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Lifespan behavioral and neural resilience in a social insect

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1 Lifespan behavioral and neural resilience in a social insect

2

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6

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24 Abstract

25 Analyses of senescence in social species are important to understanding how group living
26 influences the evolution of aging in society members. Social insects exhibit remarkable lifespan
27 polyphenisms and division of labor, presenting excellent opportunities to test hypotheses
28 concerning aging and behavior. Senescence patterns in other taxa suggest that behavioral
29 performance in aging workers would decrease in association with declining brain functions.
30 Using the ant *Pheidole dentata* as a model, we found that 120 day-old minor workers, having
31 completed 86% of their laboratory lifespan, showed no decrease in sensorimotor functions
32 underscoring complex tasks such as alloparenting and foraging. Collaterally, we found no age-
33 associated increases in apoptosis in functionally specialized brain compartments or decreases in
34 synaptic densities in the mushroom bodies, regions associated with integrative processing.
35 Furthermore, brain titers of serotonin and dopamine - neuromodulators that could negatively
36 impact behavior through age-related declines - increased in old workers. Unimpaired task
37 performance appears to be based on the maintenance of brain functions supporting olfaction and
38 motor coordination independent of age. Our study is the first to comprehensively assess lifespan
39 task performance and its neurobiological correlates and identify constancy in behavioral
40 performance and the absence of significant age-related neural declines over the worker lifespan.

41

42 Keywords

43 Senescence, neurodegeneration, biogenic amines, task performance, ants

44

45 **Introduction**

46 Evolutionary theories of senescence predict behavioral declines in old age [1–3] in
47 association with physiological and genetic processes that deteriorate over the lifespan [4,5].
48 Aging nervous systems may functionally decline, leading to sensory and motor deficits [6,7] due
49 to neurodegeneration, manifest as apoptosis (programmed cell death), which may increase with
50 age [8], disease [9], or development [10]. Few studies have examined the influence of sociality
51 on aging and life history [11–13], and little is understood about cognitive decline and
52 neurobiological changes accompanying senescence in social animals, apart from humans [12,14–
53 16]. Social insects have striking lifespan polyphenisms [17–20]: queens often live more than a
54 decade, whereas workers may live only several months [21], indicating that the differentiation of
55 reproductive and sterile castes has had profound effects on senescence, which may be influenced
56 by the social organization of colony labor. The relationship of task performance to age-related
57 changes in the worker brain, however, is not well understood.

58 The ant *Pheidole dentata* has served as a model to explore the evolution and ecology of
59 social structure, as well as the neurobiological underpinnings of age-related worker behavior
60 [22–27]. Minor workers expand their task repertoires from eclosion to approximately three
61 weeks of age [23] while their brains undergo synaptic remodeling [28] and serotonergic systems
62 that influence behavioral development and task performance mature [25,29–31]. How do
63 workers age behaviorally, and do age-related and brain-based neuroanatomical and
64 neurochemical changes occur that could negatively impact lifespan task performance? Using
65 individuals of known age and robust and comprehensive assays to quantify effectiveness of task
66 performance in minor workers, we tested the hypothesis that behavioral performance declines
67 across the *P. dentata* minor worker lifespan in association with age-related patterns of apoptosis

68 and cellular modifications in synaptic complexes considered significant to sensory processing,
69 integration, and motor control, and thus behavioral efficacy [32,33]. In addition, we quantified
70 levels of neuromodulators known to influence neural structure and signaling [34] that could alter
71 circuit function in an age-dependent manner to regulate task performance or alter other
72 physiological processes associated with aging. We predicted that if workers functionally
73 senesce, nursing and foraging performance should decrease, and sensory and motor processes
74 regulating worker responsiveness to social signals and cues that guide these tasks should covary
75 with age-related neurobiological declines. We localized cell death in functionally specialized
76 brain compartments – the optic lobes (OL; vision), antennal lobes (AL; olfaction), mushroom
77 bodies (MB; integration, learning and memory), central complex (CC; motor response and
78 integration), subesophageal zone (SEZ; control of mouthparts), and the remainder of the central
79 brain (RCB) [35] - to quantify neural changes that could underscore senescence and impact task
80 performance. We hypothesized that apoptosis in the brain would increase with age, and the
81 density of synaptic complexes of microglomeruli (MG) associated with cognitive function
82 [33,36] would decrease prior to or concurrent with age-related declines in task performance.
83 Finally, we predicted that brain biogenic amine titers, notably serotonin (5HT) and dopamine
84 (DA), would decrease as workers aged and compromise the functionality of systems under
85 neuromodulatory control, as well as neuroplasticity [37,38].

86

87 **Materials and Methods**

88 Ant husbandry

89 Colonies of *Pheidole dentata* were collected in and around Alachua County, Florida and
90 reared in Harris environmental chambers maintained at 25°C and 40-55% relative humidity and

91 cultured as described in Seid et al. [28]. Subcolonies with minors of known age were established
92 with 40-70 pupae within 72 hours of eclosion, the queen, brood, and approximately 150 marked
93 workers. Workers were sampled from subcolonies derived from 5-15 parent colonies and
94 assigned haphazardly to assays; numbers of subcolonies are provided for each assay below.
95 Details of subcolony establishment and marking are provided in supplemental material. Minor
96 workers of known ages (20-22, 45-47, 95-97 and 120-122 days) were selected for behavioral
97 analyses that encompass the breadth of the minor worker repertoire [22,23]. Workers reared in
98 our queenright subcolonies live up to 140 days; because few workers survived to 120 days,
99 sample sizes were smaller at this age than for other worker ages due to the diminishing return on
100 attempting to acquire samples of very old workers.

101

102 Behavioral assays

103 *Nursing effort*

104 Nursing ability was assayed in a cylindrical chamber (34 mm inner diameter) with a
105 humidified plaster-like bottom (dental stone) to prevent larvae from desiccating. Sides were
106 coated with Fluon® to prevent escape. Eight 2nd to 4th instar larvae with dark guts were added to
107 the chamber and evenly distributed along the circumference of a 20 mm circle. A cover bisected
108 by a 10 mm diameter tube was placed over the chamber, and a single minor worker was
109 introduced to the central tube using featherweight forceps. After the worker acclimated for 5
110 minutes, the lid was gently removed, allowing access to larvae. Responses were recorded for 20
111 minutes using a Canon FS400 digital camcorder placed above the assay chamber. The number
112 and duration of approaches and acts to larvae were scored manually using JWatcher Video
113 (www.jwatcher.ucla.edu) by an observer blind to the age of the ant (N=13, 16, 17, 6 for 20, 45,

114 95, and 120 day old ants respectively from 10 subcolonies). We quantified the number of times
115 a worker approached, carried, fed, piled or otherwise provided care to larvae and recorded the
116 duration of feeding, carrying, and unspecified brood-directed behavior, which could include
117 licking or other manipulation of brood items. Details are provided in supplemental material.
118 Two-way Chi-square was used for brood-care act frequencies and two-way ANOVA was used
119 for durations to test for effects of age, differences in frequencies and durations of behaviors and
120 interaction effects. Statistical analyses were performed in JMP®.

121

122 *Pheromone trail-following ability*

123 To determine if workers experienced age-related declines in sensorimotor functions
124 associated with the perception of trail pheromone and trail-following ability, individual ants were
125 presented with artificial pheromone trails at concentrations of 1, 0.1, or 0.01 pheromone glands per
126 trail [25]. Minor workers did not follow trails at 0.001 glands per trail. Details are provided in
127 supplemental material. We video recorded each assay of worker responsiveness to an artificial
128 trail and subsequent orientation using a Canon FS400 digital camcorder for 5 minutes and used
129 Ctrax [39] to quantify worker movement. Worker age was masked with a random number so
130 that analyses were conducted blind. Trail-following was assessed by tracking activity within and
131 outside a 1 cm diameter annulus digitally drawn along the chemical trail to estimate the active
132 space of the pheromone. Annulus size was determined conservatively after observing numerous
133 occurrences of trail-following behavior. Using larger annuli did not significantly affect results
134 (data not shown). Of the 23 95 day-old minors sampled, 6 were raised in a subcolony lacking a
135 queen. These ants were not significantly different in any trail-following metrics than age-

136 matched workers from queenright colonies tested at the same pheromone concentrations, and
137 data were therefore pooled.

138 Trail-following metrics (mean deviation from the trail axis [accuracy], duration and
139 distance of following activity within the annulus, and mean and maximum duration and distance
140 of individual following bouts were used to quantify the integration of sensory and motor activity.
141 Details are provided in supplemental material. We tested 20 and 95 day old minors using
142 pheromone concentrations of 1.0, 0.1, and 0.01 gland per trail (20-day: N=7, 10, 7; 95-day: N=7,
143 9, 7 for 1.0, 0.1, and 0.01 gland/trail TPC respectively, sampled from 7 subcolonies). Accuracy,
144 duration, distance, mean and maximum distance and duration of individual following bouts were
145 analyzed using a two-way ANOVA for age and pheromone concentration in JMP®; to correct
146 for multiple tests a FDR correction was applied [40].

147

148 *Worker predatory behavior*

149 Ants were isolated for two minutes in a small Fluon®-lined Petri dish. A live fruit fly,
150 tethered by its wings or abdomen end with fine watchmakers forceps affixed to a small platform,
151 was then introduced into the arena. Only flies that responded to touch with movement were
152 used. Each ant was assigned a random number, and worker response was video recorded for 2
153 minutes. Predatory response by the worker was scored on a four-point scale: 1: no aggression; 2:
154 mandible flaring; 3: latent attack (delayed or not sustained for the duration of the assay); 4:
155 attack. Because this assay was scored preliminarily in real time, it was not always possible to
156 record responses blind to the age of the ant, for instance, if only one age was being tested on a
157 day. However, videos that did not involve immediate and consistent attack, which was
158 unambiguous, were reviewed at a later date by a blinded observer. Chi-squared tests were

159 performed in JMP® to examine the effect of age (N= 59, 41, 34, 17 for each age group from 20
160 to 120 days from 15 subcolonies) on predatory response.

161

162 Age-related changes in neuroanatomy

163 *Measurement of apoptosis in the brain*

164 Brains of minor workers aged 20-22 or 95-97 days were selected for analysis by Tdt-
165 mediated dUTP-biotin nick end labeling (TUNEL). TUNEL identifies fragmented DNA
166 characteristic of apoptotic cell death [41] and serves as a primary method for identification of
167 apoptosis in a variety of tissues [42,43]. Negative controls, brain tissue not treated with the active
168 TUNEL enzyme, terminal transferase, were generated according to manufacturer instructions
169 (Roche *In Situ* Cell Death Detection Kit, Fluorescein, Cat. no. 11 684 795 910). For positive
170 controls, we adapted procedures developed for *Drosophila* to confirm assay function in *Pheidole*
171 brains. Workers of unknown age were selected for positive controls and were briefly cold
172 immobilized and then impaled in the head with a metal pin, (0.1mm diameter, Fine Science
173 Tools) between the mandibles and into the brain. Injured ants were maintained in groups with
174 food and water for 16-20 hours prior to dissection. TUNEL reactions were performed according
175 to manufacturer instructions, vibratome sectioned at 100 μm , incubated with DAPI and mounted
176 in 80% glycerol. Details are provided in supplemental material. All control brain sections were
177 imaged on an Olympus FluoView® 10i confocal microscope using a 10x objective. TUNEL-
178 positive cells were confirmed using a 60x objective and examination of nuclear morphology. A
179 set of controls and experimental brains was processed at one time; all brains were discarded from
180 any set in which positive or negative controls failed for a total of 22 usable brains (N=12, 10 for

181 20 and 95 days from 6 subcolonies). Known-age ant brain sections were scanned in entirety with
182 a 60x objective using 2 μ m optical sections.

183 Brains were assigned a random number and images were analyzed manually using
184 FluoView® software (FV10-ASW 3.0 Viewer) by an observer blind to worker age. TUNEL-
185 positive cells were identified visually by their brightly fluorescent cell bodies. In some instances,
186 only a portion of the nucleus appeared TUNEL-positive. Apoptosis was confirmed by observing
187 nuclear morphology: apoptotic nuclei often show enhanced DNA condensation [44]. Cells
188 identified as apoptotic were TUNEL-positive, co-localized with a nucleus, and exhibited atypical
189 nuclear morphology. Cells were counted and categorized according to the nearest neuropil.
190 Although we could not always be certain that cell bodies projected to an adjacent neuropil
191 region, our method reasonably estimated brain compartment variation in cell death.

192 To compare the frequency of apoptosis between functional brain regions that differ in
193 volume and cell number, we employed an unbiased stereological approach to estimate total cell
194 number. Volumes were measured directly on a subset of brains by an experimenter blind to brain
195 age, and means for each age group calculated. Apoptosis was then scaled to total cell number in
196 each brain region. Cell number per region was estimated using an optical disector [45] on one
197 brain of each age group and extrapolated for other brains. We were thus able to calculate the
198 proportion of apoptotic cells across age groups and brain regions. Proportions of apoptotic cells
199 (number of apoptotic cells/estimated cell number) were calculated for each brain region. Because
200 the total number of cells per region is large relative to the total number of apoptotic cells, small
201 errors in total cell estimation have negligible effects on results. A mixed-effects ANOVA was
202 run in SPSS to test for effects of age, brain region, and age x brain region interaction effects on
203 the log-transformed proportion of apoptotic cells with age as a between-subjects factor and brain

204 region as a within-subject factor. Differences between brain regions were identified using pair-
205 wise comparisons and Bonferroni corrections. Details are provided in supplemental material.

206

207 *Quantification of microglomeruli (MG) in the mushroom body lip*

208 MG, synaptic complexes characterized by a synapsin-immunoreactive presynaptic bouton
209 surrounded by a halo of phalloidin-labeled postsynaptic terminals, were immunohistochemically
210 labeled in 20 and 95-day old minors (N=28, 18 from 14 subcolonies) following the protocols of
211 Groh et al. [46]. Details are provided in supplemental material. To quantify age-related changes
212 in MG densities, the imaging plane where the peduncle completely bisects the calyx and the
213 collar is maximally visible was selected because it could be easily identified across samples. We
214 thus minimized potential spatial effects by selecting approximately the same location. All
215 images were taken using an Olympus FluoView® 10i confocal microscope with a 60x objective
216 and analyzed blind to brain age in ImageJ [47]. Global adjustments were made to brightness and
217 contrast in single-channel images, which were then merged and pseudocolored to count MG in
218 two circles (400 μm diameter each) drawn over the lip region. Details of placement and counting
219 criteria are provided in supplemental material. Mean counts per calyx (lateral or medial) and
220 means of both calyces were analyzed with a one-way ANOVA. Counts were converted to MG
221 per μm^3 using calyx neuropil measurements from Muscedere and Traniello [31].

222

223 Quantification of biogenic amines

224 Biogenic amine titers of individual brains were measured using high performance liquid
225 chromatography with electrochemical detection as described in Muscedere et al. [25] by an
226 experimenter blind to worker age. Details are provided in supplemental material. Volume

227 measurements of the rind, the outer region of the brain where cell bodies are located, in 20 and
228 95 day-old minors show no significant differences with age; neuropil volumes would be
229 expected to show similar patterns. Therefore, it was not necessary to correct for brain size.
230 Eleven of 98 minor workers sampled for our assessment of amine titers across age and social role
231 were from a colony lacking a queen. Neither 5HT nor DA titers in these workers differed
232 significantly from titers of age-matched minors from queenright colonies; data were therefore
233 pooled. Amine titers are reported in pg/brain for workers from 13 subcolonies (5HT: N=23, 31,
234 18, 14, DA: N=23, 29, 17, 13 for each age group from 20 to 120 days). Octopamine (OA) titers
235 were not measured because levels of this monoamine are very low and do not change with age
236 from eclosion to 20 days in *P. dentata*, a period during which 5HT and DA titers both
237 significantly increase [29]. Effects of age on amine titers were tested with an ANOVA in JMP®
238 and significant differences between groups determined with Tukey's post-hoc test.

239 Power analysis

240 To be confident that negative results biologically reflected lack-of-age effects, we
241 performed retrospective power analyses using suggested effect sizes from Cohen 1988 [48]; see
242 also [49–51]). Power, expressed as a proportion ranging from 0 to 1, reflects the probability of
243 correctly rejecting the null hypothesis [49] and therefore the ability to detect a statistically
244 significant result. Power greater than 0.8 is generally considered sufficient to detect a specified
245 effect size (Cohen 1988). We used standardized effect sizes to estimate the power of our analyses
246 and hence our ability to detect significance. For TUNEL analysis, we calculated power using a
247 simulation method for an effect size of 0.5 as well as 2.5, which we found between brain regions.
248 A 2.5 fold difference between ages was used as benchmark for detecting apoptotic cells in
249 association with negligible senescence [52] and an effect size of ~2.5 was found for age-related

250 changes in MG density [53]. Our effect sizes of 0.3 and 0.5 are thus conservative. All other
251 tests had power of minimally 0.77 for an effect size of 0.5. We are thus confident that we could
252 detect effects of relevant size in all assays. All power analyses were conducted in PASS¹⁴ (NCSS
253 Statistical Software, Kaysville, Utah).

254

255 **Results**

256 Task performance

257 We measured the efficacy of tasks performed within and outside the nest that comprise
258 the breadth of the *P. dentata* minor worker repertoire at four ages: 20-22, 45-47, 95-97 and 120-
259 122 days. Laboratory mortality of minors is 50% at 77 days and 25% at 117 days (Kaplan-Meier
260 survival estimate), with a maximum lifespan of 140 days.

261

262 *Nursing effort*

263 Behavioral components of nursing involving sensory and motor functions did not
264 significantly vary with worker age and the frequencies of behaviors were not significantly
265 different from each other (two-way Wald test, age: $\chi_{150}^2=7.53 \times 10^{-6}$, $p=1.00$, behavior:
266 $\chi_{200}^2=0.00011$, $p=1.00$, age x behavior: $\chi_{600}^2=2.54$, $p=1.00$; 20, 45, 95, and 120 days: N=13, 16,
267 17, 6, Fig. 1A). Durations of unspecified brood-directed behavior, feeding and carrying did not
268 change with age and workers spent significantly more time providing unspecified brood-directed
269 behavior than feeding (two-way ANOVA, age: $F_{3,144}=0.398$ $p=0.755$, behavior: $F_{2,144}=6.542$,
270 $p=0.0019$, age x behavior: $F_{6,144}=0.557$, $p=0.764$, N=13, 16, 17, 6; Tukey's post hoc, Fig. 1B;
271 power = 0.942 and 0.366, respectively, for 0.5 and 0.3 effect sizes).

272

273 *Responsiveness to social signals regulating foraging and other collective actions*

274 We examined the impact of worker age on fundamental sensorimotor functions (olfactory
275 responsiveness, motor activity and motion coordination) and thus the ability of workers to
276 participate in cooperative foraging by quantifying osmotropotactic pheromone-trail following.
277 Neither worker age nor trail-pheromone concentration (TPC) significantly affected trail-
278 following accuracy, without an interaction effect (two-way ANOVA, $F_{1,41}=1.444$, age $p=0.2104$,
279 TPC $F_{2,41}=2.995$, $p=0.1895$, age x TPC $F_{2,41}=0.903$, $p=0.4809$, all false-discovery rate (FDR)
280 corrected; 20-day $N=7, 10, 7$, 95-day $N=7, 9, 7$ for 1.0, 0.1, and 0.01 gland/trail TPC
281 respectively; Fig. 1D). Duration within the active space of pheromone was not significantly
282 affected by age and there was no significant effect of TPC or interaction effect (two-way
283 ANOVA, age $F_{1,41}=4.2404$ $p=0.1607$, TPC $F_{2,41}=1.899$, $p=0.20$, age x TPC $F_{2,41}=0.4644$,
284 $p=0.6318$, all p-values are FDR corrected, Fig. 1E). Mean and maximum durations of following
285 did not differ significantly with age or TPC and did not show significant interaction effects (two-
286 way ANOVA, age $F_{1,41}=0.0132$, TPC $F_{2,41}=1.388$, age x TPC $F_{2,41}=1.574$, all $p>0.2$). Older
287 workers followed trails for longer distances and minors of both ages tend to follow trails for
288 greater distances at higher concentrations (two-way ANOVA, age $F_{1,41}=9.684$ $p=0.0238$, TPC
289 $F_{2,41}=4.863$, $p=0.0889$, age x TPC $F_{2,41}=0.545$, $p=0.6318$, all p-values FDR corrected, Fig. 1F;
290 power = 0.768 and 0.366, respectively, for effect sizes of 0.5 and 0.3 for all trail-following
291 metrics).

292

293 *Age-related changes in predatory behavior*

294 Aging minors showed no decline in responsiveness to prey or predatory attack ($\chi_9^2=7.25$,
295 $p=0.61$; 20, 45, 95, and 120 days: $N=59, 41, 34, 17$, Fig. 1C; power = 0.977 and 0.728,
296 respectively, for effects sizes of 0.5 and 0.3).

297

298 Age-related neurobiological changes

299 *Minor worker age and distribution of apoptosis in brain compartments*

300 Across all ages, minor workers had very few apoptotic cells in all brain regions (Fig. 2A).
301 The proportion of apoptotic cells was highly variable and did not differ between 20 and 95 day-
302 old workers. Apoptotic cell counts differed by brain region (mixed effects ANOVA, age
303 $F_{1,78}=2.570$, $p=0.120$, brain region $F_{5,27.8}=5.403$, $p<0.001$, age x brain region $F_5=1.974$,
304 $p=0.1863$, 20 days: $N=11, 7, 9, 8, 9, 11$; 95 days: 10, 10, 10, 9, 9, 7 for the MB, AL, OL, CC,
305 RCB, and SEZ respectively, Fig. 2B). The CC and OLs had a significantly lower proportion of
306 apoptotic cells than the RCB. For an effect size of 0.5, power was 0.16 for age, 0.33 for brain
307 region and 0.20 for interaction effect. Assuming an effect size of 2.5, as we found between the
308 CC and RCB and was the smaller of our two significant differences, power was 0.79, 1.0, and
309 1.0 for age, brain region, and age x brain region.

310

311 *Density of synaptic complexes*

312 MG density did not significantly change with worker age (mean \pm standard error, 20
313 days: 0.0891 ± 0.00179 , 95 days: 0.0859 ± 0.00224 MG/ μm^3 of calyx, $N=28, 18$, ANOVA,
314 $F_{1,44}=1.211$, $p=0.28$, Fig. 2C; power= 0.9125 and 0.512 for effect sizes of 0.5 and 0.3
315 respectively).

316

317 *Brain titers of dopamine and serotonin*

318 Brain 5HT and DA levels significantly increased with age (ANOVA, 5HT: $F_{3, 82}=10.443$,
319 $p<0.0001$, $N=23, 31, 18, 14$; power = 0.973 and 0.597 for 0.5 and 0.3 effect sizes; DA:
320 $F_{3,78}=20.921$, $p<0.0001$, $N=23, 29, 17, 13$; Fig. 2D; power = 0.970 and 0.585 for effect sizes of
321 0.5 and 0.3). 5HT was significantly higher in brains of 95 and 120-day old minors than 20 and
322 45-day old minors. DA titer increased beyond 45 days of age.

323

324 **Discussion**

325 With advancing age, *P. dentata* minor workers showed no apparent deficits in the suite of
326 behaviors we assayed, no increase in apoptosis in brain compartments that regulate task
327 performance, and no decline in densities of synaptic structures considered significant to
328 cognitive ability. In aggregate, our behavioral and neurobiological results capture the pattern of
329 minor worker aging through comprehensive assays of task performance and suggest negligible
330 senescence throughout at least 85% of the minor worker lifespan. Results of behavioral assays
331 were consistent with records of low levels of apoptosis throughout the brain and particularly in
332 the MBs, and ALs, and OLs of aging workers. The age-invariance of MG density suggests
333 olfactory processing abilities remain intact in old age. Extranidal work involves individual and
334 collective actions requiring orientation and navigation, and the sampling of diverse sensory
335 environments. Responsiveness to light, which cues the performance of outside-nest work, did
336 not decline with age, and old minors showed positive phototaxis, providing evidence that light
337 level discrimination does not senesce (supplemental material, Fig S1). Our results indicate that
338 old workers retain the range of sensory abilities necessary to be behaviorally pluripotent and
339 switch tasks having different spatial distributions. Neither the efficacy nor plasticity of task

340 performance appeared to be compromised in aging workers. Our results suggest age-related
341 losses in cell numbers in the brain were extremely low and thus insignificant, and appear to have
342 no impact on behavioral functions. Therefore, processing power underscoring social signal and
343 cue perception and response appears to be maintained in old workers. The CC and OLs had
344 lower levels of apoptosis than the RCB, suggesting that brain regions critically important to
345 information processing and responsiveness to task stimuli may be particularly buffered from
346 decline. The CC is being increasingly implicated in integrative functions as well as motor output
347 [54], and maintenance of the OL neuropil with increasing age could be particularly important to
348 extranidal task performance. *P. dentata* have relatively small eyes and OLs [31] which could
349 contribute to lower levels of redundancy in these circuits and hence greater importance for their
350 protection. High variability in the proportion of apoptotic cells in the CC likely stems from the
351 small number of cells that comprise this region, causing a small absolute change in the number
352 of dying cells to more strongly influence this proportion. Nevertheless, the CC showed
353 significantly lower levels of apoptosis than the RCB. Titrers of 5HT - a neuromodulator
354 significant for olfactory social functions in *P. dentata* [25] - and DA increased with age, rather
355 than showing senescence-associated declines [37,55]. DA can have neurotrophic functions, and
356 5HT and DA may interact to promote circuit development and neuroplasticity [34] as well as
357 modulate sensory [56,57] and motor function [58,59], perhaps contributing to the preservation of
358 task performance in aging workers. In sum, we did not identify age-related deficits in behavioral
359 abilities or aminergic or cell deterioration, suggesting that *P. dentata* minor workers do not
360 exhibit senescence throughout much of their lifespan and are able to continue to effectively
361 contribute to colony labor well into their final weeks of life.

362 Older *P. dentata* minors are more efficient at nursing [24] and show a significantly
363 increased response than young minors (< ~10-15 days of age) to colony conditions that reflect
364 greater brood-care needs [23]. Our results demonstrate that minor workers retain the olfactory
365 sensitivity required to assess brood welfare and nurture growth and development, as well as
366 provide motor control to mouthparts to manipulate delicate eggs and immatures, thus allowing
367 them to remain effective alloparents late in life.

368 As in many ant species, olfaction is the predominant sensory modality in *P. dentata*.
369 Responsiveness to chemical cues and pheromones can develop with maturity [60] and decline
370 with senescence [61]. However, results suggest that old *P. dentata* minors do not decline in
371 performing foraging or nursing tasks that differ qualitatively in olfactory social cue and signal
372 arrays. Odorant inputs are transduced to trigger motor output through the SEZ to control the
373 mouthparts [62] and thoracic ganglia to direct leg movements [63]. Our assessment of predation
374 showed no effect of worker age on sensory responsiveness to prey or the motor execution of
375 attack. Minors of all ages did not appear impaired in attack mechanics, including biting behavior,
376 suggesting that mandibular muscle functions and SEZ neuropil appear well-maintained with age.
377 Again, contrary to senescence theory, we found improvements in trail following and activity
378 level (supplemental material, Fig. S1) in aging minors, suggesting continued development and/or
379 experience-dependent enhancements of behavior into old age. Workers did not decrease in trail-
380 following accuracy; surprisingly, 95-day minors followed trails for longer distances than younger
381 individuals, perhaps due to their higher activity level (Fig. S1), enhanced olfactory
382 responsiveness to trail pheromone, or both. Therefore, older minors not only did not exhibit
383 senescence, but in fact improved in certain functions, suggesting selection for effective task
384 performance throughout the lifespan. This does not imply behavioral specialization by older

385 workers on outside-nest tasks; indeed *P. dentata* do not appear to exhibit strong age-related
386 variation in worksites (Giraldo et al. *in review*). Specialized workers may in fact not be more
387 efficient in task performance in at least some taxa (*Temnothorax albipennis*; [64]).
388 Pharmacological depletion of 5HT in *P. dentata* decreases responsiveness to trail-pheromone,
389 likely through modulation of olfactory sensitivity [25]. Although declining monoamine titers
390 could cause olfactory and hence behavioral deficits, 5HT and DA titers increased with age,
391 potentially enabling olfaction to remain intact.

392 Social insect workers have been hypothesized to experience programmed death at an age
393 marginally greater than their life expectancy in nature [65], implying that task performance
394 abilities either gradually diminish or abruptly decline with age. Although worker longevity in the
395 field is not known in *P. dentata*, it is reasonably anticipated to be shorter than the 140-day
396 lifespan we recorded in the laboratory, given increased worker mortality associated with the
397 transition to extranidal tasks performed in more unpredictable environments [20,66,67].
398 Although we cannot exclude the possibility that *P. dentata* minor workers decline rapidly just
399 before death, this appears to be unlikely because precipitous senescence [68] is rare and
400 associated with unusually high investment in reproduction [69], which is absent in sterile
401 workers. The lack of functional senescence in *P. dentata* minors contrasts with the rapid aging
402 exhibited by most genetic gerontological models [70]. Studies on aging honey bees are
403 equivocal regarding functional senescence in workers [12,14–16], which do not decline in
404 responsiveness to light and sucrose, olfactory associative learning, or locomotion as they age
405 [12]. However, chronological age is only one factor influencing senescence in honey bees:
406 overwintering bees, which can be more than six months old, maintain tactile and olfactory
407 learning but show deficits in olfactory long-term memory in comparison to chronologically

408 younger foragers [71]. Our behavioral metrics encompassed measures of sensorimotor function
409 similar to those used in studies of honey bees, as well as efficacy assessments of task
410 performance inside and outside the nest required to fully evaluate worker capability in a species
411 that retains a broad task repertoire throughout the worker lifespan. Such differences in
412 hymenopteran aging phenotypes may be related to the lack of temporal caste discretization in *P.*
413 *dentata* [23,72] and contrasting nesting and foraging ecology.

414 Eusociality has had profound consequences for the evolution of behavioral development,
415 immune function, and genetic regulation of aging [17–19]. Social interactions appear to mediate
416 metabolic homeostasis and affect mortality rates [73]. Division of labor could in part lead to
417 selection for the maintenance of individual functionality throughout the sterile worker lifespan
418 [20]. In ants, specialized morphologies and task performance are key to social complexity;
419 worker polymorphism, task repertoire development, and behavioral specialization are
420 underscored by brain neuropil growth and investment patterns [31]. Complex task repertoires are
421 generated by a miniature brain that even at an extremely small size does not appear to
422 compromise cognitive ability [74], suggesting that ant brains may operate with a level of
423 metabolic efficiency that could enable conserved energy to be distributed to provide molecular
424 protection against neural and behavioral senescence. The lack of functional senescence in *P.*
425 *dentata* minor workers, underscored by the maintenance of neuroanatomical and neurochemical
426 substrates that activate and support task performance, suggests that the ant nervous system has
427 evolved robust functionality throughout the relatively short sterile worker lifespan. This
428 resilience may be associated with energy savings resulting from the absence of reproductive
429 costs of workers, reduced neuron and neural circuit size, and lower requirements for redundancy
430 and information storage that could minimize neurometabolic costs in individual worker brains

431 [75–78]. Additionally, the benefits of living in a highly integrated homeostatic social system
432 capable of collective information processing and emergent cognition may allocate cost
433 reductions in brain metabolism to neural systems operational maintenance and thus effective
434 lifetime behavioral performance.

435

436 **Data Accessibility**

437 Datasets for this article have been uploaded to Dryad.

438

439 **Competing Interests**

440 We have no competing interests.

441

442 **Authors' Contributions**

443 YMG conceived the study, designed and performed experiments, analyzed data, performed
444 statistics, and drafted and edited the manuscript. JFK was involved in experimental design, data
445 analysis, and manuscript editing for the apoptosis study. VF and MM analyzed trail-following
446 assays. SJKR assisted with statistical analyses. AR, LW, AD and AK performed experiments,
447 assisted in data analysis, and contributed to drafting the manuscript. JFAT co-conceived the
448 studies, designed experiments, and drafted and edited the manuscript. All authors gave final
449 approval of the manuscript.

450

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723 **Figure Legends**

724 **Fig. 1.** Number (**A**) and duration (**B**) of nursing behaviors (boxes show quartiles and whiskers
725 95% confidence intervals) of minor workers of different ages are maintained from 20 to 120 days
726 (N=13, 16, 17, 6 for 20, 45, 95, and 120 day-old minors respectively). **C.** Percentage of each age
727 group (20, 45, 95, and 120 days; N= 59, 41, 34, 17) exhibiting each type of predatory response.
728 Number of minors for each response type and age are indicated. **D-F:** Worker trail-following
729 improves with age. **D.** Trail-following accuracy by 20- and 95-day old minor workers. **E.**
730 Duration within the active space of an artificial trail. **F.** Trail-following distance (20 day: N=7,
731 10, 7; 95 day: N=7, 9, 7 for 0.01, 0.1 and 1 TPC respectively). Significant effect of age on trail-
732 following distance is indicated by an asterisk. All data points shown. Boxes reflect first and third
733 quartiles and whiskers show 95% confidence intervals.

734

735 **Fig. 2.** Neuroanatomical and neurochemical metrics. **A.** Representative apoptotic cell in the MB
736 rind (cell body region) in a 95-day old minor. Cell bodies labeled with DAPI (blue); TUNEL-
737 positive cell (green). Inset: TUNEL-positive cell co-localized showing condensed nucleus. **B.**
738 Proportion of apoptotic cells in relation to total cells/brain region (95% confidence intervals
739 [whiskers]) in 20 and 95-day old ants (20 days: N= 11, 7, 9, 8, 9, 11; 95 days: 10, 10, 10, 9, 9, 7
740 for the MB, AL, OL, CC, RCB, and SEZ respectively). Age did not significantly affect
741 proportion of cells but brain regions differed. Brain regions are abbreviated for clarity: MB =
742 mushroom bodies, AL = antennal lobes, OL = optic lobes, CB = central body, RCB = remainder

743 central brain, SEZ =subesophageal zone. **C.** Representative micrographs illustrating densities of
744 MG in a 20- (left) and 95-day old minor (right). Brains are pseudocolored; phalloidin (green),
745 synapsin (magenta). **D-E.** Brain titers of 5HT (**D**) and DA (**E**) significantly increase with age
746 ($p < 0.001$; 5HT: N=23, 31, 18, 14; DA: N=23, 29, 17, 13, for 20, 45, 95, and 120 day-old minors,
747 respectively). Boxes show first and third quartiles; whiskers = 95% confidence intervals.
748



