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Animal behaviour

Octopamine increases individual and collective foraging in a neotropical stingless bee

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The biogenic amine octopamine (OA) is a key modulator of individual and social behaviours in honeybees, but its role in the other group of highly eusocial bees, the stingless bees, remains largely unknown. In honeybees, OA mediates reward perception and affects a wide range of reward-seeking behaviours. Thus, we tested the hypothesis that OA increases individual foraging effort and collective food source exploitation in the neotropical stingless bee *Plebeia droryana*. OA treatment caused a significant increase in the number of bees at artificial sucrose feeders and a 1.73-times higher individual foraging frequency. This effect can be explained by OA lowering the sucrose response threshold and, thus, increasing the perceived value of the food source. Our results demonstrate that, similar to its effects on honeybees, OA increases both individual and collective food source exploitation in *P. droryana*. This suggests that, despite having evolved many complex behaviours independently, OA might have similar regulatory effects on foraging behaviours in the two groups of highly eusocial bees.

1. Introduction

Biogenic amines play crucial roles by regulating neurophysiological responses and, ultimately, many behaviours [1–4]. For example, a large body of research has revealed that octopamine (OA) plays important roles in the central nervous system and peripheral sensory systems of invertebrates, including in arthropods, annelids, nematodes and molluscs [2–6]. Honeybees (*Apis mellifera*) have been particularly well studied regarding the role that OA plays in regulating individual and social behaviours. For example, OA reduces the responsiveness to light stimuli [7], enhances the appetitive learning ability [8,9] and increases recruitment communication, most likely via its effects on reward perception [10,11]. These effects of OA are relatively short term, i.e. treatment can affect behaviours within minutes. However, more profound changes in social behaviours have also been observed: OA modulates temporal polytheism by accelerating the transition from in-hive worker to outside forager [12–14].

Stingless bees are the only other group of highly eusocial bees and with more than 500 tropical and subtropical species they represent the largest group of social bees [15]. They live in perennial colonies and are the most important group of pollinators in many tropical habitats [16]. However, it is largely unknown whether and how OA modulates behaviour and physiology in stingless bees. Honeybees (Apini) and stingless bees (Meliponini) separated about 80 Mya [17] and have evolved many complex social traits independently. The two groups vary considerably in their division of labour [18], their recruitment communication [19] and stingless bees differ from honeybees in how they respond to some neuroactive chemicals [20]. This raises the question whether

Table 1. Ten pairs of colonies. Control and treatment colonies were categorized by colony size, which was estimated based on foraging traffic (average number of bees per minute entering a colony in 2 min, measured three times on a day with good foraging conditions). Colonies in italics were excluded from the experiment, either due to rain (colony 10 and 20) or robber bee attacks (colony 18).

pair	control	foraging traffic ± s.e.	octopamine-treated	foraging traffic ± s.e.
1	colony 15	6.00 ± 1.29	colony 5	4.83 ± 0.60
2	colony 19	3.50 ± 0.85	colony 17	3.17 ± 1.14
3	colony 9	8.83 ± 1.28	colony 14	9.50 ± 1.23
4	colony 12	8.17 ± 0.87	colony 11	8.17 ± 1.19
5	colony 3	1.00 ± 0.45	colony 4	1.00 ± 0.37
6	colony 8	9.67 ± 1.69	colony 13	9.83 ± 1.92
7	colony 6	10.67 ± 1.52	colony 16	10.00 ± 1.83
8	colony 2	12.83 ± 1.83	colony 7	11.50 ± 2.47
9	colony 1	7.00 ± 0.97	*colony 20*	5.33 ± 0.61
10	*colony 10*	1.67 ± 0.42	*colony 18*	3.50 ± 0.89

OA plays similar roles in stingless bees as in honeybees. We are aware of only one study that has explored the effects of OA in stingless bees: Mc Cabe *et al*. [21] found that in the stingless bee *Melipona scutellaris*, workers treated with OA showed an increased sucrose responsiveness, similar to what has been found in *A. mellifera* [21,22]. They found both time- and dose-dependent effects of OA on sucrose responsiveness.

Here, we tested for the first time, whether OA modulates the individual and collective foraging behaviour in a meliponine bee. We tested the prediction that OA increases short-term foraging effort in the common Brazilian stingless bee *Plebeia droryana*. This would lead to an increased foraging tempo as well as potentially promoting the recruitment behaviour in *P. droryana* [20]. We manipulated wild *P. droryana* colonies to assess OA effects in the natural environment of this species.

2. Material and methods

We studied 20 wild nests of the stingless bee *P. droryana*, located on the campus of the University São Paulo, Ribeirão Preto, Brazil. The 20 colonies were divided into 10 pairs according to their estimated colony size, which we based on the traffic of returning foragers [18]. Traffic was counted three times on a day with normal foraging conditions (11 : 30, 14 : 30 and 17 : 30), 2 min per count. The average number of foragers in 1 min is shown in table 1. The paired colonies were tested on the same day to reduce variation. Control and treatment were allocated randomly for each pair. Due to bad weather and robber bee attacks, we had to exclude some trials (table 1).

Each trial consisted of a training phase, a treatment phase and a testing phase. During the training phase, a 35% (w/w) unscented sucrose solution, offered in artificial feeders, was used (a typical concentration of nectars collected by stingless bees) [23]. Training started in the morning and two colonies comprising a pair were trained to a feeder at *ca* 10 m from the respective hive at the same time (see [20] for more details on the training methods). On the same day and before the treatment period started, 10 trained bees per colony were marked individually while drinking at the feeder. During the treatment phase (0–60 min), cleaned feeders again offered 35% unscented sucrose solution. At the treatment feeder, we added 0.01 M OA (Sigma Adrich), whereas the control feeder did not offer OA (Colonies that were offered an OA feeder during the treatment phase will be called OA colonies, whereas colonies offered only sucrose solution will be called control colonies). This OA concentration effectively lowered the sucrose response threshold in *M. scutellaris* [21] and recruitment communication in honeybees [11]. The treatment period lasted 60 min, during which all marked and unmarked bees at the feeders were counted at 5 min intervals. The first measurement was made at 5 min. Additionally, the number of visits of marked bees was recorded to calculate the foraging frequency (visits per min). After the treatment phase, control and treatment feeder were removed simultaneously for 20 min before the testing phase started. For the testing phase (minute 80–170 since start of treatment), we offered a 30% unscented sucrose solution without OA at either feeder. We used 30% sucrose solution because it was not very attractive for *P. droryana* foragers in a previous study [20]. The first measurements were made at minute 85. During the testing phase, foragers at the feeders were counted as described for the training phase. For data collection, each feeder was observed by one observer. For each paired trial, only one (randomly chosen) observer knew which feeder was control or treatment and, during a trial, observers did not know how many bees were at the other feeder. Generally, the number of bees at a feeder was easy to count (mostly 10–20 bees per count).

All data were analysed with R 3.4.4 (http://www.R-project.org/). We used linear mixed effects models (LME) and the nlme package to analyse the data [24]. We used *treatment* (OA versus control) and *time* (treatment phase 0–60 min, testing phase 80–170 min) as fixed effects. We also tested the interaction between the two fixed effects using a likelihood ratio test (LRT). We used focal colony ID and pair ID as random effects. To reduce the variation due to differences in colony size, we standardized the count data to form proportions of foragers relative to the average number of foragers counted during the treatment period. This was done for each colony separately. Wald tests were used to test the significance of the fixed effects [24]. We used LMEs to compare the foraging frequency (visits per min) of control and treatment bees. Additionally, we used *experiment phase* (treatment versus testing) as a fixed effect to test if foraging activity differed between treatment and testing phase.

3. Results

(a) Effects of octopamine on foraging

During the treatment phase, when bees were offered 35% sucrose solution, the absolute number of bees at both feeders increased over time irrespective of whether the solutions contained OA or not (LME, treatment × time: LRT = 0.26, $p = 0.61$; time: $t = 8.79$, $p < 0.0001$). We found no effect of the OA treatment on the absolute number of bees at the feeders during the treatment phase (treatment: $t = -0.24$, $p = 0.81$). For the subsequent analyses, we used the proportion of bees at a feeder relative to the average number of bees drinking during the training phase. In the testing period, we found a significant interaction between treatment and time (LME, treatment × time: LRT = 30.16, $p < 0.0001$, figure 1*a*). In order to better assess the changes in the number of bees visiting the feeders over the time during the testing period, we analysed control and OA colonies separately. In both the OA and control colonies we found an increase in the relative number of bees visiting the feeders over time (LME, OA

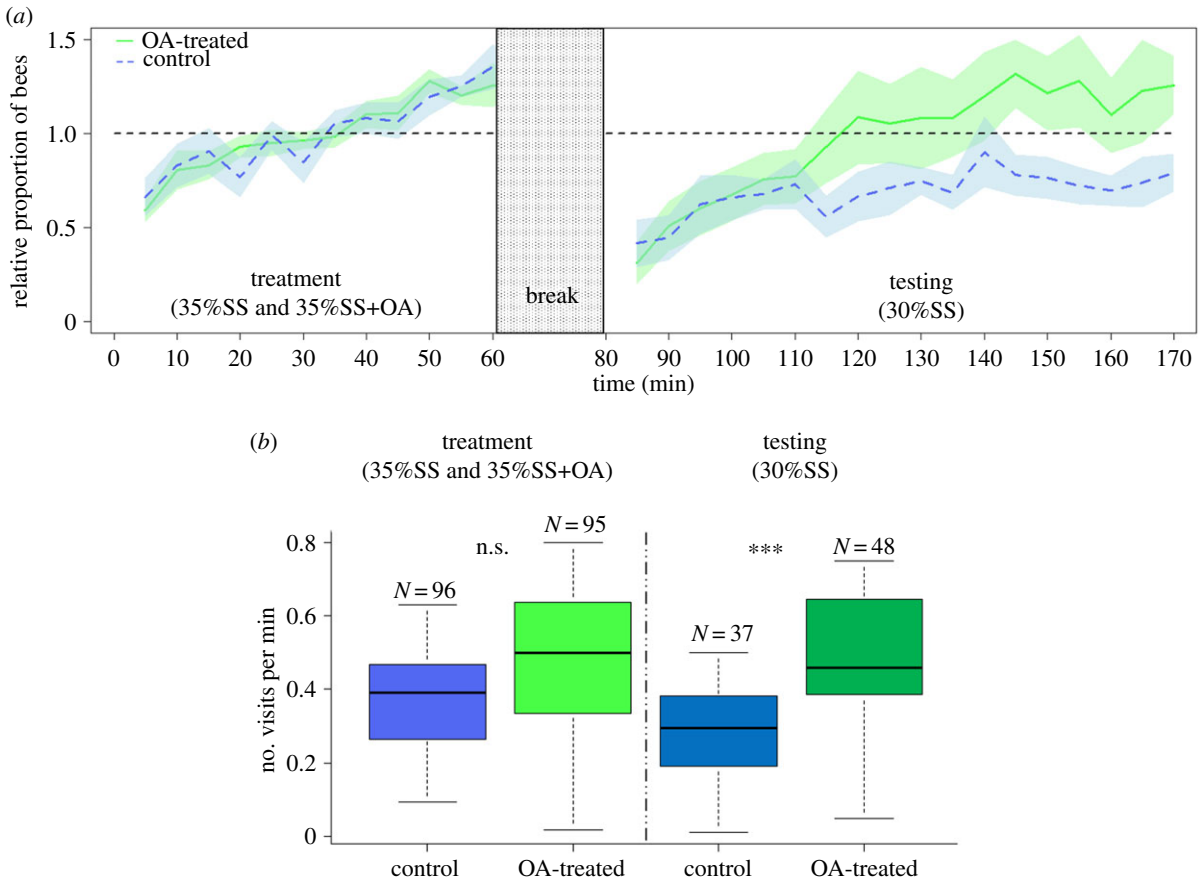

Figure 1. Effects of octopamine feeding on foraging behaviour in *P. droryana*. (*a*) Relative proportion of bees at the feeders during the treatment phase (35% sucrose solution, SS) and the testing phase (30% SS). The *y*-axis shows the proportion of bees at a feeder in relation to the average number of bees at that feeder during the treatment phase. The shaded areas represent the s.e. of the mean. (*b*) The number of visits per minute by individually marked bees during the treatment and the testing phase. The boxplots indicate the medians, the 25% and 75% quartiles. *N* represents the number of individually marked bees. *** Represents the *p*-value < 0.001, n.s. represents the *p*-value > 0.05.

colonies: $t = 11.01$, $p < 0.0001$; control colonies: $t = 5.32$, $p < 0.0001$, figure 1*a*), but this increase was stronger in OA colonies (figure 1*a*). As a result, we found a significant treatment effect at the last count of the testing the phase (OA colonies had about 60% more foragers at feeders than control colonies) (LME, treatment: $t = 2.70$, $p = 0.016$, figure 1*a*).

When comparing the foraging frequency of individually marked bees during the treatment phase, there was no difference between OA and control feeders containing 35% sucrose solution (LME, treatment: $t = 1.67$, $p = 0.11$, figure 1*b*). However, during the testing phase when both feeders offered a 30% sucrose solution, bees visiting the feeder that offered OA solution during the treatment phase had a significantly higher foraging frequency (1.73 times) than bees visiting the control feeder (LME, treatment: $t = 5.69$, $p = 0.0001$). When comparing the foraging frequency of the control group during the treatment and testing phase, we found that the foraging frequency was significantly lower during the testing phase (LME, phases: $t = 4.08$, $p = 0.0001$, figure 1*b*). On the other hand, the OA-treated bees showed no difference in foraging frequency between phases (LME, phases: $t = -0.94$, $p = 0.35$).

4. Discussion

We found that oral treatment of foragers with OA enhanced individual and collective foraging effort in *P. droryana*. During the treatment phase, OA-treated bees did not differ in their foraging effort compared to bees from control colonies, but when colonies were offered identical 30% sucrose solution during the testing phase, more foragers from OA colonies visited the feeders. Moreover, during the testing phase, the number of bees from OA colonies increased more strongly over time than at feeders visited by foragers from control colonies (figure 1*a*). The observation that the OA treatment effects only became apparent during the testing phase suggests that in *P. droryana*, it may take around 30 min before significant changes in behaviour occur, which is slightly longer than was found in honeybees, fruit flies and the stingless bee *M. scutellaris* where OA feeding affected behavioural responses within the range of minutes after uptake [10–12,14,21,25]. Furthermore, the foraging frequency was about 1.73 as high for bees treated with OA during the testing period. In honeybees, several studies have found that increasing endogenous OA levels increase the sensitivity to olfactory, visual or gustatory stimuli [8,10,26,27]. Thus, it is likely that the higher foraging frequency was mediated by an increase in reward sensitivity in *P. droryana* foragers. Foragers from control colonies significantly decreased their foraging rate during the testing phase compared to the treatment phase, whereas the foraging rate did not change in OA colonies between treatment and testing phases even though sucrose concentration was 5% lower during the latter phase (figure 1*b*). This suggests that the OA treatment compensated for the drop in sucrose concentration.

In honeybees, oral OA treatment has a positive effect on waggle dancing, resulting in more recruitment to food sources [11]. *P. droryana* is able to recruit nest-mates to nearby food sources [22]; thus, recruitment behaviour may also be influenced by the OA in *P. droryana*. This could explain the faster increase in the number of bees at the feeders of OA colonies compared to control colonies during the testing phase. Additionally, foragers that visited the feeder during the treatment period may have been more motivated to inspect the feeder after the break between the treatment and testing period. Honeybee foragers were more likely to return to food sources they perceived as more rewarding [28]. Either process leads to an increased collective exploitation of the feeder by OA colonies.

In summary, we found that OA increases foraging effort in wild colonies of a common Brazilian stingless bee. Additionally, oral OA treatment caused a substantial increase in the number of bees at a feeder. This increase in foraging motivation is likely to be linked to changes in reward sensitivity in *P. droryana* foragers [21]. Stingless bees and honeybees have been on separate evolutionary trajectories for about 80 million years and have independently evolved a highly eusocial lifestyle [17,29]. Stingless bees differ from honeybees in important aspects of their sociobiology, including many foraging behaviours and recruitment communication [30]. Our results indicate that, despite this divergence, OA has overlapping effects on the individual and collective foraging behaviours in these two groups of eusocial bees. Still little is known about the neurobiological basis of behaviour in stingless bees. A better understanding of the neurophysiological basis of stingless bee behaviour would help reveal whether there are general patterns in how neurotransmitters regulate complex behaviours in social bees. Further research could, for example, explore how biogenic amines such as OA or dopamine regulate behaviours like sleeping, learning, aggression and division of labour in stingless bee.

Data accessibility. All the raw data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.v9s4mw6rk [31].

Authors' contributions. T.F.P. and C.G. conceived and designed the study; T.F.P. and M.S. carried out the experiments and analysed the data; T.F.P. and C.G. wrote the original draft. All authors reviewed and edited the manuscript. All authors approved of the final version of this manuscript to be published and agreed to be held accountable for the content therein.

Competing interests. We declare we have no competing interests.

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