Age-related differences in learning, memory and brain plasticity in workers of the common wasp *Vespula vulgaris*

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Abstract

Learning, memory, and brain plasticity are thought to play an important role in regulating behavioural roles in social insects, as workers perform different tasks as nurses, builders, foragers and defenders. However, it remains challenging to disentangle whether neural changes regulate behaviour or arise as a consequence of it. While cognition has been extensively studied, especially in honeybees, the variation of cognitive traits remains poorly understood in social wasps. Here, we investigated age-related changes in learning, memory and neuroanatomy in workers of the common wasp *Vespula vulgaris.* We developed a Y-maze to test differential conditioning and memory of wasps and later visualised the brains using a high-resolution micro-CT imaging. We found that younger individuals exhibited slower decision-making yet made more accurate decisions compared with older individuals, revealing a pronounced speed-accuracy trade-off. Short-term memory showed only a slight decline with age. Neuroanatomical image analyses revealed that, despite a reduction in overall brain volume, key major neuropils involved in sensory processing and learning, such as mushroom bodies, optic lobes and antennal lobes, increased in relative volume with age. These findings corroborate with studies in bees and provide novel insights into how ageing influences cognitive function and brain structure in wasps.

Summary statement

Our study provides the first integrated analyses of age, cognition and brain plasticity in the common eusocial wasp, *Vespula vulgaris*.

Introduction

Social insects forage in highly dynamic and unpredictable environments, where food availability fluctuates rapidly due to displacement or depletion (Manattini et al., 2024). To cope with these challenges, they have evolved remarkable cognitive abilities, with learning and memory at the core of their behavioural flexibility (Agrawal, 2001; Giurfa, 2015; Richter, 2000; Tibbetts, 2002; Tibbetts et al., 2018). Associative learning allows individuals to link stimuli with rewards or punishments (El-Sayed et al., 2018) while spatial learning supports navigation using visual and olfactory landmarks (Moreyra et al., 2017). Together, these abilities enable social insects such as wasps (Gong et al., 2019; Richter, 2000), ants (Fenli et al., 2025; Hölldobler & Wilson, 1990) and bees (Giurfa, 2007, 2015; Kheradmand et al., 2020; Leadbeater & Chittka, 2007; Menzel & Giurfa, 2001) to efficiently exploit resources, adapt to changes in their environment, and are considered key components of their ecological and evolutionary success.

Variation in cognitive performance is shaped not only by ecological factors but also by ageing (Tonoki & Davis, 2015). In solitary insects such as the fly *Drosophila melanogaster*, senescence is typically associated with declines in learning and memory driven by synaptic degeneration, oxidative stress, and altered neurotransmission (Iliadi & Boulianne, 2010; Tonoki & Davis, 2015). By contrast, eusocial insects often display extended lifespans and division of labour, such that cognitive performance is shaped as much by behavioural role as by chronological age

(Gage et al., 2020; Giraldo et al., 2016; Scheiner & Amdam, 2009). A major driver of this role-related variation is temporal polyethism, the age-related division of labour that structures colony life (Siegel et al., 2013). Workers typically begin adult life performing tasks within the nest before transitioning to foraging, a shift often accompanied by changes in learning, memory, and sensory responsiveness (Gage et al., 2020; Scheiner & Amdam, 2009; Siegel et al., 2013). For example, in honeybees, foragers exhibit heightened sensitivity to visual and olfactory cues compared with nursing workers (Tsuruda & Page Jr, 2009), with performance further modulated by both intrinsic factors (e.g. hormonal state) and extrinsic factors (e.g. environmental conditions) (Brothers, 2021; Fahrbach & Robinson, 1996). Taken together, these findings suggest that the evolution of social complexity may buffer some age-related declines while amplifying role-specific cognitive specialisations, raising the question of whether cognitive ageing represents a conserved constraint or an adaptive trajectory in social insects.

Such behavioural changes observed in social insects are closely tied to learning and memory processes. Short-term memory (STM) forms rapidly after conditioning and typically decays within hours, whereas long-term memory (LTM) requires repeated, spaced training and can persist for days or longer (DeZazzo & Tully, 1995; Giurfa, 2015; Moreyra et al., 2017). In honeybees, associative memory underpins odour and colour discrimination as well as complex long-term learning (Chittka, 1998; Dyer & Garcia, 2014; Giurfa, 2004; Menzel, 1999), but performance varies with age and task: long-lived winter bees largely retain memory, whereas foragers show subtle impairments or division-of-labour effects on olfactory learning (Behrends & Scheiner, 2010; Maleszka et al., 2009). Ants also rely on robust visual and olfactory memories to navigate and exploit resources, supported by well-developed mushroom bodies (Gronenberg et al., 1996; Hölldobler & Wilson, 1990). Comparable flexibility occurs in wasps with solitary and eusocial lifestyles. For instance, the parasitoid jewel wasp *Nasonia vitripennis* retains olfactory associations for several days, which facilitates the localization of new hosts (Schurmann et al., 2012), the social paper wasp *Mischocyttarus* show both colour reward learning and sex and age dependent differences (da Silva et al., 2023; Lucchetta et al., 2008), and the paper wasp *Polistes fuscatus* can remarkably recognize conspecific faces (Sheehan & Tibbetts, 2008; Tibbetts et al., 2021). In fact, neurobiological studies are beginning to reveal the mechanisms underlying visual individual recognition in paper wasps, while genomic analyses point to recent selection shaping their cognitive abilities (Tumulty et al., 2023). In the eusocial species *Apis mellifera* and *Vespula vulgaris* it was shown that the individuals are even able to discriminate human faces (Avarguès-Weber et al., 2018). Collectively, these studies highlight that advanced cognitive abilities are widespread across Hymenoptera and that they are shaped by ecological as well as social pressures.

The neural basis of cognitive abilities in insects lies in a highly structured brain, where specialized neuropils process and integrate sensory information (DeZazzo & Tully, 1995; Gronenberg et al., 2007). The antennal lobes (AL; olfaction), optic lobes (OL, including medulla (Me) and lobula (Lo); vision), central complex (CX; locomotor control) and mushroom bodies (MB; sensory integration, associative learning and memory) are particularly important, with the latter supporting associative learning and memory (Ehmer & Gronenberg, 2002; Habenstein et al., 2023; Hansson & Anton, 2000; Heisenberg, 2003; Rother et al., 2021; Rybak et al., 2010). Social insects typically invest heavily in the mushroom bodies, reflecting their reliance on spatial cognition and chemical communication (Fahrbach, 2006; Heisenberg, 2003). In honeybees and bumblebees, mushroom bodies expand as workers age and transition to foraging, reflecting experience-dependent synaptic reorganization rather than neurogenesis (Fahrbach et al., 1998; Fahrbach & Robinson, 1996; Farris et al., 2001). Comparable plasticity has been observed in the paper wasps *Polistes* and *Mischocyttarus*, where social experience and dominance behaviour influence mushroom body volume and regions associated with visual processing (Jernigan et al., 2021; Molina & O'Donnell, 2008; O'Donnell et al., 2007). The same pattern was observed in solitary alkali bees, with experience and not age driving mushroom body expansion, reinforcing that adult experience is a conserved determinant of neural investment across Hymenoptera (Hagadorn et al., 2021). From a neuro-ecological perspective, variation in cognitive demands across behavioural roles is expected to drive differential brain investment, as brain tissue is metabolically costly and colony-level selection may favour optimized allocation tailored to caste- or task-specific functions (O’Donnell et al., 2018). However, neural plasticity could also arise through regulatory changes, intrinsic developmental or genetic programs that guide brain maturation, as also experience-independent phases of adult mushroom body growth have been documented in honeybees (Fahrbach et al., 1998). Because these reflective and regulatory changes often overlap, disentangling whether age- and role-related brain differences regulate behaviour or arise as its consequence remains a central challenge.

Compared with honeybees, where age- and role-related changes in cognition are well studied, far less is known about such processes in highly eusocial wasps such as *V. vulgaris*. Previous studies showed that wasps are capable of learning and memorizing associations, in *V. vulgaris* and *V. germanica* foragers perform specialized orientation flights to gather spatial, visual, and olfactory information about food sources (Collett & Rees, 1997; D'adamo & Lozada, 2003;

Lozada & D’Adamo, 2014), often forming long-lasting associations after just a single visit (D'adamo & Lozada, 2007; Moreyra et al., 2017). When food is displaced, they first search the original site before adjusting to new environmental cues (Lozada & D’Adamo, 2011, 2014; Manattini et al., 2024; Wilson-Rankin, 2015), highlighting behavioural plasticity. Wasps also exhibit robust associative learning, readily linking colours and odours with rewards (D'adamo & Lozada, 2009; El-Sayed et al., 2018; Yossen et al., 2020). Furthermore, Y-maze experiments with *V. germanica* and hornets (*Vespa velutina nigrithorax* and *Vespa crabro*) showed that they are not only capable of acquiring colour–reward associations but also can reverse them when contingencies shifted (Lacombrade et al., 2023; Moreyra & Lozada, 2021), a flexibility comparable to the visual learning capacities documented in nectar-feeding bees (Dyer & Howard, 2023).

Here, we fill the gap in the existing knowledge on learning and memory in wasps, by testing age-related differences in learning and short-term memory in *V. vulgaris* workers, and by examining their neuroanatomy using high-resolution micro-CT. Based on findings from bees and other wasps, we hypothesized that newly emerged workers would exhibit lower cognitive performance, whereas older foragers would show enhanced learning and memory associated with structural changes in key brain regions, particularly the mushroom bodies.

Material and Methods

Seven *V. vulgaris* (Linnaeus, 1758) nests were collected in the region of Leuven (Belgium; 50° 52′ 46″ N, 4° 42′ 3″ E) during the summer of 2024. Colonies were kept in wooden nestboxes (Fig. 1A) under standard conditions (28°C and 12L:12D light cycle) in the laboratory. Each nestbox was connected to a foraging box where water, sugar water (50:50), mealworms and nesting material were given *ad libitum*. Each nestbox contained a single comb with emptied cells to allow egg-laying, while larvae of different developmental stages and pupae were kept, allowing emergence, to keep natural transitions. The queen and 50–100 older workers were introduced into the nest box to promote natural behaviour. Over the following three days, newly emerged individuals (NE) from the remaining combs were checked and collected daily (NE per colony: 160–190, mean = 175), marked with a paint marker (Uniball Paint Marker PX-21), and returned to their respective colonies. These individuals were subsequently used for both associative learning trials and neuroanatomical comparisons.

Learning and memory trials

Associative learning and short-term memory were assessed in 176 workers of known ages: newly emerged (n = 47), 3-day-old (n = 44), 6-day-old (n = 34), 9-day-old (n = 30), and 12-day-old (n = 21). Under our laboratory conditions, adult *V. vulgaris* had a short life span, surviving only around 12–14 days, so 12-day-old wasps were selected as the oldest group to capture the late phase of adult life in our setup. Age categories (0d, 3d, 6d, 9d, 12d) were chosen to represent the in-nest (0–3 d) phase versus onset and progression of foraging (6–12 d) (Ferreira et al., 2023; Hurd et al., 2007).

Differential conditioning was performed using a custom 3D printed Y-maze with interchangeable coloured arms (blue and yellow), colours used in previous wasp learning experiments (Howard & Dyer, 2024) (Fig. 1B). Prior to conditioning, age-specific wasps were removed from the nestboxes and placed in isolation where they underwent starvation for two hours to increase motivation. They were then transferred to the Y-maze's holding chamber to acclimatise for 5 minutes before starting the trials. Each trial began with an exploration run, in which wasps were allowed to walk freely in the maze, without any food reward present. Their first choice of side (left or right) and colour (yellow or blue) were recorded to assess potential side or colour biases.

Following this, each wasp underwent six consecutive conditioning trials (Fig. S1, example Video 1). In each trial, sugar solution (50:50) was placed in an Eppendorf cap at the end of one coloured arm, serving as the positively conditioned stimulus (CS+). The other arm contained water, acting as a neutral conditioned stimulus (CS). Each age group included at least 10 individuals per colour condition (Table S1). The colour-reward pairing (blue or yellow as CS+) remained constant throughout the six trials for each individual. To control for spatial learning, the positions left (L) and right (R) of the coloured arms were pseudo-randomized across four switching sequences (LRRLRL, RRLLLR, LRLLRR, or RLRLRL), assigned per treatment group (Table S2).

The time taken to find the reward was measured from the moment the wasp entered the maze. If the wasp failed to locate the reward within 5 minutes, the trial was ended. Wasps that failed to complete two consecutive trials were removed and replaced with a new individual of the same age group (Table S4). Upon finding the reward, the wasp was allowed to feed for 3 seconds before the reward was removed. It was then given the chance to explore the maze to find the non-rewarding feeding cup containing water. Once the wasps returned to the starting chamber, the sliding door was closed, and a 5-minute rest period began. This procedure was repeated for the remaining five conditioning trials. After the final conditioning trial, a two-hour break was initiated, preceding two short-term memory (STM) trials. These STM trials followed the same maze protocol as the conditioning phase, except that no reward was provided. Empty feeding cups were placed in the maze to check for antennation, which was used as an indicator of active search behaviour. Colour and side choice, as well as the time taken to reach feeding cup at the trained rewarding side was again recorded.

Age-related neuroanatomy

Additionally, we investigated age-related brain plasticity. Six wasps per age category (30 individuals in total) were randomly selected following the completion of the memory trials. The wasps were decapitated, their mandibles removed, and a small opening was made in the posterior region of the head capsule to improve reagent infiltration during subsequent preparation steps. All heads were photographed in frontal view using a Leica stereomicroscope connected to a digital camera (Leica MSV266) using the Leica Application Suite program. To estimate average head capsule depth (mm), we additionally photographed 31 randomly chosen heads in lateral view (Table S5). Images were analysed using Fiji (ImageJ 1.54p) (Schindelin et al., 2012). Head width (mm) was measured at the widest point above the antennal sockets and head height (mm) was measured from the centre of the vertex to the bottom tip of the anchor shape on the clypeus, at the level of the lower eye margin (Fig. 2A). The measurements were then used to calculate the head capsule volume (mm³) using the formula for an ellipsoid (O'Donnell, 2019):

$$Head\ capsule\ volume = \frac{4}{3}\pi * \frac{1}{2}\ head\ width\ * \frac{1}{2} head\ height\ * \frac{1}{2} head\ depth \quad (1).$$

Heads were then fixed in 4% paraformaldehyde (PFA) until further processing for Micro-CT imaging. Seven days prior to scanning, heads were washed twice in 1x PBS for 30 minutes each, then submerged in a 5% phosphotungstic acid (PTA) solution containing 3% DMSO to enhance contrast of brain tissue. Heads were scanned in 70% ethanol within Eppendorf tubes, stabilized using low-density black soundproofing material and green floral foam (Fig. 2B). Imaging was performed using a Skyscan 1272 X-ray micro-computed tomography microscope at the FIBEr facility (KU Leuven, Belgium). Scanning settings included a 1 mm Al filter, 2016 × 1344 pixel resolution, 6 µm voxel size, 0.3° rotation steps, 3-frame averaging, 89 kV voltage, and 112 µA current.

Micro-CT scans of the heads were processed and analysed using Amira software (v5.3.3). Regions of interest of the brain, including optic lobes (medulla and lobula), antennal lobes, mushroom bodies, central complex and total brain area, were manually traced for annotation. A 3D reconstruction of the brain was created, and volume measurements (mm^3) were obtained for each region (Fig. 2C,2D,2E).

Data analysis

All statistical analyses were performed in R (version 4.4.2) (R Core Team, 2022) and graphs were made using *ggplot2* (Wickham, 2016).

To assess whether wasps exhibited pre-existing biases toward colour (blue or yellow) or spatial orientation (left or right) prior to conditioning, we fitted two binomial generalized linear models (GLMs), using the *lme4* package (Bates et al., 2015). The response variable was the first colour choice or side choice, with age (scaled) as a continuous predictor to capture potential developmental effects on learning. For interpretability, predicted probabilities were computed at representative ages (0, 3, 6, 9, and 12 days) by inverse-logit transformation of the fitted log-odds. These model-derived probabilities incorporate the estimated age effect and offer a smoothed representation of the expected choice behaviour. Model fit was assessed using residual diagnostics, and Tukey post hoc comparisons between age groups and within age group comparison with random choice (0.5) were conducted using the *emmeans* package (Lenth, 2025).

To assess learning performance across different age groups, binomial generalized linear mixed models (GLMMs) were fitted using the *glmer* function in *lme4* package (Bates et al., 2015), with choice correctness (correct/incorrect) as response variable. Fixed effects included scaled continuous variables age, trial and time, as well as the categorical variable trained colour and the interaction between time and age. An offset term was added to control for any intrinsic colour bias. Colony and individual ID were included as random effects.

To analyse changes in search time across learning trials while accounting for censored observations (i.e., trials in which the reward was not located within 300 seconds), we carried out a survival analysis. Both accelerated failure time (AFT) models (*survival* package (Therneau & Grambsch, 2000)) and flexible parametric survival models (*flexsurv* package (Jackson, 2016)) were tested, with the generalized gamma model providing the best fit.

Short-term memory performance was analysed using a similar binomial GLMM approach as for learning performance, with choice correctness (binary: correct/incorrect) during memory trials as the response variable. To ensure that learning occurred, only individuals with 4 or more correct choices during the conditioning phase were included (Table S3). Alternative filters (≥5 or 6 correct choices) were tested but yielded qualitatively similar results. Fixed effects included age, trial, time and colour. We also tested whether the predicted probability of correct choice for each age groups significantly differed from random choice (0.5) using the *emmeans* package (Lenth, 2025).

Finally, to assess age-related changes in brain structure, we analysed absolute and relative neuropils volumes using linear models (lm), with age as a continuous predictor of the performance variable. For models of absolute volume, head capsule volume and total brain volume were included as covariates to control for body size. For the models of relative volume, age and head capsule volume were used as predictors, as the response variable was already normalized by total brain volume. Colony identity (three levels) was included as a random factor in the candidate GLMMs and evaluated by AICc. Models retaining the colony term had higher AICc and a near-zero variance component, and fixed-effect estimates and inferences were unchanged. Accordingly, the AICc-supported GLMMs without the colony term are reported as the final models. Model assumptions were checked via residual diagnostics. Where assumptions were violated, we applied log-transformations (e.g., for absolute lobula volume) or spline fits to account for nonlinearity (e.g., for absolute volumes of the central complex and total brain, and relative volume of the medulla).

AI tools

Artificial intelligence tools (ChatGPT, OpenAI) were used to assist with R code development and troubleshooting as well as for grammar checking of the manuscript text. All scientific content, analyses, and interpretations were designed, conducted, and verified by the authors.

Results

Colour and side biases

Each wasp was first allowed to perform an exploratory run to test for inherent biases toward either colour or side in the Y-maze. Age had a significant effect on initial colour choice (GLM: z

= -3.412, $p < 0.001$). Out of the 175 wasps tested, 95 chose blue (54%) and 80 chose yellow (46%). To test whether colour preference changed with age, we analysed the proportion of wasps choosing blue across our five age groups (0, 3, 6, 9, and 12 days, Fig. 3A). Initial colour preference significantly deviated from chance (0.5) in an age-dependent manner. At day 0, 69.8% of wasps chose blue as their first choice ($p < 0.001$). Similarly, 3-day-old wasps also showed a significant bias toward blue (60.7%, $p = 0.014$). In contrast, 6- and 9-day-old wasps showed no significant colour preference (6 d: 50.7%, $p = 0.87$; 9 d: 40.6%, $p = 0.091$). Interestingly, 12-day-old wasps showed a significant preference for yellow, with only 31.3% of wasps choosing blue as their first choice ($p = 0.014$). In contrast, side choice (left of right arm of the Y-maze) ($z = 0.47$, $p = 0.64$), and no age group differed from chance (all $p > 0.2$, Fig. 3B). Because we observed significant age-specific biases in colour preference, we corrected for this in the models for learning and memory.

Associative learning

Learning performance was evaluated across six conditioning trials using a binomial generalized linear mixed model (GLMM). The probability of making a correct choice (i.e., selecting the rewarded colour) increased significantly over trials ($\beta = 0.25 \pm 0.08$ s.e., $p < 0.01$), indicating that the wasps learned the colour-reward association over time. However, overall performance declined with increasing age ($\beta = –0.34 \pm 0.12$ s.e., $p < 0.01$, Fig. 4A). Trained colour (yellow or blue) did not have a significant influence on the probability of a correct choice ($p = 0.18$).

Longer search durations were associated with a significantly lower probability of making a correct choice ($\beta = –1.61 \pm 0.16$ s.e., $p < 0.001$), indicating that increased decision time may reflect or contribute to impaired task performance. This relationship was moderated by age as the negative effect of search time on accuracy was significantly stronger in older wasps ($\beta = –0.45 \pm 0.14$ s.e., $p < 0.01$, Fig. 4B). While younger wasps exhibited a higher overall probability of correct responses, older individuals demonstrated a more pronounced decline in choice accuracy during longer searches. Random effects in the model confirmed substantial individual- and colony-level variation, with individual ID accounting for a large proportion of the variance.

To further investigate the predictors of search time, we fitted a parametric survival model using a generalized gamma distribution (Trials 1–6). Search time decreased significantly with age ($\beta = –0.05 \pm 0.0072$ s.e., $p < 0.001$), indicating that older wasps completed the task more quickly (Fig. 4C). Similarly, search time decreased significantly across repeated trials ($\beta = –0.10 \pm$

0.018 s.e., $p < 0.001$), suggesting faster performance of all ages with repeated exposure. Trained colour had no significant effect ($p = 0.72$). Interestingly, wasps that made a correct choice had significantly longer search times than those that made incorrect choices ($\beta = 0.60 \pm 0.03$ s.e., $p < 0.001$), suggesting a speed–accuracy trade-off during decision-making.

Short-term memory

To assess memory performance following conditioning, we analysed trials 7 and 8 (MT 1-2), conducted without reward, using a generalized linear mixed model (GLMM) with a binomial error structure and individual ID as a random intercept. Only wasps that had achieved at least four correct choices during the conditioning phase were included (n = 216 observations from 108 individuals). Out of these 108 wasps, 62 (57,4%) had good memory (two correct choices), 37 (34,3%) had neutral memory (one correct choice), and 9 (8,3%) had bad memory (two incorrect choices).

To determine whether memory performance exceeded random choice levels, we compared the observed number of correct choices during memory trials (MT 1-2) to chance level (50%) using a binomial test. Wasps performed significantly better than chance in memory trials, with 160 correct choices out of 216 ($p < 0.0001$). A model-based estimate using a generalized linear mixed model (GLMM) confirmed this result, with an overall predicted probability of correct choice of 76.6%. The probability of making a correct choice during these memory trials was not significantly affected by age ($\beta = -0.29 \pm 0.18$ s.e., $p = 0.111$), trained colour ($\beta = 0.16 \pm 0.18$ s.e., $p = 0.366$), or trial number ($\beta = -0.28 \pm 0.18$ s.e., $p = 0.125$) (Fig. 5). All ages were significantly different from random choice (0-3-6d: $p < 0.001$; 9d: $p = 0.04$), except 12day old wasps ($p = 0.24$).

Search time was a strong predictor of performance with longer search durations being associated with a significantly reduced probability of making a correct choice ($\beta = -1.20 \pm 0.27$ s.e., $p < 0.001$). Random effects accounted for individual-level variation ($\sigma^2 = 0.022$). These results suggest that rapid decisions during memory tests were more likely to be correct, while longer latencies to choose reflected uncertainty or memory decay. Since these results are based on the group of wasps making four or more correct choices during the conditioning trials, we also checked for a stricter filter only including wasps making five or six correct choices. This resulted in a total of 61 (4 bad, 18 neutral and 39 good) and 25 wasps (4 neutral and 21 good), respectively. did not support.

Neuroanatomy

All Micro-CT images of brains were reconstructed into 3D models comprising six distinct neuropils, which were used to perform volume measurements. We report both absolute and relative brain region volumes. Absolute volumes provide baseline anatomical information and capture individual variation, while relative volumes reveal how investment in specific neuropils changes independently of overall brain size. We found a significant positive relationship between total brain volume and head capsule volume ($R^2 = 0.12$, $p = 0.036$), indicating that individuals with larger heads tend to have larger brains. Head capsule volume did not vary significantly with age ($p = 0.745$), suggesting that head size remains stable across the studied age range.

Effects of age, head capsule volume (HCV), and total brain volume (TBV) were analysed on the absolute volumes of the major neuropils. For the medulla, TBV had a significant positive effect on volume ($F_{(1,26)} = 11.44$, $p = 0.002$), while the effect of age was marginally non-significant ($F_{(1,26)} = 3.93$, $p = 0.058$) and HCV had no significant effect ($F_{(1,26)} = 0.0010$, $p = 0.975$) (Fig. 6A). For the lobula (log transformed model), both age ($F_{(1,26)} = 6.37$, $p = 0.018$) and TBV ($F_{(1,26)} = 5.59$, $p = 0.026$) had significant positive effects, whereas HCV was not significant ($p = 0.62$) (Fig. 6B). The optic lobes (medulla and lobula combined) showed a significant increase in volume with both age ($F_{(1,26)} = 6.48$, $p = 0.017$) and TBV ($F_{(1,26)} = 12.95$, $p = 0.001$), while HCV was again not a significant predictor ($p = 0.876$) (Fig. 6C). In contrast, the volume of the central complex (spline model TBV) was not significantly affected by age ($p = 0.72$), HCV ($p = 0.42$), or TBV ($p = 0.94$) (Fig. 6D). Similarly, antennal lobe volume showed no significant effect of age ($p = 0.18$), HCV ($p = 0.49$), or TBV ($p = 0.065$) (Fig. 6E). For the mushroom bodies, both HCV ($F_{(1,26)} = 9.36$, $p = 0.005$) and TBV ($F_{(1,26)} = 11.03$, $p = 0.003$) had highly significant positive effects, while age was not significant ($p = 0.108$) (Fig. 6F). Finally, total brain volume (spline model for age) itself decreased significantly with age ($F_{(1,26)} = 19.16$, $p < 0.001$) and increased with HCV ($F_{(1,26)} = 6.48$, $p = 0.017$) (Fig. 6G). Although age did not show a significant effect for most of these individual neuropils, there seems to be a general increasing trend except for the central complex.

To examine whether this increasing trend is relevant, we investigated the effects of age and HCV on the relative volumes of these neuropils. We found a significant positive effect of age for the relative volume of all neuropils except the central complex. For the medulla (spline model for HCV), age led to a significant increase in relative volume ($F_{(1,26)} = 10.21$, $p = 0.004$) (Fig. 7A), whilst HCV had no significant effect ($F_{(1,26)} = 0.24$, $p = 0.62$). Age had a significant positive effect on the relative volume of the lobula ($F_{(1,27)} = 16.37$, $p < 0.001$), whereas HCV was not significant ($p = 0.39$) (Fig. 7B). The optic lobes showed a significant increase in relative volume with both age ($F_{(1,27)} = 16.88$, $p < 0.001$) and HCV was again not significant ($p = 0.47$) (Fig. 7C). Similarly to the absolute volume, the relative volume of the central complex was not significantly affected by age ($p = 0.33$) nor HCV ($p = 0.79$) (Fig. 7D). Age also had a significant positive effect on the relative antennal lobe volume ($F_{(1,27)} = 9.90$, $p = 0.004$) and HCV was not significant ($p = 0.78$) (Fig. 7E). Lastly, the relative volume of the mushroom bodies was also positively significantly affected by age ($F_{(1,27)} = 11.27$, $p = 0.002$) and HCV ($F_{(1,26)} = 6.71$, $p = 0.015$) (Fig. 7F). Overall, all examined neuropils increased in relative volume with age, with the central complex as the sole exception; only the mushroom bodies showed and HCV effect.

Discussion

In this study, we investigated whether age influences learning and memory performance in workers of the eusocial wasp *V. vulgaris* and whether behavioural changes correspond with structural brain changes. While younger workers (0–3 days old) learned visual colour-reward associations more effectively than older foragers (9–12 days old), short-term memory remained broadly preserved across ages, except for the oldest individuals. Neuroanatomical analyses revealed age-related shifts in relative neuropil volumes, suggesting brain plasticity associated with adult behavioural maturation.

Age-related colour preference

We first asked whether wasps displayed an inherited bias towards particular colours, as such bias could influence associative learning outcomes. Initial colour biases varied with age. Newly emerged wasps (0-3 days old) strongly preferred blue, while older wasps (12 days old) favoured yellow. This transition likely reflects dynamic changes in perceptual or motivational state. A default bias toward short-wavelength colours, as documented in naïve *Bombus* and *Apis* species (Briscoe & Chittka, 2001; Raine & Chittka, 2007), may explain the early blue preference. Older free-flying workers of honeybees and the common wasp also showed an initial preference for blue (Howard & Dyer, 2024). Alternatively, the relatively darker blue arm of the Y-maze may mimic nest-like conditions, which are preferred by young in-nest workers. The later emergence of a yellow bias could correspond to increased responsiveness to ecologically relevant cues associated with foraging (Papiorek et al., 2016), or simply to perceived brightness mimicking outdoor light conditions. This behavioural shift parallels structural changes in the brain, with the relative volume of the optic lobes (OL), the neuropil for visual processing, which significantly increased with age. Experience-dependent modulation of colour preference has been observed in other hymenopterans (Chittka & Raine, 2006; Muñoz-Galicia et al., 2021), suggesting that early neural development and the onset of foraging jointly shape perceptual biases.

Evidence for age-related decline in learning

Our results indicate a pronounced age-related decline in colour-reward associative learning. Although all age groups have demonstrated visual associative learning, improving in accuracy and reducing search time across trials, consistent with appetitive conditioning studies in wasps and bees (Dyer & Garcia, 2014; Dyer & Howard, 2023; Giurfa, 2004), younger wasps learned faster and reached higher accuracy than older workers, contrary to our initial hypothesis. This indicates that newly emerged wasps possess functional neural circuits for learning shortly after emergence, and that cognitive decline in older workers is more likely due to senescence than lack of experience. This is also consistent given the average longevity of an adult *V. vulgaris* wasp is two weeks (Spradbery, 1971). Spatial memory may have interfered with colour learning, particularly when maze arm positions were switched. As shown in *V. germanica*, wasps often rely on the spatial position of a reward rather than colour alone (Moreyra et al., 2017; Moreyra & Lozada, 2021). We switched the coloured arms to promote visual learning instead of spatial learning, which made it more challenging for wasps to find the reward. This was particularly observed in wasps trained under treatments Y2 and B2, where the maze configuration remained unchanged for three consecutive trials. Rapid choices toward previously rewarded positions in our trials suggest that older wasps may prioritize familiar spatial cues over new associations.

In contrast to finding an increase in learning abilities with age, as found previously in *M. cerberus* (da Silva et al., 2023), we did not find evidence for increased learning abilities in older workers. However, in *Polistes* wasps, young individuals exhibit early recognition of nestmates, even in the absence of olfactory cues (Signorotti et al., 2014). This could suggest an alternative trend of learning abilities early on, followed by an age-related cognitive decline as observed across animal taxa (Iliadi & Boulianne, 2010; Tonoki & Davis, 2015). Alternatively, older foragers may face cognitive constraints due to increased memory load (Moreyra & Lozada, 2021; Scheiner & Amdam, 2009). However, given the limited foraging range in our setup, true memory overload appears less likely, and reduced plasticity may instead reflect intrinsic age-related changes in brain function.

Furthermore, there seemed to be a difference in strategy between young and old workers, indicating a speed-accuracy trade-off. Older wasps completed trials faster but with lower accuracy, suggesting a shift toward a fast but error-prone decision strategy (Chittka et al., 2009; Stroeymeyt et al., 2010). Individual and within-individual speed-accuracy trade-offs have been previously found in bumblebees (Chittka et al., 2003). Such behaviour could reflect foraging-related pressures in natural contexts, where rapid decisions may outweigh perfect accuracy. In many social insects, ageing and task specialization are deeply intertwined, complicating interpretation. For example, Scheiner et al. (2009) demonstrated that cognitive performance in honeybees is more closely tied to foraging experience than chronological age. However, without controlling for individual foraging experience, the influence of experience versus age remains unclear (Ben-Shahar & Robinson, 2001).

In our study, we examined the effects of age on learning performance but did not directly assess task specialization, as in *V. vulgaris* there is an increase in foragers related to age in wasps reared in captivity (Ferreira et al., 2023). Similarly, our experimental conditions may have promoted accelerated behavioural maturation, given the small artificial colony size and limited brood, workers may have transitioned to foraging roles more rapidly than under natural conditions. While this design allowed us to include newly emerged individuals and knowing the ages and subject the wasps to similar environmental conditions, it lacked ecological validity compared with testing free-flying foragers. Furthermore, we used water as a neutral control, but using an aversive stimulus such as quinine could have enhanced learning performance, as shown in *Vespula* wasps (Dyer & Howard, 2023).

Short-term memory remains largely intact with ageing

By contrast, STM formation appeared largely resilient to aging effects. Despite lower learning performance, STM retention across two hours was largely consistent among age groups, with only 12-day-old wasps failing to perform above chance. This suggests that short-term memory remains relatively robust, although a 6-9days old declining trend was observed. These results mirror findings in honeybees and ants, where some cognitive functions remain resilient to ageing, while others, particularly new associative learning, decline (Behrends & Scheiner, 2010; Giraldo et al., 2016). Longer decision times predicted poor memory performance, with older wasps being faster but less accurate. This suggests individual, experience-based foraging decisions rather than coordinated recruitment (Lozada & D'Adamo, 2006, 2014; Moreyra et al., 2017) (Lozada & D'Adamo, 2006, 2014; Moreyra et al., 2017), highlighting the importance of memory in their natural behaviour, regardless of age.

Selective plasticity in the ageing brain

At the neural level, ageing was associated with increased MB and OL volumes, but these changes did not directly mirror cognitive performance. Despite the observed decline in the absolute total brain volume with age, our neuroanatomical analyses revealed the opposite trend at the structural level: relative volumes of most major neuropils (OL, AL, MB) increased, with the central complex (CX) remaining stable. The same pattern was less noticeable in absolute volume which showed only a non-significant positive trend with age, which suggests that age-related differences in relative investment are not driven by changes in total brain size. This pattern points to selective investment in sensory and integrative regions, likely reflecting the energetic costs of maintaining and expanding neural tissue involved in sensory processing or learning, which may in turn lead to compensatory reductions in less behaviourally relevant brain regions (Poissonnier et al., 2023). Similar region-specific plasticity has been reported in *Apis* and *Bombus*, where MB and OL growth is both age- and experience-dependent (Fahrbach et al., 1998; Jernigan et al., 2021; Jones et al., 2013; Withers et al., 1995). Work on solitary alkali bees further shows that experience rather than age enlarges MB subregions, reinforcing the primacy of adult experience (Hagadorn et al., 2021).

Since *V. vulgaris* workers start off in the nest and only transition to foraging tasks after a few days (Ferreira et al., 2023), we expected regions such as the optic lobes and mushroom body to increase in volume with age. Interestingly, the high early learning ability in young workers implies that MB and OL are already functionally competent before full structural maturity, consistent with findings in honeybees and *Polistes* wasps (Maleszka et al., 2009; Signorotti et al., 2014). Still, there was also a significant increase of the relative volume of the MB, similar to previous studies in honeybees, where the first week after emergence is marked by substantial structural plasticity in the mushroom body (Fahrbach et al., 1998; Withers et al., 1995). In this species, MB growth occurs both through intrinsic developmental processes and through experience-dependent mechanisms linked to foraging (Fahrbach et al., 1998; Withers et al., 1995). However, MB expansion alone does not necessarily predict improved learning performance (Maleszka et al., 2009), which may explain why older wasps, despite larger relative neuropils, exhibited reduced learning capacity. Prior studies have found that increases in MB volume are more experience-driven and not strictly age-related, as larger MB expansion is observed in foragers compared with age-matched nurse bees (Maleszka et al., 2009). Comparable patterns of early MB maturation were observed in *Bombus* bumblebees and *Polistes* wasps (Jernigan et al., 2021; Jones et al., 2013), and may represent a common strategy among insects with shorter lifespans and accelerated task transitions, as the case of the common wasp. Together with evidence from solitary alkali bees, these patterns suggest that experience-dependent MB plasticity is widespread and likely ancestral, while in complex eusocial species the age-related division of labour makes neural maturation more predictably age-correlated, driven by experience rather than chronological age per se (Fahrbach et al., 1998; Hagadorn et al., 2021).

Similarly to the MB, OL and AL showed a significant age-related change in relative volume, indicating that olfactory and visual processing centres are already developed early on in life, but seem to increase in importance with age. Again, this is similar to findings in honeybees where antennal lobe volume increased in an activity-dependent manner and was associated with improved associative learning performance (Sigg et al., 1997; Winnington et al., 1996). Analogous experience- and age-related expansions of visual neuropils have been documented in *Bombus* and *Apis* species, where increasing foraging activity drives sensory system plasticity (Fahrbach et al., 1998; Jones et al., 2013). Furthermore, Jernigan et al. (2021) also found that mature individuals had relatively larger visual regions of newly emerged workers in *P. fuscatus* (Jernigan et al., 2021). In contrast to all other brain regions, the CX did not vary with age, indicating that central integration centres remain stable across early adult development in *V. vulgaris*.

In conclusion, our study shows that in the common wasp *V. vulgaris*, visual learning ability declines with age while short-term memory remains comparatively stable. Although these findings did not support previous studies in *M. cerberus* showing increasing learning abilities with age (da Silva et al., 2023), it does suggest that it’s not the chronological age but rather the experience that controls learning and memory capacities (Scheiner & Amdam, 2009). Brain plasticity appears region-specific, supporting early functional readiness followed by selective structural growth in visual and olfactory centres. Due to our laboratory setup, individuals experienced a simplified environment and restricted foraging opportunities compared with individuals under natural conditions. This reduced exposure to navigational, sensory, and environmental challenges likely limited experience-driven neuroplasticity, particularly in brain regions such as the mushroom bodies or optic lobes. Studies in other social insects demonstrate that environmental complexity and experience strongly influence neural development: honeybee workers reared in social isolation and darkness show constrained mushroom body growth (Fahrbach et al., 1998), and exposure to multisensory stimuli enhances brain development in Bombus impatiens (Jones et al., 2013). Additionally, foraging under natural conditions exposes workers to nutritional variability that can influence brain development, as pollen availability shapes brain amino acid profiles thought to support neurodevelopment and neural plasticity across early adulthood in honeybees (Gage et al., 2020). These factors may therefore moderate the magnitude of age-related changes in brain volume observed in our study. At the same time, the controlled conditions allowed us to monitor all age classes and disentangle age-related effects from variation arising through task specialization or differing foraging experiences.

To our knowledge, this is the first study to simultaneously examine age-related variation in both learning and short-term memory and their neuroanatomy correlates in *V. vulgaris*. By integrating behavioural and neuroanatomical perspectives, our results highlight wasps as a valuable comparative model for understanding cognitive ageing in social insects. More specifically, future work could examine long-term memory and incorporate appetitive-aversive conditioning, which could result in more apparent differences between differently ages workers. Controlling for task specialization and foraging experience in a natural setup will also be critical for disentangling the roles of chronological age versus behavioural experience in shaping cognition. This will allow a critical test of whether cognitive ageing reflects a conserved constrained or an adaptive trajectory tuned by ecological demands and social organization.

List of abbreviations

AFT	Accelerated failure time model
AL	Antennal lobes
Al	Aluminium
CAL	Calyces

CS	Conditioned stimulus
CX	Central complex
DMSO	Dimethyl sulfoxide
GLM	Generalized linear model
kV	Kilovolt
L	Left
LA	Lamina
LO	Lobula
Lm	Linear model
LTM	Long-term memory
MB	Mushroom bodies
ME	Medulla
µA	Microampere
NB	Newborn
OC	Ocelli synaptic plexi
OL	Optic lobes
PBS	Phosphate Buffered Saline
PED	Peduncle
PFA	Paraformaldehyde
R	Right
S	Sugar water
STM	Short-term memory
W	Water

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Competing interests

The authors declare no conflict of interest.

Author contributions

AM and CAO conceived, TW and FSN supervised the study. AM, HC, KD, VDP and HMF were responsible for wasp nest collection. KD designed and constructed the Y-maze. Learning and memory trials were performed by AM and HC. Micro-CT scanning was done by AM, segmentation and manual annotation of the brain regions was done by HC with assistance of AM. Data analysis was done by HC with assistance of AM and TW. AM, HC, VDP and HMF wrote the first draft of the manuscript. All authors contributed to the revision and proofreading of the manuscript and approved the final version for submission.

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Data Availability

All datasets, supplementary material and R-script are available online at https://doi.org/10.17632/wxxkwjk9hm.3.

References

Agrawal, A. A. (2001). Phenotypic plasticity in the interactions and evolution of species. *Science*, *294*(5541), 321-326. https://doi.org/10.1126/science.1060701

Avarguès-Weber, A., d'Amaro, D., Metzler, M., Finke, V., Baracchi, D., & Dyer, A. G. (2018). Does holistic processing require a large brain? Insights from honeybees and wasps in fine visual recognition tasks. *Frontiers in Psychology*, *9*, 1313. https://doi.org/10.3389/fpsyg.2018.01313

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of statistical software*, *67*, 1-48. https://doi.org/10.18637/jss.v067.i01

Behrends, A., & Scheiner, R. (2010). Learning at old age: a study on winter bees. *Frontiers in Behavioral Neuroscience*, *4*, 1563. https://doi.org/10.3389/fnbeh.2010.00015

Ben-Shahar, Y., & Robinson, G. E. (2001). Satiation differentially affects performance in a learning assay by nurse and forager honey bees. *Journal of Comparative Physiology A*, *187*, 891-899. https://doi.org/10.1007/s00359-001-0260-z

Briscoe, A. D., & Chittka, L. (2001). The evolution of color vision in insects. *Annual Review of Entomology*, *46*(1), 471-510. https://doi.org/10.1146/annurev.ento.46.1.471

Brothers, D. J. (2021). Aculeate Hymenoptera: phylogeny and classification. In *Encyclopedia of Social Insects* (pp. 3-11). Springer. https://doi.org/10.1007/978-3-319-90306-4_1-1

Chittka, L. (1998). Sensorimotor learning in bumblebees: long-term retention and reversal training. *Journal of Experimental Biology*, *201*(4), 515-524. https://doi.org/10.1242/jeb.201.4.515

Chittka, L., Dyer, A. G., Bock, F., & Dornhaus, A. (2003). Bees trade off foraging speed for accuracy. *Nature*, *424*(6947), 388-388. https://doi.org/10.1038/424388a

Chittka, L., & Raine, N. E. (2006). Recognition of flowers by pollinators. *Current opinion in plant biology*, *9*(4), 428-435. https://doi.org/10.1016/j.pbi.2006.05.002

Chittka, L., Skorupski, P., & Raine, N. E. (2009). Speed–accuracy tradeoffs in animal decision making. *Trends in ecology & evolution*, *24*(7), 400-407. https://doi.org/10.1016/j.tree.2009.02.010

Collett, T. S., & Rees, J. A. (1997). View-based navigation in Hymenoptera: multiple strategies of landmark guidance in the approach to a feeder. *Journal of Comparative Physiology A*, *181*, 47-58. https://doi.org/10.1007/s003590050092

D'adamo, P., & Lozada, M. (2003). The importance of location and visual cues during foraging in the German wasp (Vespula germanica F.)(Hymenoptera: Vespidae). *New Zealand Journal of Zoology*, *30*(3), 171-174. https://doi.org/10.1080/03014223.2003.9518336

D'adamo, P., & Lozada, M. (2007). Foraging behavior related to habitat characteristics in the invasive wasp Vespula germanica. *Insect Science*, *14*(5), 383-388. https://doi.org/10.1111/j.1744-7917.2007.00165.x

D'adamo, P., & Lozada, M. (2009). Flexible foraging behavior in the invasive social wasp Vespula germanica (Hymenoptera: Vespidae). *Annals of the Entomological Society of America*, *102*(6), 1109-1115. https://doi.org/10.1603/008.102.0620 da Silva, R. C., Aguiar, J. M. R. B. V., Oi, C. A., Batista, J. E., Giurfa, M., & do Nascimento, F. S. (2023). Sex and lifestyle dictate learning performance in a neotropical wasp. *Iscience*, *26*(4). https://doi.org/10.1016/j.isci.2023.106469

DeZazzo, J., & Tully, T. (1995). Dissection of memory formation: from behavioral pharmacology to molecular genetics. *Trends in neurosciences*, *18*(5), 212-218. https://doi.org/10.1016/0166-2236(95)93905-D

Dyer, A. G., & Garcia, J. E. (2014). Color difference and memory recall in free-flying honeybees: forget the hard problem. *Insects*, *5*(3), 629-638. https://doi.org/10.3390/insects5030629

Dyer, A. G., & Howard, S. R. (2023). Aversive reinforcement improves visual discrimination learning in free-flying wasps (Vespula vulgaris). *Behavioral ecology and sociobiology*, *77*(9), 101. https://doi.org/10.1007/s00265-023-03375-x

Ehmer, B., & Gronenberg, W. (2002). Segregation of visual input to the mushroom bodies in the honeybee (Apis mellifera). *Journal of Comparative Neurology*, *451*(4), 362-373. https://doi.org/10.1002/cne.10355

El-Sayed, A. M., Jósvai, J. K., Brown, R. L., Twidle, A., & Suckling, D. M. (2018). Associative learning of food odor by social wasps in a natural ecosystem. *Journal of chemical ecology*, *44*, 915-921. https://doi.org/10.1007/s10886-018-0984-7

Fahrbach, S., Moore, D., Capaldi, E. A., Farris, S. M., & Robinson, G. E. (1998). Experience-expectant plasticity in the mushroom bodies of the honeybee. *Learning & Memory*, *5*(1), 115-123. https://doi.org/10.1101/lm.5.1.115

Fahrbach, S. E. (2006). Structure of the mushroom bodies of the insect brain. *Annu. Rev. Entomol.*, *51*, 209-232. https://doi.org/10.1146/annurev.ento.51.110104.150954

Fahrbach, S. E., & Robinson, G. E. (1996). Juvenile hormone, behavioral maturation, and brain structure in the honey bee. *Developmental neuroscience*, *18*(1-2), 102-114. https://doi.org/10.1159/000111474

Farris, S. M., Robinson, G. E., & Fahrbach, S. E. (2001). Experience-and age-related outgrowth of intrinsic neurons in the mushroom bodies of the adult worker honeybee. *Journal of Neuroscience*, *21*(16), 6395-6404. https://doi.org/10.1523/JNEUROSCI.21-16-06395.2001

Fenli, E., Mert, Ö., & Aksoy, V. (2025). The Role of Associative Learning in Ant Learning and Memory. *Journal of Insect Behavior*, *38*(1), 7. https://doi.org/10.1007/s10905-025-09871-4

Ferreira, H. M., Di Pietro, V., Wenseleers, T., & Oi, C. A. (2023). Conserved role of juvenile hormone in regulating behavioural maturation and division of labour in a highly eusocial wasp. *Animal Behaviour*, *200*, 59-69. https://doi.org/10.1016/j.anbehav.2023.03.013

Gage, S. L., Calle, S., Jacobson, N., Carroll, M., & DeGrandi-Hoffman, G. (2020). Pollen alters amino acid levels in the honey bee brain and this relationship changes with age and parasitic stress. *Frontiers in neuroscience*, *14*, 231. https://doi.org/10.3389/fnins.2020.00231

Giraldo, Y. M., Kamhi, J. F., Fourcassié, V., Moreau, M., Robson, S. K., Rusakov, A., Wimberly, L., Diloreto, A., Kordek, A., & Traniello, J. F. (2016). Lifespan behavioural and neural resilience in a social insect. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1822), 20152603. https://doi.org/10.1098/rspb.2015.2603

Giurfa, M. (2004). Conditioning procedure and color discrimination in the honeybee Apis mellifera. *Naturwissenschaften*, *91*, 228-231. https://doi.org/10.1007/s00114-004-0530-z

Giurfa, M. (2007). Behavioral and neural analysis of associative learning in the honeybee: a taste from the magic well. *Journal of Comparative Physiology A*, *193*(8), 801-824. https://doi.org/10.1007/s00359-007-0235-9

Giurfa, M. (2015). Learning and cognition in insects. *Wiley Interdisciplinary Reviews: Cognitive Science*, *6*(4), 383-395. https://doi.org/10.1002/wcs.1348

Gong, Z., Tan, K., & Nieh, J. C. (2019). Hornets possess long-lasting olfactory memories. *Journal of Experimental Biology*, *222*(13), jeb200881. https://doi.org/10.1242/jeb.200881

Gronenberg, W., Ash, L. E., & Tibbetts, E. A. (2007). Correlation between facial pattern recognition and brain composition in paper wasps. *Brain Behavior and Evolution*, *71*(1), 1-14. https://doi.org/10.1159/000108607

Gronenberg, W., Heeren, S., & Hölldobler, B. (1996). Age-dependent and task-related morphological changes in the brain and the mushroom bodies of the ant Camponotus floridanus. *Journal of Experimental Biology*, *199*(9), 2011-2019. https://doi.org/10.1242/jeb.199.9.2011

Habenstein, J., Grübel, K., Pfeiffer, K., & Rössler, W. (2023). 3D atlas of cerebral neuropils with previously unknown demarcations in the honey bee brain. *Journal of Comparative Neurology*, *531*(11), 1163-1183. https://doi.org/10.1002/cne.25486

Hagadorn, M. A., Johnson, M. M., Smith, A. R., Seid, M. A., & Kapheim, K. M. (2021). Experience, but not age, is associated with volumetric mushroom body expansion in solitary alkali bees. *Journal of Experimental Biology*, *224*(6), jeb238899. https://doi.org/10.1242/jeb.238899

Hansson, B. S., & Anton, S. (2000). Function and morphology of the antennal lobe: new developments. *Annual Review of Entomology*, *45*(1), 203-231. https://doi.org/10.1146/annurev.ento.45.1.203

Heisenberg, M. (2003). Mushroom body memoir: from maps to models. *Nature Reviews Neuroscience*, *4*(4), 266-275. https://doi.org/10.1038/nrn1074

Hölldobler, B., & Wilson, E. O. (1990). *The ants*. Harvard University Press.

Howard, S. R., & Dyer, A. G. (2024). Quantity misperception by hymenopteran insects observing the solitaire illusion. *Iscience*, *27*(2). https://doi.org/10.1016/j.isci.2023.108697

Iliadi, K. G., & Boulianne, G. L. (2010). Age-related behavioral changes in Drosophila. *Annals of the new York Academy of Sciences*, *1197*(1), 9-18. https://doi.org/https://doi.org/10.1111/j.1749-6632.2009.05372.x

Jackson, C. (2016). flexsurv: a platform for parametric survival modeling in R. *Journal of statistical software*, *70*, 1-33. https://doi.org/10.18637/jss.v070.i08

Jernigan, C. M., Zaba, N. C., & Sheehan, M. J. (2021). Age and social experience induced plasticity across brain regions of the paper wasp Polistes fuscatus. *Biology Letters*, *17*(4), 20210073. https://doi.org/10.1098/rsbl.2021.0073

Jones, B. M., Leonard, A. S., Papaj, D. R., & Gronenberg, W. (2013). Plasticity of the worker bumblebee brain in relation to age and rearing environment. *Brain, Behavior and Evolution*, *82*(4), 250-261. https://doi.org/10.1159/000355845

Kheradmand, B., Cassano, J., Gray, S., & Nieh, J. C. (2020). Influence of visual targets and landmarks on honey bee foraging and waggle dancing. *Insect Science*, *27*(2), 349-360. https://doi.org/10.1111/1744-7917.12651

Lacombrade, M., Doblas-Bajo, M., Rocher, N., Tourrain, Z., Navarro, E., Lubat, C., Vogelweith, F., Thiéry, D., & Lihoreau, M. (2023). Flexible visual learning in nectar-foraging hornets. *Behavioral ecology and sociobiology*, *77*(7), 76. https://doi.org/10.1007/s00265-023-03349-z

Leadbeater, E., & Chittka, L. (2007). Social learning in insects—from miniature brains to consensus building. *Current Biology*, *17*(16), R703-R713. https://doi.org/10.1016/j.cub.2007.06.012

Lenth, R. (2025). *emmeans: Estimated Marginal Means, aka Least-Squares Means_R package version 1.11.1*. In https://cran.r-project.org/web/packages/emmeans/index.html

Lozada, M., & D'Adamo, P. (2006). How long do Vespula germanica wasps search for a food source that is no longer available? *Journal of Insect Behavior*, *19*, 591-600. https://doi.org/10.1007/s10905-006-9045-0

Lozada, M., & D’Adamo, P. (2011). Past experience: a help or a hindrance to Vespula germanica foragers? *Journal of Insect Behavior*, *24*(2), 159-166. https://doi.org/10.1007/s10905-010-9244-6

Lozada, M., & D’Adamo, P. (2014). Learning in an exotic social wasp while relocating a food source. *Journal of Physiology-Paris*, *108*(2-3), 187-193. https://doi.org/10.1016/j.jphysparis.2014.05.006

Lucchetta, P., Bernstein, C., Théry, M., Lazzari, C., & Desouhant, E. (2008). Foraging and associative learning of visual signals in a parasitic wasp. *Animal Cognition*, *11*, 525-533. https://doi.org/10.1007/s10071-008-0144-5

Maleszka, J., Barron, A. B., Helliwell, P. G., & Maleszka, R. (2009). Effect of age, behaviour and social environment on honey bee brain plasticity. *Journal of Comparative Physiology A*, *195*, 733-740. https://doi.org/10.1007/s00359-009-0449-0

Manattini, M. C., Buteler, M., & Lozada, M. (2024). Cognitive abilities related to foraging behavior in Vespula vulgaris (Hymenoptera: Vespidae). *Current Research in Insect Science*, *6*, 100088. https://doi.org/10.1016/j.cris.2024.100088

Menzel, R. (1999). Memory dynamics in the honeybee. *Journal of Comparative Physiology A*, *185*(4), 323-340. https://doi.org/10.1007/s003590050392

Menzel, R., & Giurfa, M. (2001). Cognitive architecture of a mini-brain: the honeybee. *Trends in Cognitive Sciences*, *5*(2), 62-71. https://doi.org/10.1016/S1364-6613(00)01601-6

Molina, Y., & O'Donnell, S. (2008). Age, sex, and dominance-related mushroom body plasticity in the paperwasp Mischocyttarus mastigophorus. *Developmental Neurobiology*, *68*(7), 950-959. https://doi.org/10.1002/dneu.20633

Moreyra, S., D'adamo, P., & Lozada, M. (2017). Long-term spatial memory in Vespula germanica social wasps: the influence of past experience on foraging behavior. *Insect Science*, *24*(5), 853-858. https://doi.org/10.1111/1744-7917.12366

Moreyra, S., & Lozada, M. (2021). Spatial memory in Vespula germanica wasps: A pilot study using a Y-maze assay. *Behavioural Processes*, *189*, 104439. https://doi.org/10.1016/j.beproc.2021.104439

Muñoz-Galicia, D., Castillo-Guevara, C., & Lara, C. (2021). Innate and learnt color preferences in the common green-eyed white butterfly (Leptophobia aripa): experimental evidence. *PeerJ*, *9*, e12567. https://doi.org/10.7717/peerj.12567

O'Donnell, S., Donlan, N., & Jones, T. (2007). Developmental and dominance-associated differences in mushroom body structure in the paper wasp Mischocyttarus mastigophorus. *Developmental Neurobiology*, *67*(1), 39-46. https://doi.org/10.1002/dneu.20324

O'Donnell, S. (2019). Head-to-body size allometry in wasps (Vespidae): does brain housing constrain the evolution of small body sizes? *Insectes Sociaux*, *66*(4), 647-651. https://doi.org/10.1007/s00040-019-00715-x

O'Donnell, S., Bulova, S., Barrett, M., & von Beeren, C. (2018). Brain investment under colony-level selection: soldier specialization in Eciton army ants (Formicidae: Dorylinae). *BMC Zoology*, *3*, 1-6. https://doi.org/10.1186/s40850-018-0028-3

Papiorek, S., Junker, R. R., Alves-dos-Santos, I., Melo, G. A., Amaral-Neto, L. P., Sazima, M., Wolowski, M., Freitas, L., & Lunau, K. (2016). Bees, birds and yellow flowers: pollinator-dependent convergent evolution of UV patterns. *Plant biology*, *18*(1), 46-55. https://doi.org/10.1111/plb.12322

Poissonnier, L.-A., Tait, C., & Lihoreau, M. (2023). What is really social about social insect cognition? *Frontiers In Ecology And Evolution*, *10*, 1001045. https://doi.org/10.3389/fevo.2022.1001045

R Core Team. (2022). *R: A language and environment for statistical computing. R Foundation for Statistical Computing*. https://www.R-project.org/

Raine, N. E., & Chittka, L. (2007). The adaptive significance of sensory bias in a foraging context: floral colour preferences in the bumblebee Bombus terrestris. *PloS one*, *2*(6), e556. https://doi.org/10.1371/journal.pone.0000556

Richter, M. R. (2000). Social wasp (Hymenoptera: Vespidae) foraging behavior. *Annual Review of Entomology*, *45*(1), 121-150. https://doi.org/10.1146/annurev.ento.45.1.121

Rother, L., Kraft, N., Smith, D. B., El Jundi, B., Gill, R. J., & Pfeiffer, K. (2021). A micro-CT-based standard brain atlas of the bumblebee. *Cell and Tissue Research*, *386*, 29-45. https://doi.org/10.1007/s00441-021-03482-z

Rybak, J., Kuß, A., Lamecker, H., Zachow, S., Hege, H.-C., Lienhard, M., Singer, J., Neubert, K., & Menzel, R. (2010). The digital bee brain: integrating and managing neurons in a common 3D reference system. *Frontiers in systems neuroscience*, *4*, 30. https://doi.org/10.3389/fnsys.2010.00030

Scheiner, R., & Amdam, G. V. (2009). Impaired tactile learning is related to social role in honeybees. *Journal of Experimental Biology*, *212*(7), 994-1002. https://doi.org/10.1242/jeb.021188

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., & Schmid, B. (2012). Fiji: an open-source platform for biological-image analysis. *Nature methods*, *9*(7), 676-682. https://doi.org/10.1038/nmeth.2019

Schurmann, D., Sommer, C., Schinko, A. P., Greschista, M., Smid, H., & Steidle, J. L. (2012). Demonstration of long-term memory in the parasitic wasp Nasonia vitripennis. *Entomologia experimentalis et applicata*, *143*(2), 199-206. https://doi.org/10.1111/j.1570-7458.2012.01253.x

Sheehan, M. J., & Tibbetts, E. A. (2008). Robust long-term social memories in a paper wasp. *Current Biology*, *18*(18), R851-R852. https://doi.org/10.1016/j.cub.2008.07.032

Siegel, A. J., Fondrk, M. K., Amdam, G. V., & Page, R. E. (2013). In-hive patterns of temporal polyethism in strains of honey bees (Apis mellifera) with distinct genetic backgrounds. *Behavioral ecology and sociobiology*, *67*, 1623-1632. https://doi.org/10.1007/s00265-013-1573-y

Sigg, D., Thompson, C. M., & Mercer, A. R. (1997). Activity-dependent changes to the brain and behavior of the honey bee, Apis mellifera (L.). *Journal of Neuroscience*, *17*(18), 7148-7156. https://doi.org/10.1523/JNEUROSCI.17-18-07148.1997

Signorotti, L., Cappa, F., d’Ettorre, P., & Cervo, R. (2014). Novel insights into the ontogeny of nestmate recognition in Polistes social wasps. *PloS one*, *9*(5), e97024. https://doi.org/10.1371/journal.pone.0097024

Spradbery, J. (1971). Seasonal changes in the population structure of wasp colonies (Hymenoptera: Vespidae). *The Journal of Animal Ecology*, 501-523. https://doi.org/10.2307/3259

Stroeymeyt, N., Guerrieri, F. J., van Zweden, J. S., & d'Ettorre, P. (2010). Rapid decision-making with side-specific perceptual discrimination in ants. *PloS one*, *5*(8), e12377. https://doi.org/10.1371/journal.pone.0012377

Therneau, T. M., & Grambsch, P. M. (2000). The cox model. In *Modeling survival data: extending the Cox model* (pp. 39-77). Springer. https://doi.org/10.1007/978-1-4757-3294-8_3

Tibbetts, E. A. (2002). Visual signals of individual identity in the wasp Polistes fuscatus. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1499), 1423-1428. https://doi.org/10.1098/rspb.2002.2031

Tibbetts, E. A., Pandit, S., & Nondorf, D. (2018). Developmental plasticity and the origin of novel communication systems: Individual recognition in Polistes wasps. *Evolution*, *72*(12), 2728-2735. https://doi.org/10.1111/evo.13613

Tibbetts, E. A., Pardo-Sanchez, J., Ramirez-Matias, J., & Avarguès-Weber, A. (2021). Individual recognition is associated with holistic face processing in Polistes paper wasps in a species-specific way. *Proceedings of the Royal Society B*, *288*(1943), 20203010. https://doi.org/10.1098/rspb.2020.3010

Tonoki, A., & Davis, R. L. (2015). Aging impairs protein-synthesis-dependent long-term memory in Drosophila. *Journal of Neuroscience*, *35*(3), 1173-1180. https://doi.org/10.1523/JNEUROSCI.0978-14.2015

Tsuruda, J. M., & Page Jr, R. E. (2009). The effects of foraging role and genotype on light and sucrose responsiveness in honey bees (Apis mellifera L.). *Behavioural brain research*, *205*(1), 132-137. https://doi.org/10.1016/j.bbr.2009.07.022

Tumulty, J. P., Miller, S. E., Van Belleghem, S. M., Weller, H. I., Jernigan, C. M., Vincent, S., Staudenraus, R. J., Legan, A. W., Polnaszek, T. J., & Uy, F. M. (2023). Evidence for a selective link between cooperation and individual recognition. *Current Biology*, *33*(24), 5478-5487. e5475. https://doi.org/10.1016/j.cub.2023.11.032

Wickham, H. (2016). Data analysis. In *ggplot2: elegant graphics for data analysis* (pp. 189-201). Springer. https://doi.org/10.1007/978-3-319-24277-4_9

Wilson-Rankin, E. E. (2015). Level of experience modulates individual foraging strategies of an invasive predatory wasp. *Behavioral ecology and sociobiology*, *69*(3), 491-499. https://doi.org/10.1007/s00265-014-1861-1

Winnington, A. P., Napper, R. M., & Mercer, A. R. (1996). Structural plasticity of identified glomeruli in the antennal lobes of the adult worker honey bee. *Journal of Comparative Neurology*, *365*(3), 479-490. https://doi.org/10.1002/(SICI)1096-9861(19960212)365:3%3C479::AID-CNE10%3E3.0.CO;2-M

Withers, G. S., Fahrbach, S., & Robinson, G. (1995). Effects of experience and juvenile hormone on the organization of the mushroom bodies of honey bees. *Journal of neurobiology*, *26*(1), 130-144. https://doi.org/10.1002/neu.480260111

Yossen, M. B., Buteler, M., & Lozada, M. (2020). Foraging experience modulates response to aversive odour cues in social wasps. *Animal Behaviour*, *164*, 113-122. https://doi.org/10.1016/j.anbehav.2020.04.007

Figures

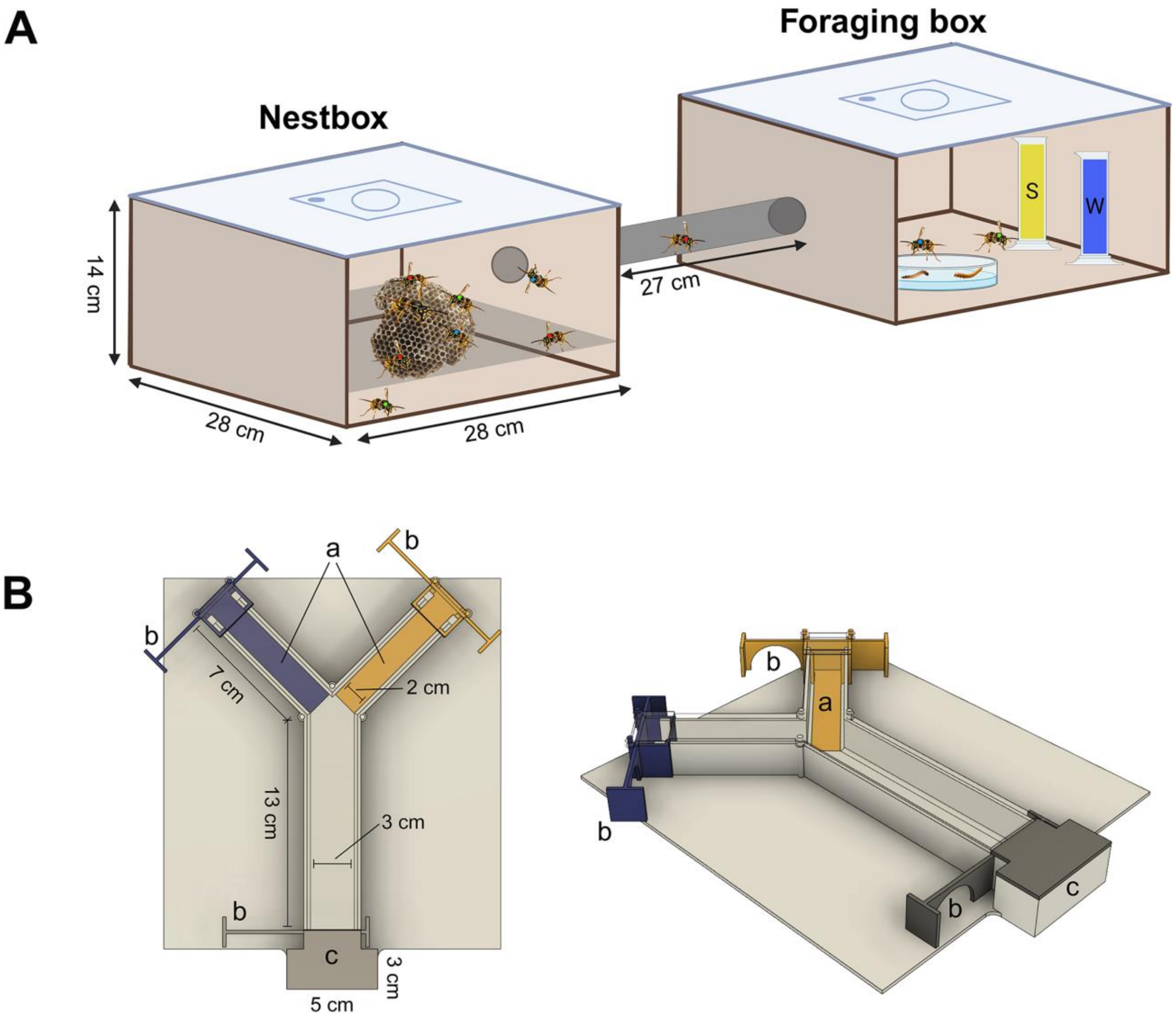


Fig. 1. (A) Laboratory colony setup for *Vespula vulgaris*. Each colony was housed in a wooden nestbox containing a single comb, the queen, 50–100 older workers and marked newly emerged workers. The nestbox was connected via a plastic tube to a foraging box supplied ad libitum with sugar water (S), water (W), mealworms, and nesting material. **(B)** 3D printed Y-maze including interchangeable coloured sides (a), sliding doors (b), and a holding chamber (c). The top is covered with plexiglass, allowing observation of behaviour and easy removal for cleaning in-between runs.

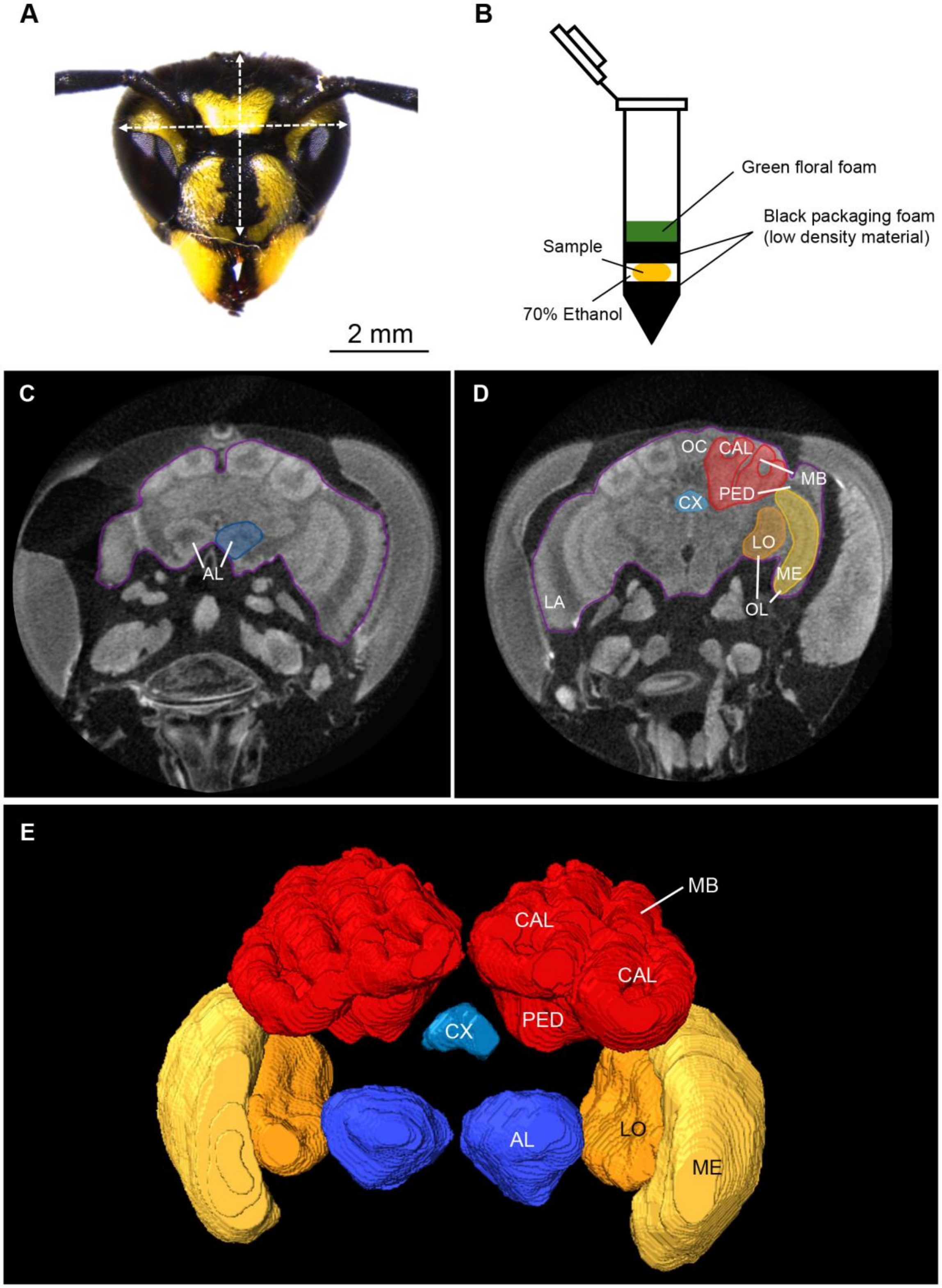

A
B
2 mm
Green floral foam
Black packaging foam
(low density material)
Sample
70% Ethanol
C
AL
D
OC
CAL
MB
PED
CX
LO
ME
LA
OL
E
MB
CAL
CAL
PED
CX
AL
LO
ME

Fig. 2. Overview of Micro-CT preparation and analysis of *Vespula vulgaris worker brains*. (A) Frontal view of a *Vespula vulgaris* worker head showing landmarks for head width and height measurements. Taken above the antennal sockets and from vertex to clypeus margin, respectively. **(B)** Micro-CT sample preparation in an Eppendorf tube, with heads submerged in 70% ethanol and stabilized using low-density black soundproofing material and green floral foam to prevent movement without interfere with image quality. **(C)** Frontal 2D slice at the level of the antennal lobes (AL). **(D)** Frontal 2D slice showing the position of the central brain within the head capsule, with neuronal tissues labelled, include the lamina (LA) and three ocellar synaptic plexi (OC), of which only one is visible. The purple contour outlines the total brain area used for total brain volume calculations. **(E)** 3D surface reconstruction of the brain from a frontal perspective, with neuronal tissue between the central complex (CX, light blue) and AL (dark blue) removed for visibility. Other neuropils: optic lobes (OL) including the lobula (LO, orange) and medulla (ME, yellow), mushroom bodies (MB, red) including two calyces (CAL) and a peduncle (PED).

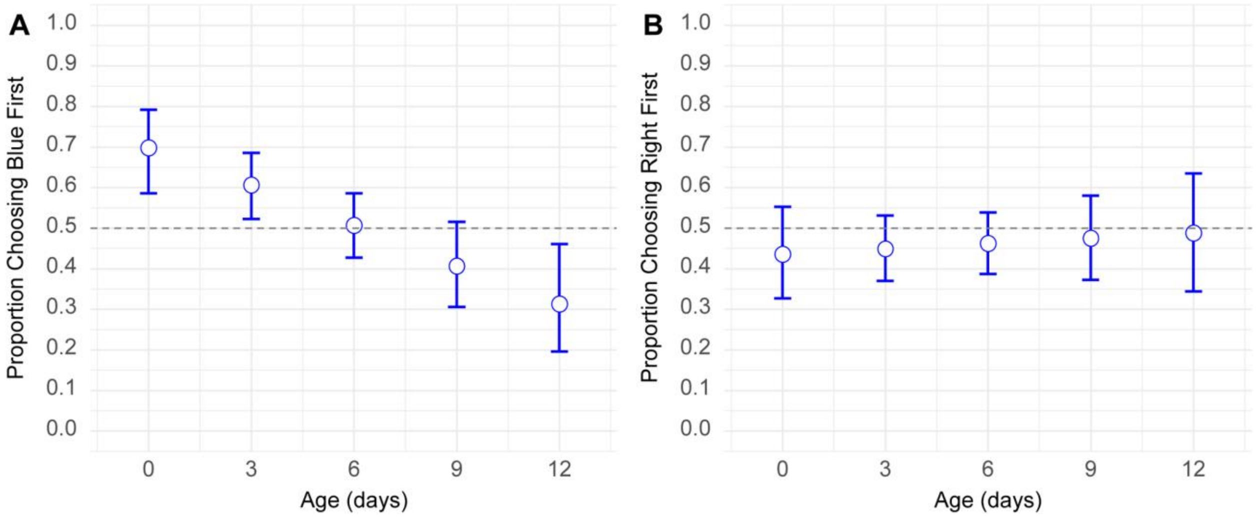


Fig. 3. Inherent bias in colour and side preference across ages. (A) Younger wasps (0 and 3d, n = 47 and n = 44, respectively) showed a significant bias toward blue (binomial GLM, *p* < 0.001), while 12-day-old wasps (n = 21) preferred yellow (bias against blue, *p* = 0.014). In contrast, 6-day-old and 9-day-old wasps (n = 34 and n = 30, respectively) showed no significant colour preference (binomial GLM, *p* = 0.091). **(B)** No inherent bias was observed in side choice across any age group (*p* = 0.64). Error bars indicate 95% confidence intervals. Significance levels based on comparisons to random choice (0.5).

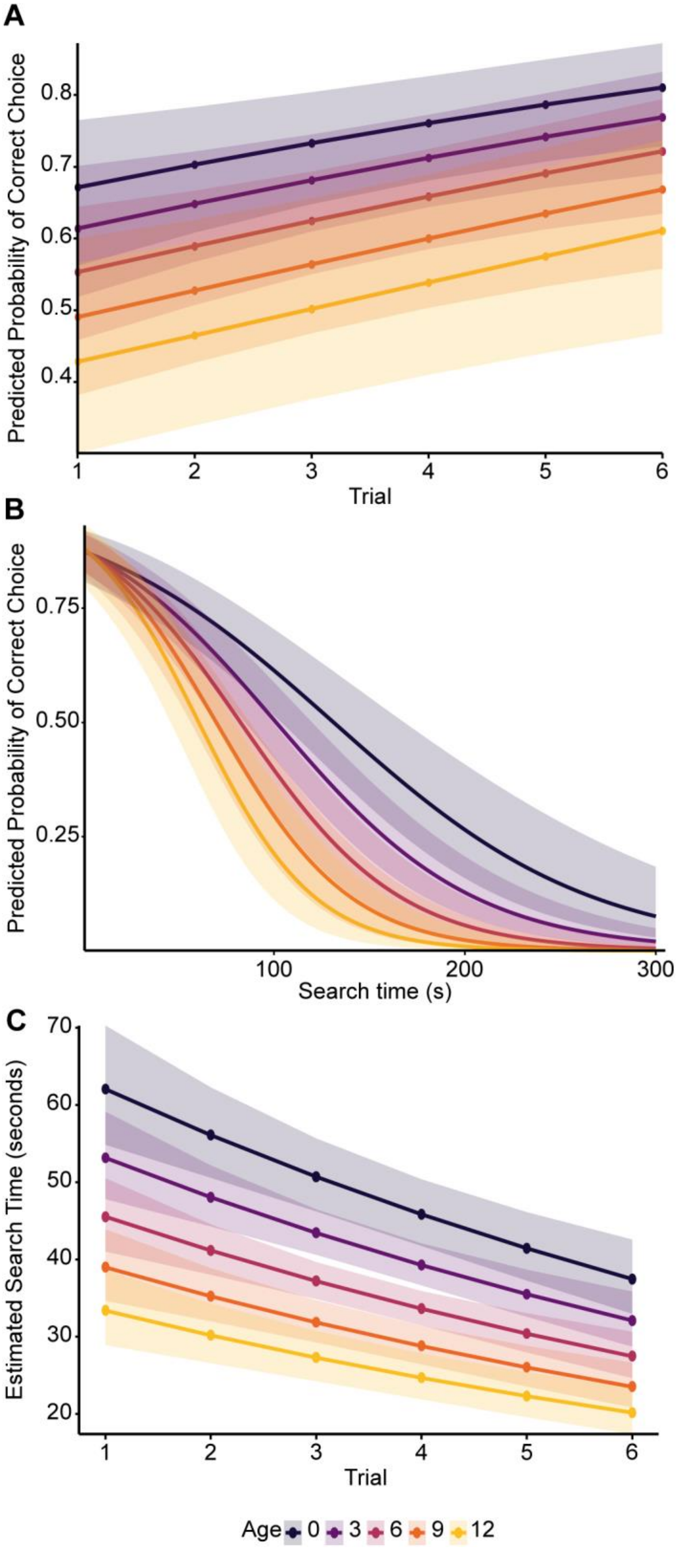

A
Predicted Probability of Correct Choice
0.8
0.7
0.6
0.5
0.4
1
2
3
4
5
6
Trial
B
Predicted Probability of Correct Choice
0.75
0.50
0.25
100
200
300
Search time (s)
C
Estimated Search Time (seconds)
70
60
50
40
30
20
1
2
3
4
5
6
Trial
Age
0
3
6
9
12

Fig. 4. Learning performance and search time during Y-maze conditioning trials in *Vespula vulgaris* workers (n = 176). Shaded areas indicate 95% confidence intervals. (A) Predicted probabilities of correct colour choice from a binomial GLMM show that accuracy increased with trial number but was lower in older workers ($p < 0.01$). (B) Probability of correct choice declined with increasing search time ($p < 0.001$), with a stronger negative effect in older individuals ($p < 0.01$). (C) Estimated search times from a parametric survival analysis decreased significantly across trials ($p < 0.001$) and with age ($p < 0.001$). Search time was measured from departure from the holding chamber to reward consumption.

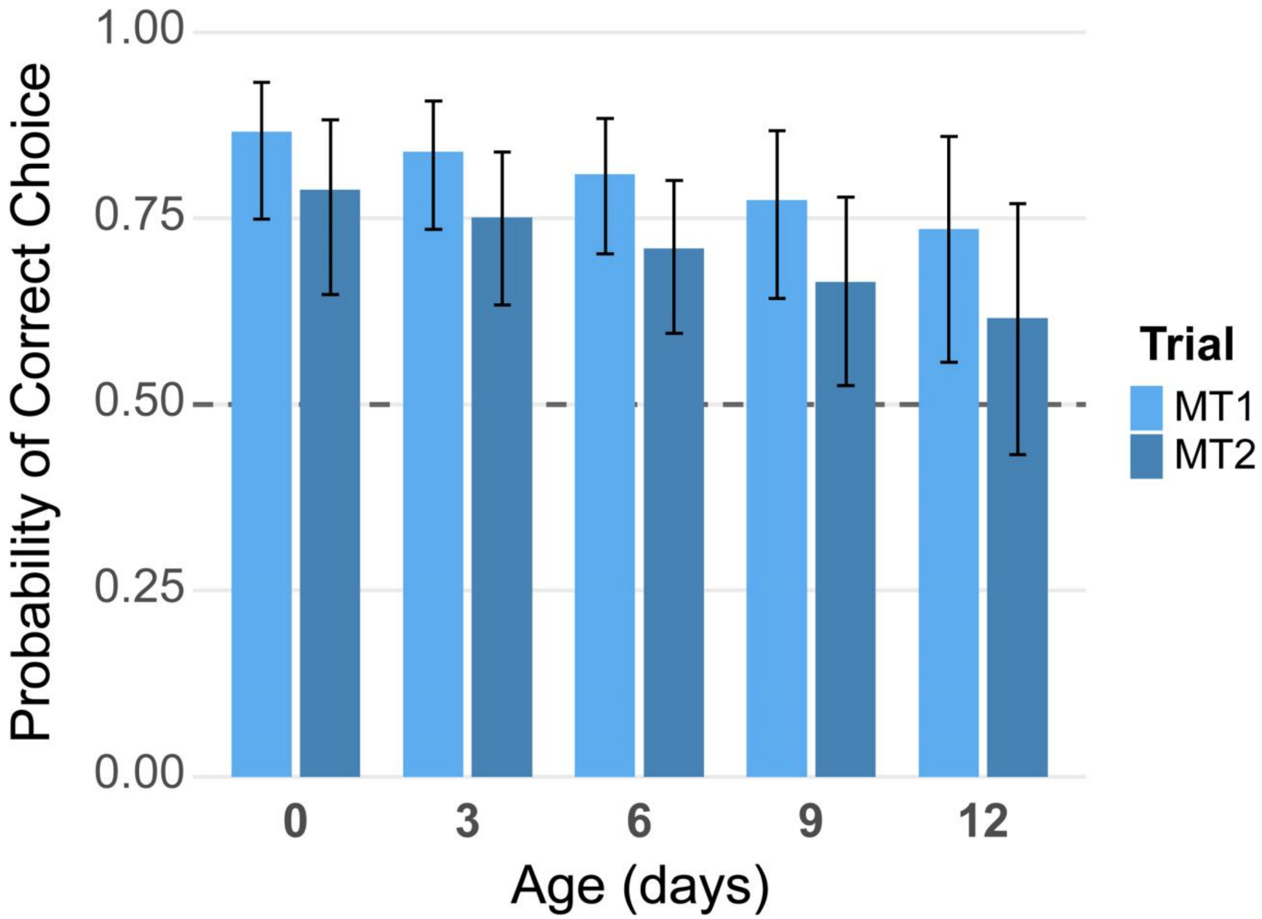


Fig. 5. Memory performance across age groups in *Vespula vulgaris*. Only individuals with ≥4 correct choices during conditioning were included (n = 108). Probability of correct choice was overall higher than chance (binomial GLMM, 76.6%, $p < 0.0001$), indicated by the dashed line. No significant differences in memory performance were detected across age groups ($p = 0.111$) or memory trial ($p = 0.125$). All ages were significantly different from random choice (0-3-6days old: $p < 0.001$; 9days old: $p = 0.04$), except 12day old ($p = 0.24$). Error bars represent 95% confidence intervals. Dashed line represents 50% chance level.

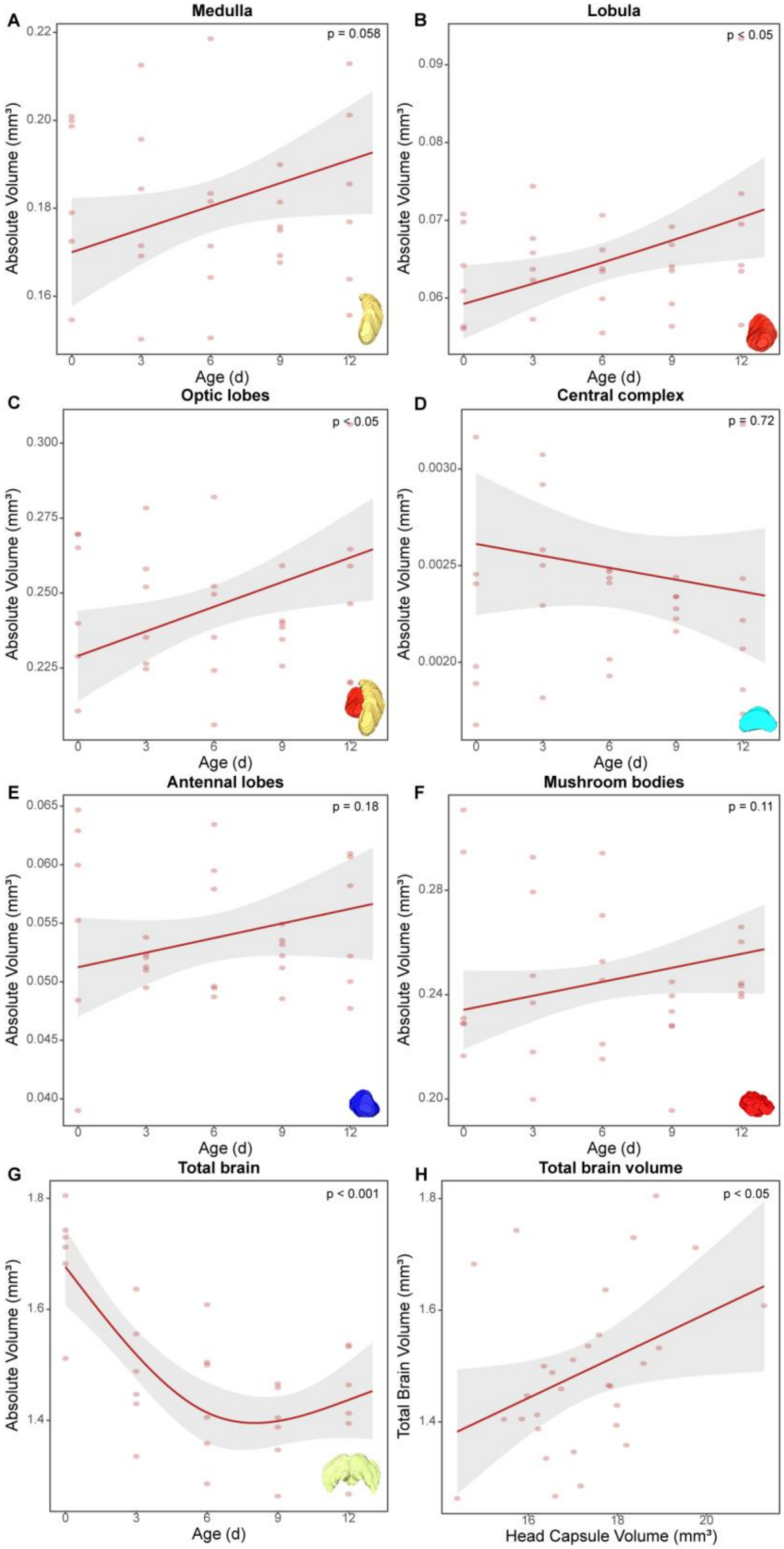

A
Medulla
p = 0.058
Absolute Volume (mm³)
0.22
0.20
0.18
0.16
Age (d)
0
3
6
9
12
B
Lobula
p < 0.05
Absolute Volume (mm³)
0.09
0.08
0.07
0.06
Age (d)
C
Optic lobes
p < 0.05
Absolute Volume (mm³)
0.300
0.275
0.250
0.225
Age (d)
D
Central complex
p = 0.72
Absolute Volume (mm³)
0.0030
0.0025
0.0020
Age (d)
E
Antennal lobes
p = 0.18
Absolute Volume (mm³)
0.065
0.060
0.055
0.050
0.045
0.040
Age (d)
F
Mushroom bodies
p = 0.11
Absolute Volume (mm³)
0.28
0.24
0.20
Age (d)
G
Total brain
p < 0.001
Absolute Volume (mm³)
1.8
1.6
1.4
Age (d)
H
Total brain volume
p < 0.05
Total Brain Volume (mm³)
1.8
1.6
1.4
16
18
20
Head Capsule Volume (mm³)

Fig. 6. Effect of age on the absolute volume (mm^3) of major neuropils in *Vespula vulgaris* (n = 30). Shown are model predictions from linear models (lm). **(A)** Medulla: marginally non-significant increase ($p = 0.058$). **(B)** Lobula: significant increase ($p < 0.05$). **(C)** Optic lobes: significant increase ($p < 0.05$). **(D)** Central complex: no significant result ($p = 0.72$). **(E)** Antennal lobes: no significant increase ($p = 0.181$). **(F)** Mushroom bodies: no significant increase ($p = 0.11$), and **(G) & (H)** Total brain volume, which decreased significantly with age ($p < 0.001$) and increased significantly with HCV ($p < 0.05$). 95% confidence intervals are given and each data point corresponds to an individual wasp.

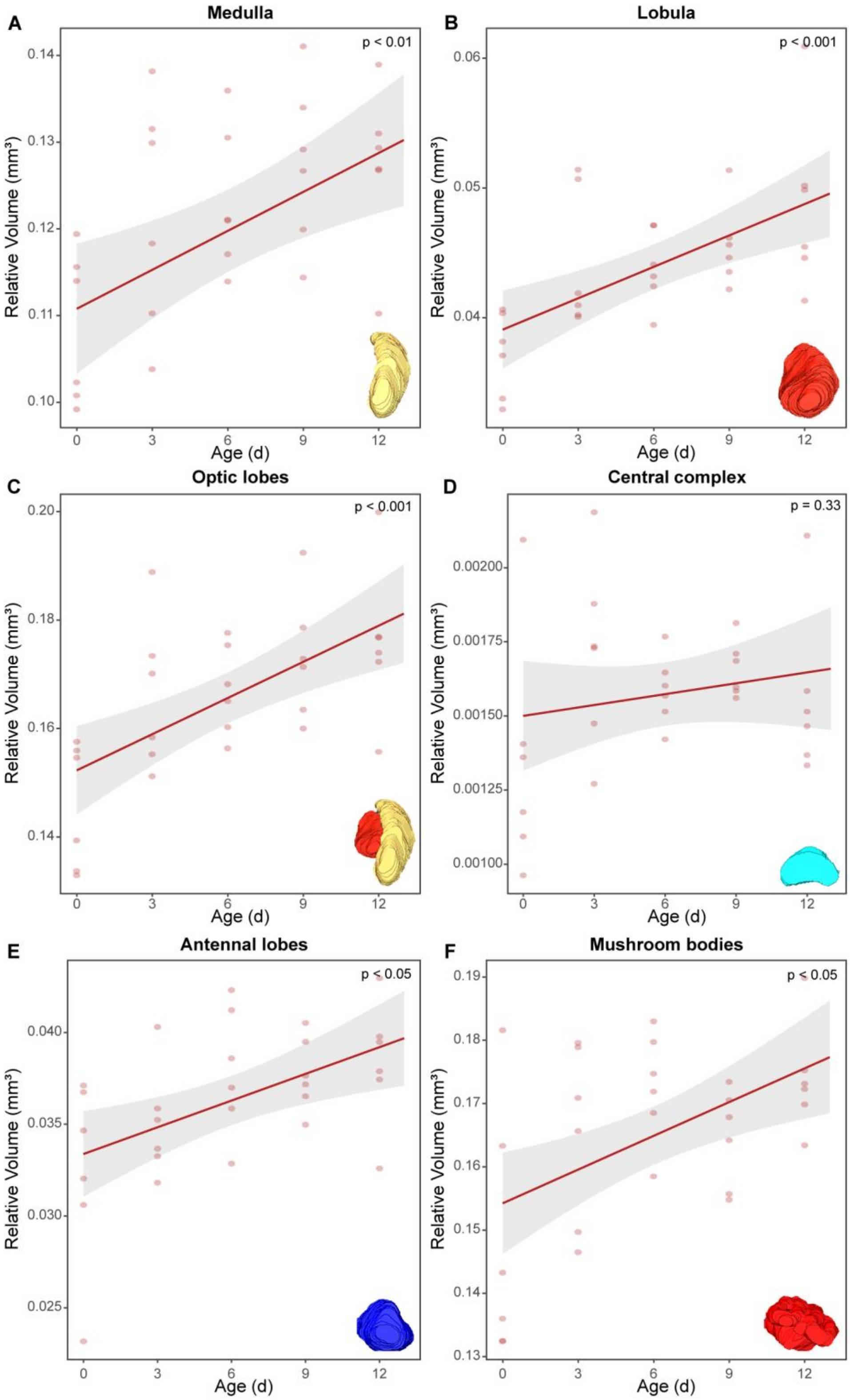

A
Medulla
p < 0.01
Relative Volume (mm³)
0.14
0.13
0.12
0.11
0.10
0
3
6
9
12
Age (d)
B
Lobula
p < 0.001
Relative Volume (mm³)
0.06
0.05
0.04
0
3
6
9
12
Age (d)
C
Optic lobes
p < 0.001
Relative Volume (mm³)
0.20
0.18
0.16
0.14
0
3
6
9
12
Age (d)
D
Central complex
p = 0.33
Relative Volume (mm³)
0.00200
0.00175
0.00150
0.00125
0.00100
0
3
6
9
12
Age (d)
E
Antennal lobes
p < 0.05
Relative Volume (mm³)
0.040
0.035
0.030
0.025
0
3
6
9
12
Age (d)
F
Mushroom bodies
p < 0.05
Relative Volume (mm³)
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0
3
6
9
12
Age (d)

Fig. 7. Effect of age on the relative volume (mm³) of major neuropils in *Vespula vulgaris* (n = 30). Shown are model predictions from linear models (lm). **(A)** Medulla: significant increase (p = 0.002). (**B)** Lobula: significant increase (p < 0.001). **(C)** Optic lobes: significant increase (p < 0.001). **(D)** Central complex: no significant result (p = 0.33). **(E)** Antennal lobes: significant increase (p = 0.004). **(F)** Mushroom bodies: significant increase (p = 0.002). 95% confidence intervals are given and each data point corresponds to an individual wasp.

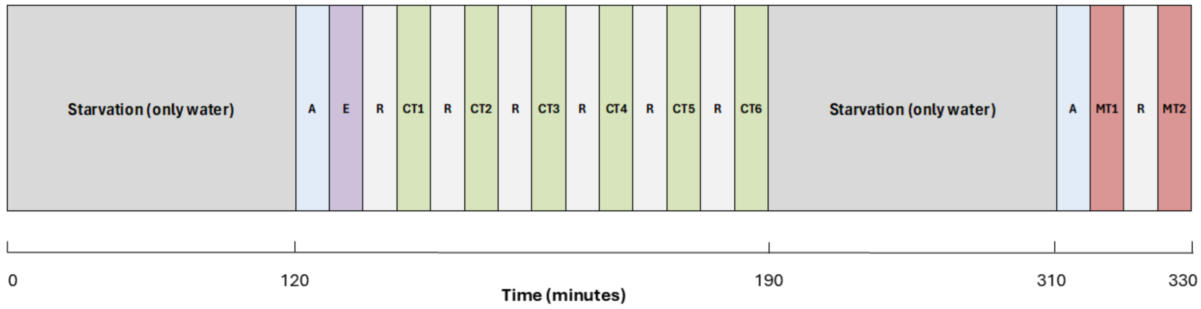


Fig S1. Visual conditioning procedure. Schedule showcasing the order of consecutive conditioning trials and memory testing for each individual wasp. First, the individual was isolated and starved for two hours (water available). Then, 5-minute-long periods of acclimatising (A), an explorative trial (E), conditioning trials (CT) and rest (R) followed each other. During periods of A or R, while the wasp was in the holding chamber, the maze was sprayed with ethanol to remove pheromone trails and the coloured arms were switched if necessary, according to the assigned treatment. Time in minutes is presented on a non-linear timescale.

Table S1. Number of workers trained with yellow or blue as the rewarded colour. Each age group was trained with both colour conditions, with at least 10 individuals per colour. The total number of trained wasps was 176.

Age	**Trained Colour**		
	B	Y	**Total**
NB	24	23	**47**
3	18	26	**44**
6	19	15	**34**
9	15	15	**30**
12	10	11	**21**
Total	**86**	**90**	**176**

Table S2. Number of wasps trained per treatment. All age group were trained across the eight treatments, except for the 12-day-old group, as insufficient individuals reached that age. The total number of trained individuals per age group was as follows: newborns (n = 47), 3 days (n = 44), 6 days (n = 34), 9 days (n = 30), 12 days (n = 21).

Age	**Treatment**							
	Y1	Y2	Y3	Y4	B1	B2	B3	B4
NB	8	6	6	3	8	6	6	4
3	4	8	4	10	4	8	3	3
6	4	2	3	6	4	4	4	7
9	2	6	4	3	2	6	4	3
12	5	/	4	2	3	/	3	4
Total	**23**	**22**	**21**	**24**	**21**	**24**	**20**	**21**

Table S3. Number of wasps categorized as learner or non-learner. Wasps with more than four correct choices during the conditioning trials were seen as learner, wasp with less than four correct choices were non-learners. Overall, there were more learners than non-learners for each age group. Only wasps that were seen as a learner were used for the subsequent analysis of the memory data.

Age	**Trained Colour**		
	Non-learners	Learners	**Total**
NB	16	31	**47**
3	20	24	**44**
6	12	22	**34**
9	11	19	**30**
12	7	14	**21**
Total	**66**	**110**	**176**

Table S4. Number of wasps removed during conditioning trails. Wasps that failed to perform two consecutive trials within 5 minutes were removed and replaced by new individuals of the same age group. These wasps either did not walk around or did not show any interest in the sugar reward, showing a lack of motivation. We show the number of wasps removed per total amount of wasps tested per age category.

Age	**Number of wasps removed after failing two trials**
NB	1/48
3	1/45
6	4/38
9	5/35
12	2/23

Table S5. Selection and measurement of head depth of 31 workers. Out of all wasps that completed the conditioning trials, 31 were randomly selected. Head depth was measured using Fiji (ImageJ 1.54p). All age groups are represented in this calculation, including 4 newly emerged individuals, 11 three-day-olds, 8 six-day-olds, 5 nine-day-olds, and 3 twelve-day-olds.

Wasp ID	Head depth (mm)
D3_18	2.086
D3_19	2,274
D3_21	2,182
D3_22	2.045
D3_17	2.120
D3_29	2.322
D3_30	2,201
D3_38	2,135
D3_39	2.442
D3_41	1.900
D3_42	2.036
D6_11	2.060
D6_12	1.828
D6_14	2.157
D6_19	2.074
D6_16	2.288
D6_18	2.284
D6_20	2.560
D6_28	2.338
D9_8	1.853
D9_15	2.244
D9_16	2.594
D9_17	2.642
D9_19	2.639
D12_10	2.545
D12_12	2.588
D12_14	2.610
NB_39	2.250
NB_45	2.430
NB_46	2.169
NB_47	2.209
Average	**2.261**

Dataset 1.

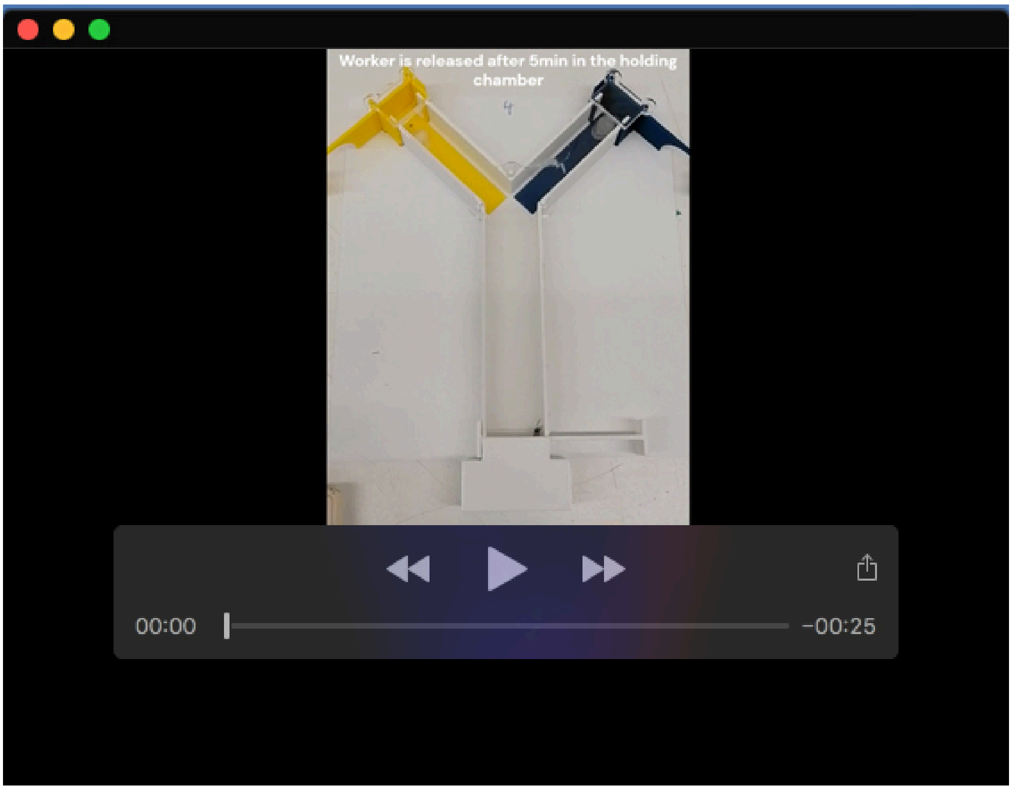
Available for download at
https://journals.biologists.com/jeb/article-lookup/doi/10.1242/jeb.251673#supplementary-data

Dataset 2. This file contains data from the learning and memory trials.

Available for download at
https://journals.biologists.com/jeb/article-lookup/doi/10.1242/jeb.251673#supplementary-data

Dataset 3. This file contains data from the measurements of the head, thorax and volume of the brain regions.

Available for download at
https://journals.biologists.com/jeb/article-lookup/doi/10.1242/jeb.251673#supplementary-data



Worker is released after 5min in the holding chamber

Movie 1. This video shows a conditioning trial of a 9-day-old *Vespula vulgaris* worker trained to associate the visual cue blue with a sugar reward. The wasp was confined in the holding chamber for 5 minutes before the start of the trial. When the door was opened, the search time was recorded from the moment the wasp fully entered the maze. Upon locating the sugar reward, the wasp was allowed to feed for 3 seconds, after which the reward cup was removed. The wasp could then continue exploring the maze until it returned to the holding chamber. Once inside, the door was closed, initiating another 5-minute resting period before the next trial.