Received: 19 May 2020 | Revised: 17 November 2020 | Accepted: 26 November 2020

DOI: 10.1111/gbb.12718

Genes, Brain and Behavior

ORIGINAL ARTICLE

Correlation between octopaminergic signalling and foraging task specialisation in honeybees

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Funding information
Fondo para la Investigación Científica y Tecnológica, Grant/Award Number: PICT 2017_2688; PROG. COOP. BILATERAL CONICET-DAAD 2018, Grant/Award Number: 24820180100042CO01

Abstract

Regulation of pollen and nectar foraging in honeybees is linked to differences in the sensitivity to the reward. Octopamine (OA) participates in the processing of reward-related information in the bee brain, being a candidate to mediate and modulate the division of labour among pollen and nectar foragers. Here we tested the hypothesis that OA affects the resource preferences of foragers. We first investigated whether oral administration of OA is involved in the transition from nectar to pollen foraging. We quantified the percentage of OA-treated bees that switched from a sucrose solution to a pollen feeder when the sugar concentration was decreased experimentally. We also evaluated if feeding the colonies sucrose solution containing OA increases the rate of bees collecting pollen. Finally, we quantified OA and tyramine (TYR) receptor genes expression of pollen and nectar foragers in different parts of the brain, as a putative mechanism that affects the decision-making process regarding the resource type collected. Adding OA in the food modified the probability that foragers switch from nectar to pollen collection. The proportion of pollen foragers also increased after feeding colonies with OA-containing food. Furthermore, the expression level of the *AmoctαR1* was upregulated in foragers arriving at pollen sources compared with those arriving at sugar-water feeders. Using age-matched pollen and nectar foragers that returned to the hive, we detected an upregulated expression of a TYR receptor gene in the suboesophageal ganglia. These findings support our prediction that OA signalling affects the decision in honeybee foragers to collect pollen or nectar.

KEYWORDS

brain, division of labour, honeybee, octopamine, pollen foragers, receptor gene expression, subesophageal ganglion, task specialisation, task switching, tyramine

1 | INTRODUCTION

Task specialisation and division of labour are essential features for the ecological success of insect societies.[1,2] Division of labour enables different activities to be performed simultaneously by groups of specialized individuals of the worker caste.[1,3] Workers performing different tasks have different physiological states, distinct neurochemical and hormonal profiles and often differ in how they perceive and respond to task-related stimuli.[4,5] In many social insects, these different internal states are linked to the age of the workers. In the honeybee *Apis mellifera*, for example, workers make a transition from in-hive tasks to the search and collection of resources outside at an age of 2–3 weeks.[6]

The biogenic amines octopamine (OA) and tyramine (TYR) are important drivers of the regulation of honeybee division of labour.[7-9] OA increases in the bee brain soon before the onset of foraging,[10] but it does not change during periods of foraging inactivity or with different amounts of foraging experience.[11] Foragers present higher OA and TYR titres and have an upregulated gene expression of some OA (AmoctαR1) and TYR (*Amtyr2*) receptors compared with nursing bees.[9,12-14] By functioning as a neuromodulator,[15-19] OA enhances behavioural responsiveness to both gustatory[20] and olfactory stimuli,[21-24] a modulation that might enable bees to better assess foraging-related stimuli.[25]

Because of the effects of OA on bees' chemosensory responsiveness[20-24] it might affect division of labour among foragers. In honeybees, the collection of food sources, mainly protein and carbohydrates, is achieved by individuals specializing in pollen or nectar foraging. It is well known that the tendency to forage for pollen or nectar is predicted by the sensitivity to sucrose.[26] In behavioural bioassays, the offering of increasing concentrations of sugar solutions showed that pollen foragers exhibit significantly lower sucrose response thresholds (SRT) than nectar foragers[26] indicating that both groups differ in how they perceive and evaluate the quality of nectar resources.[27-30] Pharmacological activation of OA and TYR signalling has been shown to increase the gustatory responsiveness of nectar foragers to the level of pollen foragers,[9,20] which raises the question whether OA and TYR and their receptors affect the behavioural regulation between pollen and nectar foragers.

OA and TYR are derived from L-tyrosine (aromatic amino acid) and achieve their effects on behaviour through binding to specific membrane proteins that belong to the family of G-protein-coupled receptors (GPCRs) in areas of the brain with functions in the processing and integration of information.[7] Five OA receptors have been described for *A. mellifera*: one α-adrenergic-like and four β-adrenergic-like OA receptors.[31,32] Receptor AmoctαR1 leads to an increase in the Ca^{2+} signalling, whilst β receptors increase intracellular cyclic adenosine monophosphate (cAMP) levels when activated. Changes in concentrations of both intracellular second messengers modulate the activity of a variety of kinases, phosphatases and/or transcription factors that act on effector proteins to modulate the cell's signalling properties. Distribution of the AmoctαR1 includes the antennal lobes (ALs), the calyces, pedunculus, vertical and medial lobes of the mushroom body (MB), optic lobes, subesophageal ganglion and the central complex.[33] In honeybees, β receptors include AmOctβR1, mOctβR2, AmOctβR3 and AmOctβR4. For TYR there are at least two receptors.[34,35] TYR activates AmTyr1 that leads to the inhibition of adenylyl cyclase, resulting in a decrease of cAMP)[36-38] and its mRNA was expressed in most parts of the adult worker honeybee brain.[37] AmTyr2 increases cAMP by combination with nanomolar concentrations of TYR or micromolar concentrations of OA.[35] In comparison to the other six receptors, AmTyr2 has been less studied.

Behavioural experiments suggest that OA influences the type of material collected by foragers, since OA-treated foraging bees were more likely to collect water than non-treated foragers.[39] Tyramine administration leads to an intermediate response. Schulz and coworkers[40] explored the link between the amount of OA and the probability of bees to forage either pollen or nectar. They quantified OA levels in the brain of honeybees selected for high and low pollen-hoarding[41] and found that despite an increasing level of OA with age, there were no differences between both strains. Similar results were obtained from the MBs of non-selected (wild type) pollen and nectar foragers.[42] On the other hand, Scheiner et al.[43] found that pollen foragers displayed significantly higher mRNA expression of the *Amtyr1* in the brain compared with nectar foragers.

Tasks specialisation for nectar and pollen is not fixed, as some foragers can switch from one resource type to the other in response to sudden changes in environmental or colony conditions.[30,44] Whilst neurochemical factors like OA and TYR seem to work more proximally to foraging initiation,[11] early endocrine factors (like higher levels of juvenile hormone [JH] and vitellogenin [Vg] protein at adult emergence), might be responsible for the control of forager development over a longer time scale.[40] There is evidence that the temporal dynamics of JH and Vg production, both related to reproductive maturation of insects, promotes pollen collection,[45] a resource fundamental for brood rearing.[46]

Here we explore the effect of OA on the specialisation of food collection. We hypothesize that OA affects forager preferences for pollen and nectar by means of changing gustatory responsiveness. To elucidate to what extent OA signalling affects pollen and nectar foragers, we focused on three levels. At the individual response level, we investigated whether OA administration influences the probability to switch from sucrose to pollen collection. To this end, we quantified the percentage of bees switching from sucrose solution to pollen feeders when the sugar concentration was decreased experimentally, whilst the quality/availability of the pollen source remained unaltered. We expected OA-treated foragers to show a higher probability of switching behaviour than control bees. At the colony level, we explored whether OA influences foraging activity patterns. We assessed changes in the ratio of incoming pollen and non-pollen foragers before and after offering either sugar solution or sugar solution with OA inside hives. We reasoned that the feeding of OA would increase the proportion of pollen foragers. Finally, we assumed that pollen and nectar foragers perceive the resources differently, in part, due to naturally different levels of OA signalling. Even when there is no evidence for differences in biogenic amine titres between nectar and pollen foragers, regulation of foraging division of labour could be related to sensitivity to, rather than the amount of, circulating biogenic amines. Therefore, we compared gene expression of five OA and one TYR receptors in the brain of foragers that have been collecting pollen or nectar whilst controlling either for their foraging motivation or the age. Receptor expression was quantified in the MBs, the ALs and the suboesophageal ganglia (SOG), neuropils highly involved in the processing of odours and gustatory information.[47-49]

2 | MATERIALS AND METHODS

2.1 | Study site, bees and hives

Behavioural experiments were carried out during the summer seasons of 2018/2019 in the Experimental Field of the School of Exact and Natural Sciences of the University of Buenos Aires (34°32'S,

58°26'W). For these experiments, we used *A. mellifera ligustica*. For the individual foraging response, we trained bees from two colonies to visit artificial feeders that offered 30% wt/wt sucrose solution. The feeders were located approximately 120 m from the hives. To study the collective foraging response, we used fourteen 10-frame Langstroth hives (about 15,000 worker bees), all containing a mated queen, 4–5 brood frames and 1–2 frames with food reserves.

Receptor gene expression was quantified in *A. mellifera carnica*, from colonies located on the campus of the Johannes-Gutenberg University in Mainz, Germany. In a first experimental series, we trained foragers from three different colonies to collect at feeding stations that simultaneously offered pollen and sugar solutions. In a second experimental series, we introduced marked newly emerged bees into a hive, which were then captured 18 days later when they returned to the colony with either nectar or pollen.

All experiments complied with the animal care guidelines of the National Institute of Health (1985) and the current laws of Argentina and Germany.

2.2 | OA effect on switching behaviour from sugar solution to pollen feeders

In this experiment, we studied how oral administration of OA influences the transition of bees between sucrose and pollen collection. To this end, we quantified the percentage of nectar foragers that switched to pollen gathering as the profitability of the sugar solution they were collecting steadily deteriorated.[30] Switches were measured during four tests (T30%, T10%, T3%, and T1%) during which the concentration of sugar solution offered at the feeder decreased from 30% to 1% wt/wt (Figure S1(A)).

Before the evaluations, honeybees previously trained to forage at the artificial feeder, were reactivated for 30 min. to a feeder that offered 30% wt/wt sucrose solution. During reactivation, foragers were marked with acrylic paint to follow them during the whole experiment. According to the experimental design (see below), reactivated bees were offered to collect a sucrose solution that did or did not contain OA (0.01 M) for another 30 min. Here, and in the following experiments, we used octopamine hydrochloride (DL-octopamine ≥95%; Sigma-Aldrich). Afterwards, tests began. Each testing phase (T30%, T10%, T3% and T1%) lasted 40 min (Figure S1(A)). Throughout these tests, an *ad libitum* pollen feeder (Petri dish of 9 cm in diameter) containing commercially available multiflora crushed bee-collected pollen (7 g), was presented next to the sugar solution feeder. Colour-marked foragers were considered to have switched to the pollen feeder as soon as they gathered pollen and formed incipient pollen loads on their hind legs. Bees that switched were captured, killed in the freezer (−18°C) and inspected for colour marks. Unmarked bees were discarded as we could not confirm that they belonged to the focal group. Once T30% finished, we removed the pollen feeder and replaced the content of the feeder with a 10% wt/wt sugar solution. By decreasing the profitability of the sucrose source, we expected a reduction in the number of bees that keep on foraging under the new rewarding condition, but also an increased likelihood to switch to pollen.[26] Before T10% started, we re-labelled the bees with a second colour for 10 min in order to count the number of bees that due to their higher sensitivity to the sucrose, continued foraging on the 10% sugar solution (Figure S1(A)). Once all the remaining bees were counted, the pollen feeder was presented again for T10% to initiate. Test3% and T1% were carried out following the same procedure. Switching behaviours were obtained from 14 independent groups of bees, each group tested on different days. Seven groups were fed sucrose solution with OA and seven groups sucrose solution without OA. On average, groups contained 104.7 ± 19.9 bees. Three independent groups fed sucrose solution containing OA and three groups fed sucrose solution alone came from the same hive, the other groups came from the second hive.

2.3 | OA effect on the rate of incoming foragers

Here we addressed whether the administration of OA diluted in the food of the colonies altered the incoming rate of pollen and non-pollen foragers (presumably nectar foragers). To this end, we evaluated the foraging activity patterns for colonies that were fed sucrose solution (30% wt/wt) with or without OA (0.01 M). Sucrose solution (80 ml) was offered by means of entrance feeders, a 5 cm × 20 cm × 1 cm plastic container that was slid through the entrance of the hive to its interior. It took the colonies 60–100 min to empty the feeder (Figure S1(B)). The number of incoming bees was obtained from videos taken with a digital camera (SONY) at the entrances of 14 hives. Ten-minute videos were recorded immediately before and 10 min after the food either containing OA (7 hives) or not (7 hives) was finished (Figure S1(B)). Incoming bees carrying pollen loads on their hind legs were identified as pollen foragers. Based on the rates of pollen and non-pollen foragers obtained before, the initial rate - (*iR*) and after the treatment, the final rate (*fR*) we calculated the ratio of incoming bees before and after treatment as *fR/iR*.

2.4 | OA receptor gene expression in pollen and nectar foragers

2.4.1 | Sampling pollen and nectar foragers at the beginning of the foraging visit

We trained bees of unknown age to visit a feeding station 30 m from the colony that simultaneously provided sucrose solution (40% wt/wt) and pollen (Figure S1(C)). As soon as the foragers showed their preference for either pollen or nectar, a few seconds after landing, they were captured and frozen in liquid nitrogen and shortly afterwards, stored in a −80°C freezer until brains were dissected. Using this procedure, we aimed to control for the motivation of the bees that, when captured, were still largely empty and motivated to forage. At the same time, using this procedure we controlled for the potential effect of the location of the feeders and the resources quality (i.e., the feeder contained 40% sucrose solution).

2.4.2 | Sampling age-matched pollen and nectar foragers that returned to the hive

Because the age of the worker might affect OA and TYR titres and their receptors in the brain,[9-14] we designed an experiment to control for the age of the foragers. About 4000 colour-marked newly emerged bees were introduced into a host hive (Figure S1(D)). Newly emerged bees were obtained from 3 sealed brood frames taken from three different colonies, placed in an incubator at 32°C, 55% RH and darkness.[50] Every day about 1000, 1-day-old workers were collected from the frames and labelled with the traceable colour on the thorax to control for their age and soon afterwards introduced into the host colony. This procedure was repeated for four consecutive days. In the following weeks, we started watching the entrance of the hive looking for bees of 18 days of age that became foragers. Labelled bees that entered the hive were captured in plastic tubes. Pollen foragers were identified as they carried pollen loads on their hind legs. Nectar foragers were recognized as they exhibited a distended abdomen and regurgitated their gut content as soon as they were gently squeezed. Empty bees were discarded, and we kept only those bees that carried nectar of 15% wt/wt sugar or more (measured with a hand-held refractometer).

2.4.3 | Brain dissections, RNA isolation, cDNA synthesis and qPCR

Bee heads were cut off with dissection scissors and immediately fixed on a small piece of dental wax on an ice-cooled Petri dish. We opened the head capsule with a scalpel by cutting and removing the cuticle from the front of the head capsule. All glands, membranes and trachea covering the brain were carefully removed to expose the AL, the SOG, MB calyces. During dissection, the brain remained immersed in cooled phosphate-buffered saline and over ice. AL, SOG and MBs calyces were dissected in less than 5 min. Only the calyces of the MBs were taken. We used sharp tweezers (FST, Canada) to remove the calyces of MBs from their bottom. The subesophageal ganglia was dissected once the ALs were removed. It was obtained by clamping the structure with both arms of a sharp tweezers and by producing a slight torsion to separate the ganglia from the rest of the brain.

The paired AL, MB calyces and the SOG were transferred into different vials with TRIzol® (Invitrogen) for RNA extraction. For each sample, we pooled the brains of either three pollen or three nectar foragers in order to reduce the variability among different samples.

The RNA extraction was performed with RNeasy Mini Kit (Qiagen, Germany) according to the manual. The Quanti Tect Reverse Transcription Kit (Qiagen, Germany) was used to remove the genomic DNA from the previously isolated RNA. The Kit was also used to synthesize the cDNA of the genes we were interested in. All qPCR reactions were performed on the mic qPCR cycler (Bio Molecular Systems, Australia). The thermal cycling protocol comprises 40 cycles of denaturation at 95°C for 5 s and annealing at 60°C for 20 s. We focused on the receptor genes *AmoctαR1*, *AmoctβR1*, *AmoctβR2* and *Amtyr1*,[13,36] as well as the housekeeping genes *GAPDH* and *eiF3-S8*. To investigate AmoctβR3 and AmoctβR4 receptors, which originate from one gene by alternative splicing[32] we performed a common approach (i.e., using a single common primer[14] under the name of *AmOctβR3/4*. Primer sequences for receptors and reference gene were taken from the published literature[51-53] and are reported in Table S1. The $2^{-\Delta CT}$ method was used for calculating the relative gene expression.[54] Further information describing the amount and control of the quality of RNA was provided in Supporting Information.

2.5 | Statistics

All data were analysed using generalized linear mixed models (GLMM[55,56]; or generalized linear models (GLM) in the R environment (http://www.R-project.org/). Differences in switching behaviour were assessed by means of GLMM with binomial distributions.[57] Here we explored the role of two fixed effects on switching behaviour, "treatment (i.e., bees that collected sugar solution with or without OA)" and "testing phase (T30%, T10%, T3% and T1%)". The day of the experiment was considered as a random effect, specified via the model formula. We checked for overdispersion.[58] We used the glmer function of the lme4 package.[59,60] The lme4 package (glmer function) uses Wald Z-tests to approximate *p*-values for GLMMs.[56]

The ratio of incoming bees before and after treatment was analysed by means of GLMM with normal distribution. Here we analysed the effect of "treatment (offering of sugar solution or sugar solution with OA)" as a fixed effect. Colonies were considered as a random factor. Homoscedasticity and normality assumptions (Levene and Shapiro–Wilk tests, respectively) were met after modelling the variance with "VarIdent".[58]

Relative mRNA expression was analysed by means of GLMM with gamma distribution. To test the differences in biogenic amine receptor gene expression, we explored the impact of two fixed effects, "forager type (pollen or nectar)" and "brain part (AL, SOG and MB)". To compare the expression of biogenic amine receptor genes between treatments and brain parts, pairwise comparisons were performed, and a sequential Bonferroni correction was applied to adjust *p*-values for multiple testing (multcomp package in R).

3 | RESULTS

3.1 | OA increased switching behaviour from sugar to pollen feeders

The percentage of foragers that switched from the sugar feeder to the pollen feeder increased through the successive testing phases, which means that as the concentration of the sucrose solution offered at the feeder decreased, bees were more likely to switch from sucrose to pollen. More importantly, switching behaviour was influenced by the OA administration (Figure 1), as the percentage of bees that switch during single testing phases was higher if they previously collected sugar solutions containing OA. Consistent with these results,

the analysis showed a significant interaction between the tested factors (treatment*test: $F_{3,9}$ = 3.442, p = 0.006). Main effects confirmed that switching behaviour of OA treated foragers was higher than controls in T30% (Z = 3.904, p = 0.0001), T10% (Z = 2.480, p = 0.013) and T3% (Z = 1.986, p = 0.047). In other words, OA-treated foragers were more likely to switch from nectar to pollen foraging than control bees, as it was found at least, for the first three testing phases.

3.2 | OA effect on the rate of incoming foragers

Additional evidence for the role of OA was found when looking at the colony response. Colonies fed sucrose solution containing OA exhibited a higher ratio of incoming pollen foragers than colonies fed sucrose solution alone (treatment: Chiq = 5.641, p = 0.017; Figure 2 (A)). This suggests that the rate of incoming pollen-loaded bees increased more after the offering of food with OA than without OA. Interestingly, the ratio of incoming non-pollen foragers did not differ between colonies treated with or without OA (treatment: Chiq = 0.439, p = 0.507; Figure 2(B)). These results indicate that feeding sucrose solution containing OA affected the individual foraging preferences for pollen and modified the pollen foraging activity of the colony.

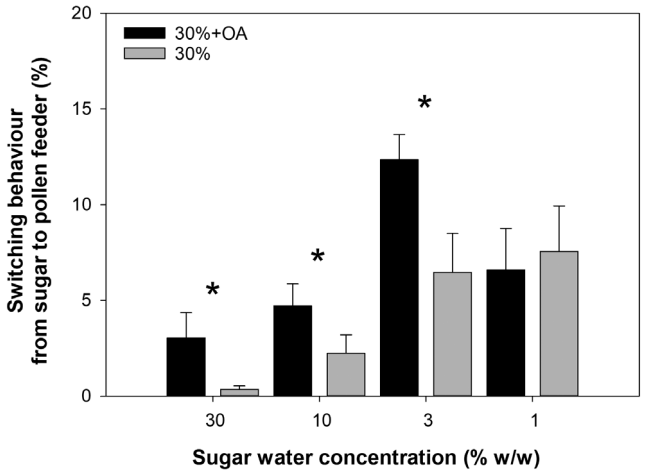

FIGURE 1 Switching behaviour from sucrose to pollen feeders. Percentage of labelled honeybees that changed their foraging preferences to pollen throughout four phases. Switching behaviour was quantified in foragers that had access either to a 30% wt/wt sugar solution or to a 30% wt/wt sugar solution with octopamine (OA) (0.01 M). Bars show medians ± SE of seven independent groups of bees for each treatment. Asterisks indicate statistically significant differences (*p < 0.05; Tukey's test)

3.3 | Variation in expression of OA and TYR receptors between pollen and nectar foragers

When the expression of receptor genes was studied in pollen and nectar foragers captured at the very beginning of a foraging visit, we detected significantly higher mRNA levels for *AmoctαR1* in the brain of pollen foragers compared with nectar foragers (forager type: $F_{1,66}$ = 5.336, p = 0.024; Figure 3(A)) irrespective of the part of the brain. No differential expression linked to forager type was found for *AmoctβR1*, *AmoctβR2*, *AmoctβR3/4* or *Amtyr1* receptor genes (Figure 3(B-D)).

When expression was analysed in age-matched pollen and nectar foragers captured at the entrance of the hive (end of the foraging visit), a significant difference was found for the receptor gene *Amtyr1*, with pollen foragers having an up-regulated expression compared with nectar foragers. Regarding the expression of this gene (*Amtyr1*), our analysis showed a significant interaction between the factors (forager type*brain part: $F_{2,65}$ = 2.972, p = 0.05; Figure 4(E)). The difference between foraging groups was explained by a higher receptor gene expression in the SOG of pollen foragers (Dunnett's test; pollen vs. nectar foragers: Z = −2.280, p = 0.0226, Figure 4(E)). Expression of *AmoctαR1*, *AmoctβR1*, *AmoctβR2* and *AmoctβR3/4* was not affected by foraging type (Figure 4(A-D)).

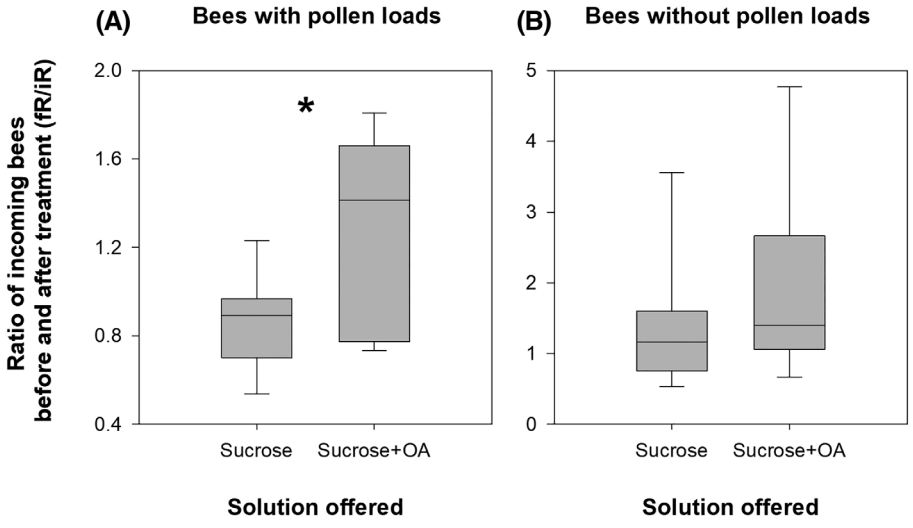

FIGURE 2 Ratio of incoming bees before and after treatment (final rate/initial rate) with (A) and without (B) pollen loads obtained from colonies fed either sucrose solution or sucrose solution with octopamine (OA) (0.01 M). Box plots show medians, quartiles, and 5th and 95th percentiles from 14 hives. Asterisks indicate statistical differences (*p < 0.05)

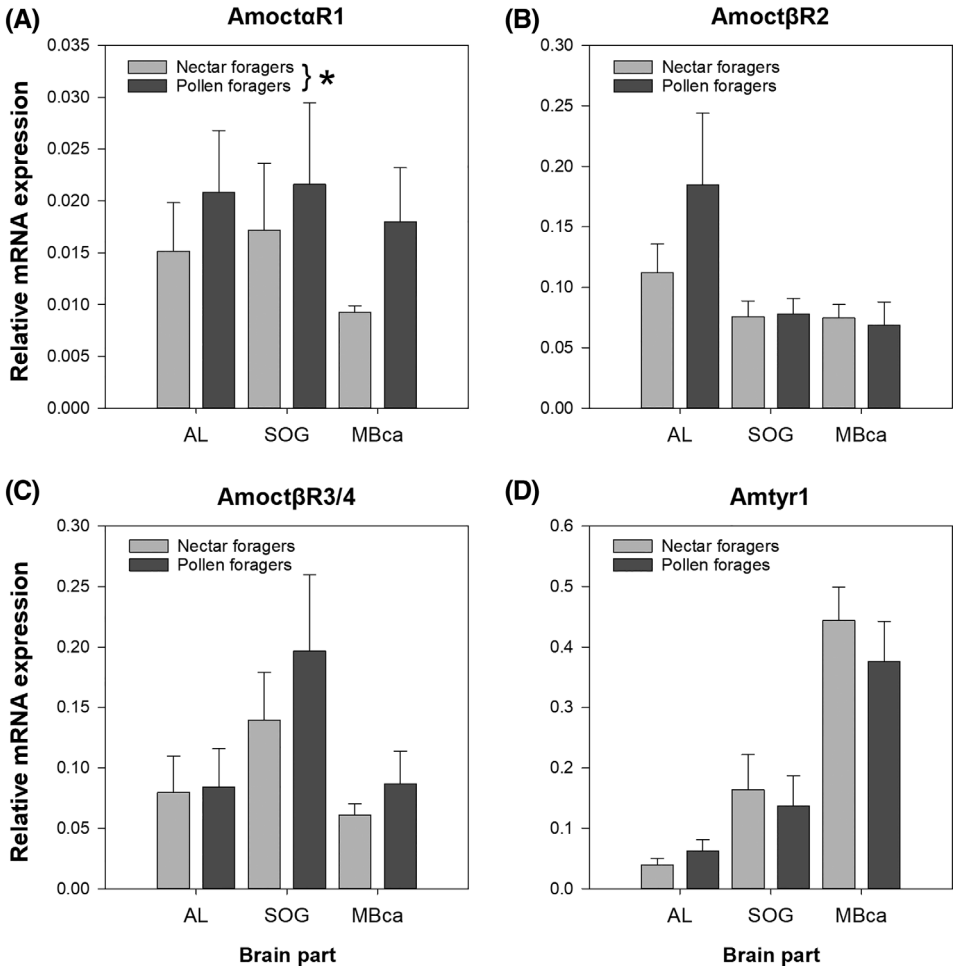

FIGURE 3 Biogenic amine receptor gene expression in different parts of the bee brain of nectar and pollen foragers captured when arriving at artificial feeders. Bars show mean expression levels relative to the two reference genes (*GAPDH* and *eiF3-S8*) ± SE. Each bar shows the mean for 10–12 samples, each one made by pooling three different bees from the same hive and foraging sub-caste. Asterisk indicates an overall significant difference between nectar and pollen foragers (*$p < 0.05$). AL, antennal lobe; MBca, calyces of mushroom bodies; SOG, suboesophageal ganglia

4 | DISCUSSION

The combined experimental approaches performed in this study suggest that OA and TYR signalling are involved in the short-term regulation of foraging division of labour in honeybees. At the individual level, foraging bees treated with OA were more likely to switch from nectar to pollen than bees of the control group. OA also impacted on the collective response, in which OA-treated colonies showed higher rates of incoming pollen foragers than control colonies. The behavioural choice regarding the preferred resource type correlated with an overall difference in the expression of receptor gene *AmoctαR1* in the brain of bees as they started to collect either pollen or sucrose solution and, consistent with Scheiner et al,[43] with a change in the expression of receptor gene *Amtyr1* in the SOG of bees that returned to the hive either carrying nectar or pollen.

4.1 | Octopamine influences individual foraging preferences for pollen

Our results indicate that OA treatment is involved in the regulation of the transition of foragers between nectar and pollen collection. Arenas and Kohlmaier[30] recently observed that switching between resource types can be an active decision of the bees in response to changes in sugar profitability of the feeding site. In general terms, bees that persisted in visiting the feeding station when it offered low-quality sucrose solutions, presumably due to lower sugar response thresholds, were more likely to switch to pollen than those bees foraging only on highly concentrated solutions. Our results are consistent with previous findings[30] and go further showing that OA-treated bees are more likely to switch than controls, probably through OA effects on the perception of sugar and pollen reward-related stimuli.[20] Because high OA levels in the brain modulate response thresholds for stimuli like odours[21] and sucrose,[20] it is plausible that OA-treated bees were more sensitive and responsive to certain chemosensory cues of pollen, such as volatiles and tastes,[61,62] which are responsible for attracting the bees and eliciting pollen foraging behaviour.[63,64] Furthermore, because bees showing lower SRTs are less demanding regarding the reward,[61] they might also learn better with nutritional and non-nutritional compounds available in the pollen.[65]

As expected, not all OA-treated bees became pollen foragers. This suggests that this behavioural plasticity is not only controlled by OA, but probably depends on the interplay between endocrinal and neurochemical factors.[40] Regarding foraging specialisation, it has been suggested that the temporal dynamics of different endocrinal factors, like higher levels of JH and Vg protein at adult emergence, promote pollen collection.[32] Behavioural development under the control of

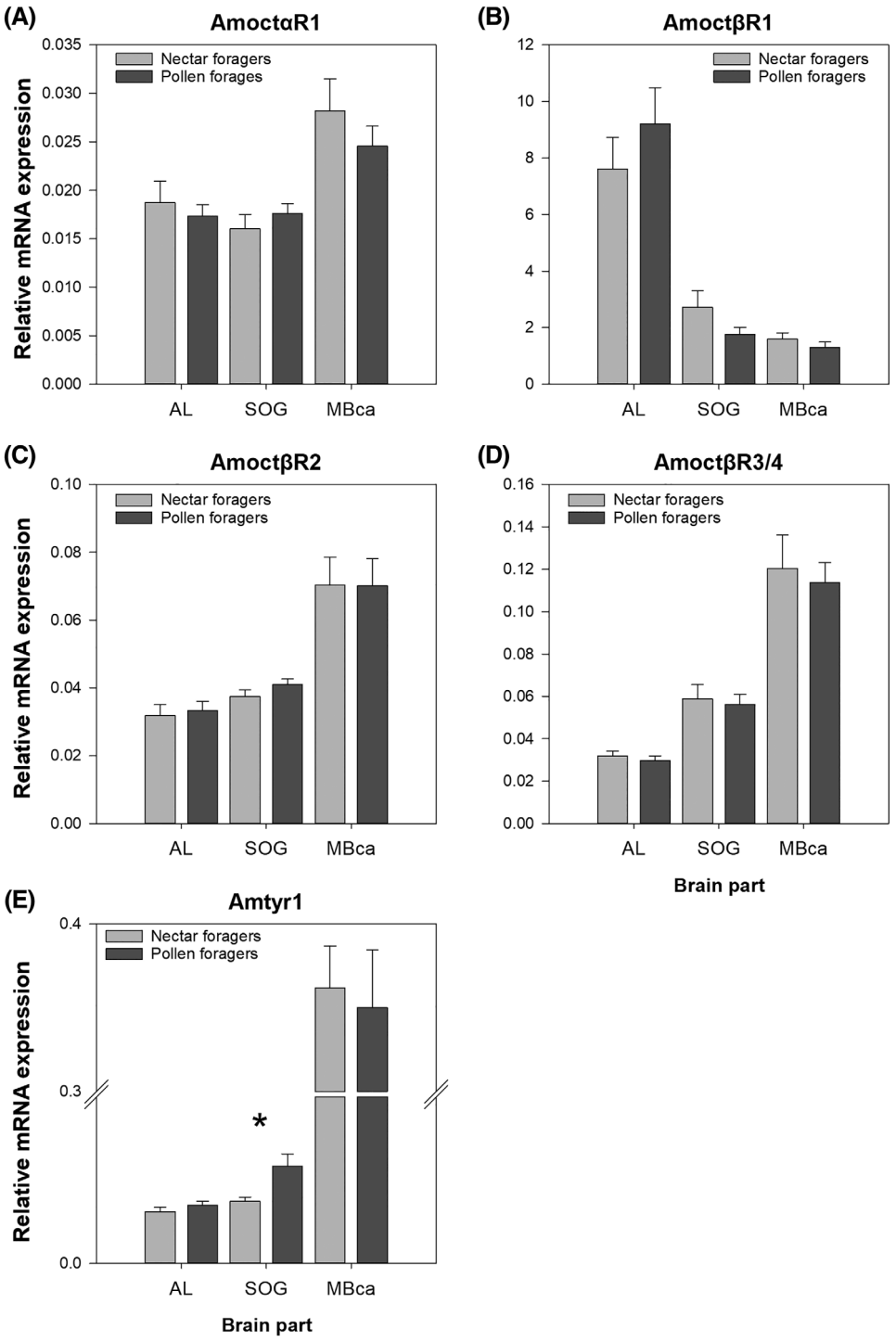

FIGURE 4 Biogenic amine receptor gene expression in different parts of the bee brain of aged-matched nectar and pollen foragers, captured at the entrance of the hive. Bars show mean expression levels relative to the two reference genes (*GAPDH* and *eiF3-S8*) ± SE. Each bar shows the mean for 11–12 bees of 18 days of age, that returned to the colony loaded either with pollen or nectar. Asterisk indicates an overall significant difference between nectar and pollen foragers (*$p < 0.05$). AL, antennal lobe; MBca, calyces of mushroom bodies; SOG, suboesophageal ganglia

early endocrine events and changes in OA signalling might ultimately drive foraging preference for either pollen or nectar.

4.2 | Octopamine affects pollen-foraging activity of colonies

Consistent with changes in individual switching behaviour at the foraging site, we found that OA treatment also affects colony foraging activity towards nectar and pollen resources. Here, we observed that the ratio of bees carrying pollen increases after feeding the colonies sucrose solution with OA. This result is in line with a previous finding in which foragers treated with OA were more likely to collect water than non-treated foragers.[39] Taken together, this evidence supports a role of OA in foraging regulation between nectar and resources that do not necessarily provide an immediate energy reward (e.g., pollen, water or resin).

It is known that circulation of gustatory information inside the hive modulates sugar response thresholds of workers[66,67] and is

responsible for the re-allocation of foragers among nectar sources of different profitability.[46] Furthermore, it has recently been observed that the modulation of sugar thresholds also drives a re-allocation between nectar and pollen sources. Arenas & Kohlmaier[30] observed that the ratio of pollen versus non-pollen foragers increased after feeding a colony low-quality sugar solution (3% wt/wt) and decreased after the feeding of a high-quality sugar solution (50% wt/wt). With increasing sugar responsiveness due to OA administration, we would expect more foragers to become responsive to low-quality nectars and also to pollen-related stimuli, a situation that would promote recruitment, activation of new foragers,[25] and/or reactivation of experienced foragers to pollen sources. Adjustments in the amount of pollen collected also include foragers carrying heavier pollen pellets and intensification of the frequency of their foraging bouts.[68,69] However, whether OA treatment also impacts on individual efforts remains to be tested.

In our experiments, behavioural responses were only tested with OA. However, whether other biogenic amines like dopamine or serotonin[47,70] could also affect resource selection, remains unknown. Our study and Scheiner et al.[43] suggest that TYR signalling is involved in foraging specialisation. Because OA in high concentrations can also bind, to some extent, to TYR receptors,[37,71] we cannot discard the possibility that OA administration affects the regulation of foraging behaviour via TYR receptors. TYR might also impact on motor activity,[43] which is crucial for pollen collecting manoeuvres by which foragers brush pollen with their legs from body hairs to their hind legs, where they accumulate as pellets in the corbiculae.

4.3 | OA and TYR receptor expression correlates with nectar or pollen collection

Our results from experiment 3, in which receptor gene expression was obtained from foragers captured immediately after landing at the feeder, showed that there was an overall higher expression for receptor gene *AmoctαR1* in the brain of foragers that landed at the pollen feeder. It is noteworthy that receptors of the β family did not show differences. However, we currently cannot exclude that the greater expression in pollen foragers was due to age effects as it has been reported that pollen foragers initiate foraging at slightly younger age than nectar foragers, a trait related to the accelerated behavioural development under the control of early endocrine processes.[45] Furthermore, it was recently found that AmoctαR1 is s linked to the age.[72] However, given that older bees have higher mRNA expression of OA receptors than younger bees,[13,14] we deem it is unlikely that the upregulation of *AmoctαR1* in pollen foragers were linked to age differences.

Interestingly, pollen and nectar foragers of similar age (18-days) captured at the end of their foraging bout (experiment 4) did not show differences in *AmoctαR1* expression, but for *Amtyr1* in the SOG. These findings match with a previous study that sampled pollen foragers at the entrance of the hive and found a higher expression of the *Amtyr1* in the SOG, but not in the AL or MB.[43] Because the SOG is located in the ventral nerve cord, between the brain and the thoracic and abdominal ganglia, it could serve as a relay centre for information descending and ascending along the ventral nerve cord, which might be important for both the assessment of pollen with their tarsi for the control and coordination of leg movements during pollen gathering. In addition, ventral unpaired median neurons, all octopaminergic neurons,[73] innervate different parts of the SOG and the brain, and might mediate reinforcement with pollen.[65] Together, our results suggest that TYR receptor Amtyr1 in the SOG is involved in the division of labour among pollen and nectar foragers.

The different findings of experiments 3 and 4 suggest a complex role of BAs in the regulation of resource selection. On the one hand, it is plausible that the expression of *AmoctαR1* receptor gene is higher in the bee brain at the beginning of the foraging trip but down regulated as the foragers become satiated and ready to leave the foraging site. Once inside the hive, and according to colony conditions, receptors might be upregulated again, driving the bees to resume pollen foraging. More investigations are necessary to examine whether changes in OA and TYR receptors expression relate to different phases of the pollen foraging bout.

We cannot rule out that the location of the foraging sites and the identity and/or quality of the resources were at least in part, responsible for the differences between experiment 3 and 4. In experiment 4, bees collected nectar and pollen from natural food sources. The sugar concentration in nectars sampled from the crops of returning foragers ranged from 15% to 20% wt/wt. Pollens were observed to belong to at least three different species (as showed by the different colours of the pollen pellets). Bees from experiment 3 foraged 30 m from the hives on ad libitum feeders (10 cm apart) either offering 40% wt/wt sucrose solution or crushed multifloral-bee collected pollen. These differences might have amplified differences in *AmoctαR1* receptor gene expression between foragers due to the more pronounced contrast between the profitability of both resource types. Nonetheless, our results suggest that pollen and nectar foragers differ in their assessment of gustatory and olfactory stimuli, and possibly in motor activity too, and that this is related to OA- and TYR signalling via the *AmoctαR1* and *Amtyr1* receptors.

A combination of higher receptor expression in the brain of pollen foragers plus increased OA titres would lead to a higher sensitivity, and the capacity to elicit stronger responses with small changes in the amount of biogenic amines. So far, there is no evidence for differences in OA levels neither in primary integration centres of odour (AL) and gustatory (SOG) information, nor in the higher processing centre of the bee brain (MB) between pollen and nectar foragers.[40,42] However, Schulz et al[40] discussed that a lack of differences between OA titres between high and low pollen-hoarding strains[41] could be because bees were tested before they became foragers. Furthermore, Scheiner et al[42] did not test OA titres in the AL or SOG, brain regions that are involved in the processing and evaluation of olfactory and gustatory stimuli. Thus, so far, no study analysed the amount of TYR in the brain of pollen and nectar foragers, which would allow us to link TYR levels in the brain with changes in the expression of *Amtyr1* (Scheiner et al[43] and this study). Likewise, biogenic amine titres at different stages of foraging bouts have not yet been compared.

Our study provides new insights into the underlying physiological processes and mechanisms involved in the control of resource collection. Quantitative but also qualitative differences in OA and TYR receptors among brain neuropils might reflect the complex patterns of gene expression that determine reward value representation in pollen and nectar foragers at different phases of the foraging bout. This, in turn, could affect the decision of foragers to collect either pollen or nectar.

ACKNOWLEDGEMENTS

We thank MJ. Corriale for help with statistical analyses. This study was partly supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT) to A. Arenas and by CONICET-DAAD Bilateral Cooperation Program 2018.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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How to cite this article: Arenas A, Lajad R, Peng T, Grüter C, Farina W. Correlation between octopaminergic signalling and foraging task specialisation in honeybees. *Genes, Brain and Behavior*. 2020;e12718. https://doi.org/10.1111/gbb.12718