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Experience, but Not Age, is Associated With Volumetric Mushroom Body Expansion in Solitary Alkali Bees

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RESEARCH ARTICLE

Experience, but not age, is associated with volumetric mushroom body expansion in solitary alkali bees

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ABSTRACT

In social insects, changes in behavior are often accompanied by structural changes in the brain. This neuroplasticity may come with experience (experience-dependent) or age (experience-expectant). Yet, the evolutionary relationship between neuroplasticity and sociality is unclear, because we know little about neuroplasticity in the solitary relatives of social species. We used confocal microscopy to measure brain changes in response to age and experience in a solitary halictid bee (*Nomia melanderi*). First, we compared the volume of individual brain regions among newly emerged females, laboratory females deprived of reproductive and foraging experience, and free-flying, nesting females. Experience, but not age, led to significant expansion of the mushroom bodies – higher-order processing centers associated with learning and memory. Next, we investigated how social experience influences neuroplasticity by comparing the brains of females kept in the laboratory either alone or paired with another female. Paired females had significantly larger olfactory regions of the mushroom bodies. Together, these experimental results indicate that experience-dependent neuroplasticity is common to both solitary and social taxa, whereas experience-expectant neuroplasticity may be an adaptation to life in a social colony. Further, neuroplasticity in response to social chemical signals may have facilitated the evolution of sociality.

KEY WORDS: Experience-dependent, Experience-expectant, Sociality, Mushroom body plasticity, *Nomia melanderi*

INTRODUCTION

Insect species living in cooperative societies have brains capable of changing with colony needs and in response to features of social life (Gronenberg et al., 1996; Jaumann et al., 2019; Molina and O'Donnell, 2007, 2008; O'Donnell et al., 2007; Rehan et al., 2015; Smith et al., 2010; Withers et al., 1993, 1995). This neuroplasticity, i.e. changes in neural structure and function over a lifetime (Kolb and Gibb, 2008), comes in two forms – experience-dependent and experience-expectant. Whether each form evolved prior to or in response to sociality is unknown.

Experience-dependent plasticity involves changes in brain architecture driven by experience. Mushroom bodies, paired cognitive processing centers in the insect brain, are associated with learning, memory and sensory integration (Gronenberg, 2001; Strausfeld et al., 2009). Previous studies have shown experience-based increases in mushroom body volume among insects in response to oviposition (van Dijk et al., 2017), foraging (Durst et al., 1994; Farris et al., 2001; Gronenberg et al., 1996; Ismail et al., 2006; Maleszka et al., 2009; Rehan et al., 2015; Withers et al., 1993, 1995, 2008) and social interactions (Jaumann et al., 2019; Molina and O'Donnell, 2007; O'Donnell et al., 2007; Rehan et al., 2015; Smith et al., 2010).

Experience-expectant plasticity occurs independent of experience, in anticipation of neural response to the environment. For instance, many workers in highly eusocial colonies progress through distinct behavioral phases with age (Robinson, 1992). This behavioral maturation includes an age-related shift from nest-oriented tasks, such as brood care, to work outside of the nest, such as foraging (Wilson, 1971). In various species of social bees (Durst et al., 1994; Fahrbach et al., 1998; Tomé et al., 2014; Withers et al., 1993, 1995), ants (Gronenberg et al., 1996) and wasps (O'Donnell et al., 2004), behavioral maturation is supported by changes in neural organization (e.g. mushroom body expansion) (Fahrbach et al., 1998; Fahrbach, 2006; Withers et al., 1993, 1995, 2008). These neuroanatomical changes are considered 'experience-expectant' because they occur before behavioral shifts, and can be induced in response to colony need (Durst et al., 1994; Withers et al., 1993). This type of neuroplasticity is thus considered a priming mechanism for the onset of new task performance associated with division of labor within eusocial colonies (Withers et al., 1993, 1995).

Both types of neuroplasticity occur in eusocial insects with large colonies and behavioral maturation (i.e. honey bees, stingless bees, ants and highly social paper wasps) (Durst et al., 1994; Fahrbach et al., 2003; Farris et al., 2001; Gronenberg et al., 1996; O'Donnell et al., 2004; Seid et al., 2005; Tomé et al., 2014; Withers et al., 1993, 1995), but the evolutionary relationship between experience-dependent, experience-expected plasticity, and social organization is unclear. Earlier studies suggest that experience-dependent plasticity may be a common feature of all bees and wasps, whether solitary or social. Central-place foraging, a complex task (Avarguès-Weber and Giurfa, 2013; Menzel et al., 1996; Menzel and Giurfa, 2001), is associated with mushroom body plasticity across the Hymenoptera (ants, bees, wasps) (Durst et al., 1994; Farris et al., 2001; Gronenberg et al., 1996; Ismail et al., 2006; Maleszka et al., 2009; Rehan et al., 2015; Withers et al., 1993, 1995, 2008). Honey bees exhibit mushroom body plasticity in response to foraging experience, which occurs independent of age (Durst et al., 1994; Farris et al., 2001; Withers et al., 1993) and persists after foraging ceases (Fahrbach et al., 2003). Additionally, foraging leads to mushroom body expansion in solitary (Withers et al., 2008) and facultatively social species (Rehan et al., 2015). This suggests that experience-dependent plasticity evolved prior to the evolution of eusociality.

However, life in a social colony provides unique experiences, and it is unknown whether neuronal sensitivity to social cues preceded or followed the evolution of eusociality. One of the most important

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types of experience in social insects is that coming from social interactions (Dunbar, 1998; Lihoreau et al., 2012; O'Donnell et al., 2011), whereby the need for flexible information processing may be necessary for adapting to the social environment (Menzel and Giurfa, 2001). Isolation leads to a lack of mushroom body development and behavioral impairment in carpenter ants (*Camponotus floridanus*) (Seid and Junge, 2016), and the evolutionary loss of sociality accompanies decreased mushroom body investment in the sweat bee *Augochlora pura* (Pahlke et al., 2020). Mushroom body plasticity is also associated with the maintenance of dominance in social wasps (Molina and O'Donnell, 2007; O'Donnell et al., 2007), facultatively eusocial sweat bees (Jaumann et al., 2019; Smith et al., 2010) and facultatively social small carpenter bees (Rehan et al., 2015). There is some evidence to suggest that solitary species may also exhibit neuronal sensitivity to social stimuli. For example, mushroom body plasticity accompanies high density larval rearing in *Drosophila melanogaster* (Heisenberg et al., 1995). Elucidating the relationship between neural sensitivity to social cues and social organization is important for identifying features that may have facilitated social evolution, yet this is unexplored in solitary Hymenoptera.

It is also unclear whether experience-expectant neuroplasticity is a developmental feature common across Hymenoptera or whether it is unique to species that exhibit advanced eusociality. In highly social species with large colonies and age-related task specialization, neuroanatomical changes precede the transition to foraging (Gronenberg et al., 1996; Seid and Wehner, 2009; Tomé et al., 2014; Withers et al., 1993, 1995, 2008). In honey bees, mushroom body enlargement coincides with the transition to work outside of the hive (Fahrbach, 2006; Farris et al., 2001; Ismail et al., 2006; Withers et al., 1993). However, in stingless bee workers (*Melipona quadrifasciata*), similar age-related changes occur very early in adult life, well before the behavioral transition to foraging (Tomé et al., 2014). Further, where division of labor is size-based (e.g. bumble bees), age-related plasticity happens within the first few days of life (Jones et al., 2013; Kraft et al., 2019; Riveros and Gronenberg, 2010). This indicates that experience-expectant neuroplasticity may be a common developmental feature of social insects (Withers et al., 2008), though the specific time scales vary with socioecological traits. Yet, research with facultatively social species have shown no effect of age (Jaumann et al., 2019), have not controlled for age (Smith et al., 2010), or leave doubt as to what extent changes are age-related (Rehan et al., 2015). Moreover, only one study has investigated experience-expectant neuroplasticity in a solitary Hymenoptera. In the orchard bee *Osmia lignaria*, age does not significantly impact mushroom body plasticity (Withers et al., 2008). However, because *O. lignaria* overwinter as adults, it is possible that experience-expectant change occurred before spring emergence (Withers et al., 2008). Therefore, the evolutionary relationship between eusociality and neuroplasticity remains inconclusive.

We investigated the effects of age, social environment and nesting experience on neuroplasticity in the solitary alkali bee *Nomia melanderi* (Halictidae). Several features of alkali bee biology make them well suited for investigating the relationship between neuroplasticity and social evolution. First, they overwinter as prepupa (Bohart and Cross, 1955), eliminating the possibility that age-related neuroplasticity occurs undetected in overwintering adults. Second, alkali bees belong to the subfamily Nomiinae (Wcislo and Engel, 1996), which is sister to the Halictinae, in which eusociality has evolved two or three times (Brady et al., 2006; Gibbs et al., 2012). The common ancestor of these clades was likely solitary (Danforth, 2002), thus alkali bees may harbor traits shared with the ancestor that gave rise to sociality. Alkali bees also exhibit characteristics considered to be pre-adaptations of sociality, including nesting gregariously (when individual nests are clustered close together) (Cane, 2008) and extended maternal care (tending to eggs in the nest) (Batra and Bohart, 1969; Batra, 1970). As such, alkali bees are a useful solitary model for testing hypotheses regarding the origins of eusociality (Kapheim, 2017; Kapheim and Johnson, 2017a,b).

To explore relationships between social evolution and neuroplasticity, we tested for experience-dependent and experience-expectant neuroplasticity in *N. melanderi*. If *N. melanderi* have experience-dependent plasticity, mushroom body volume will increase with foraging. Therefore, we predicted mushroom body investment to increase with foraging and nesting experience in alkali bees relative to females kept in the laboratory. Additionally, in the absence of foraging, if social stimuli influence mushroom body plasticity, we predicted that alkali bees sharing a cage with another female should have significantly increased mushroom body investment relative to bees kept alone. Finally, if *N. melanderi* exhibit experience-expectant plasticity, they should have increases in mushroom body volume with age, even when kept alone without foraging. If experience-expectant neuroplasticity is absent in alkali bees, it could indicate that this feature is unique to social taxa, and potentially evolved as an adaptive response to social complexity. We found that experience, but not age, led to increases in mushroom body volume, suggesting that neuronal sensitivity to experience preceded the evolution of sociality in bees.

MATERIALS AND METHODS

Study sites

We conducted this study in Touchet, WA, USA, between 27 May and 19 June 2016 (Experiment 1) and 29 May and 27 June 2018 (Experiment 2). In the Touchet Valley, alkali bees [*Nomia melanderi* (Cockerell 1906)] nest in large, salty soil beds near alfalfa seed fields (Cane, 2008). For Experiment 1, we collected adult female alkali bees from three previously established bee beds, all within 5 km of one another. For Experiment 2, we used two of these three bee beds (3 km apart).

Field collections

The collection and rearing methods for both experiments are identical to that of Kapheim and Johnson (2017b), but repeated here briefly. We captured newly emerged females (<24 h old) leaving their natal nests for the first time after emerging from diapause by placing traps over bee beds known to host large nesting aggregations. Nesting, reproductive females were collected in nets and identified as those carrying pollen on their hind legs, which indicates that they were provisioning offspring.

After collection, we transported bees back to the laboratory in 15 ml conical tubes placed in a cooler between single layers of cardboard flanked by ice packs to keep bees cool, but not anesthetized. In the laboratory, we kept bees in cages constructed from cylindrical, perforated plastic containers [72×113 mm (upper diameter) and ×90 mm (lower diameter)]. We provided *ad libitum* sugar water with pollen mixture [2.5 g of finely ground honey bee pollen (Betterbee, Greenwich, NY, USA) homogenized in 30 ml of 35% (w/v) sucrose solution] that we changed every other day. We maintained cages between 22 and 28°C at 40–85% relative humidity under a 13 h:11 h light:dark cycle (Kapheim and Johnson, 2017a).

Experiment 1: experience-expectant and experience-dependent neuroplasticity

Experiment 1 samples were a subset of those used in a previous study (Kapheim and Johnson, 2017b). We randomly assigned newly

Journal of Experimental Biology (2021) 224, jeb238899. doi:10.1242/jeb.238899

emerged bees to one of two treatment groups: (1) newly emerged or (2) laboratory reared. We killed newly emerged bees (N=7) upon return to the laboratory, whereas laboratory-reared bees (N=7) were kept in individual cages with *ad libitum* food for 10 days. We collected data on two additional laboratory-reared females (a total of nine). These two females, however, were given alfalfa flowers in addition to sugar water and pollen. Because this stimulus was absent in other laboratory-reared bees, we removed these samples (A18.01 and H4.03) prior to analyses to eliminate the potential for this as a confounding factor. We compared newly emerged and laboratory-reared females with nesting, reproductive females (N=7) of unknown age.

We used newly emerged bees and laboratory-reared females to assess age-related neuroplasticity. These females lacked foraging and nest-construction experience, and were unmated, as confirmed by the absence of sperm in their spermathecae. Newly emerged bees served as a baseline for volumetric measurements. Nesting females had mating (sperm present in their spermathecae), foraging and nest-construction experience. We compared nesting females with newly emerged and laboratory-reared females to explore experience-dependent neuroplasticity.

Experiment 2: socially induced experience-dependent neuroplasticity

In this experiment, we tested whether living with another bee affected neuroanatomical plasticity. We randomly assigned newly emerged bees to either the solitary (solo) or paired treatment group. Solo females (N=17) were kept alone for 10 days, and paired females (N=23) were given a nesting female cage-mate for the same duration. For paired bees, we measured neuroplasticity only in the focal (10 days old) female, and did not dissect the brains of the female cage-mates. Rearing conditions were as for Experiment 1, except that we provided bees with a daily sprig of fresh alfalfa flowers to avoid an olfactorily barren environment. We paint-marked the dorsal thorax of all bees with enamel paints (Testors Corporation, Rockford, IL, USA) for identification, including solo bees to control for the effects of paint-marking (Packer, 2005).

Sample preparation, microscopy and volumetric measurements

We chilled individuals at 4°C for 5 min prior to decapitation. For Experiment 1, we removed the mouthparts after bees were immobilized, whereas for Experiment 2 we also removed eye capsules. We preserved head capsules in 4% paraformaldehyde (PFA) (Alfa Aesar, Ward Hill, MA, USA) in 1× phosphate-buffered saline (PBS) (Ambion, Austin, TX, USA) at 4°C until dissection. We rinsed head capsules in 1× PBS (3×10 min) after removal from PFA and conducted dissections in 1× PBS using a Leica EZ4 HD stereomicroscope (Leica Microsystems, Buffalo Grove, IL, USA). Using 2% glutaraldehyde (Sigma-Aldrich, St Louis, MO, USA), we post-fixed dissected brains at room temperature for 48 h. Next, we rinsed brains in 1× PBS (3×10 min) and then bleached them in a formamide solution [1× PBS, 3% formamide (Thermo Fisher Scientific, Rockford, IL, USA), 1% Triton-X (Sigma-Aldrich) and 20% hydrogen peroxide (Fisher Scientific, Fair Lawn, NJ, USA)] (modified protocol from Zukor et al., 2010), which decreased the effects of shadowing during autofluorescence owing to residual pigment. We bleached brains from Experiments 1 and 2 for an average of 75 and 35 min, respectively. Post-bleaching, we rinsed brains in 1× PBS (3×10 min) prior to serial dehydration through a series of ascending ethanol concentrations (30%, 50%, 70%, 90%, 95% and 3×100%, 10 min each). We cleared and stored brains in methyl salicylate (Fisher Scientific) at −20°C until imaging.

We imaged whole brains using autofluorescence and laser scanning confocal microscopy at 10× magnification (Zeiss LMS 710, Jena, Germany) while mounted in methyl salicylate (Fig. 1A). Scanning included 5 µm intervals, with steps imaged in 3×2 tile scans (2867×1946 pixels) ultimately combined to form image stacks ranging from approximately 700 to 900 µm. We imaged brains simultaneously using two lasers, the first of which had a wavelength, laser power and a range of gains designated as 410–485 nm, 4.0–3.5, and between 510 and 557, respectively. The second used a wavelength of 495–538 nm, 3.5–3.0 laser power, and

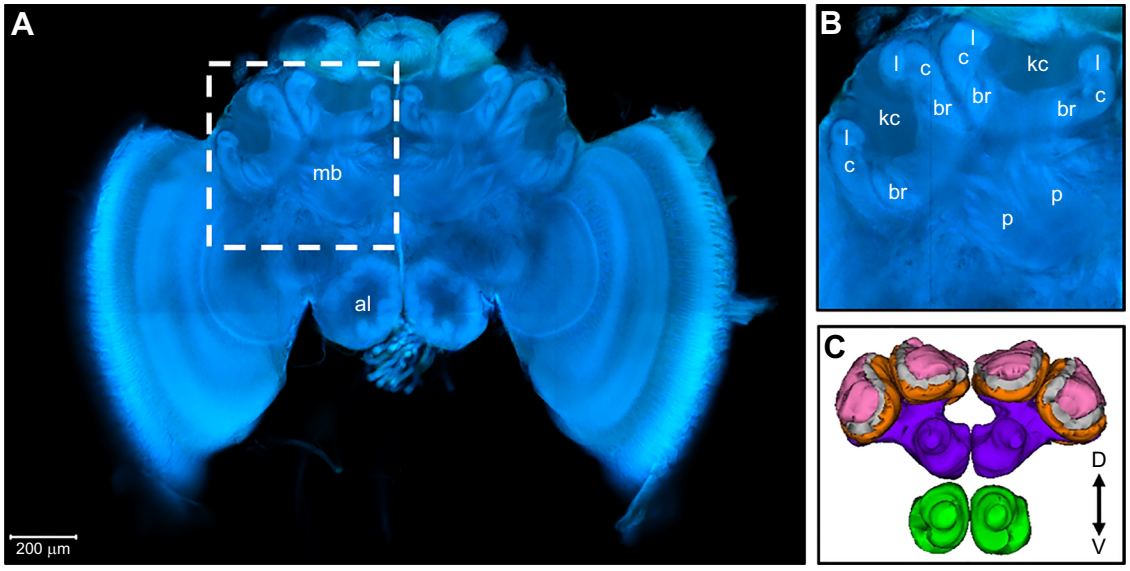

Fig. 1. *Nomia melanderi* confocal microscopy brain image. (A) A 5 µm optical slice taken in the frontal plane. Mushroom bodies (mb) and antennal lobes (al) are visible. The dotted line represents the mushroom body region enlarged in B to show the Kenyon cells (kc), lip (l), collar (c), basal ring (br) and pedunculus (p). (C) Three-dimensional serial reconstruction of individual traces. Volumetric measurements between treatments were compared for the Kenyon cells (pink), lips (gray), collar (orange), mushroom body lobes (basal ring+peduncle+ventral lobe+medial lobe; purple) and antennal lobes (green). D, dorsal; V, ventral.

Journal of Experimental Biology (2021) 224, jeb238899. doi:10.1242/jeb.238899

a gain range including 500–548. The pinhole was maintained between 6.00 and 7.00 airy units.

Mushroom bodies include Kenyon cells, calyces and lobes (Fahrbach, 2006). Kenyon cell dendrites innervate the lip and collar, calyx subregions associated with olfactory and visual input, respectively (Fahrbach, 2006; Gronenberg, 2001), and their axons form peduncles that branch into the distinct lobes (Fahrbach, 2006).

We generated volumetric measurements for the whole brain and five neuroanatomical structures, including the lip and collar, mushroom body lobes (basal ring, peduncle and lobes as one structure), Kenyon cells and antennal lobes (Fig. 1) using serial reconstruction [Reconstruct software (Fiala, 2005), Version 1.1.0.0, http://synapses.clm.utexas.edu]. The basal ring is a structure of the calyx; however, owing to image quality, and to promote consistency, this structure was traced with the peduncle and included as a component of the mushroom body lobes. Additionally, because of occasional damage to the outer edge of optic lobes, whole-brain traces always exclude the lamina and retina. Experience-expectant neuroplasticity is associated with increases in neuropil relative to Kenyon cell (N:K) volume (Fahrbach, 2006; Withers et al., 1993, 1995, 2008). Therefore, we also calculated N:K ratios.

Experiments 1 and 2 trace intervals were every 5 and 10 µm optical slice, respectively. We randomized samples and traced them blind to treatment group. For each sample, we standardized structure volumes to the whole brain by calculating structure:whole brain ratios, which is referred to as 'relative volumes'.

Statistical analyses

We used R version 3.6.1 (https://www.r-project.org/) to conduct all statistical analyses. We assessed relative volumes for each structure (lip, collar, mushroom body lobes, Kenyon cells and antennal lobes) and N:K ratios. We used Anderson–Darling normality tests (Nortest, version 1.0-4; https://CRAN.R-project.org/package=nortest) and visual inspections of qq-plots (car, version 3.0-3; Fox and Weisberg, 2019) to detect significant departures from normality. One variable – relative lip volume for Experiment 1 – failed to meet normality assumptions. Therefore, we applied a Box–Cox transformation (MASS, version 7.3-51.4; Venables and Ripley, 2002) based on the optimal value $\lambda=-0.364$. We verified homogeneity of variance using the R package car (version 3.0-3; Fox and Weisberg, 2019).

For Experiment 1, we used ANOVAs followed by Tukey *post hoc* tests (multcomp, version 1.4-10; Hothorn et al., 2008) to evaluate the effects of treatment on relative volumes of brain regions and N:K (stats, version 3.6.1). For Experiment 2, we conducted Student's *t*-tests (stats, version 3.6.1) to compare sample means between solo and paired bees for each brain region and N:K. We assessed significance at $\alpha=0.05$.

RESULTS

Experiment 1: experience-expectant and experience-dependent neuroplasticity

We found evidence for experience-dependent, but not experience-expectant, neuroplasticity in female alkali bees. Relative volume of the mushroom body neuropil ($F_{2,18}=20.50$, $P=2.29\times10^{-05}$; Fig. 2A) and N:K ($F_{2,18}=15.83$, $P=1.08\times10^{-04}$; Fig. 2B) was significantly different among groups. In both cases, nesting females had significantly larger values than newly emerged and laboratory-reared bees, but newly emerged and laboratory-reared females were not significantly different (Fig. 2). We did not find significant differences in Kenyon cell ($F_{2,18}=0.64$, $P=0.54$; Fig. 2A), antennal lobe ($F_{2,18}=3.01$, $P=0.07$; Fig. 2A) or whole brain ($F_{2,18}=0.13$, $P=0.88$) relative volumes across treatment groups.

Experience also had a significant effect on the relative volumes of calyx substructures and mushroom body lobes (includes basal ring, peduncle, ventral lobe and medial lobe). Relative lip ($F_{2,18}=30.52$, $P=1.65\times10^{-06}$), collar ($F_{2,18}=12.82$, $P=3.46\times10^{-04}$) and total calyx ($F_{2,18}=24.86$, $P=6.63\times10^{-06}$) volumes were significantly different among treatment groups (Fig. 3). Tukey's pairwise comparisons showed no significant differences in these structures between newly emerged and laboratory-reared bees, but nesting females had significantly larger relative volumes compared with the other two groups (Fig. 3). Additionally, there was a significant effect of treatment on mushroom body lobe relative volume ($F_{2,18}=10.81$, $P=8.24\times10^{-04}$; Fig. 3). Nesting females had significantly larger

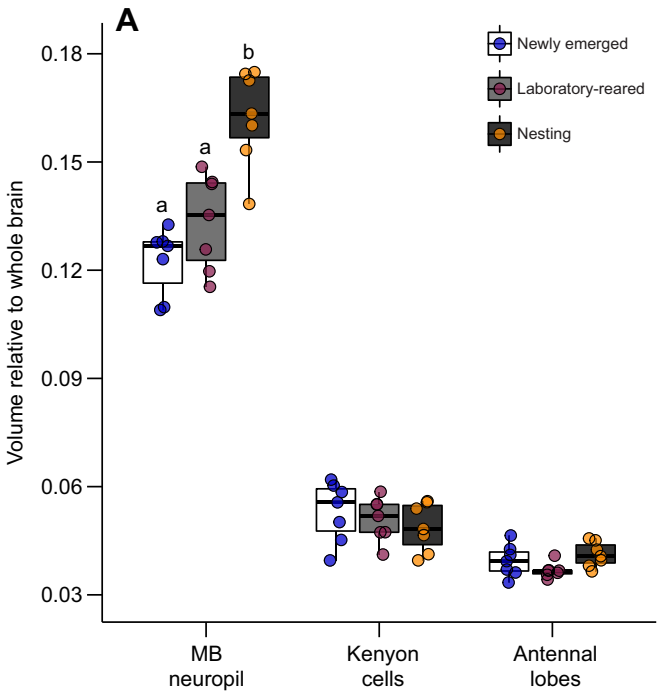

Fig. 2. Effects of age and nesting experience on alkali bee neuroplasticity. (A) Mushroom body neuropils ($F_{2,18}=20.50$, $P=2.29\times10^{-05}$) were larger in females with nesting experience, whereas no significant differences were found in the Kenyon cells (neural cell bodies) ($F_{2,18}=0.64$, $P=0.54$) or antennal lobes ($F_{2,18}=3.01$, $P=0.07$). Volumes are reported as proportions of the whole brain. (B) Neuropil:Kenyon cell ratio also varied with treatment group ($F_{2,18}=15.83$, $P=1.08\times10^{-04}$). Different letters indicate significant differences between groups ($P<0.05$ in Tukey *post hoc* tests). Boxes represent the interquartile range, with the lines as medians. Whiskers extend to 1.5 times the interquartile range. Filled circles are individual data points. Treatment groups included newly emerged (NE; white boxes; blue circles; $N=7$), 10 days laboratory-reared (LR; gray boxes; dark red circles; $N=7$) and nesting (NS; dark gray boxes; orange circles, $N=7$) females.

Journal of Experimental Biology (2021) 224, jeb238899. doi:10.1242/jeb.238899

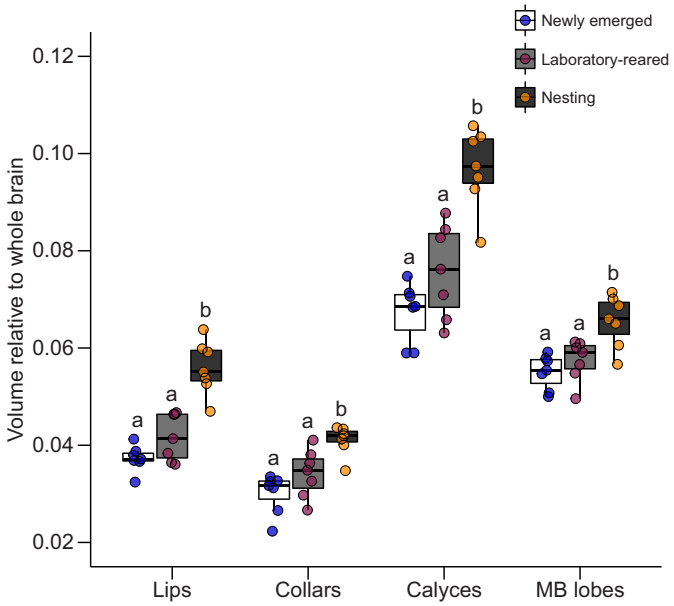

Fig. 3. Effects of age and nesting experience on alkali bee mushroom body subregions. Relative volume of the lip ($F_{2,18}$=30.52, P=1.65×10^{-06}), collar ($F_{2,18}$=12.82, P=3.46×10^{-04}), calyx (lip+collar; $F_{2,18}$=24.86, P=6.63×10^{-06}) and mushroom body lobes (basal ring+peduncle+ventral lobe+medial lobe; $F_{2,18}$=10.81, P=8.24×10^{-04}) were significantly larger in nesting (dark gray boxes, orange circles; N=7) compared with 10 days laboratory-reared (gray boxes, dark red circles; N=7) and newly emerged females (white boxes, blue circles; N=7). Volumes are reported as proportion of the whole brain. Different letters indicate significant differences between groups (P<0.05 in Tukey *post hoc* tests). Boxes represent the interquartile range, with the lines as medians. Whiskers extend to 1.5 times the interquartile range. Filled circles are individual data points.

mushroom body lobes than newly emerged and laboratory-reared bees, but the latter two groups were not significantly different (Fig. 3).

Experiment 2: socially induced neuroplasticity

We found that social environment significantly impacts brain investment in female alkali bees. Relative volumes of total neuropil (t=−1.23, d.f.=38, P=0.23), Kenyon cells (t=−0.30, d.f.=38, P=0.77), antennal lobes (t=−0.79, d.f.=38, P=0.44) and N:K (t=−1.01, d.f.=38, P=0.32) did not differ significantly between solo and paired bees (Fig. 4). Social environment did not significantly affect relative volume of the collar (t=0.22, d.f.=38, P=0.82), total calyces (t=−1.73, d.f.=38, P=0.09) or mushroom body lobes (t=0.33, d.f.=38, P=0.74) (Fig. 5). However, relative volume of the mushroom body lip was significantly larger in females housed with a cage-mate than those reared alone (t=−2.90, d.f.=38, P=0.01; Fig. 5). Mean whole brain volumes did not differ significantly between the two groups (t=−1.32, d.f.=38, P=0.19).

DISCUSSION

We found that in solitary alkali bees, as with social bees, mushroom bodies expand in response to adult experience. Remarkably, this includes social experience, which suggests that the ancestors of social bees may have been pre-wired to respond to the cues of social partners – a critical component of sociality. Females with foraging experience also had significantly enlarged mushroom bodies, a finding consistent with other bee species studied. Lastly, our results suggest that solitary bees do not have experience-expectant neuroplasticity, indicating that this phenomenon may have evolved as an adaptive response to age-related changes in task performance among highly eusocial species.

Unlike in eusocial species, where tasks are distributed across castes (Michener, 1974; Wilson, 1971), reproductively mature female solitary bees must manage multiple tasks simultaneously. This includes mating, nest construction, navigation and foraging activities, all of which may be cognitively demanding. We found that these experiences led to brain changes in alkali bees, such that free-flying, nesting females had significantly enlarged mushroom bodies relative to females with limited experiences. Our findings corroborate those of a study with solitary orchard bees (*Osmia lignaria*), which found that foraging experience significantly influenced mushroom body investment (Withers et al., 2008). Together, these results suggest that adult experience is an important driver of neuroplasticity in both solitary and social species.

Social experience also leads to neuroanatomical changes in alkali bees. We found that *N. melanderi* individuals paired with a cage-mate had significantly greater lip volume – the calyx subregion primarily associated with olfactory input (Gronenberg, 2001) – relative to those reared alone. Alkali bees from both our solo and paired treatment groups were exposed to olfactory stimuli, including natal nest odors and alfalfa in their housing containers, indicating that the increased calyx lip volume was associated specifically with stimuli present in the social environment. Though we cannot determine whether the lip expansion was driven by enhanced olfactory stimulus in general or was specific to social signals, this result does suggest that the common ancestor of solitary and social bees may have been capable of responding at the neurological level to olfactory cues from conspecifics.

While our study was not designed to differentiate between specific social stimuli, viewing the results in light of socially relevant tasks, such as communication, is intriguing. Communication is critical for coordinating social behaviors in a colony (Blum, 1996; Leonhardt et al., 2016), and social insects must be able to discriminate various recognition cues, some of which are olfactory (Leonhardt et al., 2016). Therefore, sensory systems that could recognize nestmate from non-nestmate may have been particularly important for facilitating the earliest stages of social life (d'Ettorre et al., 2017). But, as social complexity increases, communication requirements expand to include information from the social environment, such as task allocation, defense and food acquisition (Blum, 1996; Leonhardt et al., 2016). Social bees invest more in their peripheral olfactory nervous system (antennal sensilla) than their solitary relatives (Wittwer et al., 2017), presumably to facilitate chemical recognition and communication. It is thus unsurprising that social experience in our experiment led to enlargement in the mushroom body region dedicated to processing chemosensory input.

Solitary bees are similar to many other insects in that they rely on chemical cues to recognize their nests, prospective mates and potential resources (Anzenberger, 1986; Cane, 1997; Falibene et al., 2015; Guédot et al., 2006; Leonhardt et al., 2016; Shimron et al., 1985; Wcislo, 1992; Wenseleers and van Zweden, 2017). It is therefore possible that the neurological response to conspecifics we observed could represent selection on cognitive sensitivity to novel resources associated with mating, nesting, foraging or other cognitive tasks unrelated to sociality. However, alkali bees routinely encounter conspecifics. While they are non-social in that each female provisions her own nest, alkali bees live in dense aggregations up to 100 nests per square meter (Cane, 2008; Johansen et al., 1978). Hence, nesting females must be able to recognize their nest among a dense collection of others. Alkali bees use vision for nest recognition (Hackwell, 1967), but may use olfaction as well, because olfactory cues are

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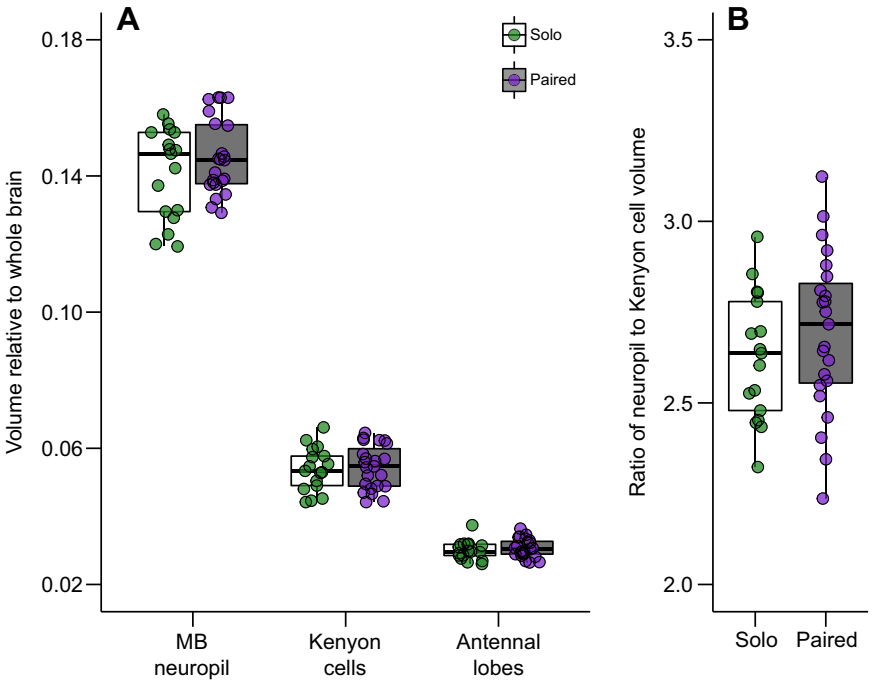

Fig. 4. Effects of social environment on alkali bee neuroplasticity. (A) Relative volumes of mushroom body neuropil (t=−1.23, d.f.=38, P=0.23), Kenyon cells (t=−0.30, d.f.=38, P=0.77) and antennal lobes (t=−0.79, d.f.=38, P=0.44) were not significantly different between females reared alone ('solo') and with a cage-mate ('paired'). Volumes are reported as proportion of the whole brain. (B) Neuropil:Kenyon cell ratio also did not differ significantly between groups (t=−1.01, d.f.=38, P=0.32). Boxes represent the interquartile range, with the lines as medians. Whiskers extend to 1.5 times the interquartile range. Filled circles are individual data points. Treatment groups included solo (white boxes, green circles; N=17) and paired (gray boxes, purple circles; N=23) females.

important for nest recognition in other densely aggregated, solitary ground-nesting bees (Shimron et al., 1985; Wcislo, 1992). Therefore, neuroplasticity in the lip region of the mushroom bodies likely represents functionally relevant neurological responses to socially associated stimuli. Thus, an interpretation of our results that emphasizes selection for response to novel olfactory cues is consistent with the hypothesis that neurological sensitivity to olfactory cues from the social environment is a pre-adaptation for the evolution of sociality.

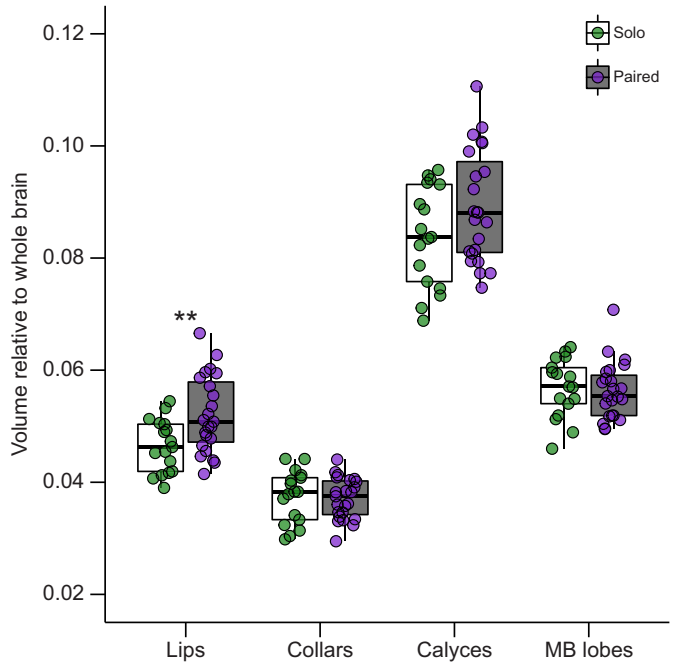

Fig. 5. Effects of social environment on alkali bee mushroom body subregions. Relative lip volume was significantly larger (t=−2.90, d.f.=38, P=0.01) in females reared with a cage-mate (paired; gray boxes, purple circles; N=23) than those kept alone (solo; white boxes, green circles; N=17). Collar (t=0.22, d.f.=38, P=0.82), calyx (lip+collar; t=−1.73, d.f.=38, P=0.09) and mushroom body lobe (basal ring+peduncle+ventral lobe+medial lobe; t=0.33, d.f.=38, P=0.74) volumes did not significantly differ between the two groups. Volumes are reported as proportion of the whole brain. Boxes represent the interquartile range, with the lines as medians. Whiskers extend to 1.5 times the interquartile range. Filled circles are individual data points. **P<0.01.

Dominance or aggressive interactions between our paired females may have also contributed to alkali bee calyx plasticity. Social dominance induces brain plasticity across social insects (Jaumann et al., 2019; Molina and O'Donnell, 2007, 2008; O'Donnell et al., 2007, 2017; Rehan et al., 2015; Smith et al., 2010), and calyx enlargement is associated with high dominance rank and increased aggression in wasps (Molina and O'Donnell, 2007, 2008; O'Donnell et al., 2007, 2017). Aggressive behaviors have been reported in alkali bees in laboratory tests (Smith et al., 2019) and while observing nesting conflict (Batra, 1970; Hackwell, 1967). However, there is no evidence that *Nomia* form dominance hierarchies, and we did not explicitly measure dominance or aggressive behaviors among our caged bees. Moreover, aggressive interactions were relatively rare in forced associations of *N. melanderi* (26% of all behaviors) (Smith et al., 2019). Thus, dominance and aggression are unlikely to be the primary drivers of the lip expansion observed in socially caged bees.

Experience-expectant neuroplasticity is likely an adaptive response to age-related systems of division of labor and task allocation, which is not possible in solitary bees. Our results complement previous research to suggest that this is a phenomenon unique to social species. Age-related plasticity was not detected in the solitary bee *O. lignaria*, suggesting that the brain may be 'pre-wired' at emergence (Withers et al., 2008). These results were inconclusive, however, because this species overwinters as adults (Bosch and Kemp, 2000), during which time neuronal reorganization could occur undetected (Withers et al., 2008). Alkali bees diapause as prepupa (Bohart and Cross, 1955; Hackwell, 1967), making them a useful species for investigating experience-expectant neuroplasticity, while facilitating robust comparisons across differing life history strategies. In our study, mushroom body neuropil volume and N:K ratio increased with age, but the differences between newly emerged and laboratory-reared

bees were not statistically significant, potentially owing to a large amount of within-group variance. Interestingly, N:K ratios in alkali (2.35:1) and orchard bees (2.31:1) at emergence were similar to those of behaviorally mature honey bee foragers (2.1:1) (Withers et al., 1993, 2008). This could indicate that solitary bees emerge with brains ready for navigation and foraging, tasks that both orchard and alkali bees perform almost immediately upon emergence (Bohart and Cross, 1955; Hackwell, 1967; Withers et al., 2008).

An alternative explanation for our findings is that experience-expectant plasticity occurs after 10 days post-emergence. However, 10 days is approximately 25% of the alkali bee 5 week adult lifespan, and encompasses the period during which most females begin nest building and provisioning (Hackwell, 1967; Pitts-Singer, 2008). This suggests that experience-expectant neuroplasticity is unlikely to occur beyond 10 days.

It is also possible that brain development may occur in the nest prior to emergence above ground (Withers et al., 2008). Rapid calyx plasticity is observed in *Drosophila* 6 h post-eclosion (Barth and Heisenberg, 1997). Adult alkali bees can spend 3–4 days hardening their exoskeleton prior to emerging from their brood cells (Bohart and Cross, 1955). Thus, this may be a period of age-related plasticity undetectable by our methods. If solitary bees do undergo intrinsically driven neuroplasticity before emergence, this would suggest that the evolution of age-related division of labor is accompanied by a shift in timing of experience-expectant plasticity.

Overall, our results suggest that experience-expectant plasticity, as seen in extant eusocial insects, may not have been present in the solitary ancestor of social halictid bees, but it may be an adaptive response to social life. It is not clear whether closely related social halictine bees exhibit experience-expectant neuroplasticity, or even the age polyethism with which it is typically associated. There is mixed evidence for age-associated neuroplasticity in the facultatively eusocial sweat bee *Megalopta genalis*, where females nest either solitarily or in a small social colony, but do not exhibit age-related task specialization (Smith et al., 2007; Wcislo et al., 2004). Young *M. genalis* females had smaller mushroom bodies relative to social queens and solitary reproductives, but age was not explicitly controlled for (Smith et al., 2010). However, a follow-up study found no effect of age on mushroom body development, contrasting the prior finding (Jaumann et al., 2019). A brain ready to engage in all tasks at emergence may be more adaptive for species that maintain totipotency, as is often associated with sociality in halictine bees (Michener, 1974, 1990). Additional research in other social halictines is needed to clarify the evolutionary relationship between sociality and experience-expectant neuroplasticity.

Conclusions

Neuroplasticity in insects is associated with foraging and many aspects of social behavior, including task specialization and dominance interactions. Because most studies have focused on understanding these relationships in social taxa, it is unknown whether neural plasticity is a pre-adaptation or adaptive response to social evolution. We did not find evidence of experience-expectant neuroplasticity in solitary alkali bees, suggesting that this form of plasticity may have evolved with sociality. Conversely, nesting and foraging experience, as well as social interactions, induce neuroplasticity in both solitary and social species. This suggests that experience-dependent plasticity is a conserved trait in bees, and that mushroom body plasticity in the area responsible for processing chemosensory stimuli may have been an important pre-adaptation to sociality.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: M.A.H., K.M.K., A.R.S., M.A.S.; Methodology: M.A.H., A.R.S., M.A.S., K.M.K.; Formal analysis: M.A.H., K.M.K.; Investigation: M.A.H., M.M.J., K.M.K.; Resources: A.R.S., M.A.S., K.M.K.; Data curation: M.A.H.; Writing - original draft: M.A.H., K.M.K.; Writing - review & editing: M.M.J., A.R.S., M.A.S., K.M.K.; Visualization: M.A.H.; Supervision: K.M.K.; Project administration: M.A.H.; Funding acquisition: M.A.H., A.R.S., M.A.S., K.M.K.

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Data availability

All volumetric data are available from Dryad (Hagadorn et al., 2021): dryad.xksn02vfc. Relevant code is stored in GitHub: www.github.com/kapheimlab/nomia_neuroplasticity.

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