton on adjacent sections. MT, mitochondria. Scale bar = 1 μm below p (applies to a–p).

volume per synapse was also significantly greater in older workers (0.046 ± 0.003 μm^3 vs 0.013 ± 0.007 μm^3 in young ants; t-test = 67.2, df = 60, $P < 0.001$). The size of individual postsynaptic densities did not differ significantly between ages (Fig. 8a, t-test = 1.6, df = 60, $P = 0.1$). The total number of postsynaptic densities in the lip region was also similar in young and old ants (t-test = 0.8, df = 4, $P = 0.45$; Table 2), as was the volume occupancy in the lip region (Fig. 8b).

Synaptic vesicles

Synaptic vesicles were grouped into two types, clear and dense-core. The clear vesicles had a continuous size distribution from 10 to 60 nm in diameter. Older workers had

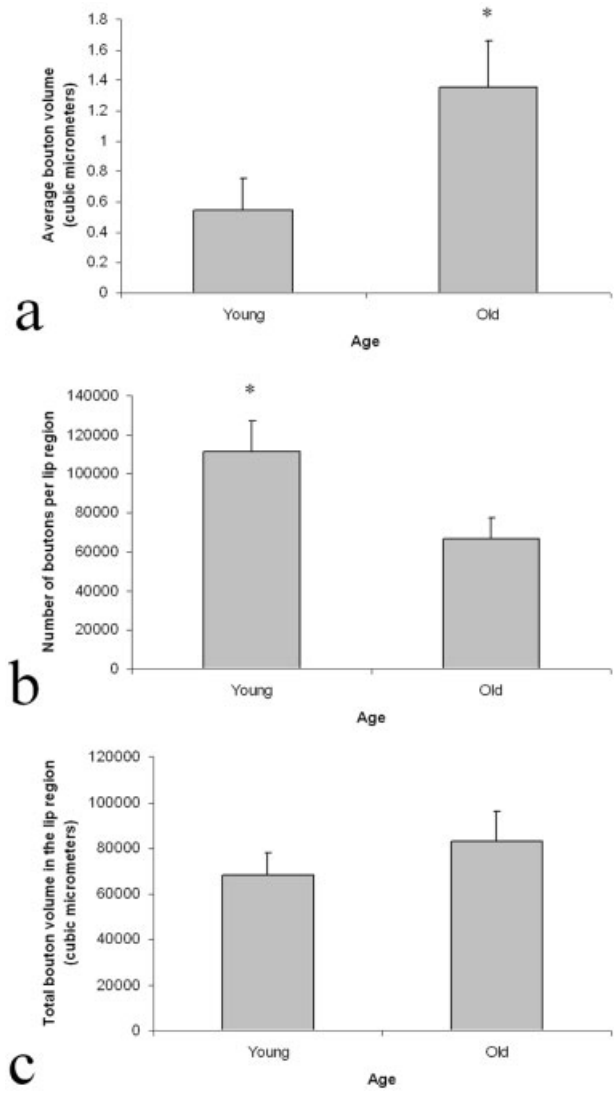

Fig. 5. Bouton and synaptic properties in the lip region of the mushroom bodies of young and old worker ants. **a:** Average bouton volume of a young and an old worker (± SD). **b:** Number of synapses per bouton. **c:** Total bouton volume in the lip region of young and the old workers (± SD;*, $P < 0.01$).

more clear vesicles per bouton (Table 1, t-test = 16.8, df = 60, $P < 0.001$) as well as a higher packing density (Table 1, t-test = 3.1, df = 60, $P < 0.001$). The number of clear vesicles per postsynaptic density was also significantly greater in older (145.2 ± 35.1) than younger (69.7 ± 35.0) workers (t-test = 8.58, df = 60, $P < 0.0001$). In addition, the total number of clear vesicles in the estimated lip volume was greater in older workers (Table 2, t-test = 5.16, df = 4, $P < 0.01$).

Dense-core vesicles ranged from 40 to 65 nm in diameter. Older workers had a greater number of dense-core vesicles per bouton (Table 1, t-test = 6.54, df = 60, $P < 0.001$), but their packing density did not differ significantly from that of young workers (Table 1, t-test = 1.18, d = 60, $P = 0.2$). The number of dense-core vesicles per postsynaptic density was significantly greater in older

TABLE 1. Characteristics of Individual Boutons at Young and Old Ages[1]

	Bouton volume		Synapses		Clear vesicles			Dense-core vesicles		
Ant	μm^2	Mean	No./bouton	Mean	No./bouton	Mean	Density/μm^3	Mean	No./bouton	Mean	Density/μm^3	Mean
Young												
1 (n = 15)	0.67 (±0.26)		28.1 (±12.3)		1407.0 (±507)		2584 (±709)		205 (±68.3)		320 (±94)	
2 (n = 10)	0.61 (±0.14)	0.54 (±0.2)	24.5 (±7.59)	25.9 (±9.7)	2187.0 (±871)	1747 (±674.1)	3633 (±1229)	3219 (±1110.0)	112 (±48.0)	137.8 (±83.4)	193 (±100)	240 (±121)
3 (n = 8)	0.45 (±0.07)		23.5 (±5.59)		1507.9 (±429)		3958 (±1000)		48 (±24.0)		152 (±100)	
Old												
1 (n = 10)	1.40 (±0.30)		42.3 (±12.10)		5747.0 (±1443)		4213 (±962)		513 (±228.0)		383 (±199)	
2 (n = 11)	1.30 (±0.30)	1.35 (±0.3)	41.9 (±7.63)	42.2 (±9.9)	5902.0 (±1306)	6135 (±1347.0)	4763 (±843)	4489 (±965.7)	233 (±78.0)	387.3 (±205.6)	200 (±66)	281 (±151)
3 (n = 8)	1.60 (±0.30)		42.6 (±10.9)		6942.0 (±1098)		4453 (±1135)		442 (±177.0)		275 (±84)	
P value		<0.001		<0.001		<0.001		<0.001		<0.001		0.200

[1]Average values (±SD) for each individual ant brain are presented with the "Mean" columns indicating the overall mean value (±SD) for all individuals in the young or old groups.

TABLE 2. Estimated Total Values (± SD) for the Lip Region of the Mushroom Bodies

Ant age	Boutons		Synapses (no./lip)	Clear vesicles (no./lip)	Dense-core vesicles (no./lip)
	No./lip	Volume (total/lip)			
Young (n = 3)	1.1×10^5 ($\pm 1.6 \times 10^4$)	6.8×10^4 ($\pm 9.7 \times 10^3$)	3.0×10^6 ($\pm 4.3 \times 10^5$)	1.6×10^8 ($\pm 2.2 \times 10^7$)	2.3×10^7 ($\pm 3.3 \times 10^6$)
Old (n = 3)	6.7×10^4 ($\pm 1.1 \times 10^4$)	8.3×10^4 ($\pm 1.3 \times 10^4$)	2.8×10^6 ($\pm 4.4 \times 10^5$)	3.5×10^8 ($\pm 5.5 \times 10^7$)	3.4×10^4 ($\pm 5.4 \times 10^6$)
P value	<0.02	0.20	0.44	<0.01	<0.05

[1]Again, equal decimal points and reduced number of places as for Table 1.

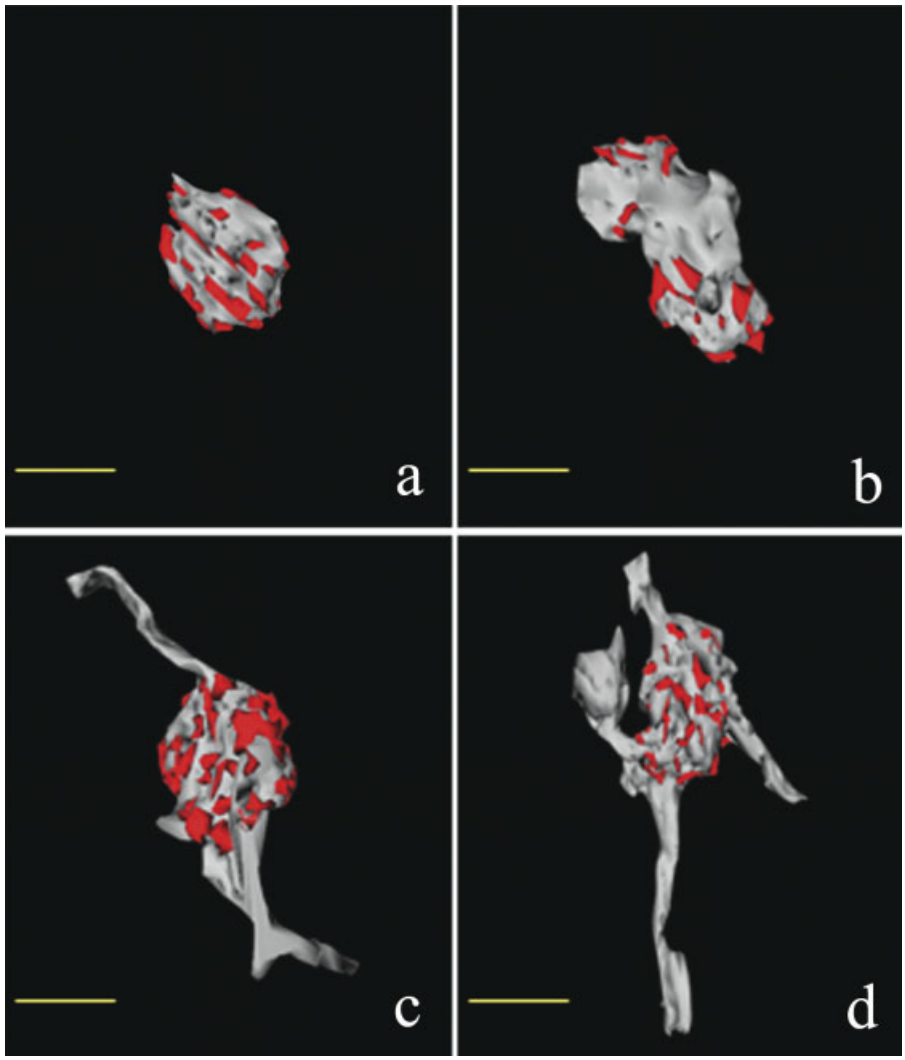

Fig. 6. Three-dimensional reconstruction of axonal boutons and synapses in the lip region of minor worker mushroom bodies. Synapses are red patches on the gray boutons. **a:** Small bouton of a young worker. **b:** Small bouton of an old worker. **c:** Large bouton of a young worker. **d:** Large bouton of an old worker. Scale bars = 1 μm in a–d.

(give 9.5 ± 5.5) than younger (5.7 ± 4.1) workers (*t*-test = 3.09, df = 60, $P < 0.01$), and the total number of dense-core vesicles in the estimated lip volume was also greater for old workers (Table 2, *t*-test = 3.15, df = 4, $P < 0.05$).

DISCUSSION

There is substantial remodeling of axons, synapses, and glia in the lip region of the mushroom bodies during behavioral maturation of *P. dentata* adult minor workers. The total number of axonal boutons and the relative volume of glia decreased, whereas the size and vesicle content of the remaining axonal boutons, the number of synapses per bouton, and the average size of postsynaptic elements all increased with worker age. These refinements in synaptic connectivity and growth suggest enhanced efficacy and can be evaluated in light of our recent

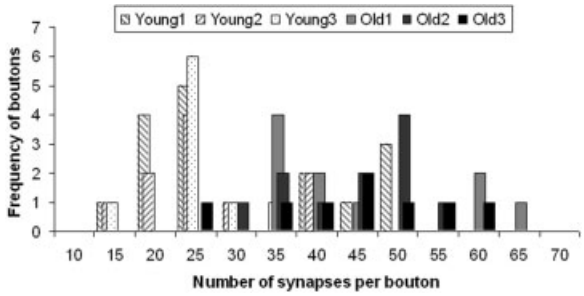

Fig. 7. Distribution of the number of synapses per bouton. **a:** All values for three young workers. **b:** All values for three old workers.

finding of age-correlated behavioral repertoire size expansion in *P. dentata* (Seid, 2004).

Our findings support an experience-expectant model of neural development. Young workers eclose with excess axonal boutons. The subsequent loss of boutons may reflect axonal pruning after adult eclosion. In the older workers, the boutons were larger and had more vesicles, consistent with a maturational increase in synaptic efficacy. Total synapse number in the lip region did not change with maturation. Thus, as the minor workers mature, some synaptic connections are strengthened by addition of synapses, whereas others are lost, thereby providing a mechanism for modulation of sensory integration and information storage with age and acquisition of new behaviors.

Selective enhancement of connectivity

The average number of synapses on each bouton increased with age, yet the total synapse number within the lip region remained relatively constant while the total bouton number decreased. This increase in synaptic efficacy at a local level, but stability in overall number, suggests that the available pool of synaptic material is maintained at a fixed level (Davis and Bezprzvanny, 2001) in young and old ants. Because Kenyon cell number remains constant with age in ants (Gronenberg et al., 1996), perhaps when axonal boutons are lost, some of the Kenyon cell postsynaptic elements switched to the surviving boutons, thereby selectively enhancing their connectivity.

In addition to the enhanced connectivity, the number of vesicles in the remaining boutons was also significantly greater in older workers. Axonal boutons with large numbers of vesicles have been shown in insects to have tonic synaptic activity (Leitinger and Simmons, 2002). Large numbers of vesicles (4,000–6,000/μm^3) occur in axonal boutons in the visual systems of both fruit flies and locusts (Meinertzhagen and O'Neil, 1991; Leitinger and Simmons, 2002). Vesicle density averaged more than 4,000/μm^3 in older workers, in contrast to less than 3,300 vesicles/μm^3 in young workers. Older workers also had on average more than 6,000 clear and 300 dense-core vesicles per axonal bouton (Table 1), which was more than three times greater than that of young workers. Furthermore, there were more than twice as many vesicles per synapse in the older workers. More vesicles at synapses in older workers suggests greater synaptic efficacy (Murthy et al., 2001). In *Drosophila* synapse number and elevated synaptic efficacy have been implicated in enhanced olfactory perception (Acebes and Ferus, 2001). Young worker ants have roughly one-third the behavioral repertoire size of older workers, suggesting that the greater number of synapses with more vesicles per synapse in older workers allows them to be more responsive to sensory input thereby providing a possible synaptic mechanism for their expanded behavioral repertoire.

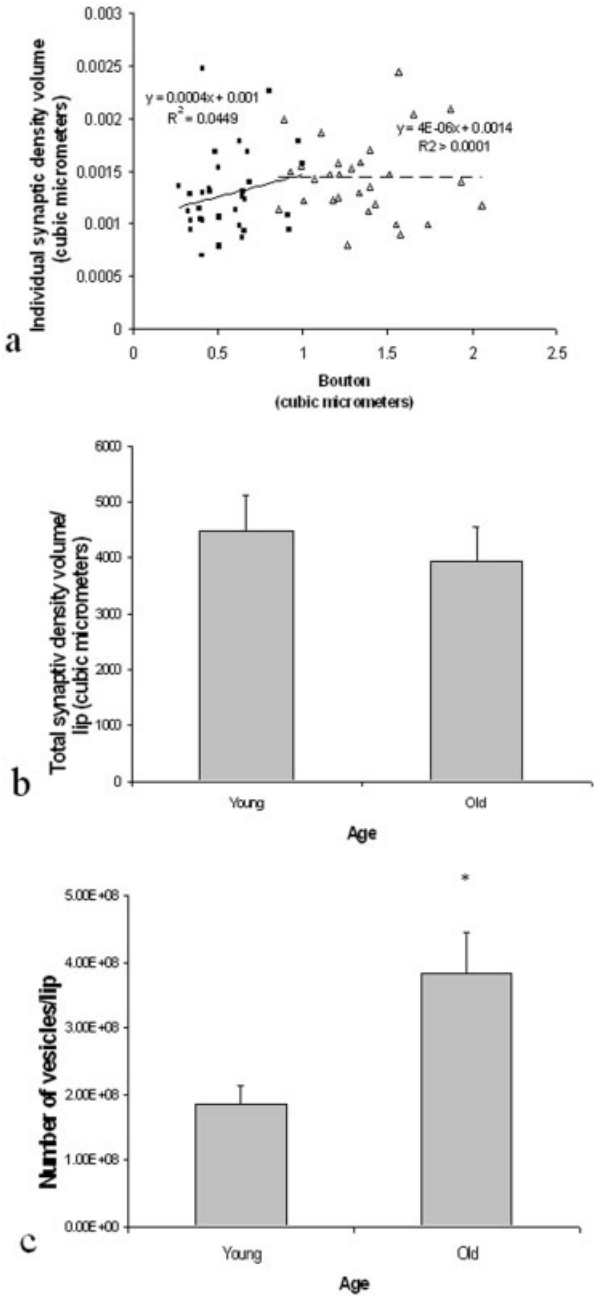

Fig. 8. **a:** Relationship between the size of the synapse and bouton size in young (squares) and old ants (triangles). R^2 values indicate that there is no relationship between bouton size and synaptic density volume for young and old ants, $P = 0.09$ and $P = 0.97$, respectively. **b:** Total postsynaptic density volume in the lip region of young (4478 ± 640 μm^3) and old (3919 ± 617 μm^3) workers. **c:** Total number of clear vesicles in the lip region for young and old workers (*, $P < 0.01$).

Synaptic maturation and models of division of labor

Although age-related task partitioning in social insects is well described (Robinson, 1992; Beshers and Fewell, 2001), little is known about the underlying neural mechanisms that govern polyethism (Gronenberg et al., 1996; Farris et al., 2001; Heisenberg, 2003). Our study provides the first description of age-related changes in synaptic connectivity and strength in the lip region of the mushroom bodies of a social insect. In social insects, physiological differences among workers are believed to regulate response thresholds to environmental and/or social stimuli (Beshers et al., 2001; Huang et al., 1994,; Huang and Robinson, 1996). Our findings support a "response threshold model" of division of labor: the increased number of synapses and vesicles per bouton in older ants could underlie the increased integration of sensory modalities important to a worker's responsiveness to specific stimuli and increase sensitivity or reduce the response threshold to stimuli associated with performance of a given task.

Ants and other social insects such as honey bees provide important model systems in which neural maturation can be uncoupled from behavioral development. By manipulating colony demographics and removing older worker cohorts, young workers will accelerate their behavioral maturation and perform tasks such as foraging at an earlier age than expected. Similarly, older workers can be induced to perform tasks normally associated with young workers by removing the younger worker cohorts (Calabi and Traniello, 1989b; Robinson, 1992). These and other manipulations, which are easily carried out in *P. dentata*, will allow an independent study of the contribution of age and synaptic changes to behavioral development. The relative accessibility and small size of the *P. dentata* nervous system provides important opportunities to study how neuronal connections are formed and modified during the behavioral maturation of individuals that live in a complex society.

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