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ORIGINAL PAPER

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Selective modulation of task performance by octopamine in honey bee (*Apis mellifera*) division of labour

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Abstract Octopamine treatment has previously been shown to increase honey bee foraging behaviour. We determined the effects of octopamine on other tasks to learn how octopamine affects division of labour in honey bee colonies. Octopamine treatment did not increase the rate of corpse removal from the hive, suggesting that elevated brain levels of octopamine do not act to increase the performance of all flight-related tasks. Octopamine treatment also did not increase attendance in the queen's retinue, suggesting that elevated brain levels of octopamine do not act to increase responsiveness to all olfactory stimuli. Consistent with these findings, octopamine treatment enhanced the foraging response to brood pheromone but not the cell capping response, a component of brood care. These results demonstrate a relatively specific form of neuromodulation by octopamine in the regulation of division of labour in honey bee colonies.

Keywords Octopamine · Honey bee · Division of labour · Behavioural state · Pheromone

Introduction

Honey bee (*Apis mellifera*) workers are morphologically indistinguishable and share a common social environment in the colony, but they manifest remarkably diverse behavioural specialisations. This results in a complex division of labour that is a key factor in the success of the social insect lifestyle (Seeley 1985; Winston 1987). Physiological differences between workers generate different 'behavioural states' (Robinson 2002) defined on the basis of discrete and predictable patterns of task performance. The most well characterised of these involve brood care ('nursing'), typically performed during the first 2 weeks of adulthood, and foraging, usually beginning when adult workers are about 3 weeks old. Previous studies have established octopamine as a modulator of division of labour, increasing foraging behaviour (Schulz et al. 2002a). However, it is not yet clear how octopamine exerts this effect.

Octopamine is an important insect neurotransmitter, neuromodulator and neurohormone (Evans 1985; Orchard et al. 1993; Roeder 1999). Within the brain, octopamine modulates diverse behavioural processes, which include learning and memory (Hammer and Menzel 1998; Mercer and Menzel 1982), olfactory (Erber et al. 1993; Pribbenow and Erber 1996), visual (Erber and Kloppenburg 1995), gustatory (Scheiner et al. 2002) and motor systems (Burrell and Smith 1995). Octopamine also acts systemically to influence muscle performance, fat metabolism and metabolic rate to prepare for greater activity (Adamo et al. 1995; Corbet 1991; Orchard et al. 1993; Roeder 1999).

The brains of forager honey bees contain more octopamine than nurses (Wagener-Hulme et al. 1999) and the difference is most pronounced in the antennal lobes (Schulz and Robinson 1999; Spivak et al. 2003). Octopamine levels are high at the onset of foraging behaviour and do not increase with subsequent foraging experience or decrease when foragers are inactive (Schulz et al. 2003). Further, an oral treatment with octopamine causes a precocious onset of foraging behaviour in young bees (Schulz and Robinson 2001) and increases foraging activity (Barron et al. 2002). How is octopamine acting to effect this behavioural change?

Octopamine acts in diverse ways to prepare insects for increased activity (Corbet 1991; Orchard et al. 1981; 1993). In locusts, neurohormonal actions of octopamine trigger a complex cascade of events to improve flight performance, mobilising lipids from fat body stores, increasing metabolism in flight muscles and modulating muscle performance to increase power output (Orchard et al. 1993). Foraging behaviour is an energetically

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demanding task for bees. Octopamine could affect foraging by preparing bees for flight activity. If this is the case, we would expect octopamine to increase performance in other tasks that are energetically demanding or involve flight. We examined this hypothesis by studying the effect of octopamine treatment on undertaking behaviour. Undertaking describes the removal of corpses from the colony; the task is typically performed by middle-aged adult bees (2–3 weeks old) who drag corpses out of the colony and fly a short distance with them before dropping them (Winston 1987).

Octopamine also is an important modulator of the insect olfactory system and has been shown to enhance the responsiveness of bees to olfactory stimuli (Hildebrandt and Muller 1995; Mercer and Menzel 1982; Spivak et al. 2003). On the other hand, in some Lepidoptera octopamine specifically modulates responses to sex pheromones (Pophof 2000; 2002). Foraging in honey bees is stimulated by many factors, including floral odours and brood pheromone (Pankiw et al. 1998; von Frisch 1967), and octopamine enhances the foraging response to brood pheromone (Barron et al. 2002). If octopamine affected foraging by generally increasing responsiveness to olfactory stimuli, we could expect octopamine treatment to affect other odour-mediated behaviour as well. To test this possibility, we studied the effect of octopamine on the workers' retinue response to the queen, which is mediated by queen mandibular pheromone (Slessor et al. 1988). In addition, brood pheromone also stimulates the performance of several other tasks including larval feeding and the capping of larval cells (Le Conte et al. 1994; 1995). If octopamine generally increases sensitivity to this pheromone, we could expect octopamine treatment to influence other responses to this pheromone. To test this hypothesis, we determined the effect of octopamine on 'cell capping' behaviour, in which adult bees place a wax capping over larvae developing in 'cells' in the honeycomb. This capping is required for larval and pupal development and so constitutes an important component of brood care.

Materials and methods

Experiments were performed during the summers of 2002 and 2003 at the University of Illinois Bee Research Facility. Honey bees were a typical North American mixture of European subspecies of *A. mellifera* (Pellett 1938) and maintained according to standard beekeeping practices.

Experimental colonies

Experiments 1 and 3 used triple-cohort colonies (TCCs), which were composed of three cohorts of bees of different ages. TCCs roughly simulate the normal range of worker ages within a colony, while controlling for variation in demography between colonies (Giray and Robinson 1994). Within a trial of an experiment, the same field colony was used as the source of bees for all TCCs to control for genetic differences between TCCs. The first cohort was 500 paint-marked 1-day-old adult bees. The second cohort comprised 400 bees that had been paint marked on emergence (with a different colour) and placed in an otherwise undisturbed colony to age normally for 1 week before collection. The final cohort consisted of 300 unmarked foragers collected returning to the entrance of a source colony. The age of the foragers is not known exactly, but we assume their average age is about 3 weeks (Michener 1974; Winston 1987). Each colony was provided with a young mated queen, one frame containing 50 g pollen and 500 ml octopamine-treated or control sucrose solution, and one frame of empty comb. All colonies were housed in Langstroth hive boxes with two frames of honeycomb.

Experiment 2 used two-frame glass-walled observation hives. Observation hives were populated by shaking the bees from three uncapped brood frames taken from a strong source colony into each observation hive. Care was taken to match the worker populations in the two observation hives in each trial as closely as possible. Different unrelated source colonies were used for each trial. Each observation hive was provided with a mated queen, 80 g pollen and 800 ml 50% sucrose in two frames of honeycomb.

Octopamine treatment

All experiments compared the behaviour of bees in octopamine-treated and untreated (octopamine-control) colonies. Octopamine-treated bees were provided with a 0.5 g/ml sucrose solution containing octopamine at a concentration of 2 mg/ml (Schulz and Robinson 2001). The sucrose solution was presented in honeycomb *ad lib*. Octopamine-control bees were fed 0.5 g/ml sucrose. This is a non-invasive method of chronically elevating octopamine levels in the brain (Schulz and Robinson 2001). This treatment elevates brain levels of octopamine in a dose-dependent manner, but does not alter levels of dopamine or serotonin (Schulz and Robinson 2001). Treatment with the octopamine precursor tyramine does not influence foraging behaviour (Schulz and Robinson 2001), suggesting that octopamine reaches the brain without degradation, and that the behavioural effects we observed are specific to octopamine treatment. Oral treatment has been used to raise brain levels of octopamine in *Drosophila* also (Schwaerzel et al. 2003).

One drawback is that the treatment likely results in all parts of the brain, and the rest of the nervous system, being exposed to increased amounts of octopamine, which precludes localization of effect. However, previous findings of a "gain of function" (precocious foraging Barron et al. 2002; Schulz and Robinson 2001) suggest that this treatment produces physiologically relevant behaviour as well as a consistent increase in brain levels. To reach the brain, octopamine would have to pass from

the gut via the haemolymph. We assume that our treatment method elevated haemolymph as well as brain levels of octopamine, but haemolymph levels were not measured directly as part of this study.

Validation of octopamine treatment

For all experiments, brain levels of octopamine in bees from octopamine-treated and octopamine-control colonies were measured using HPLC to confirm that treatments raised brain levels of octopamine. Sampling methods and HPLC protocols are described in detail in Barron et al. (2002), and Schulz and Robinson (1999). Briefly, a sample of 12–20 bees was collected directly into liquid nitrogen from each colony in each trial of each experiment. Bee heads were stored at −80°C until brain dissection and amine analysis. Whole bee heads were partially freeze dried at −10°C and 300 mTORR for 62 min to aid dissection (Müller and Altfelder 1991) and brains were dissected from the head capsule over dry ice to prevent thawing. Amines from each individual brain were extracted in 100 µl 0.2 M perchloric acid. Ten microliters of each sample were separated across a 80 mm×4.6-mm-high efficiency, reverse phase ESA Catecholamine HR-80 column, and amine content analysed with an ESA Coulochem II electrochemical detector coupled to an ESA 2-channel microdialysis analytical cell. DHBA and synephrine were included with each sample as internal standards, and the external standards dopamine, serotonin and octopamine were included at the beginning and end of each HPLC run. Identification of amines was based on peak retention times referenced to the internal and external standards using the EZChrom software package (ESA). Levels of dopamine and serotonin were not quantified here because a previous study showed no effects of octopamine treatment on these amines (Schulz and Robinson 2001).

Pheromone treatment

In these experiments we used both natural (described in experiment 3) and artificial brood pheromone to elevate the level of brood pheromone in each colony to more than 5 larval equivalents (Leq) per resident bee. This dose was chosen since it had been shown by two prior studies to be a level of artificial pheromone that influenced foraging behaviour (Pankiw et al. 1998; Barron et al. 2002). Our total dose was 6,800 Leq per TCC. The dose is large considering the adult population in each TCC was just 1,300, but since the number of brood in the brood nest of a typical colony can exceed 10,000 our dose was within the normal range experienced by bees.

The brood pheromone treatment was similar to that used by Barron et al. (2002). We used the synthetic brood pheromone mixture of ten fatty acid esters described by Le Conte et al. (1994), which is the pheromone blend produced by old larvae. The pheromone blend was dissolved in hexane and applied to a petri dish. Once the hexane had evaporated, the petri dish coated in the solid pheromone was inserted into a cut section of honeycomb to position it in a colony. We applied a dose of 5 Leq/bee (383 ng solid pheromone = 1 Leq, Le Conte personal communication). Control colonies received a hexane-washed petri dish.

Experiment 1: Effect of octopamine on undertaking behaviour

Undertaking behaviour refers to the removal of corpses from colonies by workers (Visscher 1983). Each trial of this experiment used two matched TCCs (octopamine-treated and octopamine-control). To assay undertaking behaviour, 20 freshly killed bees (with a spot of paint on the thorax) were tipped between the honeycombs in the TCCs. For the next hour, the colony entrances were observed to record when the corpses where removed. The age cohort of the bee (first, second or final) removing the corpse was also recorded. Three runs were performed for each trial on consecutive days and four trials of the experiment were carried out.

Foraging activity was also recorded in these colonies as a bioassay for the effectiveness of octopamine treatment (we expected higher foraging activity in the octopamine-treated colony, as in Barron et al. 2002). Foraging activity was estimated by recording the number of foragers entering or leaving the hive in a 10-min observation period, as in Barron et al. (2002). Each colony was observed for 10 min each hour starting 1 h after administering the brood pheromone treatments. Four observations were made of each colony each day. Foragers were identified as bees with pollen loads or swollen abdomens indicating a full crop.

Experiment 2: effect of octopamine on retinue response to the queen

This experiment used two observation hives (octopamine-treated and octopamine-control). Each colony was observed for 1 h each day over 5 days (half an hour in the morning and half an hour in the afternoon). During observations, the queen and surrounding bees were videotaped for later analysis (Sony Hi 8 digital camera). At minute intervals, the number of bees in the queen's retinue was counted; this is an established measure of the responsiveness of bees towards a queen (Slessor et al. 1988). Bees were classed as attending the retinue if they were in antennal contact with, and oriented towards, the queen. Sometimes concentric rings of attendants formed surrounding the queen, in which case we also counted bees that were motionless, oriented towards the queen and in antennal contact with a bee in the next layer closer to the queen. At each minute, one bee in the retinue was selected at random and the duration of her time in attendance was measured. Four trials of this experiment were performed.

The colonies used in this experiment were also used in unrelated experiments that required the observation hives to be housed in two adjacent sections of a large outdoor flight cage, and foragers in the octopamine-treated colony to be fed at an octopamine-treated feeder. This had the dual benefit of continually replenishing the octopamine in the octopamine-treated colony and also facilitated monitoring foraging as a bioassay of the effectiveness of the octopamine treatment.

Experiment 3: effect of octopamine on brood care and foraging responses to brood pheromone

Nurse bees build a thin wax cap over cells containing fifth instar honey bee larvae so that the larvae complete development in a sealed and stable environment (Winston 1987). This behavior is an essential component of brood care, since failure to cap or damage to the caps usually results in wing malformations during pupation. The capping response is stimulated by brood pheromone, and is an easily quantified component of brood care (Le Conte et al. 1994). To assay capping behaviour, we applied a controlled capping stimulus to each colony. This was a 10×10-cm section of honeycomb containing larvae (approx. 300) at the right developmental stage for capping. Sections were cut from a comb containing fourth and fifth instar larvae taken from a donor colony. Two 10×10 sections were cut from the same comb. Care was taken to match the numbers of larvae on each section as closely as possible and sections were assigned arbitrarily to TCCs. The number of larvae at the capping stage was counted on each section, and sections were inserted into a pre-cut area of an otherwise empty honeycomb for placement in the experimental TCCs. After 24 h in a TCC, the number of these larvae that had been capped was recorded and the proportion of capped larvae determined. The control was a section of empty cells cut from a comb taken from a donor colony.

Each trial of the experiment involved four TCCs, which were made as similar to each other as possible to facilitate comparisons of activity between colonies. One pair of TCCs was treated with octopamine (octopamine-treated) and the other pair with plain sucrose (octopamine-control). On day 1 of the experiment, one colony within each pair was treated with both the synthetic brood pheromone and the section of comb with larvae (pheromone-treated) and the other colony received a petri dish washed in hexane only and a section of empty comb (pheromone-control). Foraging observations began 1 h after adding pheromone treatments. After 24 h, pheromone treatments were removed and we recorded the proportion of larval cells on the section that had been capped.

On the following day (day 2), the above protocol was repeated, but the pheromone treatments were swapped between TCCs within the octopamine-treated and octopamine-control pairs. At the end of day 2, all colonies were provided with fresh sucrose stores and colonies were sealed for 24 h (day 3). This ensured that octopamine treatments stayed fresh in octopamine-treated colonies and also balanced food reserves between colonies.

Days 4 and 5 of the protocol repeated days 2 and 1, respectively. This schedule balanced variation between colonies treated with pheromone. But within a trial it was not possible to change which colonies were treated with octopamine because it was not clear how long the treatment persisted in the honeycombs or in the bees' crops. Therefore, we could not balance variation between the octopamine-treated *pair* and octopamine-control *pair*. This weakness was countered by making the colonies as similar as possible at the outset. We performed three trials of this experiment.

Methods for recording foraging activity were the same as for experiment 1. Four measures were recorded on each of the four observation days of each trial.

Statistical methods

All analyses were performed with the S-PLUS statistical software. For each experiment octopamine measures generated by HPLC were compared between octopamine-treated and octopamine-control colonies using unpaired *t*-tests, assuming unequal variance.

In experiment 1, the corpse removal time data had a strong right-hand skew. Further, some marked corpses were not removed during the 1-h observation period and it was unclear whether these should be classed as missing data or assigned a removal time of >1 h. For these reasons, corpse removal times were analysed using survival analysis (Collett 1994; Kalbfleisch and Prentice 1980), which can accommodate data of this structure. Differences in distribution of corpse removal times between octopamine-treated and octopamine-control colonies were tested with the Peto–Peto modification of the Wilcoxon test (Collett 1994).

Bees removing marked corpses were identified to age cohort and the proportions of undertaking acts performed by bees in the first and second cohorts in the octopamine-treated and octopamine-control colonies compared with Chi-square contingency-table analyses. Within a trial, Chi-square statistics from each run were summed to give an overall comparison for each trial.

For each trial of experiment 2, the distribution of queen attendance times showed a very strong right-hand skew, resembling an exponential distribution rather than normal. Seeley (1979) observed a similar distribution for queen attendance data. We used Kolmogorov-Smirnov two-sample tests to compare the distribution of attendance times in octopamine-treated and octopamine-control colonies in each trial (Siegel and Castellan 1988). Since the Kolmogorov-Smirnov statistic is approximately Chi-square distributed for samples greater than 25, we asked if there was an overall difference in queen attendance time across the four trials by summing the individual statistics and deriving a *P* value from

chi-square tables at three degrees of freedom. The distribution of retinue size data was also non-normal, and was analysed in the same way.

For the analysis of the foraging response to brood pheromone in experiment 3, we used the method developed by Barron et al. (2002). The premise was that if we monitored two colonies that differed only in whether brood pheromone was added or not, the difference in foraging activity between them would be a measure of the response to brood pheromone. While it is impossible to have two identical colonies in the field, our TCCs were made from the same source colonies, were established with identical populations and demographies, and were provided with the same food and comb resources. Consequently, we believe that within a trial, our TCCs were similar enough to each other to justify this analysis. Response to brood pheromone was estimated for each pair of colonies (octopamine-treated or octopamine-control) by subtracting activity in the pheromone-control colony from activity in the pheromone-treated colony for each hour of observation. Each trial yielded 16 measures of the response to brood pheromone for each pair of colonies (octopamine-treated and octopamine-control). We then compared the response to brood pheromone in the octopamine-treated pair and octopamine-control pair using a Wilcoxon rank-sum test (Fowler and Cohen 1990).

To determine the effect of octopamine treatment on the capping response in experiment 3, we compared the proportion of larval cells capped in octopamine-treated versus octopamine-control colonies. Each trial of the experiment yielded four measures for each treatment. These data were analysed using a generalised linear model (Collett 1991; Venables and Ripley 1994), treating the number of larvae capped and number of larvae uncapped as a binomial response variable and colony type and trial as explanatory factors.

Results

In all three experiments, octopamine treatment significantly raised brain levels of octopamine (Table 1). Experiments 1 and 3 sampled 10-day old bees from the comb inside the hive, and here both brain levels and the magnitude of the octopamine treatment are comparable to those reported by Schulz and Robinson (2001). We observed extremely high brain levels of octopamine in the octopamine-treated group in experiment 2, perhaps because here we sampled foragers that could only feed from a feeder containing an octopamine-treated sucrose solution. Our data confirm the finding of Schulz and Robinson (2001) that both hive bees and foragers treated with octopamine show elevated brain levels of octopamine.

Experiment 1: effect of octopamine on undertaking behaviour

Table 2 summarises the effect of octopamine treatment on corpse removal. In eight comparisons, there was no significant difference in the rate of corpse removal between octopamine-treated and octopamine-control colonies. The octopamine-treated TCC was significantly faster to remove corpses on two occasions and significantly slower on one.

Figure 1 summarises the proportions of undertaking acts performed by young bees (first or second cohort) in octopamine-treated and octopamine-control colonies. In three out of four trials, the younger cohorts performed a significantly greater proportion of undertaking acts in the octopamine-treated colonies. A caveat to this conclusion is that undertakers were not individually marked, and so the same individual may have been scored more than once. Our observation is that, at the colony-level, the undertaking force was on average younger in octopamine-treated colonies.

Figure 2 summarises the effect of octopamine treatment on foraging behaviour in these TCCs. Foraging activity was significantly greater in octopamine-treated than octopamine-control colonies in four out of four trials.

Experiment 2: effect of octopamine on responsiveness to the queen

Figure 3a summarises the effect of octopamine treatment on how long workers spent in the queen's retinue. In one trial, median attendance time was significantly lower in the octopamine-treated colony than the octopamine-control, but in three out of four trials there was no difference, and no difference overall ($\chi^2 = 0.655$, df = 3, $P = 0.88$).

Figure 3b summarises the effect of octopamine treatment on the size of the retinue. Kolmogorov-Smironov analyses revealed differences, but there was no consistent effect of octopamine treatment across the four trials. In trials 1 and 4, the retinue was significantly

Table 1 Effect of octopamine treatment on brain levels of octopamine in all experiments

	Mean octopamine-treated colony (pg/brain)	Mean octopamine-control colony (pg/brain)	*t*-test
Experiment 1	1,392 s.e = 236 $N = 17$	784 s.e = 71 $N = 17$	$t = 2.33$, $P = 0.031$ d.f. = 19
Experiment 2	4,317 s.e = 400 $N = 16$	1,706 s.e = 126 $N = 20$	$t = 6.31$, $P < 0.001$ d.f. = 18
Experiment 3	1194 s.e = 163 $N = 16$	692 s.e = 38 $N = 15$	$t = 2.98$, $P = 0.008$ d.f. = 17

Table 2 Effect of octopamine on speed of corpse removal

Mean undertaking time in octopamine-treated and octopamine-control colonies calculated using the Kaplan-Meier estimator of survival. Undertaking was compared between colonies using the Peto–Peto Wilcoxon test. For each trial, an overall statistic was obtained by summing individual Chi-square values and obtaining *P* values at two degrees of freedom. Significant differences between colonies are highlighted in bold type

Trial	Day	Mean octopamine-treated (min)	Mean octopamine-control (min)	χ^2	*P*
1	1	39.1	31.5	1.2	0.268
	2	30.6	25.6	0.1	0.755
	3	51.2	49.4	0.1	0.709
Overall				1.4	*0.498*
2	1	19.8	11.3	0.1	0.704
	2	9.8	16.8	3.9	**0.040**
	3	17.6	14	0.1	0.778
Overall				4.1	0.128
3	1	31.2	22.2	2.1	0.145
	3	33.8	39.9	0.6	0.436
Overall				2.7	0.100
4	1	16.45	8.45	6.9	**0.008**
	2	16.6	31.9	3.3	0.067
	3	11.0	33.8	19.8	**< 0.001**
Overall				30	**< 0.001**

larger in octopamine-treated colonies, in trial 3, there was no difference and in trial 2, the retinue was significantly smaller. There was no overall difference across trials ($\chi^2 = 1.31$, df = 3, $P = 0.72$).

Experiment 3: effect of octopamine on nursing and foraging responses to brood pheromone

Figure 4 summarises the effect of octopamine treatment on the foraging response to brood pheromone. The change in foraging activity in response to brood pheromone treatment was significantly greater in octopamine-treated colonies than octopamine-control colonies in trials 1 and 2 and a trend in the same direction was observed in trial 3 (Fig. 4).

Figure 5 summarises the effect of octopamine treatment on the capping response to brood pheromone. In contrast to the foraging response, octopamine treatment caused a significant reduction in the brood capping response (Fig.5 and Table 3). There was also variation in the capping response between trials and a small, but significant, variation in the size of the octopamine effect between trials (Table 3).

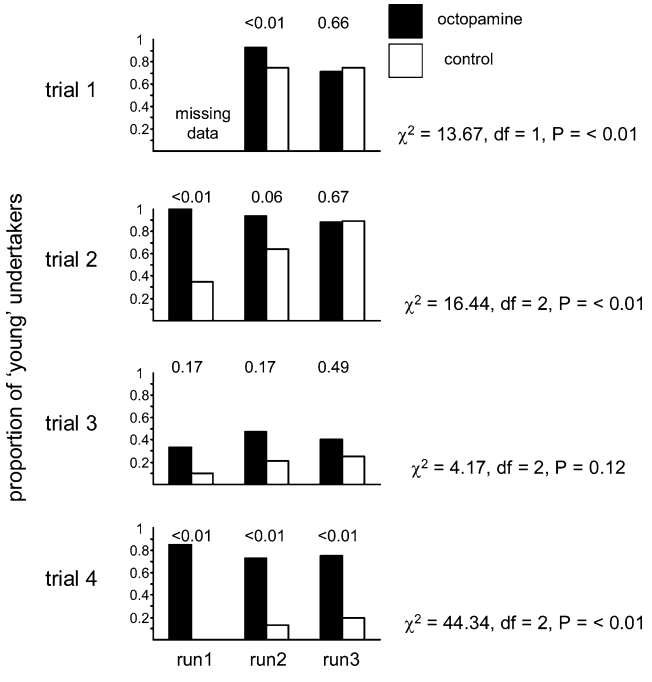

Fig. 1 Effect of octopamine on age of undertakers. Percentage of undertaking acts performed by bees in the two youngest cohorts in octopamine-treated and octopamine-control triple-cohort colonies (TCCs). Data from four trials shown; three runs were performed on consecutive days within each trial (data for run 1, trial 1 are missing). Numbers above bars are *P* values calculated from Chi-square contingency analyses. Overall Chi-square anlayses for each trial are also shown

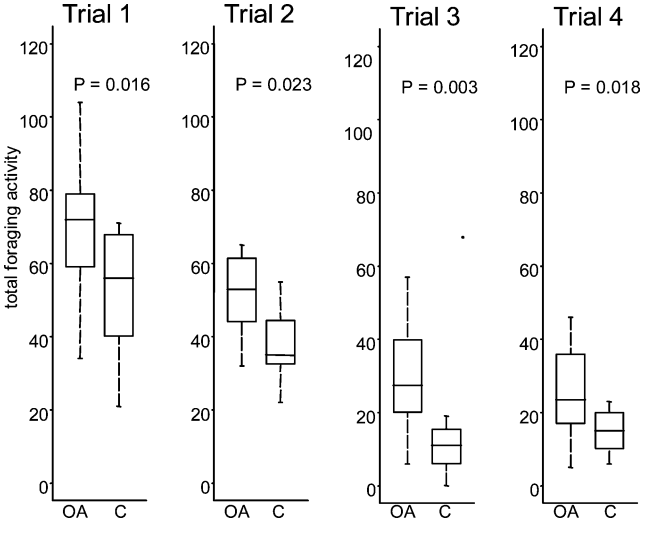

Fig. 2 Effect of octopamine on foraging activity. Total foraging activity (entering and leaving) during the 12 10-min observation periods for octopamine-treated and octopamine-control colonies. Data from four trials shown (same experiment as Fig. 1). The line within each boxplot marks the median, boxes extend to the upper and lower quartiles and whiskers extend to 1.5 × interquartile distance. *P* values refer to Wilcoxon rank-sum tests of the null hypothesis that activity did not differ between the octopamine-treated (OA) and octopamine-control (C) colonies. Activity was significantly greater in octopamine-treated colony than octopamine-control colony in four out of four trials

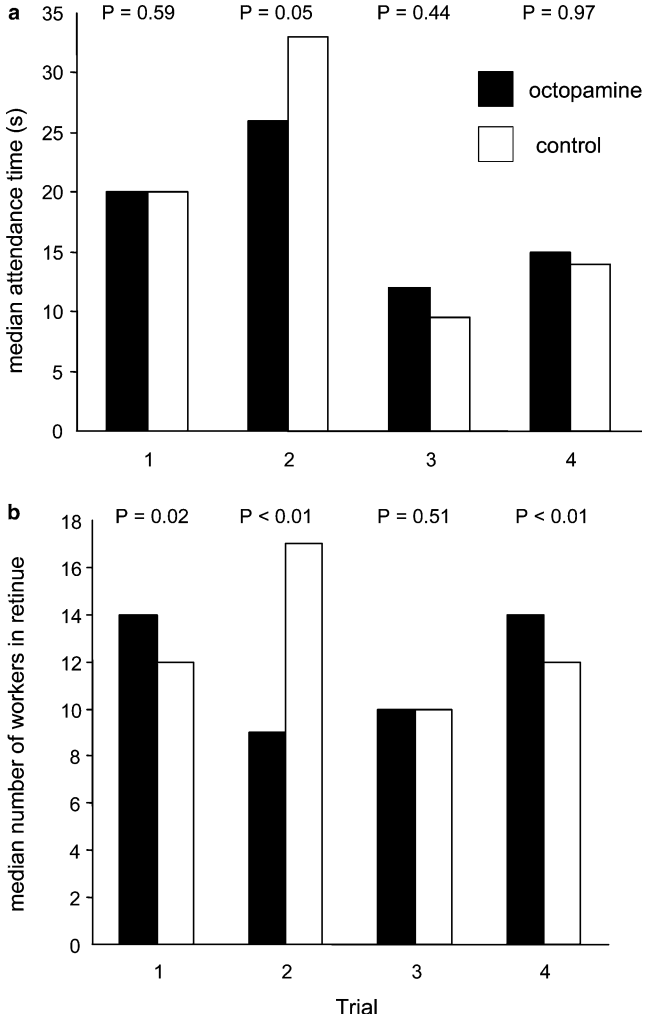

Fig. 3 Effects of octopamine on queen retinue behaviour. **a** Median time workers attend the retinue in octopamine-treated and octopamine-control colonies. Data from four independent trials shown. **b** Median number of workers in the retinue in octopamine-treated and octopamine-control colonies. Neither data set was normally distributed, therefore error bars are not shown. *P* values refer to Kolmogorov-Smirnov two-sample tests

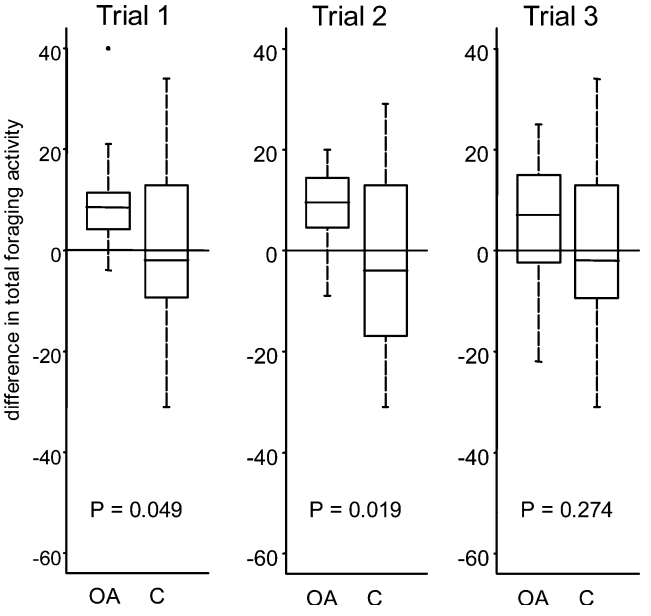

Fig. 4 Effects of octopamine on responsiveness to brood pheromone. Each box shows the difference in total foraging activity between a colony exposed to pheromone and one that was not in both octopamine-treated (OA) and octopamine-control (C) colony pairs. The line within each boxplot marks the median, boxes extend to the upper and lower quartiles and whiskers extend to 1.5 × interquartile distance. Data from three independent trials shown. *P* values refer to Wilcoxon rank-sum tests of the null hypothesis that the response to brood pheromone did not differ between octopamine-treated and octopamine-control colony pairs. The effect of brood pheromone treatment on activity was significantly greater in octopamine-treated colonies than octopamine-control colonies in two out of three trials

Discussion

These experiments show that octopamine selectively modulates task performance in the context of honey bee division of labour. Octopamine increased foraging activity, but did not change the rate of corpse removal, which suggests octopamine does not affect all tasks involving flight. Octopamine did not change the retinue response to the queen. Since retinue behaviour is mediated by queen mandibular pheromone, this suggests that octopamine does not cause a general increase in responsiveness to odour stimuli. This is consistent with the finding that octopamine changed how bees responded to brood pheromone, increasing the foraging response to the pheromone but decreasing the capping response. The selective modulation of different responses to brood pheromone suggests that octopamine does not generally increase sensitivity to brood pheromone. These results demonstrate greater specificity for neurochemical modulation of pheromone-mediated behaviour than observed previously.

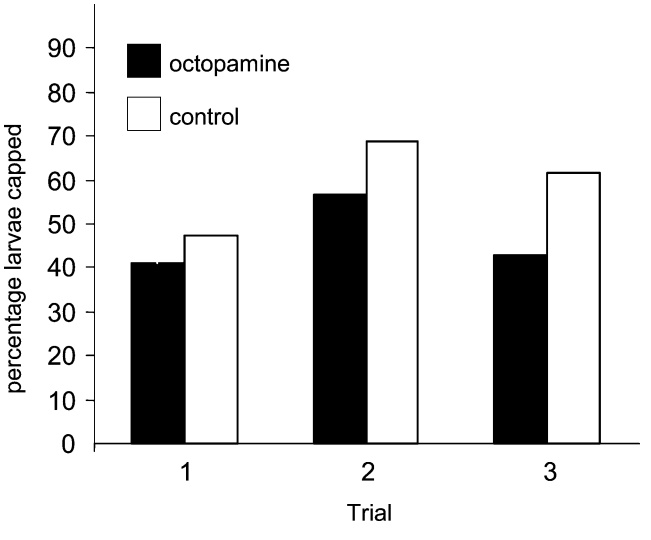

Fig. 5 Percentage capping of larvae in octopamine-treated and octopamine-control colonies. Data from three trials shown. Mean capping calculated from the four runs performed in each trial. Statistical analyses presented in Table 3

Table 3 ANODEV table for GLM testing the null hypothesis that capping of larvae did not differ with octopamine treatment and between trials

	Residual deviance	Residual df	Δ deviance	Δ df	$P(\chi^2)$
Intercept	470.912	23			
Octopamine treatment	424.914	22	46.04	1	< 0.001
Trial	358.081	20	66.83	2	< 0.001
Treatment: trial interaction	350.759	18	7.321	2	0.025

The model assumed a binomial error structure and used a logit link function to linearise the capping proportions. The explanatory variables, treatment (octopamine-treated and octopamine-control) and trial number, were expressed in the model as factors. Only the minimum adequate model is shown

One possible interpretation of our brood pheromone experiment is that octopamine treatment caused so many bees to forage that there were no bees left to cap cells containing larvae. We consider this interpretation unlikely. Capping of cells is expected to be performed by the youngest cohort (Winston 1987), who were almost never seen foraging; the octopamine-induced boost in foraging activity came from the second and third age cohorts. Therefore, the potential force of bees available to cap should have been unchanged by the octopamine treatment.

Our experiments have tested the effect of only one dose of octopamine on behaviour. It is possible that other doses could have had different behavioural effects. However, Schulz and Robinson (2001) reported that while the dose we used (2 ug/ml) had a robust effect on foraging behaviour, lower doses did not, and toxic effects were observed at higher doses. Our results show that octopamine treatment, at a dose that stimulated foraging, did not also stimulate capping of brood or the retinue response.

Octopamine is a key modulator of olfactory based behaviour in honey bees. Octopamine immunoreactive neurons are present within the honey bee antennal lobes (Spivak et al. 2003). Octopamine treatment improves learning of olfactory stimuli in laboratory assays (Mercer and Menzel 1982; Menzel 2001) and also improves performance in nestmate recognition and removal of diseased brood from the colony (hygienic behaviour), both of which require fine olfactory sensitivity and discrimination (Robinson et al. 1999; Masterman et al. 2001; Spivak et al. 2003). These results, and those presented here, indicate that octopamine modulates some, but not all, olfactory mediated behavioural responses for honey bees.

Honey bees have several different behavioural responses to brood pheromone. For example, short-term exposure causes an increase in cell capping by young bees, and an increase in foraging activity by bees already competent to forage (Le Conte et al. 1990; Pankiw et al. 1998; Barron et al. 2002) while long-term exposure delays the age at onset of foraging (Le Conte et al. 2000). Our study is notable because octopamine modulated these brood pheromone effects selectively. In Lepidoptera, octopamine acts both centrally and peripherally to increase the likelihood that males respond to female sex pheromones with an upwind flight (Grosmaitre et al. 2001; Linn and Roelofs 1986; Pophof 2000, 2002). In Lepidoptera and honey bees, octopamine can act directly on olfactory neurons to change sensitivity to olfactory stimuli (Pophof 2000; Spivak et al. 2003). Octopamine may influence the sensitivity of olfactory neurons to brood pheromone components, but the selective modulation of different behavioural responses to brood pheromone we observed suggests effects on specific circuits.

Studying the effects of octopamine treatment on EAG responses to brood pheromone and queen mandibular pheromone would reveal whether octopamine affected peripheral sensitivity to these pheromones, but exploring where in the central brain octopamine acts to modulate specific behavioural responses is more difficult. Local microinjection of amines into brain regions has only been performed on harnessed bees, who cannot participate in normal social interactions. Developing a technique for targeted drug delivery in free roaming bees would be a significant advance towards understanding neurochemical regulation of social behaviour.

Octopamine treatment increased foraging activity but did not affect the rate of corpse removal, a task, like foraging, that involves flight and leaving the hive. This finding suggests that octopamine did not increase the rate of all flight-dependent behaviour. Treatment did, however, affect the demographic structure of the undertaker group, with a higher proportion of younger bees involved in octopamine-treated colonies. Undertaking is typically performed by a group of mostly middle-age behavioural 'specialists' that perform undertaking for one or more days prior to foraging (Trumbo et al. 1997). Because we did not observe an increase in the rate of corpse removal, we speculate that the effect on undertaking by younger bees was an indirect effect of the increased recruitment of older bees to foraging activity. However, we cannot rule out the possibility of more direct effects, especially in light of known effects of octopamine on hygienic behaviour, which is functionally related to corpse removal (Spivak et al. 2003).

We have consistently observed that not all bees in an octopamine-treated colony forage (Barron et al. 2002; Schulz and Robinson 2001; Schulz et al. 2003, 2002b). We propose a high level of octopamine in the brain makes a bee more responsive to foraging-related stimuli and more *likely* to forage, but a bee must still be exposed to a superthreshold foraging stimulus before initiating foraging. There are many stimulators and inhibitors of foraging in a honey bee colony, many of which are mediated socially (Beshers and Fewell 2001; Huang and Robinson 1996; Leoncini et al. 2004; Pankiw and Page 2000; Pankiw et al. 1998; Seeley 1995). Social structure and colony state as well as brain neurochemistry will influence the behaviour of an individual bee.

Octopamine is also known to modulate performance in laboratory-based reward-learning tasks (Farooqui et al. 2003; Hammer and Menzel 1998; Mercer and Menzel 1982), sucrose responsiveness (Scheiner et al. 2002), nest mate recognition (Robinson et al. 1999) and hygienic behaviour (Spivak et al. 2003). Octopamine may act through different neuroanatomical regions, or different circuits in the brain to modulate these different responses. It is also possible that octopamine modulates aspects of sensory performance or motivation that facilitate the expression of several different behavioural responses. For example, increased sucrose sensitivity and reward-seeking motivation would likely facilitate foraging behaviour. Increased olfactory acuity would also benefit a forager, but would likely facilitate expression of hygienic behaviour and nest mate recognition as well.

The new results reported here are 'negative': no effects of octopamine treatment on undertaking, queen attendance or the cell capping response to brood pheromone. However, these results are important in light of previously shown effects on other bee behaviours, including foraging (confirmed again here). Selective modulation of task performance suggests complex and varied pathways of octopamine action in the bee brain. A better understanding of the octopaminergic pathways and circuits in the honey bee brain will give a clearer picture of how octopamine modulates behaviour.

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