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Comparing injection, feeding and topical application methods for treatment of honeybees with octopamine

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Abstract

Entomologists have used a range of techniques to treat insects with neuroactive compounds, but it is not always clear whether different treatment methods are equally effective in delivering a compound to a target organ. Here, we used five different techniques to treat honeybees with ^{3}H-octopamine (^{3}H-OA), and analysed the distribution of the ^{3}H radiolabelled compound within different tissues and how it changed over time. All treatment methods, including injection of the median ocellus, resulted in ^{3}H-OA detection in all parts of the honeybee. Injection through the median ocellus was the most effective method for delivering ^{3}H-OA to the brain. Topical application of ^{3}H-OA dissolved in dimethylformamide (dMF) to the thorax was as effective as thoracic injections of ^{3}H-OA in delivering ^{3}H-OA to the brain, but topical applications to the abdomen were less so. Most of the ^{3}H-OA applied topically remained associated with the cuticle and the tissues of the body segment to which it had been applied. For all treatment methods, ^{3}H-OA was rapidly lost from the brain and head capsule, and accumulated in the abdomen. Our findings demonstrate the value of thoracic topical treatment with compounds dissolved in dMF as an effective non-invasive method for short-term, systemic pharmacological treatments.

Keywords: Honeybee; Apis mellifera; Octopamine; Pharmacology; Biogenic amines

1. Introduction

Neuroethologists aiming to identify the molecular pathways involved in natural behaviour frequently use pharmacological treatments to manipulate behaviour and test hypotheses. Further, the types of behaviour that interest neuroethologists often demand that manipulations be as non-invasive as possible, and allow the animal to be free to move. This is especially true for studies of social behaviour.

It is not always clear how different treatment strategies compare in the delivery of drugs to a target organ. Here, we treated honeybees (Apis mellifera) with the biogenic amine octopamine (OA) using injection, oral and topical methods of drug administration, and compared the distribution of OA through the tissues of the honeybee over time.

The biogenic amines are important modulators of behaviour in vertebrates and invertebrates. In insects, the principal biogenic amines are OA, dopamine and serotonin. These three neurochemicals have been shown to play important roles in insect learning and memory (Schwaerzel et al., 2003; Unoki et al., 2005) as well as modulating states of arousal (Adamo et al., 1995) and behavioural state (Kravitz and Huber, 2003; Roeder, 2005). Further, in honeybees, OA modulates division of labour (Barron and Robinson, 2005; Barron et al., 2002; Schulz and Robinson, 2001), hygienic behaviour (Spivak et al., 2003) and nest mate recognition (Robinson et al., 1999). For these

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reasons, the biogenic amine systems in the honeybee brain have been a target for researchers exploring the neuromolecular mechanisms of many aspects of honeybee behaviour.

Different researchers have used different strategies to manipulate neurochemical systems in the bee brain. Microinjection of compounds directly into regions of the brain is a highly targeted method of drug delivery. Microinjection has been used successfully with bees harnessed for the proboscis extension response assay, an important assay for associative learning in honeybees (Hammer and Menzel, 1998). These studies demonstrated that OA is an important modulator of associative learning (Hammer and Menzel, 1998). Microinjection allows for precise and targeted delivery of OA to the brain, but since it requires that the bee's head be fixed and the head capsule opened, microinjection is incompatible with behavioural assays that require bees to move freely and interact socially. Compounds can also be introduced to the brain by microinjection through the median ocellus (Mercer and Menzel, 1982), as the ocellar nerve will conduct compounds into the brain close to the mushroom bodies (Mercer and Menzel, 1982; Pan and Goodman, 1977). Ocellar injection can be achieved by piercing or removing the lens of the median ocellus. This is a relatively subtle way of introducing compounds directly into the bee brain, compared to exposing and directly injecting the mushroom bodies.

Compounds injected through the cuticle of the thorax or abdomen have similar behavioural effects to compounds injected directly into the brain. Even complex social interactions can be modulated by octopaminergic drugs delivered in this way (Robinson et al., 1999). Typically, bees are briefly anaesthetized over ice prior to injection. It is presumed that the drugs move through the body via the haemolymph to affect their target organs. While abdominal or thoracic injections potentially expose all tissues in the bee body to the drug, they are simpler, faster and less invasive than brain microinjection, and so can be used in a wider range of circumstances.

Compounds can also be delivered topically in a solvent that allows them to penetrate the cuticle. Solvents used include acetone (Robinson, 1987; Sigg et al., 1997), dimethylformamide (dMF, Si et al., 2005) and dimethyl sulphoxide (dMSO, Guez et al., 2001; Sampson et al., 2005). This method provides a less invasive alternative to injection. A controlled dose can be quickly delivered to an individual bee's thorax or abdomen, and this technique has been used successfully to assess the effects of compounds on freely foraging bees (Si et al., 2005) by treating bees during the brief period when they are stationary on a sucrose feeder. Topical application is perhaps less precise than injection in that the amount of the drug penetrating the cuticle will vary depending on the drug and solvent used, and there will undoubtedly be inter-individual variation depending on the precise positioning of the solvent droplet, condition of the cuticle, ambient temperature and other factors. Despite these drawbacks, topical application is technically easier and less stressful to the animal than injection. Further, topical application carries less risk of infection than injection (Kucharski and Maleszka, 2003), and does not require that the animal be anaesthetized. Injection and anaesthesia cause an immune response, and affect behavioural responses (Barron, 2000; Mallon et al., 2003; Pankiw and Page, 2003).

Finally, biogenic amines have also been delivered orally to bees. Typically, compounds are dissolved in sucrose solutions or honey. This can then be fed to bees harnessed for proboscis extension assays (Pankiw and Page, 2003; Scheiner et al., 2002; Spivak et al., 2003), or even whole colonies can be treated with this technique (Barron and Robinson, 2005; Barron et al., 2002; Schulz and Robinson, 2001). Oral administration is particularly useful for chronically treating a very large number of bees with a neurochemical, and has been used to examine the effects of OA on colony-level traits such as division of labour and task specialization (Schulz and Robinson, 2001). An obvious drawback is that there will be variation in dose between bees, depending on the amount of food consumed by each bee.

Different studies demand different methods of drug delivery, but how do these methods compare in their efficiency of drug delivery to the target organs? Drugs topically applied to, or injected into, the thorax or abdomen must reach nervous tissues via the haemolymph. Insect nerves and ganglia are covered by a layer of perineural and glial cells linked by many tight and septate junctions that form an effective 'blood-brain' barrier blocking the paracellular passage of molecules between haemolymph and nervous tissues (Carlson et al., 2000). Clearly, some small neurotransmitters can cross this barrier. Maleszka et al. (2000) have shown that glutamate injected into the thorax was detected in the brain after 2–3 min. How glutamate crosses the blood-brain barrier is not clear, and few other studies have tested whether drugs applied somatically can actually reach the brain. Further, haemolymph is rapidly filtered by the Malpighian tubules, and many compounds would be expected to be rapidly cleared and excreted (Beyenbach, 2003; Pannabecker, 1995). Compounds applied orally would additionally need to survive digestion, and escape the gut into the haemolymph, which would reduce further the amount of an orally applied drug reaching nervous tissues.

These factors no doubt explain why the effective dose of a drug varies with the treatment method used, and perhaps why different drug application methods can sometimes give differing behavioural results. For example, Scheiner et al. (2002) observed that dopamine injected through the thorax reduced the responsiveness of bees to sucrose, whereas dopamine fed to bees had no significant behavioural effect. Dopamine is unstable in aqueous solution, and assuming that it takes longer for orally administered dopamine to reach the brain, it would seem likely that much of the orally applied dopamine could have broken down before reaching its target organ.

Here, we have compared the efficiency of different methods for delivering OA to the bee brain. We treated bees with ^{3}H-OA, and followed the dispersal of the radiolabel through the bee's body over time. We also used gas chromatography coupled to mass spectroscopy to confirm that ^{3}H-OA administered via topical treatment reached the brain without degradation.

2. Materials and methods

Experiments were performed at the University of Illinois Bee Research Facility at Urbana-Champaign and The Australian National University Research School of Biological Sciences, Canberra. Bees used were the standard commercially available hybrid of various European races available in North America and Australia, and reared according to standard bee keeping practices. A frame of capped brood was removed from a colony, and stored in a cage within a 33 °C humidified incubator overnight. Bees that emerged overnight were collected the following morning (newly emerged bees) for treatment. Newly emerged bees were used for the majority of the treatment studies to facilitate rapid dissection.

DL-Octopamine [1-^{3}H] hydrochloride (^{3}H-OA) was obtained from American Radiochemicals Inc (specific activity 50 Ci/mmol) dissolved in ethanol at a concentration of 1 mCi/ml.

2.1. Injection

OA was dissolved in insect ringer (Bicker, 1995) at a concentration of 1 μg/μl (5.25 mM). One μl of ^{3}H-OA in ethanol was added to 20 μl of this solution to provide sufficient radiolabelled material for later detection. One μl of this radiolabelled solution was injected into <24 h old bees using a Hamilton syringe.

Thoracic injections were made through the scutal fissure at the base of the mesonotum (Snodgrass, 1956) to the right of the midline (Maleszka et al., 2000). Immediately prior to injection, bees were briefly anaesthetized over ice. Post-injection bees were placed in small cages with access to 2 M sucrose ad libitum.

For injection of the median ocellus, bees were first mounted in metal cartridges, and secured with a 2 mm wide strip of duct tape, as if prepared for the proboscis extension assay (Maleszka and Helliwell, 2001), but ensuring that the tape immobilized the head. The lens of the median ocellus was removed with a microscalpel, exposing the photoreceptor layer beneath. A 1 μl drop of the ^{3}H-OA solution was placed onto this area, and was slowly absorbed by the tissue over a few minutes (Mercer and Menzel, 1982).

2.2. Oral treatment

Newly emerged bees were secured in a metal cartridge using a narrow strip of duct tape, and positioned so that they could move their antennae and extend their proboscis. Bees were starved for 4 h from 6 a.m. to 10 a.m.

OA was dissolved in a 2 M sucrose solution at a concentration of 2 mg/ml (10.5 mM). One μl of ^{3}H-OA in ethanol was then added to 100 μl of this solution. Harnessed bees were each fed 10 μl of this solution (Pankiw and Page, 2003; Schulz and Robinson, 2001).

2.3. Topical treatment

OA was dissolved in 20 μl dMF at a concentration of 2 μg/μl (10.5 mM), and 1 μl of ^{3}H-OA in ethanol was then added. One μl of this solution was applied to either the thorax or abdomen of newly emerged bees using a Drummond glass microcapillary pipette. The 1 μl drop was applied to the centre of the dorsal thorax taking care that it did not spread into the neck, the petiole or around the wing hinges (Si et al., 2005). For abdominal treatment, the 1 μl drop was placed on the centre of the dorsal abdomen (segment IV, Snodgrass, 1956). Bees were held immobile for 30 s after treatment, to allow the solvent to penetrate the cuticle.

2.4. Sampling and scintillation assay

Bees were sampled 15 and 60 min after treatment with ^{3}H-OA. Bees were killed by chilling. Haemolymph was sampled by gently piercing the membrane between abdominal segments III and IV (Snodgrass, 1956), and collecting the droplet that formed using a Drummond micropipette. Between 1.5 and 10 μl were sampled from each bee. The body was then dissected into head, thorax and abdomen. The head capsule was opened to remove the brain, which was analysed separately from the rest of the head capsule.

A small number of bees that had been topically treated with ^{3}H-OA on either the thorax or abdomen, were killed 60 min after treatment, and dissected more comprehensively. An additional haemolymph sample was taken from the thorax by piercing at the base of the mesonotum to the left of the midline. The gut was removed from the abdomen, and the flight muscles dissected from the thorax. Each sample was pulverised in 10 μl insect ringer, and then added to 5 ml OptiPhase HiSafe 2 scintillation cocktail (Wallac) in a glass scintillation vial. ^{3}H was quantified using a Tri-Carb 2800TR Liquid Scintillation Analyzer from Perkin Elmer. To consider background radioactivity, we included 10 μl insect ringer as a negative control with each run of samples. By comparing total counts recovered from all tissues from a single bee with counts recorded from an equivalent amount of ^{3}H-OA added directly to scintillation cocktail, we estimate our recovery of ^{3}H-OA to be between 75% and 80%.

2.5. Analysis of brain amine content by gas chromatography and mass spectroscopy

The measures of scintillation counts tracked the dispersal of radiolabelled compounds through the body of the bee,

but this technique would not show whether the ^{3}H-OA had been altered or degraded during its passage through the body. Several studies (Barron et al., 2002; Schulz and Robinson, 2001; Schulz et al., 2002) have used HPLC measurements of brain amine content to show that there was a dose dependent increase in brain level of OA following oral treatments of OA. To confirm that topical treatments of OA also elevated brain levels of OA, bees foraging at a sucrose feeder in a 20 m outdoor flight cage were treated with 1 μl 10.5 mM OA in dMF to either the dorsal thorax or abdomen. Bees were treated while they drank sucrose from the feeder, and without being restrained in any way. The treated bees continued to forage normally. Treated bees were captured within 60 min of treatment, and immersed immediately in liquid N_2. Bees were stored at −80 °C until dissection. The central brain (minus optic lobes) was dissected from the head capsule over dry ice without allowing the tissue to thaw. Brains were then placed in silanized glass 100 μl vial inserts and immersed in methanol.

Internal standard, 4-hydroxy-3-methoxybenzylamine hydrochloride (4.04 ng in methanol; Aldrich, Milwaukee, WI), was added to each 100 μl vial insert containing a bee brain and methanol. The methanol from the bee brain extract was transferred by pipette to another silanized glass insert held in a 2 ml vial. The methanol was passively evaporated just to dryness under a stream of nitrogen. The sample was then placed under house vacuum for 3 h to remove residual water. Dry toluene (50 μl, freshly distilled with $LiAlH_4$; Fisher Fair Lawn, New Jersey), 0.1 M triethylamine (20 μl; Pierce, Rockford, IL), and heptafluorobutyric acid anhydride (2.5 μl; Pierce, Rockford, IL) were added to the vial insert. The vial was capped, vortexed vigorously, and then continuously shaken on a shaker table for 1 h. Sodium phosphate tribasic, pH 6.0 buffer (50 μl, 1 M; Fisher, Fair Lawn, New Jersey) was added to the insert and vortexed for 30 s. The sample was then centrifuged for 5 min, and the organic phase pipetted into another silanized glass insert held in a 2 ml gas chromatograph (GC) autosampler vial. The components of interest in the sample were separated using a GC (Agilent 6890N, Palo Alto, CA) equipped with a 30 m × 0.25 mm i.d. DB-1 fused silica capillary column (Agilent J&W Scientific, Folsom, CA). Helium was used as the carrier gas. The GC oven was programmed from 120 to 300 °C at 10 °C/min, holding at 300 °C for 2 min. Eluting components were detected and analysed on an Agilent Mass Selective Detector (5973 Network, Palo Alto, CA) using electron impact (EI) and single ion monitoring modes (SIM). The GC-MS transfer line was set at 285 °C, the source was at 230 °C and the MS quadruples set at 150 °C. The eluting components were identified based on unique retention times and fragmentation ions. The unique ions for OA (Aldrich, Milwaukee, WI) are 317, 487, 515, and 528. Those for the internal standard are 332, 333, 348, and 545. The OA in each sample was quantified using a standard curve prepared just prior to running each set of samples.

2.6. Analysis

To compare how the distribution of ^{3}H-OA among different tissues in the honeybee varied with treatment method, we expressed the scintillation count values for each tissue sample as percentages of the total counts recorded from all samples from each individual bee. This approach corrected for the different absolute amounts of ^{3}H-OA applied in different treatment methods. We explored whether the relative amount of ^{3}H-OA recovered from each part of the bee varied with treatment method and time since treatment using two-way ANOVA. Percentage values were log-transformed to improve the fit of the data to a Gaussian distribution, and ANOVA was performed on the transformed values.

3. Results

All treatment methods resulted in recovery of ^{3}H-OA from all parts of the honeybee (Fig. 1). Minimal counts were recovered from the negative control; a sample of insect saline (mean count negative control $\pm$ SE = 25.67 ± 1.20) equivalent to $0.028 \pm 0.003\%$ (mean $\pm$ SE) of the total counts recovered from a bee. This is significantly less than counts recovered from any bee sample.

Treatment method influenced the relative amount of ^{3}H-OA in all parts of the honeybee except haemolymph from the abdomen (Fig. 1, Table 1). The relative amount of ^{3}H-OA also changed significantly with time in the abdomen, thorax and brain (Table 1). The lack of a significant effect of treatment in the haemolymph samples (Fig. 1e) could be due to the very high variation in these samples and the small sample sizes involved in this study.

Large differences in the distribution of ^{3}H-OA were observed between all treatment methods. We were surprised by the differences between bees topically treated on the abdomen and those topically treated on the thorax. We had assumed that topical treatments would release ^{3}H-OA into the haemolyph wherein the compounds could move freely through the bee. We had also assumed that all areas of the cuticle would be equally permeable to dMF. Our data showed that most of the ^{3}H-OA was recovered from the body segment to which it had originally been applied, and relatively more ^{3}H-OA was recovered from the brains and head capsule of thoracic-treated bees than abdominal-treated bees (Fig. 1).

To explore these differences further, two bees treated on the abdomen and two bees treated on the thorax were dissected more thoroughly. Data are shown in Fig. 2. Given this limited sample size, these data were not analysed statistically, but they support the conclusion that ^{3}H-OA applied topically did not spread consistently through the bee, instead remaining largely in the body segment to which it had been applied. Most of the ^{3}H-OA was recovered from the cuticle of the body segment at the point of application, but relatively more ^{3}H-OA was recovered from the abdominal haemolymph and gut of bees treated

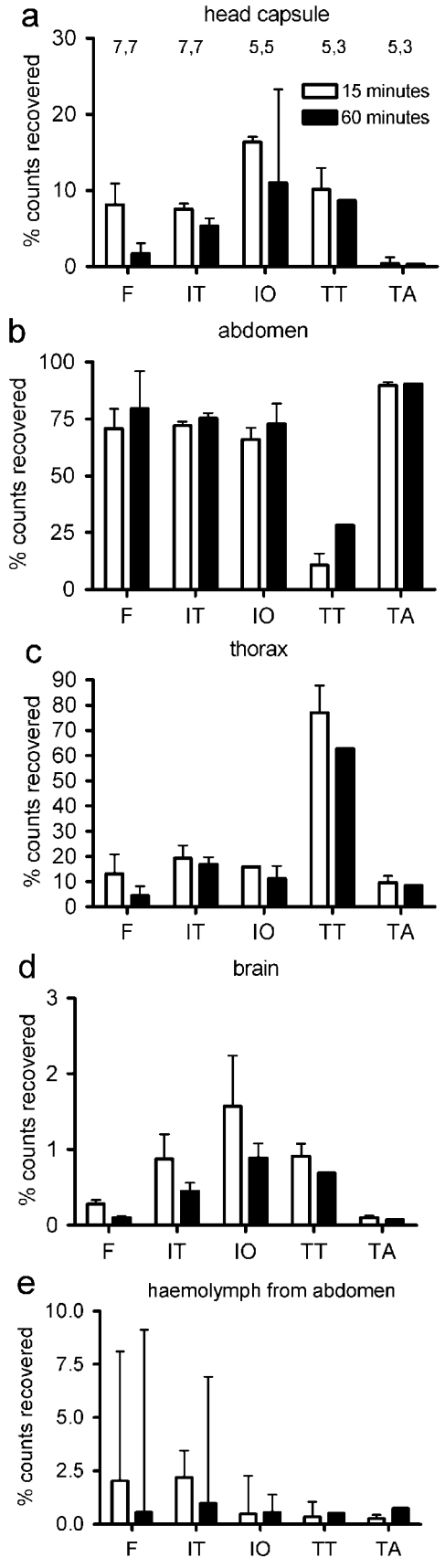

Fig. 1. Relative amount of ^{3}H-OA recovered (median ± interquartile distance) from different parts of honeybees treated with ^{3}H-OA. Bees were treated using five different treatment methods. F—feeding, IT—injection to the thorax, IO—injection to the median ocellus, TT—topical application to the thorax, TA—topical application to the abdomen. Bees were sampled at two different time points: 15 min (white bars) and 60 min (black bars) after treatment. Percentages were calculated by dividing counts recovered from a tissue sample by the total counts recovered from the individual bee. Sample sizes shown above bars.

on the abdomen than bees treated on the thorax. Similarly, relatively more ^{3}H-OA was recovered from the thoracic haemolymph and flight muscle of bees treated on the thorax. Further, relatively more ^{3}H-OA was recovered from the head capsule and brain of bees topically treated on the thorax than from bees topically treated on the abdomen (Fig. 2).

GC-MS measures of OA from the brains of foragers topically treated with OA in dMF on the thorax or abdomen confirmed that thoracic treatment was more effective than abdominal treatment in delivering OA to the brain (Fig. 3). When bees were treated on the thorax, more OA was found in the central brains of OA-treated bees than dMF-treated control bees (Mann–Witney test: $U = 27.10$, $df = 8, 12$, $P = 0.054$), but when bees were treated on the abdomen, similar amounts of OA were observed in the brains of OA-treated and dMF-treated bees (Mann–Witney test: $U = 14.00$, $df = 6, 6$, $P = 0.5887$). These findings are consistent with our experiments tracing the distribution of ^{3}H-OA through bees (Figs. 1 and 2), but further show that at least some of the topically applied OA reaches the brain as OA.

4. Discussion

The five treatment methods we compared varied enormously in how the applied compound became distributed through the bee. Unsurprisingly, in our study ocellar injection yielded the highest proportional recovery of ^{3}H-OA from the bee brain, but both thoracic injection and topical application to the thorax yielded a reasonable recovery of ^{3}H-OA from the brain. About 1.61% of total counts were recovered from the brains of bees treated with ^{3}H-OA by ocellar injection, 0.84% for bees treated by thoracic injection and 0.71% for bee treated by thoracic topical application. While these proportions are low, the OA content of the central brain (without optic lobes) is in the range 0.784–1.706 ng (Barron and Robinson, 2005; Fig. 3), and we observed that topical application of 2 μg OA to the thorax approximately increased the amount of OA in the central brain from 1.5 ng in untreated control bees to 5.1 ng in OA-treated bees (Fig. 3). These analyses were performed with field-treated foragers. We might expect OA penetration of the cuticle to be lower in foragers than newly emerged bees, because the cuticle of foragers is thicker than that of newly emerged bees, and field treatment is less precise than treating bees in the lab.

Table 1
Summary of two-way ANOVA testing the null hypothesis that the percentage of ^{3}H-OA recovered from different parts of the bee did not vary with treatment method or time since treatment

	Source of variation		
	Time	Treatment	Interaction
Head capsule	P = 0.174 % total variance = 1.05	P < **0.0001** % total variance = 63.39	P = **0.0185** % total variance = 7.29
Abdomen	P = **0.0003** % total variance = 5.28	P < **0.0001** % total variance = 67.06	P = **0.002** % total variance = 6.61
Thorax	P = **0.003** % total variance = 4.82	P < **0.0001** % total variance = 60.84	P = **0.0372** % total variance = 5.49
Brain	P = **0.0013** % total variance = 4.60	P < **0.0001** % total variance = 73.23	P = 0.0747 % total variance = 3.55
Haemolymph from abdomen	P = 0.4677 % total variance = 0.97	P = 0.1020 % total variance = 14.91	P = 0.6245 % total variance = 4.75

ANOVA were performed on log-transformed percentages. P-values significant at the 0.05 confidence level are in bold type.

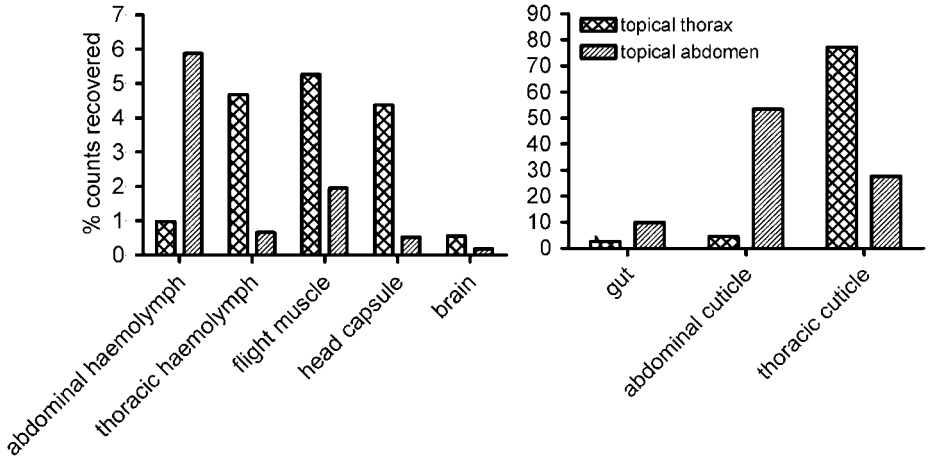

Fig. 2. Mean relative amount of ^{3}H-OA recovered from different tissues of honeybees treated with a 1 μl drop of ^{3}H-OA in dMF applied to the dorsal thorax or abdomen. Bees were sampled 60 min after treatment. Two bees were sampled at each time point in this small study.

Consequently, our data show that thoracic topical treatment is a viable method for the field treatment of bees with octopamine, where the intention is to deliver octopamine to the brain.

Feeding bees a single dose of ^{3}H-OA rapidly increased the amount of ^{3}H-OA detected in the abdominal haemolymph and the brain. This observation is concurrent with very rapid changes in behaviour observed following feeding OA to honeybees (Pankiw and Page, 2003; Spivak et al., 2003). Chronic feeding of OA in 2 M sucrose has been shown to increase OA levels in the central brain to 1.3–4.3 ng (Barron and Robinson, 2005), but prior to this study it was not clear whether the elevation in brain OA observed after oral treatment was a result of fed OA entering the brain, or fed OA triggering an increase in the endogenous level of OA (Schulz and Robinson, 2001). Our data clearly show that ^{3}H-OA can pass from the gut into the haemolymph and can also cross the bee blood-brain barrier into the brain.

In mammals, many substances with a molecular weight less than 500 Da can cross the blood-brain barrier, but most larger molecules are blocked unless they are actively transported (Nicolazzo et al., 2006). Maleszka et al. (2000) reported that glutamate can rapidly cross the honeybee blood-brain barrier, and given that caffeine, cocaine, tyramine, cGMP, and manganese and zinc ions also rapidly affect bee behaviour when delivered orally (Barron and Maleszka pers. obs., Ben-Shahar et al., 2002, 2004; Scheiner et al., 2002), and that the cockroach ventral nerve cord is permeable to small fatty acids and aliphatic alcohols (Eldewafi and O'Brian, 1966, 1967), it may be that the properties of the honeybee blood-brain barrier are similar to those of mammals, and that this structure is not generally an effective barrier to small molecules less than 500 Da. If this is the case, then other small molecules such as the biogenic amines serotonin and dopamine may also be able to pass from the haemolymph into the brain and nervous tissues, but care should be taken extrapolating our findings to other compounds. The effectiveness of topical treatment will also depend on the interaction between the compound, the solvent used and the cuticle, and the effectiveness of oral treatments will critically depend on

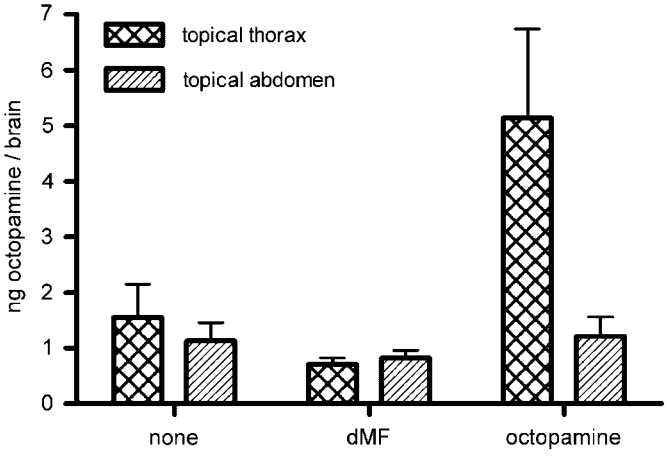

Fig. 3. GCMS measures (mean ± standard error) of OA from brains of forager bees treated on either the dorsal thorax or abdomen with 1 μl dMF or 10.5 mM OA dissolved in dMF, or sham-treated controls. Chequered bars are bees topically treated on the abdomen, crossed bars are bees topically treated on the thorax. For the thoracic-treated bees, brain levels of OA were higher in OA-treated bees than dMF-treated controls (Mann–Whitney: $U = 27$, $P = 0.054$, $df = 8,12$). For abdominal-treated bees, brain levels of OA did not differ between OA-treated bees and dMF-treated controls (Mann–Whitney: $U = 14$, $P = 0.588$, $df = 6,6$).

the ability of the compound to pass unchanged from the gut into the haemolymph. It is still not clear what transport mechanism OA uses to do this.

The distribution of ^{3}H-OA in the honeybee changed with time. For all treatment methods, ^{3}H-OA accumulated in the abdomen and haemolymph within the abdomen over time, and was lost (generally) from the brain, head capsule and thorax. We do not know where in the abdomen ^{3}H-OA was accumulating, but it is possible that ^{3}H-OA and its breakdown products were being filtered by the Malpighian tubules and accumulating in the hindgut. For most of our treatment methods, the amount of ^{3}H-OA recovered from the brain approximately halved between 15 and 60 min post delivery. From this data we might infer that the affects of a single OA treatment on the brain could be quite transient (measured in hours), although Guez et al. (2001) and Si et al. (2005) both report enduring effects of single imidacloprid and caffeine treatments, which they attribute to neuroactive metabolites of the primary compound, or lasting structural changes caused to the brain.

The majority of ^{3}H-OA applied topically in dMF was recovered from the body segment to which it had been applied. It is clear that the majority of the ^{3}H-OA applied topically remained on (or in) the cuticle, but even the ^{3}H-OA that did enter the bee's body did not circulate freely but appeared to remain predominantly within tissues within that body segment (Fig. 2). Insects are described as having an open circulatory system, and prior to this study we had assumed that compounds delivered into the haemolymph would be dispersed rapidly throughout the bee. Our findings suggest this may not be the case in honeybees, since compounds applied to a body segment remained principally within that body segment even after 1 h. Topical treatments to the thorax were more effective in elevating brain levels of ^{3}H-OA than topical treatments to the abdomen, and we recommend the thorax as a preferable body part for topical application when targeting the brain.

In summary, these experiments have shown that simple non-invasive treatment methods like oral and topical administration are effective in delivering octopamine to the honeybee, but these methods will expose all tissues to the compound, and different treatment methods will result in different distributions of OA through the bee.

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