The Science of Nature (2019) 106:49
https://doi.org/10.1007/s00114-019-1644-7

SHORT COMMUNICATION

Brain differences between social castes precede group formation in a primitively eusocial bee

Sarah Pahlke[1] · Sarah Jaumann[1] · Marc A. Seid[2] · Adam R. Smith[1]

Received: 9 April 2019 / Revised: 20 July 2019 / Accepted: 5 August 2019

Abstract

Social interactions may shape brain development. In primitively eusocial insects, the mushroom body (MB), an area of the brain associated with sensory integration and learning, is larger in queens than in workers. This may reflect a strategy of neural investment in queens or it may be a plastic response to social interactions in the nest. Here, we show that nest foundresses—the reproductive females who will become queens but are solitary until their first workers are born—have larger MBs than workers in the primitively eusocial sweat bee *Augochlorella aurata*. Whole brain size and optic lobe size do not differ between the two groups, but foundresses also have larger antennal lobes than workers. This shows that increased neural investment in MBs precedes social group formation. Larger MBs among foundresses may reflect the increased larval nutrition provisioned to future queens and the lack of social aggression from a dominant queen upon adult emergence.

Keywords Social evolution · Social brain hypothesis · Neural plasticity · Dominance · Mushroom body

Introduction

Reproductive division of labor is one of the defining characteristics of eusociality: queens reproduce and their sterile worker daughters gain indirect fitness by helping to provision the queen's offspring, who are their sisters (Michener 1974). Physiological differences between queens and workers also extend to the brain (Molina and O'Donnell 2008, O'Donnell et al. 2007, 2017, Smith et al. 2010, Rehan et al. 2015). In insects, a brain area responsible for sensory integration, learning, and memory is the mushroom body (MB) (Fahrbach 2006). In bees and wasps that are primitively eusocial, meaning queens establish and maintain worker sterility through physical dominance, the queens have larger MBs than workers (Rehan et al. 2015; O'Donnell et al. 2017). This relationship is not seen in advanced eusocial species, such as honeybees, where reproductive division of labor is maintained through pheromonal signals (Roat and da Cruz Landim 2008). The larger MBs of queens in primitively eusocial insects may be a plastic response to the social interactions involved in establishing dominance, or result from developmental differences between queens and workers *before* a social colony is established.

Adult foraging experience and social interactions can lead to enlargement of the MBs (Heisenberg et al. 1995, Gronenberg et al. 1996, Withers et al. 2008, Rehan et al. 2015, Seid and Junge 2016). Age may also play a role. In some, but not all, species of bees, ants, and wasps studied, MBs continued enlarging throughout adult life (Withers et al. 1993; Withers et al. 2008; Molina and O'Donnell 2008; Seid and Junge 2016). Thus, queen-worker MB differences may reflect queens' increased age and experience rather than social status per se. In one previously studied species, the bee *Ceratina australensis*, social groups are comprised of same-age sister pairs, rather than mother-daughter associations. Dominant females had larger MBs than subordinate ones, although it is not clear if size differences preceded or resulted from social dominance interactions (Rehan et al. 2015).

Here, we compare early-season foundresses of a primitively eusocial bee, *Augochlorella aurata*, with late-season workers. An *A. aurata* foundress emerges, mated, from

Communicated by: Paula Roig Boixeda

✉ Adam R. Smith
adam_smith@gwu.edu

[1] George Washington University, Washington, DC, USA

[2] Program in Neurobiology, University of Scranton, Scranton, PA, USA

Published online: 27 August 2019

diapause in the spring or early summer. She initiates a new nest as a solitary female and forages for pollen and nectar to provision her first generation of daughters. These daughters mature into workers and assume foraging duties. The foundress is now a queen and remains in the nest laying eggs to be provisioned by the foraging efforts of her workers. Thus, females foraging in early season are foundresses, while those in mid-summer are workers. Foundresses sometimes die during the summer, in which case one worker becomes a reproductive replacement queen (Mueller 1996). Thus, foundresses and workers both have foraging experience.

We predict that if queen-worker differences result from experience, there should be no differences between worker and foundress MBs because both forage. If social interactions per se influence MB development, then workers, who are part of a social group, should have larger MBs than solitary foundresses. If queen-worker MB differences result from developmental differences between queens and workers, then foundresses should have larger MBs than workers. To test these predictions, we measured the volume of the MB calyces, as well as the optic lobes (OL) and antennal lobes (AL), to test if differences are reflected in these sensory neuropils (Fig. 1). In addition, we measured the central complex (CX), which is also involved in sensory integration and learning and may increase in volume with sensory experience (Grob et al. 2017).

Although foundresses and workers each has foraging experience, foundresses are much older, having overwintered underground in diapause as adults from the previous summer, which may confound our analyses. Overwintering, non-diapausing honeybees showed no increase in MB development (Fahrbach et al. 2003), but the effect of diapause has never been explicitly tested (Withers et al. 2008). This is the first study to compare the brain morphology of foundresses,

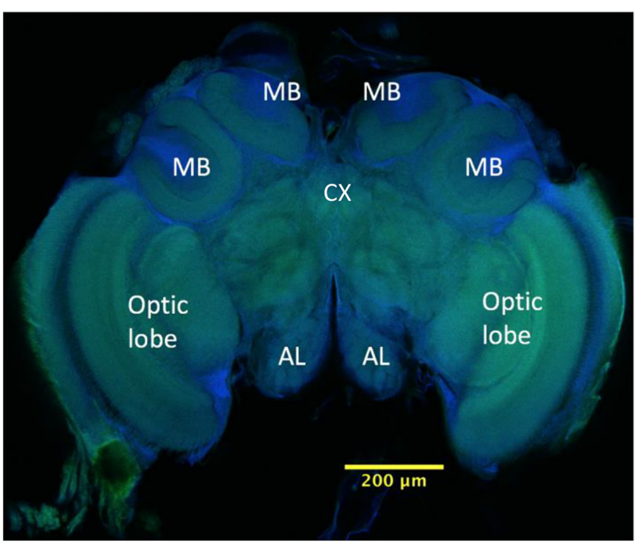

Fig. 1 Confocal micrograph of an *A. aurata* brain showing mushroom bodies (MB), antennal lobes (AL), optic lobes, and central complex (CX)

rather than active queens, with that of workers, in a primitively eusocial species.

Materials and methods

We collected 8 foundresses (between 4 and 14 June 2017 and 12 April–15 June 2018) and 8 workers (between 27 July and 19 August 2018) of *A. aurata* while they were foraging at flowers in and near Washington DC, USA (38.9299° N, 77.1143° W). We used autofluorescence through confocal microscopy and calculated volume from the resulting micrographs using serial reconstruction. We also scored ovary development (1–5; following Michener (1974)) and wing wear (number of nicks or tears on the wing) and measured body size (length of costa vein in right forewing) for all bees. Bees were divided into foundresses and workers based on collection date, confirmed by ovary dissections. See ESM for detailed methods.

Results

Foundresses were larger than workers ($t = 3.09$, $p = 0.004$; foundress wing length mean = 2.39 mm ± 0.11 SD, worker = 2.21 mm ± 0.13). All early-season bees (foundresses) had reproductive ovaries (scores 3–5) except for one individual collected 12 April with undeveloped ovaries (score = 1). All later-season bees (workers) had undeveloped ovaries (scores 1–2) except for one individual collected 3 August with developed ovaries (score = 4). Ovary development score significantly differed between foundresses (4.13 ± 1.46) and workers (1.75 ± 1.04; Mann-Whitney $U = 7.5$, $p = 0.008$). Wing wear did not significantly differ between foundresses (3.38 ± 2.77 nicks) and workers (2.88 ± 2.23; Mann-Whitney $U = 30$, $p = 0.87$).

Whole brain size was similar between foundresses and workers ($t = 0.45$, $p = 0.66$; Fig. 2a). Foundresses of *A. aurata* had significantly larger MB calyces relative to whole brain than workers ($t = 1.78$, $p = 0.0007$; Fig. 2b). There was no correlation between body size and MB calyx to whole brain ratio ($r = 0.18$, $p = 0.52$). The foundress with undeveloped ovaries did not have small MB calyces (rank = 8, out of 16) and was collected a month earlier than any other foundress. The worker with developed ovaries had the smallest MB calyces and the second smallest body size in the study. Foundresses showed greater investment in ALs as well (AL:whole brain, $t = 3.50$, $p = 0.008$; Fig. 2c). There was neither a difference in OL size between foundresses and workers (OL:whole brain ratio, $t = 1.15$, $p = 0.27$; Fig. 2d) nor a difference in CX size (CX:whole brain ratio, $t = 0.27$, $p = 0.79$; Fig. 2e).

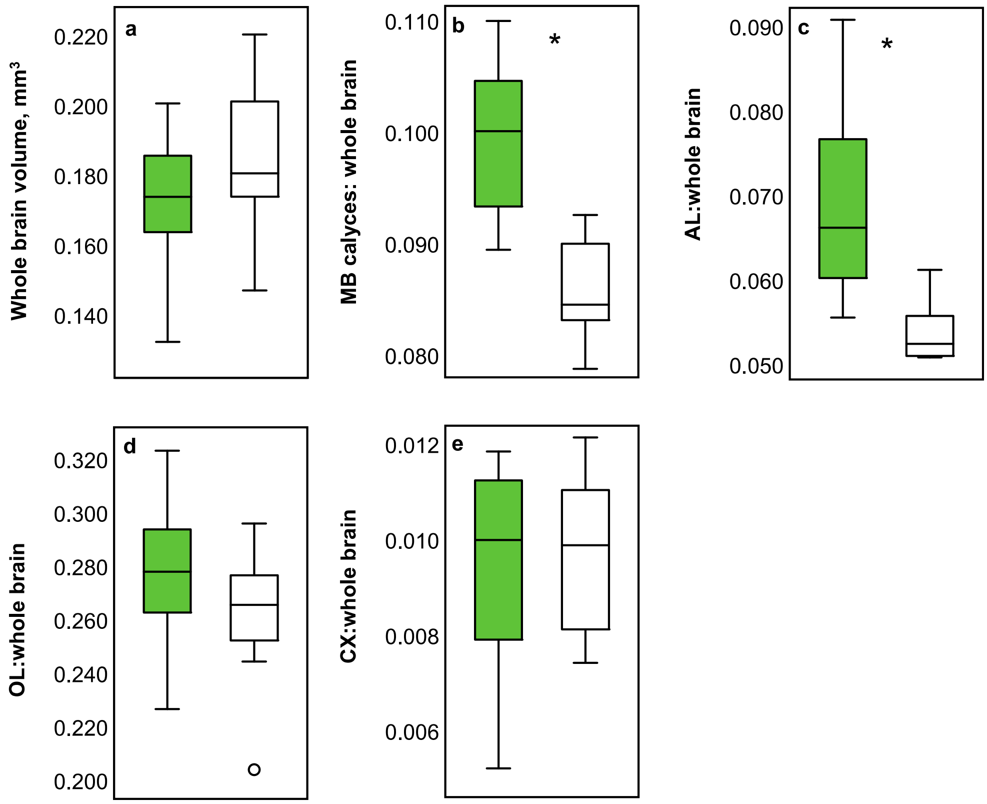

Fig. 2 Foundress data are shown in green and workers in white. **a** Size-corrected whole brain volume. Mushroom body calyces (**b**), antennal lobes (**c**), optic lobes (**d**), and the central complex (**e**), as a ratio to whole brain volume. Asterisks (*) indicate significant differences between foundresses and workers

Discussion

Our results show that foundresses of *A. aurata* invest more neural tissue in MB calyces than workers do. Similar queen-worker differences in MB size have been shown in other bees and wasps, but in all previous cases, queens and workers were collected from existing social groups (reviewed in O'Donnell et al. 2017). However, in our study, foundresses were collected before any workers emerged. This means that those individuals had no social interactions influencing their MB development.

It is not clear why foundresses have larger ALs than workers (Fig. 2c). Keeping track of nestmates and maintaining dominance in addition to the demands of foraging may be cognitively challenging (Tibbetts et al. 2018) and require increased investment in chemical communication (Wittwer et al. 2017), but this remains to be tested.

One foundress had undeveloped ovaries. She may have recently emerged from diapause and not yet enlarged her ovaries; the fact that she was collected more than a month earlier than any other foundress supports this interpretation. One worker had developed ovaries. She may have been a replacement queen from a colony with a weak or deceased foundress queen, which is consistent with her small body size (Mueller 1996). Our foundress-worker MB calyx comparisons remain statistically significant even if these individuals are excluded.

Wing wear data suggest that foraging experience is similar between groups. Queens were much older than the foragers, due to diapause. No study has tested whether time in diapause affects MB size (Withers et al. 2008), although honeybee workers that overwintered in the hive showed no MB size differences relative to younger nestmates with similar foraging experience (Fahrbach et al. 2003). Unlike workers, foundresses also mate (at the end of the previous season) and initiate nests, as well as forage for pollen and nectar. We do not know if this expanded behavioral repertoire is associated with increased MB investment.

The developmental history of foundresses and workers is quite different. Although we do not know the details for *A. aurata*, in other sweat bees, worker females are reared on significantly smaller pollen provisions compared with reproductives that are provisioned with extra nutrition for diapause (Brand and Chapuisat 2012). Larval nutrition affects brain morphology in honeybees, with queens having larger and more rapidly growing brains in the larval stage (Moda et al. 2013) and food-restricted workers having smaller MB calyces (Steijven et al. 2017), but this has not been studied in primitively eusocial groups. Moreover, workers are typically subject to aggressive dominance from the queen during their first days of adult life (Michener and Brothers 1974; Kapheim et al. 2016). Differences in nutrition and social aggression dramatically affect physiology, producing the reproductive queen and sterile worker phenotypes (Kapheim 2017). Brain development may also be affected by the increased larval nutrition provided to foundresses and the social environment free of maternal domination enjoyed by foundresses. Experimental manipulations of larval nutrition and queen removal experiments to create replacement queens from the worker ranks (Mueller 1996), as well as comparisons in other primitively eusocial species, will illuminate caste-based developmental differences in the brain.

Funding information This work was supported by NSF grant #17-1028536545 to ARS and MAS.

Compliance with ethical standards

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

Brand N, Chapuisat M (2012) Born to be bee, fed to be worker? The caste system of a primitively eusocial insect. Front Zool 9:35

Fahrbach SE (2006) Structure of the mushroom bodies of the insect brain. Annu Rev Entomol 51:209–232

Fahrbach SE, Farris SM, Sullivan JP, Robinson G (2003) Limits on volume changes in the mushroom bodies of the honey bee brain. J Neurobiol 57:141–151

Grob R, Fleischmann PN, Grübel K, Wehner R, Rössler W (2017) The role of celestial compass information in *Cataglyphis* ants during learning walks and for neuroplasticity in the central complex and mushroom bodies. Front Behav Neurosci 11:226

Gronenberg W, Heeren S, Holldobler B (1996) Age-dependent and task-related morphological changes in the brain and the mushroom bodies of the ant *Camponotus floridanus*. J Exp Biol 199:2011–2019

Heisenberg M, Heusipp M, Wanke C (1995) Structural plasticity in the Drosophila brain. J Neurosci 15:1951–1960

Kapheim KM (2017) Nutritional, endocrine, and social influences on reproductive physiology at the origins of social behavior. Curr Opin Insect Sci 22:62–70

Kapheim KM, Chan T, Smith A, Wcislo WT, Nonacs P (2016) Ontogeny of division of labor in a facultatively eusocial sweat bee *Megalopta genalis*. Insect Soc 63:185–191

Michener CD (1974) The social behavior of the bees: a comparative study. Harvard University Press, Cambridge

Michener C, Brothers D (1974) Were workers of eusocial hymenoptera initially altruistic or oppressed. Proc Natl Acad Sci U S A 71:671–674. https://doi.org/10.1073/pnas.71.3.671

Moda LM, Vieira J, Freire ACG, Bonatti V, Bomtorin AD, Barchuk AR, Simoes ZLP (2013) Nutritionally driven differential gene expression leads to heterochronic brain development in honeybee castes. PLoS One 8:e64815

Molina Y, O'Donnell S (2007) Mushroom body volume is related to social aggression and ovary development in the paperwasp *Polistes instabilis*. Brain Behav Evol 70:137–144

Molina Y, O'Donnell S (2008) Age, sex, and dominance-related mushroom body plasticity in the paperwasp *Mischocyttarus mastigophorus*. Devol Neurobiol 68:950–959

Mueller UG (1996) Life history and social evolution of the primitively eusocial bee *Augochlorella striata* (Hymenoptera: Halictidae). J Kansas Entomol Soc 69:116–138

O'Donnell S, Donlan N, Jones T (2007) Developmental and dominance-associated differences in mushroom body structure in the paper wasp *Mischocyttarus mastigophorus*. Dev Neurobiol 67:39–46

O'Donnell S, Bulova SJ, DeLeon S, Barrett M, Fiocca K (2017) Caste differences in the mushroom bodies of swarm-founding paper wasps: implications for brain plasticity and brain evolution (Vespidae, epiponini). Behav Ecol Sociobiol 71:116

Rehan SM, Bulova SJ, O'Donnell S (2015) Cumulative effects of foraging behavior and social dominance on brain development in a facultatively social bee (*Ceratina australensis*). Brain Behav Evol 85: 117–124

Roat TC, da Cruz Landim C (2008) Temporal and morphological differences in post-embryonic differentiation of the mushroom bodies in the brain of workers, queens, and drones of *Apis mellifera* (Hymenoptera, Apidae). Micron 39:1171–1178

Seid MA, Junge E (2016) Social isolation and brain development in the ant *Camponotus floridanus*. Sci Nat 103:42

Smith AR, Seid MA, Jimenez LC, Wcislo WT (2010) Socially induced brain development in a facultatively eusocial sweat bee *Megalopta genalis* (Halictidae). Proc Biol Sci 277:2157–2163

Steijven K, Spaethe J, Steffan-Dewenter I, Härtel S (2017) Learning performance and brain structure of artificially-reared honey bees fed with different quantities of food. PeerJ 5:e3858

Tibbetts EA, Injaian A, Sheehan MJ, Desjardins N (2018) Intraspecific variation in learning: worker wasps are less able to learn and remember individual conspecific faces than queen wasps. Am Nat 191: 595–603

Withers GS, Fahrbach SE, Robinson GE (1993) Selective neuroanatomical plasticity and division of labour in the honeybee. Nature 364: 238–240

Withers GS, Day NF, Talbot EF, Dobson HE, Wallace CS (2008) Experience-dependent plasticity in the mushroom bodies of the solitary bee *Osmia lignaria* (Megachilidae). 68:73–82

Wittwer B, Hefetz A, Simon T, Murphy LEK, Elgar MA, Pierce NE, Kocher SD (2017) Solitary bees reduce investment in communication compared with their social relatives. Proc Natl Acad Sci U S A 114:6569–6574