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ORIGINAL ARTICLE

Age, worksite location, neuromodulators, and task performance in the ant *Pheidole dentata*

Ysabel Milton Giraldo[1,2] **· Adina Rusakov**[1] **· Alexandria Diloreto**[1] **· Adrianna Kordek**[1] **·**
James F. A. Traniello[1]

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Abstract
Social insect workers modify task performance according to age-related schedules of behavioral development, and/or changing colony labor requirements based on flexible responses that may be independent of age. Using known-age minor workers of the ant *Pheidole dentata* throughout 68 % of their 140-day laboratory lifespan, we asked whether workers found inside or outside the nest differed in task performance and if behaviors were correlated with and/or causally linked to changes in brain serotonin (5HT) and dopamine (DA). Our results suggest that task performance patterns of individually assayed minors collected at these two spatially different worksites were independent of age. Outside-nest minors displayed significantly higher levels of predatory behavior and greater activity than inside-nest minors, but these groups did not differ in brood care or phototaxis. We examined the relationship of 5HT and DA to these behaviors in known-age minors by quantifying individual brain titers. Both monoamines did not increase significantly from 20 to 95 days of age. DA did not appear to directly regulate worksite location, although titers were significantly higher in outside-nest than inside-nest workers. Pharmacological depletion of 5HT did not affect nursing, predation, phototaxis, or activity. Our results suggest that worker task capabilities are independent of age beyond 20 days, and only predatory behavior can be consistently predicted by spatial location. This could reflect worker flexibility or variability in the behavior of individuals collected at each location, which could be influenced by complex interactions between age, worksite location, social interactions, neuromodulators, and other environmental and internal regulators of behavior.

Significance statement
Physiological changes associated with worker age are considered to be important regulators of task performance in social insects. The extent to which worker age is correlated with behavior, and the degree to which developmental changes in neuromodulators control worker labor, however, are poorly understood. We found that ant workers are behaviorally flexible in respect to task performance at a given age, but differ in predatory response and activity level based on their worksite location (within or outside of the nest). Dopamine level was higher in outside-nest workers; serotonin did not appear to be causally linked to any behavior we examined. Our findings suggest that worker task performance can be independent of age, and workers maintain task efficacy throughout their lifespan while differing in some aspects of behavioral performance that may change with worksite location. Relationships between age, worksite, and neuromodulator titers appear to be complex.

Keywords Social insect · Division of labor · Biogenic amines · Behavioral development

Introduction

The complex organization of social insect colonies involves behavioral transitions by sterile workers (Wilson 1985;

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✉ Ysabel Milton Giraldo
ygiraldo@caltech.edu

[1] Department of Biology, Boston University, Boston, MA 02215, USA

[2] Present address: The California Institute of Technology, Division of Biology and Biological Engineering, Pasadena, CA 91125, USA

Hölldobler and Wilson 1990, 2009; Gordon 2010; Mersch et al. 2013) often in accordance with age-related developmental and physiological processes (Robinson 1992; Beshers and Fewell 2001). Transitions among sets of functionally related tasks may be correlated with, but not constrained by, chronological age (Robinson et al. 1994; Beshers and Fewell 2001; Toth and Robinson 2007; Duarte et al. 2011), and task repertoires may expand as workers mature (Seid and Traniello 2006). Honey bee workers transition through cell cleaning, nursing, food processing and comb building, and foraging, distinct behavioral roles performed by discrete "temporal castes" (Seeley 1982; Johnson 2007), resulting in marked spatial separation of work (Baracchi and Cini 2014). Age-based division of labor is in part regulated by variation in response thresholds (Beshers and Fewell 2001; Rueppell et al. 2006; Tsuruda et al. 2008; Tsuruda and Page 2009), which are influenced by hormones (Marco Antonio et al. 2008; Dolezal et al. 2012), experience (Ravary et al. 2007), social interactions (Ito and Higashi 1991; Powell and Tschinkel 1999; Greene and Gordon 2007; Gordon 2010; Pinter-Wollman et al. 2013), genotype (Tsuruda and Page 2009; Constant et al. 2012), and epigenetic factors (Simola et al. 2016).

Ants are a highly diverse taxon exhibiting an impressive range of social phenotypes. Typically, workers progress from inside- to outside-nest tasks with increasing age (Hölldobler and Wilson 1990). Intranidal and extranidal tasks may be performed by distinct worker groups (Kwapich and Tschinkel 2013; 2016; Mersch et al. 2013) or by behaviorally totipotent workers (Traniello 1978; Seid and Traniello 2006). Spatial fidelity likely influences task selection, which could be driven by differences in worker age, genetics, or other factors (Pamminger et al. 2014) that may covary with age. The role of worker age in task performance is therefore complex. Research on temporal polyethism in ants has focused on behavioral development from eclosion to the onset of foraging (e.g., Thomas and Elgar 2003; Seid and Traniello 2006) or on tasks performed by foragers (Schmid-Hempel and Schmid-Hempel 1984; Burd 1996; Wolf 2008; Grüter et al. 2011; Arnan et al. 2011). Few studies have considered worker aging and long-term patterns of task performance across the worker lifespan in ants (Lenoir 1979; Mersch et al. 2013; Giraldo and Traniello 2014; Giraldo et al. 2016). Furthermore, the regulation of task performance involves mechanisms of neural organization that may vary with worker age (Muscedere et al. 2011; Stieb et al. 2012; Giraldo et al. 2013) and be controlled by biogenic amines (Schulz and Robinson 2001; Seid and Traniello 2005; Jones et al. 2009; Muscedere and Traniello 2012; Muscedere et al. 2013, 2016; Kamhi and Traniello 2013; Smith et al. 2013; Kamhi et al. 2015). Brain titers of OA, DA, and 5HT have been found to be either correlated with or causally linked to diverse social behaviors, including polyethism in honey bees (Barron and Robinson 2005; Scheiner et al. 2006) and behavioral development, subcaste-related division of labor, and colony foundation in ants, as well as motor output (Akasaka et al. 2010; Mustard et al. 2010). OA in ants, for example, has been correlated with division of labor by polymorphic workers in the leafcutter *Acromyrmex echinatior* (Smith et al. 2013) and causally linked to major worker aggression in the Australian weaver ant *Oecophylla smaragdina* (Kamhi et al. 2015). OA may also influence the transition from nursing to foraging in *Formica polyctena* (Wnuk et al. 2011) and appears to underscore behavioral transitions during colony foundation in *Veromessor pergandei* queens (Muscedere et al. 2016). Task performance in *Acromyrmex echinatior* (Smith et al. 2013) is associated with brain levels of DA. 5HT titer correlates with the onset of outside-nest activity and is causally linked to pheromone perception and trail-following behavior (*P. dentata*; Muscedere et al. 2012), feeding (*Camponotus mus*; Falibene et al. 2012), and is correlated with aggression among colony founding queens (*V. pergandei*: Muscedere et al. 2016). More than one amine may modulate the same behavior, and a single amine may have multiple behavioral effects (Barron and Robinson 2005; Scheiner et al. 2006; Kamhi and Traniello 2013).

The completely dimorphic ant genus *Pheidole*, and *P. dentata* in particular, have served as important models for understanding age-related task performance (Wilson 1976, 2003; Seid and Traniello 2006; Muscedere et al. 2009) and its neuroethological underpinnings (Seid and Traniello 2005; Seid et al. 2005, 2008; Muscedere and Traniello 2012; Muscedere et al. 2012, 2013; Ilieş et al. 2015). Mature *P. dentata* minor workers (>20 days) exhibit a pluripotent repertoire of tasks including brood care, nest maintenance, foraging, and defense and are more responsive to colony demands for nursing than young workers (Seid and Traniello 2006). Furthermore, minor workers that mature approximately 3 weeks after eclosion are more efficacious nurses than their newly eclosed nestmates (Muscedere et al. 2009). Behavioral development, and the inclusion of outside-nest acts in the repertoire, is correlated with increased titers of 5HT and DA in minors in contrast to OA levels, which are consistently low and do not vary in relation to minor worker age (Seid and Traniello 2005 and unpublished data). Minor workers can live up to 140 days in the laboratory, and their task performance efficacy does not appear to be compromised in old age; indeed, monoamine levels, which likely control olfactory sensitivity (Muscedere et al. 2012), increase in the brain with increasing age (Giraldo et al. 2016). How tasks are allocated among minors beyond the time of behavioral maturity at 3 weeks when a complete task repertoire develops remains unknown, and the neurochemical and spatial correlates of lifetime labor profiles are unexplored. Using known-age workers and a suite of robust behavioral assays coupled with quantification of brain monoamine titers and pharmacological manipulations, we examined the roles of age and neuromodulators in shaping task performance by aging *P. dentata* minor workers.

Given that changes in worker task performance during the first 20 days of life are correlated with increases in titers of 5HT and DA (Seid and Traniello 2005, 2006), we hypothesized that minor worker task performance changes over the lifespan, is correlated with the location where work is performed, and is under aminergic regulation.

Materials and methods

Ant collection and culture

Queenright monogynous *P. dentata* colonies collected in Alachua County, FL, were raised in the laboratory in temperature- and humidity-controlled environmental chambers (25.5 °C and 55 % humidity; Harris Inc.). Monoandry appears to be the rule in *Pheidole* (Huang et al. 2013), although quantitative data does not exist for *P. dentata*. Nests consisted of test tubes partially filled with water and plugged tightly with cotton and placed in a plastic box lined with Fluon® (Insect-a-slip, Bioquip Inc.). Colonies were fed sugar or honey water and varied protein (fruit flies, meal worms, earthworms, scrambled eggs) every other day.

Known-age minor workers were generated by establishing subcolonies with 40–70 pupae that were within 72 h of adult eclosion, as identified from their yellow pupal coloration. Pupae were reared with the queen, brood, and approximately 150 additional workers of mixed age and subcaste demography (5–10 % major workers), within natural demographic distributions (Oster and Wilson 1978; Johnston and Wilson 1985). These non-focal minors were distinguished by the loss of all or a portion of one leg, which we removed. When healed, they were able to move and interact socially without any apparent impairment. This method of rearing minor workers of known age allowed us to identify focal workers without creating potential artifacts associated with marking individuals whose behavioral performance was to be assessed. To maintain colony size, dead workers were removed and replaced with marked minor and major workers three times per week; individuals sacrificed for neurobiological assessment were similarly replaced. Pupae were removed every 7–10 days to prevent additional unmarked ants from eclosing. Marked workers were age-matched with dead or removed workers based on cuticular pigmentation, which is a reliable indicator of age for approximately the first three weeks following eclosion (Seid and Traniello 2005). It was therefore not possible to know the exact age of workers older than 20 days that were added to the colony. However, field colonies (Calabi and Traniello 1989) and unmanipulated laboratory colonies show high demographic variability, and our manipulations were consistent with observed ranges of worker age distributions. Each subcolony was constructed from a single queenright parent colony. Three parent colonies were used to create more than one subcolony for some experiments, with a minimum of 3 months between using colonies in assays to allow parent colonies to produce brood and grow. Workers were sampled from subcolonies derived from 5–15 parent colonies and assigned haphazardly to assays; numbers of subcolonies are provided for each assay below. At any given time, 1–4 subcolonies were maintained and the ages of the subcolonies were staggered over the duration of the study (2012–2014). On a particular day of testing, workers of 1 or more age groups may have been assayed.

Minor workers at 20–22, 45–47, and 95–97 days of age were selected for quantification of brain monoamine levels and assessments of task performance that encompass the breadth of the minor worker behavioral repertoire (Wilson 1976; Seid and Traniello 2006), focusing on ants near maturity (20 and 45 days) or entering old age (95 days). Workers raised in these lab subcolonies can live up to 140 days (50 % survival at 77 days; Kaplan-Meier survival estimate, unpublished data) and 95-days represents 68 % of the maximum laboratory lifespan. This is likely significantly longer than worker lifespan in the field. Behavioral development occurs during the first three weeks following eclosion (Seid and Traniello 2006); changes in task repertoire could occur after the onset of foraging and would be detectable in our first two worker age cohorts, and continue with advancing age. Known-age workers in each of our three age groups were categorized based on their spatial location, which is considered to be a significant correlate of division of labor in *Camponotus fellah* (Mersch et al. 2013). We refer to minors collected outside of the nest as *outside* workers and minors inside the nest as *inside* workers based on a single observation of worksite location. This classification is not intended to suggest that the labor of these minors was restricted to these spatial locations, but rather to capture the fluidity of the spatial structure of work that we anticipated from the pluripotent task performance of minor workers. The wide array of tasks performed by *P. dentata* minors more than 20 days of age (Seid and Traniello 2006) could be achieved through individual behaviorally flexibility. Our sampling made no assumption about the consistency of worker behavior at any worksite location; to record such detail would require tracking individuals over time, which was not feasible in our current study. Although minor workers inside the nest spent most of their time caring for brood or inactive on brood piles and only outside workers collected food presented outside of a nest tube, we did not classify these workers as nurses and foragers, and thus do not imply they constitute discrete social roles. Furthermore, our spatial categorization scheme facilitated selection of minors for assays without requiring behavioral observation of every worker while enabling us to include this important covariate of age in our analysis. Indeed, the repertoire expansion model suggests that the association between spatial location and age in *P. dentata*, excluding minors immediately after eclosion, is not strong (Seid and Traniello 2006; Ibarrarán Viniegra 2015). Therefore, we are confident that our selection criteria are appropriate given what is understood of age-based behavior in *P. dentata*. Minors located inside the nest were far more numerous than those found outside the nest at all ages in our subcolonies, constraining our ability to sample large and equal numbers of workers from each spatial location.

Behavioral assessments

We selected a suite of assays that reflected the breadth of the minor worker repertoire and measured the effectiveness of task performance.

Nursing efficacy

Brood-care performance of inside- and outside-nest workers was assayed in cylindrical plastic chambers (34 mm inner diameter) with humidified dental stone filled bottoms and Fluon®-coated sidewalls to prevent escape. Eight medium to large larvae (2nd to 4th instar) with dark digestive tracts were added to the chamber evenly spaced along the circumference of a 20 mm circle. A transparent plastic cover bisected by a 10 mm diameter tube was placed over the chamber and a single minor worker was introduced to the central tube using featherweight forceps. After a 5-minute acclimation period, the lid was gently removed allowing the worker access to larvae. Assays were video recorded with a Canon FS400 digital camcorder for 20 min. The camera was positioned above the chamber to provide an overhead view of the focal ant and larvae, maximizing our ability to observe individual acts of brood care

The number and duration of approaches to larvae and subsequent nursing acts were scored manually by an observer blind to ant age and worksite location (ten subcolonies constructed from eight parent colonies) using JWatcher Video (www.jwatcher.ucla.edu). We recorded the number of times a worker approached, fed, carried, piled, or otherwise interacted with and/or provided care to larvae, as well as the duration of feeding, carrying, and other unspecified brood-care acts. An approach was scored when a minor worker oriented toward larvae located approximately 1.5 mm away from a worker; in some instances this lead to an act of brood care. Brood piling was classified as the placement of one larva in close proximity to another, thereby assembling larvae into piles (Muscedere et al. 2009). Although camera placement prevented direct observation of worker mouthparts, we used the position of the worker's body axis and antennae to identify nursing behaviors (Cassill and Tschinkel 1995). Feeding larvae in *P. dentata* is often characterized by an antero-posterior body positioning (worker and larva heads in opposite directions) with antennae pointed inwards toward the larval mouthparts, and we scored larval feeding accordingly. Workers were also observed providing care to larvae when they did not assume this feeding posture. They likely groomed or licked larvae, but because we could not observe the maxillary palps, we conservatively recorded this behavior as "unspecified brood-directed behavior." Two-way ANOVA was used to identify the significance of differences in brood-care acts and durations between inside and outside workers of all ages.

Worker predatory behavior

To assess minor worker predatory behavior, we used fruit flies as model prey. When dropped into a foraging arena, flies are immediately attacked, often pinned by several ants, and typically transported to the nest. We therefore predicted that outside, but not inside, workers of any age would be adept at attacking such live prey. Focal ants were isolated in a small Fluon®-lined Petri dish for 2 min prior to initiating the assay. A live fruit fly was tethered by its wings or posterior abdomen with fine watchmaker's forceps and affixed to a plastic block. Only flies that were alive and responded with movement to touch were used. The fly was introduced into the arena, and worker response was video-recorded for 2 min. Predatory behavior was scored on a four-point scale: 1, no response, avoidance, or only olfactory investigation; 2, mandible flaring; 3, latent attack (delayed or not sustained for the duration of the assay); and 4, attack (immediate and persistent for the duration of the assay). Minors that exhibited mandible flaring but no higher level of predatory response at any time during the assay were scored as level 2. Only minors that immediately attacked the prey at the first encounter and maintained attack for the 2-min assay were scored as level 4. Each worker was assigned a random number to mask its spatial location and age. However, because only one age group may have been tested on a given day and the most aggressive responses were scored in real time, it was not always possible to be blind to worker age. Videos of responses that did not involve immediate and consistent attack, which was unambiguous, were scored at a later date by an observer blind to the ant's age and spatial location. Minors that showed no aggression sometimes retreated from flies or engaged only in olfactory investigation. Chi-squared tests were performed in JMP® used to examine the effect of social role across age groups on predatory response (15 subcolonies created from 14 parent colonies).

Phototaxis

The transition to outside-nest activity is characterized by leaving the dark nest interior to engage in diurnal tasks such as foraging. Phototactic response was therefore selected as a measure of the visual sensory responsiveness necessary for initiating extranidal activity (Narendra et al. 2010) and served as a proxy for worksite preference, if any. We thus examined if the spatial organization of minor worker behavior was associated with sensitivity to and preference for ambient light level in the environment. Phototaxis was tested in a tripartite maze. Workers were placed with featherweight forceps in an isolation dish for 2 min prior to start of the assay and then transferred to the base of an opaque Y-shaped tunnel (each arm 80 mm in length and 15 mm in diameter) with an opaque cover to block extraneous light sources. A 15-mm diameter hole at the end of each arm allowed the worker to move toward either the bright or dim arm of the Y and the observer to determine the worker's selection. Workers also had the option to remain within the dark portion of the test apparatus, indicating negative phototaxis, or move toward light sources of different intensity, showing positive phototaxis in response to varying levels of light intensity. Minors did not exhibit signs of stress such as agitated and/or accelerated random movement; therefore, we are confident that worker choices were in response to presented stimuli rather than assay conditions. Because trials were terminated when minors exited the maze or 5 min had elapsed, we could not determine if some individuals might have returned to the maze interior after an initial exit. An LED lamp (MikeLite 350–2) was placed over one end creating light levels at one exit similar to direct sunlight (approximately 97,000 lx; Nahar et al. 2004); the exit at the opposite end approximated light conditions on an overcast day (140 lx). All other portions of the maze, except for the two exits, were dark. The latency to reach either end of the maze and preference were recorded. Light level in the left or right arm of the maze was selected haphazardly. If a worker did not exit the maze, "darkness" was scored as the preference and latency set at 5 min. It was necessary to score this assay in real time because the difference in the lighting conditions would have made video recording problematic. Therefore, although all ants had been assigned a random number, the observer sometimes knew the age, but never the spatial location, of the worker being tested. However, due to the unambiguous nature of scoring behavior (a worker either did or did not exit the maze), observer bias was extremely unlikely. Two-way ANOVA was used to test differences in latency and a chi-squared for the light level decision in inside and outside workers of all age groups (nine subcolonies created from eight parent colonies).

Activity level

An activity assay was used as a measure of general locomotor function and tempo, which could be associated with age, spatial location, or behavior, and controlled by neuromodulators. A focal worker was isolated for 2 min prior to the start of the assay and then placed into a 34-mm diameter Petri dish with featherweight forceps. The dish was divided visually into four equal quadrants by a crosshair pattern drawn beneath the bottom. Worker movement was video-recorded for 5 min, and the number of quadrants entered was counted by an observer blind to the age and worksite location of the worker. Movement between quadrants was only scored when the worker passed the line with her full body length. Two-way ANOVA was used to compare activity for inside and outside workers of 20 to 95 days of age (16 subcolonies constructed from 15 parent colonies).

Quantification of biogenic amine neuromodulators and pharmacological manipulation of brain 5HT

Biogenic amine titers of individual brains were measured using high-performance liquid chromatography with electrochemical detection (HPLC-ED) as described in Muscedere et al. (2012). Our ESA system (now made by Thermo Scientific™ Dionex™, Chelmsford, MA) was composed of a model 584 pump, MD-150 (3 × 150 mm) reversed-phase analytical column, a 5011A dual-channel coulometric analytical cell or a 5014B microdialysis cell, and a Coulochem III electrochemical detector. Brains were dissected in ice-cold HEPES-buffered saline (150 mM NaCl, 5 mM KCl, 5 mM $CaCl_2$, 25 mM sucrose, 10 mM HEPES), typically following behavioral assessments. Excess buffer was removed carefully with a Kimwipe™, and brains were homogenized in 55 μl of mobile phase. Mobile phase was prepared using a formulation optimized for *Pheidole* brains (Muscedere et al. 2012): 50 mM citrate/acetate buffer, 1.4 mM sodium dodecyl sulfate, 0.01 % triethylamine, and 24 % acetonitrile in MilliQ water. Brains were kept on ice prior to measurement, centrifuged at 15,000 rpm at 2 °C, and 50 μl of supernatant injected into a Rheodyne 975i manual injector. Electrode potentials were set for channels 1 and 2, respectively, at −125 and 225 mV for the 5011a cell and −75 and 125 mV for the 5014b cell. 5HT and DA were detected on channel 2. Amine titers are reported in pg/brain. Volume measurements of the cell body rind in 20- and 95-day-old minors show no significant differences with age (unpublished data); neuropil volumes for these age groups are unknown for *P. dentata*. To the best of our knowledge, there is only one dataset in ants (*Camponotus floridanus*) that compares rind and neuropil volumes (Gronenberg et al. 1996). Total *C. floridanus* brain volume increases dramatically between eclosion and 10 days of age and then does not significantly change thereafter. Based upon mushroom body rind and neuropil volumes, this appears to be driven by more rapid increases in neuropil. Assuming that the mushroom body pattern holds for the whole brain (such data are unavailable) and that *P. dentata* minors exhibit the same growth pattern as *C. floridanus*, we corrected brain titers for neuropil volume increases and found the same statistical patterns as those for uncorrected amine titers. Therefore, it was not necessary to correct for brain size differences, and for clarity, we present raw titers. OA titers were not measured because levels of this monoamine are very low in *P. dentata* and do not change from eclosion to 20 days of age when 5HT and DA titers both significantly increase (Seid and Traniello 2005). Furthermore, although we have optimized the sensitivity of our HPLC system, we are working near the limit for detecting OA in the minute *P. dentata* minor worker brains, which would make quantifying decreases in OA titer virtually impossible. Eleven of 98 minor workers sampled for our assessment of amine titers across age and spatial location were from a subcolony lacking a queen. Neither 5HT nor DA titers in these workers differed significantly from titers of age-matched minors from queenright colonies; data were therefore pooled. Sampled workers were coded with a random number that was revealed only after data collection. Effects of age and spatial location on amine titers were tested with two-way ANOVA in JMP® (13 subcolonies created from 13 parent colonies). 5HT and DA titers were not directly compared, as a previous study (Seid and Traniello 2005) and our own work has shown higher absolute levels of DA in all individuals.

Brain 5HT was depleted using the serotonin synthesis inhibitor alpha-methyl-D-tryptophan (AMTP), a treatment successfully used to lower brain titers of 5HT in *P. dentata* (Muscedere et al. 2012). To pharmacologically manipulate brain 5HT, experimental and control subcolonies were established, each with 45 outside workers and 10 larvae for predation assays, or 25 outside workers and 8–10 larvae for all other trials. Observations of both subcolony sizes indicated no significant differences in the mortality of minors in the subcolonies with fewer workers; the smaller subcolony size was therefore used. Any subcolony that experienced high mortality was excluded from study. Both AMTP and control subcolonies were discarded; therefore, this would have not biased results. Outside-nest workers were selected to establish subcolonies because prior studies and pilot research indicated that 5HT was higher in these workers. As it was not possible to generate sufficient numbers of known-age minors for these trials, worker age was estimated as greater than 20 days based on cuticular pigmentation (Seid and Traniello 2006). Nevertheless, because AMTP treatment is highly effective at lowering 5HT titers (Muscedere et al. 2012), small differences in initial amine titer were very unlikely to override the effect of the treatment. Treated workers in subcolonies were fed a 4 mg/ml solution of AMTP in 2 M sucrose whereas control colonies were fed 2 M sucrose every other day for 10 days (days 1, 3, 5, 7, and 9). Outside-nest minors were behaviorally assayed for predation and brood-care assays and sacrificed on day 10 for HPLC verification of effective drug treatment. Workers were taken from both outside and inside the subcolony nest for phototaxis and activity level assays, but there was no significant difference between these groups, so spatial location was pooled. A *t* test was used to examine the effects of AMTP on amine titers. Behavioral effects were assessed using *t* tests or chi-squared tests, as appropriate. All statistics were performed in JMP®.

The efficacy of pharmacological interventions varies widely and seemingly unpredictably among ant species, perhaps due to differences in uptake, metabolism, and drug processing (YMG personal observation and J. F. Kamhi, personal communication). We also attempted to depress DA levels by using the inhibitor 3-iodo-tyrosine, which lowers the rate-limiting enzyme in DA biosynthesis (Neckameyer 1996). However, we were unable to consistently deplete DA titers using this method and were thus unable to experimentally vary brain DA.

Power analyses

We performed retrospective power analyses using suggested effect sizes from Cohen (1988); see also, e.g., Thomas (1997), Nakagawa and Foster (2004), and Smith et al. (2011). Power, expressed as a proportion ranging from 0 to 1, reflects the probability of correctly rejecting the null hypothesis (Thomas 1997) and therefore the ability to detect a statistically significant result. Power greater than 0.8 is generally considered sufficient to detect a specified effect size (Cohen 1988). Effect size reflects the magnitude of a difference in samples that can be detected and can be defined as the standard deviation of the means divided by the standard deviation within a group of subjects (PASS^{14} Power Analysis and Sample Size Software 2015). We used standardized effect sizes to estimate the power of our analyses and hence our ability to detect significance; for ANOVAs, we used 0.4 and 0.25 for large and medium effect sizes, 0.5 and 0.3 for χ^2 tests, and 0.8 and 0.5 for *t* tests, respectively (Cohen 1988). For example, if we assume a standard deviation of 5 for each group and group A approached brood ten times on average and group B approached brood an average of six times, the effect size would be 0.56. All our tests had power of minimally 0.61 for large effect sizes. All power analyses were conducted in PASS^{14}.

Results

Behavioral profiles of inside- and outside-nest minors

Nursing

Neither age nor spatial location significantly affected any brood-care metrics, and there was no interaction effect (two-way ANOVA, mean ± standard error of the mean (SEM), F-statistics, sample sizes and *p* values are given in Table 1). However, a retrospective power analysis using standardized effect sizes of 0.4 and 0.25 (Cohen 1988) revealed that we did not have sufficient power to detect effects of age or spatial

Table 1 Number and duration of brood care acts (means ± SEM) for inside and outside workers 20, 45, and 95 days of age

	Age (days)						Statistical Evaluation		
	20		45		95				
	Inside ($N = 7$)	Outside ($N = 6$)	Inside ($N = 13$)	Outside ($N = 3$)	Inside ($N = 9$)	Outside ($N = 8$)	Age	Location	Age × location
Power (effect size 0.4, 0.25)							0.646, 0.276	0.756, 0.364	0.646, 0.276
Approaches	42.29 ± 10.02	21.83 ± 6.48	40.54 ± 4.82	40.33 ± 4.41	31.22 ± 2.95	34.5 ± 2.24	$F_{2,40} = 0.920$ $p = 0.407$	$F_{1,40} = 1.248$ $p = 0.271$	$F_{2,40} = 2.206$ $p = 0.123$
Feeding brood	1.43 ± 0.69	1.83 ± 0.70	3.92 ± 1.28	3.67 ± 2.73	1.0 ± 0.44	1.25 ± 0.31	$F_{2,40} = 2.603$ $p = 0.0865$	$\mathrm{F}_{1,40} = 0.0188$ $p = 0.892$	$F_{2,40} = 0.0366$ $p = 0.964$
Carrying brood	1.0 ± 1.0	0.67 ± 0.33	3.92 ± 1.14	0.67 ± 0.67	1.67 ± 0.76	3.88 ± 1.52	$F_{2,40} = 1.351$ $p = 0.271$	$F_{1,40} = 0.189$ $p = 0.666$	$F_{2,40} = 2.221$ $p = 0.122$
Brood piling	0.429 ± 0.43	0.333 ± 0.21	0.923 ± 0.38	0.333 ± 0.33	0.556 ± 0.34	1.38 ± 0.56	$F_{2,40} = 0.870$ $p = 0.427$	$F_{1,40} = 0.0129$ $p = 0.910$	$F_{2,40} = 1.165$ $p = 0.322$
Unspecified brood-directed behavior	17.29 ± 4.86	13.50 ± 3.64	22.00 ± 3.75	15.67 ± 6.01	15.22 ± 1.44	18.75 ± 3.29	$F_{2,40} = 0.290$ $p = 0.750$	$F_{1,40} = 0.400$ $p = 0.531$	$F_{2,40} = 0.793$ $p = 0.460$
Feeding duration (s)	8.51 ± 4.22	34.59 ± 15.55	74.11 ± 42.91	57.73 ± 38.84	12.68 ± 5.97	13.40 ± 6.98	$F_{2,40} = 1.183$ $p = 0.317$	$F_{1,40} = 0.0147$ $p = 0.904$	$F_{2,40} = 0.170$ $p = 0.845$
Carrying duration (s)	31.41 ± 31.41	271.52 ± 183.28	94.84 ± 33.08	6.57 ± 6.57	97.35 ± 53.91	116.49 ± 59.18	$F_{2,40} = 0.694$ $p = 0.505$	$F_{1,40} = 0.752$ $p = 0.391$	$F_{2,40} = 2.034$ $p = 0.144$
Unspecified brood-directed behavior duration (s)	131.59 ± 50.52	202.36 ± 87.95	182.41 ± 45.94	186.18 ± 90.29	178.34 ± 56.64	162.19 ± 39.84	$F_{2,40} = 0.0354$ $p = 0.965$	$F_{1,40} = 0.138$ $p = 0.713$	$F_{2,40} = 0.278$ $p = 0.759$

Behaviors are explained in detail in the text location on brood care in the 46 workers tested (power for age 0.646, 0.276; spatial location 0.756, 0.364; age × spatial location 0.646, 0.276; all for effect sizes of 0.4 and 0.25, respectively). Effect sizes of 0.5 had power of greater than 0.8 (age 0.837, spatial location 0.908, age × spatial location 0.837).

Response to prey

Outside workers were significantly more likely to respond to attack experimental prey than minors collected while working inside the nest (two-way χ^2, age $\chi^2_{2,134} = 41.124$, $p < 0.0001$; Table 2, Fig. 1). Age did not significantly affect predatory response and there was no interaction effect (two-way χ^2, location $\chi^2 = 0.306$, $p = 0.2897$, age × location $\chi^2_{2,134} = 2.26$, $p = 0.323$; power = 0.996 and 0.773 for effect sizes of 0.5 and 0.3).

Phototaxis

Age and spatial location were not significantly associated with negative or positive phototaxis; both inside and outside workers generally preferred brighter light conditions (two-way χ^2, age $\chi^2_{4,53} = 4.15$, $p = 0.3867$, spatial location $\chi^2_{2,53} = 2.76$, $p = 0.2512$, age × spatial location $\chi^2_{4,53} = 4.87$, $p = 0.3009$, Table 3). A retrospective power analysis indicated that we had a power of 0.84 and 0.38 for 0.5 and 0.3 effect sizes, respectively (PASS^{14}; Cohen 1988), indicating that sampling of 53 ants was sufficient to detect large effects on phototactic response. Forty-five-day-old minors collected inside the nest tended to be more negatively phototactic than minors of any other age, although this effect was not significant. Decision latency for minors that exited the maze (i.e., chose low or high light) was not significantly affected by age or spatial location (two-way ANOVA, age $F_2 = 0.6624$, $p = 0.522$, spatial location $F_1 = 0.425$, $p = 0.518$, age × spatial location, $F_2 = 0.121$, $p = 0.886$; power for age 0.612, 0.269; spatial location 0.726, 0.356; age × spatial location 0.612, 0.269, all for effect sizes of 0.4 and 0.25, respectively; Table 4). Of the minors that exited the maze, 79 % made a selection within 60 s. Inclusion of latencies for minors that did not exit the maze did not change our results: there was no significant effect of age, spatial location, or interaction (two-way ANOVA, age $F_2 = 0.156$, $p = 0.856$, spatial location $F_2 = 2.28$, $p = 0.138$, age × spatial location $F_2 = 0.710$, $p = 0.497$).

Activity level

Both age and spatial location significantly affected activity level without an interaction effect (two-way ANOVA, age $F_2 = 3.79$, $p = 0.025$, location $F_1 = 27.73$, $p < 0.0001$, age × location $F_2 = 4.08$, $p = 0.019$; power: age 0.992, 0.751, spatial location 0.997, 0.838, age × spatial location 0.992, 0.751, for 0.4 and 0.25 effect sizes; Fig. 2). Outside-nest workers were significantly more active than inside-nest workers, and activity level increased with age in inside but not outside workers (mean movement ± SEM, 20 days: inside 33.65 ± 2.85, outside 74.90 ± 5.68, $N = 52$, 11; 45 days: inside 43.60 ± 4.39, outside 55.50 ± 7.03, $N = 30$, 8; 95 days: inside 55.52 ± 4.35, outside 73.91 ± 5.49, $N = 29$, 11; inside, $R^2 = 0.139$, outside, $R^2 = 0.00264$). However, age-matched minors from either spatial location exhibited considerable variation in activity level; for inside minors, only 14 % of the variation in activity was explained by age.

Monoamine levels, worker age, and spatial location

Brain 5HT and DA levels did not significantly increase with age, from 20 to 95 days (5HT: $F_2 = 2.91$, $p = 0.061$, power = 0.902, 0.502 for effect sizes of 0.4 and 0.25; DA: $F_2 = 3.035$, $p = 0.0541$, power = 0.890, 0.486 for effect sizes of 0.4 and 0.25; Figs. 3 and 4). Spatial location was a significant correlate of DA, but the effect was not significant for 5HT (two-way ANOVA 5HT: $F_1 = 3.79$, $p = 0.067$, power = 0.949, 0.613 for 0.4 and 0.25 effect sizes; DA: $F_1 = 18.022$, $p < 0.001$, power = 0.941, 0.597 for 0.4 and 0.25 effect sizes). 5HT did not show a significant interaction effect between age and worksite location, but this interaction effect was significant for DA: outside-nest workers appear to show a greater increase in DA titers than inside-nest workers (two-way ANOVA 5HT: $F_2 = 2.45$, $p = 0.093$, power = 0.902, 0.502 for effect sizes 0.4 and 0.25; DA: $F_2 = 3.57$ $p = 0.033$, power = 0.890, 0.486 for 0.4 and 0.25 effect sizes).

Table 2 Percentages of inside- and outside-nest workers of 20, 45, and 95 days of age exhibiting one of four levels of predatory response

Age (days)	Worksite	No aggression	Mandible flaring	Latent attack	Attack	*N*
20	Inside	37.0 %	50.0 %	10.9 %	2.2 %	46
	Outside	0 %	7.7 %	46.2 %	46.2 %	13
45	Inside	51.6 %	32.3 %	16.1 %	0 %	31
	Outside	10.0 %	0 %	40.0 %	50.0 %	10
95	Inside	42.3 %	23.1 %	23.1 %	11.5 %	26
	Outside	12.5 %	37.5 %	0 %	50.0 %	8

Percentages may not sum to exactly 100 % for each row due to rounding

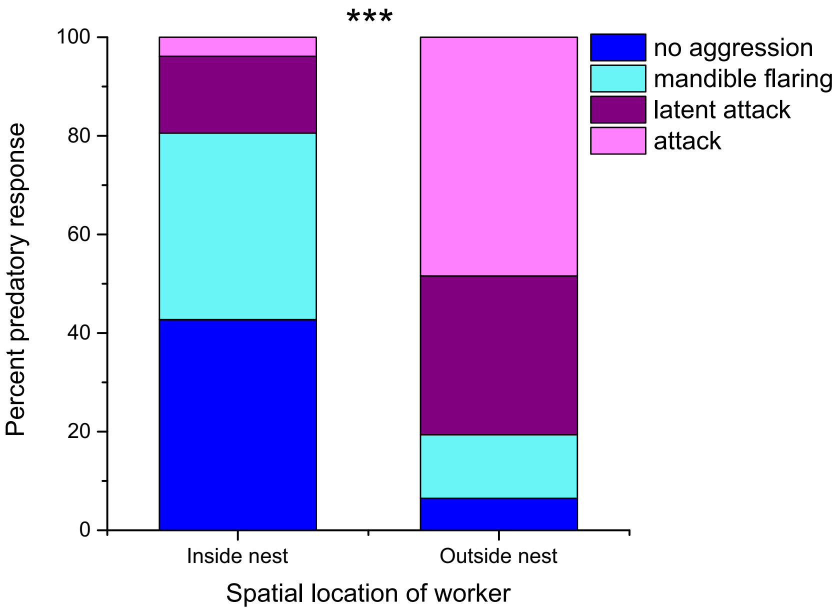

Fig. 1 Predatory response of inside-nest and outside-nest workers for all worker ages combined (***$p < 0.0001$)

Behavioral performance of pharmacologically manipulated workers

AMTP lowered the 5HT level of outside minors used in nursing assays by 59 %, whereas DA titers remained unaffected (pg/brain [mean ± SEM], 5HT: control 48.28 ± 1.61, AMTP: 19.76 ± 2.34; *t* test: $t_{28} = -10.04$, $p < 0.0001$; DA: control 101.51 ± 4.62, AMTP: 91.43 ± 4.76; *t* test: $t_{28} = -1.51$, $p = 0.143$; $N = 15$, 15). Brood care by outside-nest workers was not significantly affected by 5HT depletion (*t* tests, approach: $t_{28} = -1.199, p = 0.241$, feeding: $t_{28} = -1.70, p = 0.100$, carrying: $t_{28} = -1.703, p = 0.099$; piling: $t_{28} = -1.62, p = 0.116$; unspecified brood-directed behavior: $t_{28} = -1.89$, $p = 0.070$; control vs. AMTP, $N = 15$, 15). The number of approaches to brood and subsequent nursing acts did not differ between treatment groups (mean ± SEM; approaches: control 33.93 ± 4.11, AMTP: 27.53 ± 3.41; feeding: control 1.40 ± 0.51, AMTP: 0.47 ± 0.19; carrying: control 2.80 ± 0.82, AMTP: 1.33 ± 0.27; piling: control 1.0 ± 0.43, AMTP: 0.27 ± 0.15; unspecified brood-directed behavior: control 13.6 ± 2.34, AMTP: 8.47 ± 5.41). Durations of nursing acts were not significantly different following AMTP treatment (sec [mean ± SEM], feeding duration: control 27.06 ± 15.98, AMTP: 4.43 ± 2.20; carrying duration: control 144.30 ± 57.60, AMTP: 129.00 ± 42.41; unspecified brood-directed behavior duration: control 80.44 ± 20.14, AMTP: 46.74 ± 10.91; *t* tests: feeding duration: $t_{28} = -1.403$, $p = 0.172$; carrying duration: $t_{28} = -0.214$, $p = 0.832$; unspecified brood-directed behavior duration: $t_{28} = -1.47$, $p = 0.152$). Power was 0.988 and 0.753 for 0.8 and 0.5 effect sizes, respectively.

In our predatory behavior assay, AMTP treatment significantly lowered 5HT by 69 % in outside-nest minors, significantly below the 5HT brain titer exhibited by any unmanipulated minors (*t* test, $t_{73} = 16.19$, $p < 0.0001$; pg/brain [mean ± SEM], control 37.07 ± 1.29, AMTP: 11.33 ± 0.94, $N = 37$, 38), but did not change DA titers (*t* test, $t_{73} = 0.0617$, $p = 0.951$, pg/brain [mean ± SEM], control 60.06 ± 2.33, AMTP: 59.87 ± 2.01). Response to prey by outside workers was unaffected by AMTP treatment (percentage response, control 5.41 % no response/olfactory investigation, 0 % mandible flaring, 8.1 % inconsistent biting, 86.49 % immediate and persistent biting; AMTP: 7.89 % no response/olfactory

Table 3 Phototactic responses of inside and outside workers of different ages

		Bright light	Dim light	Darkness	
Age (days)	Worksite				*N*
20	Inside	53.9 %	23.1 %	23.1 %	13
	Outside	100 %	0 %	0 %	3
45	Inside	23.1 %	38.5 %	38.5 %	13
	Outside	50.0 %	50.0 %	0 %	2
95	Inside	78.6 %	14.3 %	7.1 %	14
	Outside	62.5 %	25.0 %	12.5 %	8

Percentage of responses to high light, dim light, and darkness are given and sum to 100 % for each age and spatial location. Percentages may not sum to exactly 100 % for each row due to rounding

Table 4 Latency (in seconds, mean ± SEM) of phototactic decisions

	Inside nest	Outside nest	*N* (inside, outside)
Age (days)			
20	27.8 ± 7.4	27.7 ± 7.2	10, 3
45	49.3 ± 27.1	32.5 ± 13.5	8, 2
95	60.3 ± 15.2	40.9 ± 12.7	13, 7

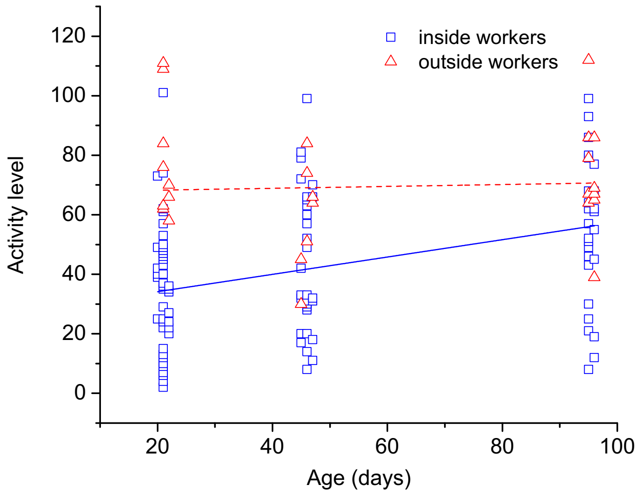

Fig. 2 Activity level (*frequency of line crosses*) by inside workers (*blue squares*) and outside workers (*red triangles*) 20, 45, and 95 days of age. Each data point represents an individual minor. *Lines* indicate linear regressions for inside-nest (*solid*) and outside-nest (*dashed*) workers

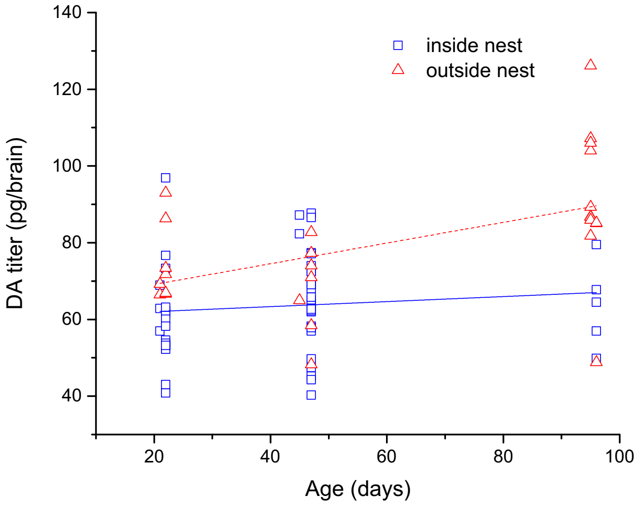

Fig. 4 DA brain titers for 20-, 45-, and 95-day-old inside nest workers (*blue squares*) and outside nest workers (*red triangles*), and linear regressions for inside (*solid*) and outside (*dashed*) workers. Each point represents a single worker

investigation, 2.63 % mandible flaring, 2.63 % inconsistent biting, 86.84 % immediate and persistent biting; $\chi_{3,75}^2 = 2.20$, $p = 0.532$). Power to detect differences between AMTP-treated and control workers in predatory behavior was 1.0 for 0.8 effect size and 0.966 for 0.5 effect size.

AMTP treatment significantly lowered 5HT by 57 % in minors used in phototaxis and activity level assessments and did not significantly affect DA titers (*t* test, 5HT: $t_{27} = 10.31$, $p < 0.0001$, DA: $t_{26} = 1.89$, $p = 0.0694$; pg/brain [means ± SEM], 5HT: control 43.13 ± 1.49, AMTP: 18.40 ± 1.85, $N = 14$, 15; DA: control 77.73 ± 7.04, AMTP: 61.93 ± 4.81, $N = 13$, 15). Phototactic responses (percentage response, control: 78.6 % bright light, 21.4 % dim light, AMTP: 86.7 % bright light, 13.3 % dim light, $N = 14$, 15, $\chi^2_{29,1} = 0.334$, $p = 0.564$) and response latency (sec [mean ± SEM], control 25.00 ± 5.48, AMTP 31.33 ± 10.7, $N = 14$, 15, *t* test, $t_{27} = -0.514$, $p = 0.611$) were unaffected by drug treatment. Power for light preference was 0.768 and 0.365 for 0.5 and 0.3 effect sizes, and decision latency also exhibited sufficient power (0.8: 0.983; 0.5: 0.721). Activity level was unaffected by 5HT manipulations (mean ± SEM control 84.36 ± 16.50, AMTP 94.00 ± 17.61, t-test, $t_{27} = -0.398$, $p = 0.694$, $N = 14$, 15). Neither phototaxis nor activity was affected by sampling control and AMTP minors from either inside or outside of the nest (movement: two-way ANOVA, treatment: $F_1 = 0.0.061$, $p = 0.807$; spatial location: $F_1 = 3.18$, $p = 0.087$, treatment × spatial location: $F_1 = 0.502$, $p = 0.485$; phototaxis choice: two-way Wald χ^2, treatment: $\chi^2 = 4.88 \times 10^{-5}$, $p = 0.994$; spatial location: $\chi^2 = 8.17 \times 10^{-5}$, $p = 0.993$, treatment × spatial location: $\chi^2 = 4.77 \times 10^{-5}$, $p = 0.995$; response latency: two-way ANOVA, treatment: $F_1 = -0.79$, $p = 0.437$; spatial location: $F_1 = -0.39$, $p = 0.698$; treatment × spatial location: $F_1 = 1.59$, $p = 0.124$); therefore, data were pooled. Activity level power was 0.987 and 0.721 for 0.8 and 0.5 effect sizes.

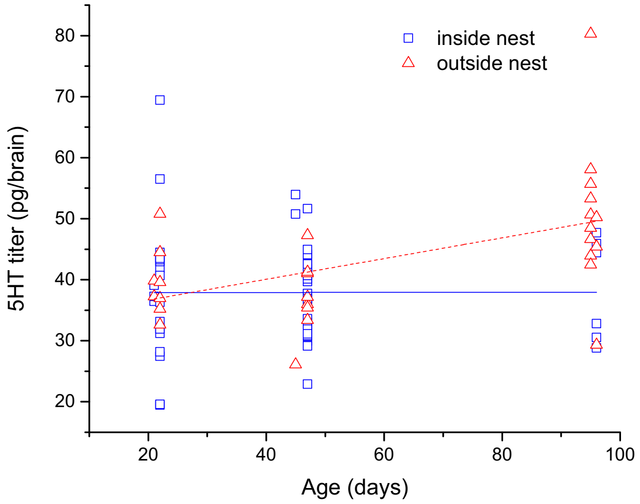

Fig. 3 5HT brain titers for inside (*blue squares*) and outside workers (*red triangles*) of 20, 45, and 95 days of age. Each point represents a single minor. *Lines* show linear regression for workers from each spatial location (inside: *solid*; outside: *dashed*)

Discussion

Worker division of labor in ants has been shown to be correlated with or determined by developmental programs (Seid et al. 2008; Muscedere et al. 2009), spatial location, and social interactions (Gordon 2010; Mersch et al. 2013; Pamminger et al. 2014) and appears to be regulated by internal drivers including genes (Ben-Shahar et al. 2002; Libbrecht et al. 2013; Lucas et al. 2015), epigenetic regulation (Simola et al. 2016), neuromodulators (Muscedere et al. 2012; Kamhi et al. 2015), and their interactions. We examined whether variation in task performance and sensorimotor function in *P. dentata* minor workers over a substantial portion of their laboratory lifespan is related to age, worksite location, and levels of DA and 5HT. We anticipated that spatial location, a potential covariate of worker chronological age, could shape behavior patterns either as a result of intrinsic differences in individuals that select different worksites or due to social interactions and/or task experience and labor demands in those locations. By quantifying brood care, predation, phototaxis, and activity level, we examined whether individually assayed minor workers change with age in task performance and/or sensorimotor function, potentially due to behavioral development and/or senescence, and whether titers of 5HT and DA are associated with age and behavior. By using known-age workers at identified worksite locations, we were able to track how both internal and external factors correlate with task performance. Although workers assayed in groups may behave differently than individually assayed workers, our extensive experience with *P. dentata* gives us confidence that data reported here are consistent with those obtained from group assays (e.g., Seid and Traniello 2006; Muscedere et al. 2009) and that our behavioral measures are therefore robust.

Minors working in or outside the nest showed considerable overlap in most tasks at all ages, suggesting that social roles do not become more or less discrete across the large portion of the adult lifespan over which we measured behavior. Large-scale tracking of ant colony worker populations has identified spatial location as well as age as important predictors and possible drivers of social interactions (Mersch et al. 2013), although the role of age, the site of task performance and neuromodulators as orchestrators of behavior in concert have not been explored. Our study specifically examined the interaction of age and worksite location on task performance and sensorimotor function, and their relationship to neurotransmitter levels. We found that spatial location appears to only correlate with predatory behavior and activity level, both of which should be significant to the effectiveness of outside-nest work, and that workers show no change in performing nursing and foraging tasks throughout much of their lifespan (Giraldo et al. 2016). However, for brood care and the decision latency involved in phototaxis, we had limited power, particularly with respect to age, to detect differences all but very large (>0.4 effect size) differences between groups. As such, we cannot exclude the possibility that age and/or worksite influences the performance of nursing behavior or the decision latency in response to light, particularly for smaller effect sizes.

Our findings appear to be generally consistent with the repertoire expansion model (REM) of polyethism (Seid and Traniello 2006). The REM describes how extranidal tasks are added to an existing array of inside-nest tasks as minor workers age, resulting in a pluripotent repertoire affording flexible adjustment in nursing- and foraging-related tasks according to colony needs. The REM originally described age-related task repertoire augmentation for mature workers of indeterminate age (>20 days of age). Our results suggest that once minors attain behavioral maturity (at ~20 days), they continue to perform a wide array of tasks effectively. However, we did not measure repertoire size, and therefore cannot conclude if behavioral repertoires change beyond 20 days. Although our study—by design—did not determine whether individual minors were found consistently in one spatial location or moved between worksites, individually identifiable 8- to 10-day-old *P. dentata* minors behaviorally tracked until days 33–35 of age showed that the same workers were not found consistently inside or outside of the nest (S. Ibarrarán Viniegra, personal communication). This also suggests *P. dentata* has a flexible system with weak spatial fidelity in which individual responsiveness to task stimuli may change independently of worker age (Greene and Gordon 2007; Pinter-Wollman et al. 2013). Although our data do not allow us to assess the fidelity of minors to an inside- or outside-nest worksite, the higher activity and predatory behavior of outside-nest workers suggests that sampling at a single time point is informative. However, repeated sampling of individuals, which would not have been feasible with our experimental design, could have revealed different patterns of behavioral performance than those observed.

Previous studies indicate that task responsiveness encompasses behavioral development initially following eclosion (Seid and Traniello 2006; Muscedere et al. 2009), and effective labor contributions from minors appear to continue beyond 20 days well into “old age” at 120 days (Giraldo et al. 2016). We also sampled minors at 120 days of age from inside and outside of the nest, but given the difficulty of obtaining workers of this age and thus limited samples, we did not include these data in the statistical analyses we present. Nevertheless, inclusion of these data did not change our results and inferences. We are confident that we tested workers throughout a portion of their lifespan sufficiently long enough to allow us to examine effects of age, and our inclusion of worksite location adds depth to our study.

Based on the REM, we hypothesized that nursing performance would remain constant with age due to the critical importance of brood care to colony fitness. Outside-nest workers were expected to show greater positive phototaxis than workers found within the nest, although light sensitivity might be expected to decline with age if the visual system senesces (honey bee oxidative damage: Seehuus et al. 2006). Neither age nor spatial location appeared to influence brood-care behavior, although our limited power, particularly for age, indicates that more subtle behavioral effects might be undetectable. Phototactic response did not vary with age or worker spatial location: inside minors were not more likely than outside minors to select dark or dimly lit environments at any age. This could suggest that mature minors respond flexibly to light conditions associated with different worksites, and accordingly, the task-related stimuli they would likely encounter. Alternatively, individuals may be consistent in their responses, although we detected no differences between groups. Because we assessed workers in a single instance and for a brief time period (up to 5 min), we cannot rule out the possibility that some minors may have exited the maze, but would have returned to the dark maze interior if given the opportunity. Age and spatial location do not appear to regulate or be proxies for phototactic response, although we had limited power to detect differences in the latency to exit the maze. Moreover, if worker social roles are discreet, we would expect a difference between minors of different ages or collected inside or outside of the nest.

Minors collected at different worksites appeared to have similar efficacies in nursing performance but differed in predatory behavior and overall activity. Independent of age, outside workers were consistently more active and responsive to live prey than inside workers. Therefore, all minors may not respond equally to prey stimuli, indicating that mature workers may not be completely interchangeable in task performance. Minors outside the nest also exhibited higher activity levels than inside-nest workers, with the greatest differences at younger ages, whereas only the latter increased in activity level with advancing age. The absence of age-related increases in activity in outside-nest workers could be due to the modulation of behavior by environmental factors such as CO_2 levels (Ziesmann 1996; Guerenstein and Hildebrand 2008) or circadian regulation (Bloch and Robinson 2001; Ingram et al. 2009). In any case, the correlation between age and activity level in inside-nest minors was weak, and the control mechanisms of activity remain unidentified.

Workers collected outside the nest were equally capable of nursing, although foraging-related stimuli, including those of prey, could be more salient behavioral triggers than brood signals and cues. Performance of one task could influence responsiveness in other contexts. For example, nursing could lower predatory aggression, compromising responsiveness to prey, but the reverse pattern need not occur. Physiology and task performance could interact to shape behavioral profiles in minor workers.

Brain titers of 5HT and DA did not significantly increase with worker age, although their patterns differed. These patterns were unchanged whether or not we adjusted for estimated changes in brain size based on work on *Camponotus floridanus* (Gronenberg et al. 1996), which provides the most applicable data set for ants. Our previous work has shown that known-age workers, irrespective of worksite from 20 to 120 days, increase in 5HT and DA titers (Giraldo et al. 2016). Although we found no significant effect of age on amine titers in the current study, together, these results suggest that age-related differences in amine titers could be driven by the oldest age group, 120-day-old minors. Furthermore, we had limited power to detect differences in titer for medium effect sizes, potentially obscuring less dramatic age-related changes. Worker spatial location did not significantly correlate with brain 5HT level. However, worker age and spatial location showed a significant interaction effect for DA: inside-nest minor DA titers increased with age with a steeper slope than DA titers in outside-nest workers. DA was significantly higher in outside nest workers than inside nest workers. The pattern we found is broadly consistent with previous work on *P. dentata* showing that 5HT and DA increased with age from newly eclosed to "older" minors only known to be greater than approximately 20 days of age (Seid and Traniello 2005). When we included our small sample of 120-day-old minors (particularly outside-nest workers), amine titers significantly increased with age for both groups (two-way ANOVA, $p < 0.001$). It is thus possible that the nonsignificant age effect on amine level we found ($p = 0.061$, $p = 0.054$ for 5HT and DA, respectively) would become significant with a larger sampling of 120-day-old workers, which have aged to 86 % of the maximum laboratory lifespan. In any case, there is no indication that amine levels decrease in old workers, suggesting that aminergic systems in *P. dentata* are maintained throughout the minor worker lifespan and do not show senescence-associated declines (Meltzer et al. 1998; Giraldo et al. 2016). Although DA was significantly higher in outside-nest workers, and because minors at either location can be of any age, it appears unlikely that DA directly modulates worksite foci and associated differences in activity level and predatory behavior. Spatial distribution based on in situ hybridization and quantitative expression of putative 5HT and DA receptors in brains of 5-day-old *P. dentata* workers do not show differences between inside and outside workers (Ibarrarán Viniegra 2015). Only DA receptor 2 showed a significant age-related decrease between a small sample of 1 and 20+ day old minors. Because we tested older ants, we cannot directly compare datasets. However, these findings together suggest that DA and 5HT receptor profiles may not be driving task performance differences in *P. dentata*.

Our ability to significantly lower 5HT (>50 %) suggests that a lack of effect was likely not due to an insufficient decrease in monoamine titers. 5HT is known to modulate trail following (Muscedere et al. 2012): AMTP treatment depleting brain 5HT by 43 % caused a 15 % decline in trail-following ability. Brain titers of 5HT (Seid and Traniello 2005) and serotonergic cell bodies in the optic lobes (Seid et al. 2008) increase with age in *P. dentata*. We therefore predicted that predatory behavior, which like trail following is primarily controlled by olfaction, and phototactic response, which could be modulated by serotonergic processes in the optic lobes (Seid et al. 2008), would be affected by 5HT depletion. Depletion of 5HT, however, had no effect on brood care, predatory behavior, activity level, or phototaxis. Moreover, olfactory responsiveness does not appear to be broadly modulated by 5HT: trail-following behavior increases with increasing 5HT titer as workers age and decreases due to 5HT depletion (Muscedere et al. 2012), but brood care, which is also dependent on olfactory cues, remained unaffected. Serotonergic circuits could nevertheless regulate task performance if inhibitory and excitatory effects cancel each other through depletion of 5HT. In honey bees, amine titers can change with age and/or social role but vary by brain region; the mushroom bodies show only age-related increases in amines, and the antennal lobes show both age- and social role-related correlations (Schulz and Robinson 1999). Although a study that quantified amine titers in each brain compartment would be difficult given the small size of the *P. dentata* brain (1.45×10^7 μm^3 [Seid et al. 2008]), results from honey bees suggest that aminergic modulation at lower levels of functional organization than the whole brain (Hammer and Menzel 1998), such as circuits or individual neurons (Haehnel and Menzel 2012), can significantly shape behavior.

Although we were unable to establish causal links between some amine titers and specific behaviors, biogenic amines could nevertheless influence the function of signaling systems to gradually shape developmental processes as well as rapid behavioral state changes in *P. dentata*. The behavioral differences we describe between inside- and outside-nest workers are likely regulated by relatively rapid processes such as gene expression or enzyme activity (Ingram et al. 2005; Lucas and Sokolowski 2009; Lucas et al. 2015), corpulence (Blanchard et al. 2000; Robinson et al. 2009, 2012), hormone profile changes (Corona et al. 2007; Dolezal et al. 2012), or aminergic modulation (e.g., Alekseyenko et al. 2014), without being detectable from whole-brain monoamine levels. Neuromodulatory changes can also operate without changes in amine titers through variation in the location and number of receptors, which in social insects has been primarily examined by receptor gene expression (McQuillan et al. 2012a, b), although data in *P. dentata* suggest that receptors do not substantially change with worker age or spatial location (Ibarrarán Viniegra 2015). These internal drivers of behavior may generate variation in response thresholds and interaction rates (Gordon 2010) and/or worksite location, which could together shape individual task performance profiles. *P. dentata* minors may be primed aminergically or by other physiological changes for foraging and thus stimulated at lower thresholds by social interactions, causing them to remain outside of the nest.

High levels of forager mortality have been documented in several ant taxa (Porter and Jorgensen 1981; Schmid-Hempel and Schmid-Hempel 1984; Kwapich and Tschinkel 2013). Colonies could therefore benefit substantially from flexible patterns of behavioral responsiveness to compensate for lost foragers, although some ant taxa are unlikely to respond flexibly to demographic changes (Kwapick and Tschinkel 2016). Our findings could suggest that the *P. dentata* workforce is composed of behaviorally flexible workers, although repeated measurements of marked individuals would be needed to test this hypothesis. Such work in younger minors suggests this flexibility to be the case (S. Ibarrarán Viniegra, personal communication). Higher DA is found in outside-nest workers, but a simple causal relationship appears unlikely. Nevertheless, worksite location is correlated with differences in predatory behavior and activity level. Although causal linkages between brain monoamine titers have been recently demonstrated in ants (Muscedere et al. 2012; Kamhi and Traniello 2013; Kamhi et al. 2015), the context-related differences we describe here do not allow us to establish a direct relationship between DA and the behaviors we measured, which could involve complex relationships between neuromodulators and patterns of gene expression (Lucas and Sokolowski 2009; Andrews et al. 2014) and/or epigenetic regulation (Simola et al. 2016) of social organization.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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