Current Zoology, 2021, 67(5), 561–563
doi: 10.1093/cz/zoaa073
Advance Access Publication Date: 27 November 2020
Article

Article

Classical olfactory conditioning promotes long-term memory and improves odor-cued flight orientation in the South American native bumblebee *Bombus pauloensis*

Denise Nery[a,b], Florencia Palottini[a,b], and Walter M. Farina [a,b,*]

[a]Laboratorio de Insectos Sociales, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina and [b]Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), CONICET-Universidad de Buenos Aires, Buenos Aires, Argentina

*Address correspondence to Walter M. Farina. E-mail: walter@fbmc.fcen.uba.ar

Handling editor: Volker Nehring

Received on 2 September 2020; accepted on 10 November 2020

When collecting a flower, bumblebees learn to associate floral features with reward, preferring, and specializing in the most profitable ones they visit (Chittka et al. 1999). The proboscis extension reflex (PER) occurs naturally in the feeding context allowing individuals to access the floral nectaries. Under controlled conditions, the PER paradigm is a well-established controlled learning procedure used in honeybees (Takeda 1961), and some bumblebees of the genus *Bombus* (Laloi et al. 1999). The assessment of cognitive capacities in an operant context has been studied mainly in *B. terrestris* and *B. impatiens* (Chittka 1998; Mirwan and Kevan 2014). However, little is known about the ability to evoke associative memories in an operant context, which had been acquired in a classical one. In turn, on the contrary, the *Bombus* genus also has the capacity to form long-term memories (Chittka 1998; McAulay et al. 2015). In particular, the South American bumblebee *B. pauloensis* (sin. *B. atratus*) can learn a pure odor when it is presented in a paired association with a sugar reward within a PER paradigm (Palottini et al. 2018). As other species, this one is a commercially reared pollinator that is increasingly managed in pollination services of some South American countries and they could be important for increasing production of crops (Palottini et al. 2018). The present research aimed to examine the long-term memory capabilities of *B. pauloensis* in an olfactory classical PER conditioning procedure and testing its memory 24 and 48 h after training. We also evaluated its capacity to transfer the information acquired by training the bumblebees in a classical PER conditioning context and then submitting them to a choice experiment in a flying cage (FC). Methodological details can be found in the Supplementary materials (Supplementary Figure S1). Briefly, we performed a long-term memory assay 24 and 48 h after a PER training for the first experiment (Long-Term Memory Assay), and for the second experiment (Information Transfer Assay) we trained bees in a PER conditioning and later tested them in an FC were they had to choose between the learnt and a novel floral odor, both without reward.

Bumblebees were able to form an association between the conditioned odor and sucrose reward during their PER training, regardless of the scent used ($z = -0.755$, $P = 0.4299$). In the training phase, the proportion of bumblebees ($n = 62$, 10 ± 7 bumblebees per colony) responding to the conditioned stimulus (CS) increased with successive trials, reaching 32.3% of conditioned responses at the eighth trial (T8) (z-range [Trials 2–8] = -1.4 to 5.04, P [trial] $= 6.63^{-11}$). In the short memory testing (15 mT) phase, the percentage of responses reached 40%, however, no significant differences were found between the last trial and the test ($z = 1.745$, $P = 0.081$). In the 24-h testing (24hT) phase, the response level was of 35.5%. No differences were found between the 24hT and T8 ($z = 0.521$; $P = 0.861$) or 15 mT ($z = -0.489$; $P = 0.876$). Lastly, the percentage of responses for the 48-h test (48hT) phase reached 52.6%; again, no significant differences were found with T8 ($z = 1.04$, $P = 0.29$) or the 15 mT ($z = 1.01$, $P = 0.27$; Figure 1A). In the Information Transfer Assay the proportion of bumblebees ($n = 17$) responding to the CS increased with successive conditioning trials, reaching 64.7% of conditioned responses at the eight trial (z-range [Trials 2–8] = -1.55 to 3.89, $P = 6.46^{-6}$). In the short memory testing phase (15 mT), the percentage of responses reached 88%, whereas the percentage of correct first choices made by each bumblebee at the FC performed 2 h after the 15 mT was of 70.6%, without significant differences ($z = 1.25$, $P = 0.21$; Figure 1B), but different than random ($P = 0.013$, CI 0.57–0.98; Supplementary Figure S2). The average searching time (time bumblebees spent flying in the cage before

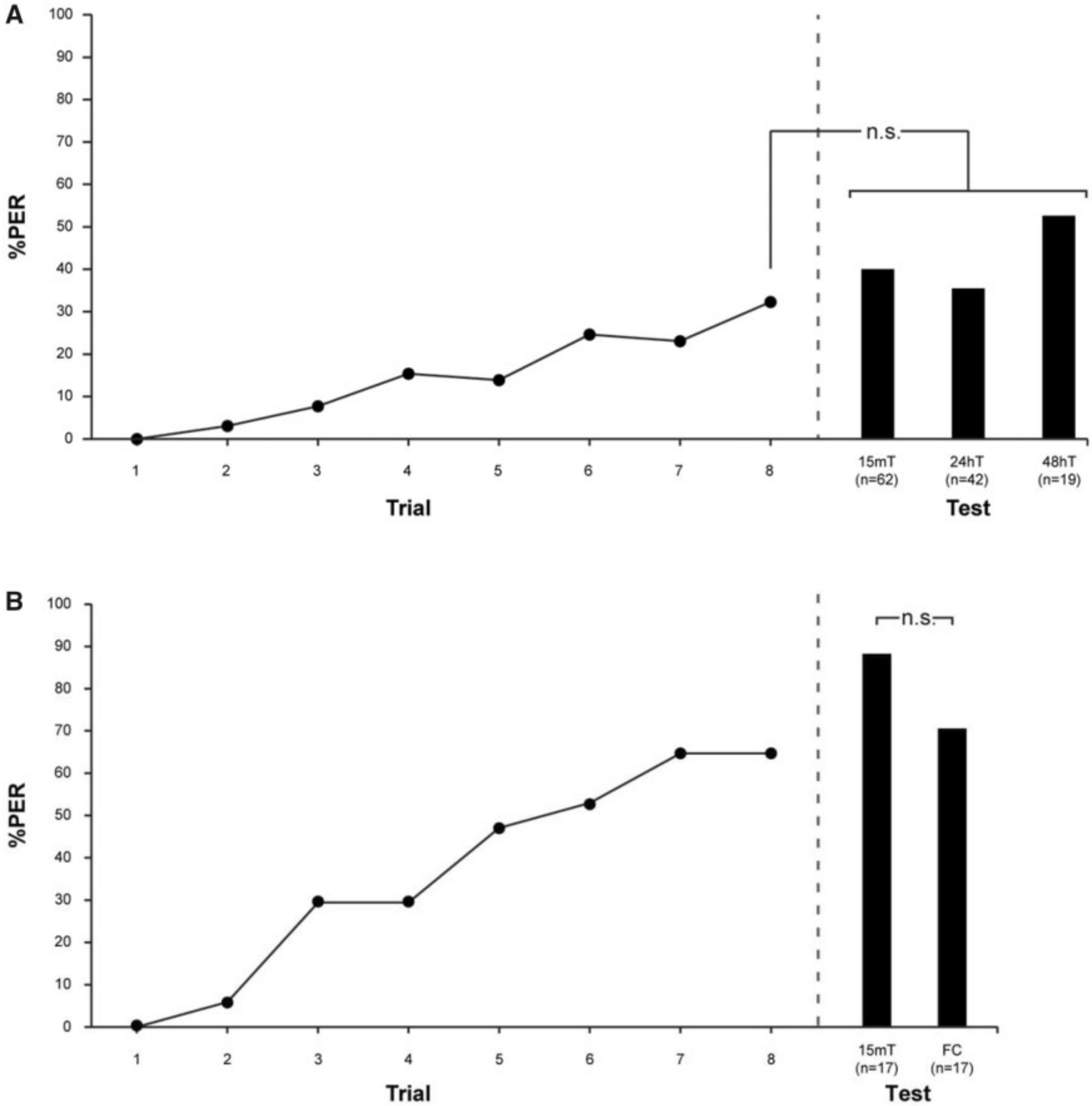

Figure 1. (**A**) *Bombus pauloensis* forms long-term memory after an olfactory PER conditioning. Percentage of bees that extended the proboscis as a response to the odorant (%PER) during training (left) and bees that responded during a testing period (right) 15 min after training (15 mT), 24 h after training (24hT), or 48 h after training (48hT). (**B**) *Bombus pauloensis* can transfer an associative memory acquired in a classical context to an operant context. Percentage of bees that extended the proboscis as a response to the odorant (%PER) during training (left), bees that responded during a testing period (right) 15 min after training (15 mT) to the conditioned odor, and bees that chose the trained odor during the FC test. Sample sizes are indicated in brackets. n.s., non-significant.

choosing a flower) was 25.2 min overall. Time spent on the correct flowers ranged from 16 s to 5 min, the average being 76.8 s, whereas time spent on the wrong flowers by those bumblebees that chose incorrectly was 10 and 28 s. In total, 3 bumblebees (17.65%) did not make a choice in the flying arena (Supplementary Figure S2). In the case of the bumblebees that chose correctly ($n = 12$), 9 extended their probosces at the artificial flower (Supplementary Figure S2). Moreover, 2 bumblebees chose the incorrect flower, but their probosces were not extended when they landed at the flower (Supplementary Figure S2). Statistical analyses were performed to confirm that there was no effect of the odor ($z = -0.002$, $P = 0.09$) or the side ($z = -0.002$, $P = 0.06$) when choosing the odor learnt during the PER training.

Our study deepens the knowledge of the cognitive abilities of the South American bumblebee *B. pauloensis*. We show that workers of this species, when trained under the PER conditioning paradigm, can learn an odor regardless of its identity and the memories can be recalled 24 and 48 h later correctly. We also found that this bumblebee can evoke an associative memory acquired in a classical context, in an operant 1 where they have to choose between the learnt and a novel odor. Summarizing, the bumblebees still make the same choice independently of the testing context. Bumblebees were not only able to choose the correct flower (learnt odor) at the FC, some of them also extended their probosces when doing so. Bumblebees can learn scents and even a few contacts with the conditioned scent led to a preference for that scent 3 days later (McAulay et al. 2015). Our study is the first one to show long-term memory in this South American bumblebee in a PER paradigm. Previous works show information transfer in operant contexts testing foraging preference of the bumblebees for an odor in an FC to which they were previously exposed in different ways inside their colony (Molet et al. 2009; McAulay et al. 2015). In this study, we show that bumblebees that were trained to an odor in a classical PER context can transfer the acquired information to an operant context in which they have to choose between the learnt odor and a novel odor. Our results are in line with a similar experiment performed with honeybees by Chaffiol et al. (2005), where after training them in a PER context to different odors they were able to investigate the honeybees olfactory cued orientation in a wind tunnel. These results suggest that a bee could learn information about the odors it can use to navigate and search for food not only during foraging, but also within the hive before a foraging bout either by contacting a nestmate or filled honeypots (Chaffiol et al. 2005; Molet et al. 2009; McAulay et al. 2015). As a summary, our results show that the South American bumblebee *B. pauloensis* is a suitable experimental model when it comes to studying learning and memory in insects, more specifically in native bees that are being increasingly reared for pollination services as an alternative to exotic and invasive species of bumblebees. Future research will focus on learning associations of scents and food within the bumblebee nest, where information transfer takes place, to evaluate its social learning capacity.

Funding

This study was partly supported by grants from ANPCYT (PICT 2016 2084), University of Buenos Aires (UBACYT 2018 20020170100078BA), and CONICET (PIP 112-201501-00633) to W.M.F. We are grateful to Biobest Argentina S.A. for providing the bumblebee colonies during the experimental period.

Authors' Contributions

D.N., F.P., and W.M.F. conceived the ideas and designed the study, wrote a first draft of the manuscript and gave final approval for publication. D.N. and F.P. collected the data, analyzed the data.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

Acknowledgments

We thank the CONICET and the University of Buenos Aires for support.

Conflict of interests

The authors declare that they have no conflict of interest.

References

Chaffiol A, Laloi D, Pham-Delègue M-H, 2005. Prior classical olfactory conditioning improves odour-cued flight orientation of honey bees in a wind tunnel. *J Exp Biol* **208**: 3731–3737.

Chittka L, 1998. Sensorimotor learning in bumblebees: long-term retention and reversal training. *J Exp Biol* **201**: 515–524.

Chittka L, Thomson JD, Waser NM, 1999. Flower constancy, insect psychology, and plant evolution. *Naturwissenschaften* **86**: 361–377.

Laloi D, Sandoz JC, Picard-Nizou AL, Marchesi A, Pouvreau A et al., 1999. Olfactory conditioning of the proboscis extension in bumble bees. *Entomol Exp Appl* **90**: 123–129.

McAulay MK, Otis GW, Gradish AE, 2015. Honeypot visitation enables scent learning and heightens forager response in bumblebees *Bombus impatiens*. *Learn Motiv* **50**: 22–31.

Mirwan HB, Kevan PG, 2014. Problem solving by worker bumblebees *Bombus impatiens* (Hymenoptera: Apoidea). *Anim Cogn* **17**: 1053–1061.

Molet M, Chittka L, Raine NE, 2009. How floral odours are learned inside the bumblebee *Bombus terrestris* nest. *Naturwissenschaften* **96**: 213–219.

Palottini F, Barcala MCE, Farina WM, 2018. Odor learning and its experience-dependent modulation in the South American native bumblebee *Bombus atratus* (Hymenoptera: apidae). *Front Psychol* **9**: 1–10.

Takeda K, 1961. Classical conditioned response in the honey bee. *J Insect Physiol* **6**: 168–179.