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RESEARCH ARTICLE

Octopamine mobilizes lipids from honey bee (*Apis mellifera*) hypopharyngeal glands

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ABSTRACT

Recent widespread honey bee (*Apis mellifera*) colony loss is attributed to a variety of stressors, including parasites, pathogens, pesticides and poor nutrition. In principle, we can reduce stress-induced declines in colony health by either removing the stressor or increasing the bees' tolerance to the stressor. This latter option requires a better understanding than we currently have of how honey bees respond to stress. Here, we investigated how octopamine, a stress-induced hormone that mediates invertebrate physiology and behavior, influences the health of young nurse-aged bees. Specifically, we asked whether octopamine induces abdominal lipid and hypopharyngeal gland (HG) degradation, two physiological traits of stressed nurse bees. Nurse-aged workers were treated topically with octopamine and their abdominal lipid content, HG size and HG autophagic gene expression were measured. Hemolymph lipid titer was measured to determine whether tissue degradation was associated with the release of nutrients from these tissues into the hemolymph. The HGs of octopamine-treated bees were smaller than control bees and had higher levels of HG autophagy gene expression. Octopamine-treated bees also had higher levels of hemolymph lipid compared with control bees. Abdominal lipids did not change in response to octopamine. Our findings support the hypothesis that the HGs are a rich source of stored energy that can be mobilized during periods of stress.

KEY WORDS: Honey bee, Hypopharyngeal gland, Lipid, Octopamine, Stress, Hemolymph

INTRODUCTION

Insect pollinators provide both a foundational ecological service and a commercial asset for agricultural systems around the world. Populations of the most commercially utilized pollinator, the honey bee (*Apis mellifera*), have experienced rapid declines in the past 15 years due to a synergy of stressors called 'the four Ps': poor forage, pathogens, parasites and pesticides. Two major goals of honey bee research are to identify the presence and magnitude of these stressors and to develop ways to mitigate their negative effects. This latter goal requires a deep knowledge of the various mechanisms that honey bees use to respond to stress, an area that is currently underdeveloped (Even et al., 2012). A more complete understanding of how bees manage individual or multiple stressors at the individual and colony level might shed light on strategies to increase their overall resilience.

Honey bee workers take on different roles in the hive, with the most obvious and well-studied distinction being between in-hive and out-of-hive duties. At around one week of age, workers nourish brood, young bees and the queen with secretions from their hypopharyngeal and mandibular glands, otherwise known as jelly. The hypopharyngeal glands (HGs) are paired structures in the head that produce the major protein fraction of jelly. They are found throughout the Hymenoptera, and become more developed in form and function with increasing levels of sociality (Cruz-Landim, 1967). The HGs of honey bees are small in newly emerged adults, but reach maximal size and function within ∼3–8 days in a hive worker (Crailsheim and Stolberg, 1989; Knecht and Kaatz, 1990). Afterwards, the glands degrade as the workers take on foraging tasks. The normal age at first forage is highly variable, but occurs at ∼2 weeks of age (Winston, 1987). The typical nurse-to-forager transition is accelerated during stress; stressed bees can begin to forage as early as 4–7 days of age (Goblirsch et al., 2013; Schulz et al., 1998). Stressed bees have smaller HGs and depleted abdominal lipids (Corby-Harris et al., 2019; De Smet et al., 2017; DeGrandi-Hoffman et al., 2018; Heylen et al., 2011). Experimental reductions in abdominal lipids induce precocious foraging (Toth et al., 2005), suggesting that abdominal lipid content may drive this behavioral transition. Precocious foraging has negative consequences to individual and hive health. Forager searching behavior is compromised and colony failures increase when foragers leave earlier than normal (Perry et al., 2015; Ushitani et al., 2016). Although the colony-level consequences of precocious foraging have been examined, the underlying mechanism behind stress-induced aging in worker bees has yet to be fully elucidated.

Octopamine is a biogenic amine related to the vertebrate hormone noradrenaline that enables invertebrates to respond rapidly to stressful stimuli (Adamo et al., 1995; Even et al., 2012; Johnson, 2017; Orchard et al., 1993; Roeder, 1999). Octopamine has a short half-life and increases within minutes in stressed insects (Adamo et al., 1995; Goosey and Candy, 1982; Harris and Woodring, 1992). This rapid increase in octopamine activates the catabolism of energy stores, fueling energetically expensive behaviors such as flight, muscle contraction or enhanced locomotion (Arrese and Soulages, 2010; Fields and Woodring, 1991; Li et al., 2016; Orchard et al., 1982, 1993; Roeder, 1999, 2005; Sherer and Certel, 2019; Tao et al., 2016; Yang et al., 2015). It does this by acting on the fat body directly or indirectly via adipokinetic hormone (AKH) (Downer et al., 1984; Orchard et al., 1982, 1993; Zeng et al., 1996).

In honey bees, octopamine is a key element of the nurse-to-forager transition because it induces foraging and increases forager sensitivity to foraging-related hive cues (Barron and Robinson, 2005; Barron et al., 2002; Mayack et al., 2019; Schulz et al., 2002a).

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Octopamine also increases sucrose responsiveness, a behavioral marker that is higher in foragers than nurses (Scheiner et al., 2002, 2017b). Foragers have higher levels of octopamine in their antennal lobes compared with nurses, which may explain why foragers learn odors better than nurse bees (Scheiner et al., 2017b; Schulz and Robinson, 1999; Wagener-Hulme et al., 1999). Octopamine may also mediate the bee's physiological response to stress (Even et al., 2012). Harris and Woodring (1992) showed that stressed foragers have higher levels of octopamine in their brains. Bees infected with *Nosema* at emergence have higher whole-body expression of the octopamine precursor and the octopamine receptor Octβ2R genes (Mayack et al., 2015) and higher levels of octopamine in their brain at 7 days of age (Gage et al., 2018). Octopamine also enhances the colony response to alarm pheromone (Rittschof et al., 2019) and is elevated in bees performing hygienic behavior against pathogens (Spivak et al., 2003). The role of octopamine in the honey bees' physiological response to stress, specifically lipid mobilization, is less clear. Because the nurse-to-forager transition is induced by octopamine and because this transition is associated with physiological responses such as abdominal lipid depletion and HG degradation, one prediction is that octopamine induces the degradation of these tissues and the release of lipids in order to fuel the stress response.

We asked whether octopamine caused abdominal and HG lipid depletion and the release of lipids into the hemolymph. Nurse-aged workers were treated with octopamine or a negative control and their abdominal lipid content, HG size and hemolymph lipid levels were compared. Pollen consumption of treated and control bees was measured to determine if any physiological changes could be explained by diet. In addition, although we know that the abdominal lipid levels of nurse-aged honey bees can be quite high (Corby-Harris et al., 2019; Keller et al., 2005; Toth et al., 2005), the lipid content of the HGs was not clear, so HG lipid content was measured. We expected that octopamine-treated bees would have decreased abdominal lipids, smaller HGs and elevated hemolymph lipid titers. HG autophagy gene expression was also measured. Pollen-deprived bees exhibit HG degradation and increased expression of evolutionarily conserved autophagy genes (*atg6* and *atg9*) (Corby-Harris et al., 2016, 2019; Singh and Cuervo, 2011) that are critical for lipid mobilization in mammals (Koga et al., 2010; Singh et al., 2009). If HG degradation is important for lipid mobilization under stress, we hypothesized that *atg6* and *atg9* expression would increase with octopamine treatment. Our overall goal in performing these experiments was to provide insight into the mechanisms underpinning the honey bee stress response.

MATERIALS AND METHODS

Hypopharyngeal gland and head capsule lipid content

The abdomen of young nurse-aged workers contains a large amount of stored lipids, especially when those workers are fed pollen (Corby-Harris et al., 2019; Keller et al., 2005; Toth et al., 2005). In contrast, the lipid content of the HGs in young workers was not clear. We therefore assayed the lipid content of the head, separating the HGs from the remaining head capsule that contained all of the remaining (non-HG) head structures, such as the mandibular glands and brain. Five hives (N=5) were selected from apiaries at Carl Hayden Bee Research Center, each containing ≥10 frames of bees and both sealed and unsealed brood. All hives were headed by *Apis mellifera ligustica* queens from a commercial queen breeder in California. From each hive, >20 bees were brushed off a frame of open brood and cold anesthetized at 4°C. The heads of these 20 immobilized bees were separated from their respective thoraces. The HGs were dissected from the heads and placed into a pre-weighed 1.5 ml screw cap microcentrifuge tube containing 0.5 mm diameter silica beads, pooled by colony (i.e. each sample contained the glands of 20 bees from the same colony). The remnant head capsules were pooled into another similar pre-weighed tube. The pooled tissue samples (N=5 samples containing HGs or head capsules of 20 bees) in the pre-weighed tubes were dried (Human et al., 2013) and reweighed to obtain the dry mass of the tissue. The samples were twice subjected to a Folsch extraction and assayed for lipid content using the sulfuric acid–vanillin–phosphoric acid assay (Corby-Harris et al., 2019; Van Handel, 1985). Sample absorbance was evaluated against a standard curve containing vegetable oil. The mass (in µg) of total lipids in each sample and the mass of lipids in µg per mg of dry tissue mass were calculated.

Source bees for hormone treatments

Ten- or twenty-frame colonies headed by *Apis mellifera ligustica* queens from a commercial queen breeder in California supplied the bees for these experiments. Sealed brood frames from these five colonies were placed in a hive box in a temperature-controlled room (33±1°C). All adults that emerged over an 18 h period were placed into plastic cages (11.5×7.5×16.5 cm) and fed water, 30% w/v sucrose and pollen patty *ad libitum*. Pollen patty contained equal parts bee-collected pollen (Great Lakes Pollen; www.bulkfoods.com), table sugar (sucrose; Domino Foods, Inc.) and Drivert® sugar (8% sucrose and fructose+92% sucrose; Domino Foods, Inc.) mixed with water to a thick but pliable consistency. Three replicate cages containing approximately 150 bees per cage were set up for each treatment age. The cages were maintained at 32±1°C and 50% humidity with no light.

Octopamine treatment

Octopamine was dissolved in dimethylformamide (dMF) at a concentration of 2 µg µl^{-1} (Barron et al., 2007); dMF without hormone was the negative control. In preparation for treatment, the bees were immobilized by chilling the entire cage at 4°C (90 min on average). 1 µl of either octopamine or the control was applied topically to the thorax. The bees were marked with different colors of water-based paint (Posca USA) on the abdomen according to treatment. For each cage, approximately 50 bees were treated with either the treatment or the control (100 total per cage). The bees were then placed back into their original cage where they recovered in the incubator at 32±1°C and 50% humidity with *ad libitum* access to pollen patty (see above), water and 30% w/v sucrose. Bees chilled for this amount of time typically recover and are active again after 5 min in the incubator. Bee mortality was negligible in all of the cages and did not differ for bees treated with octopamine or the control. The treatments were replicated in three cages for each treatment age (treated at 5, 6 or 7 days).

Hypopharyngeal gland measurements

HGs are smaller in stressed bees (Corby-Harris et al., 2019; De Smet et al., 2017; DeGrandi-Hoffman et al., 2018; Heylen et al., 2011), so we hypothesized that the HGs may be smaller in bees treated with the stress hormone octopamine. To investigate HG size in the presence of this hormone, the gland acini were measured under a microscope to provide a robust and sensitive assessment of each individual bee's gland size (Corby-Harris and Snyder, 2018). Bees were treated at 5, 6 or 7 days post-emergence and returned to their cage as described above until they reached 8 days of age (i.e. 1, 2 or 3 days post-treatment). For each treatment age (5, 6 or 7 days), 30 bees of either treatment were collected from each of the three

replicate cages (10 bees per cage), flash frozen in liquid nitrogen and maintained at −80°C until their glands were measured. The HGs of each bee were dissected and measured as in Corby-Harris and Snyder (2018). The area (mm^2) of a minimum of 10 but up to 12 randomly selected acini per bee was measured at 60–80× using the Leica Applications Suite v.3.8.0 software. Only acini with clear borders were used. Acini areas were averaged for each individual. The data were log transformed to fit the assumptions of a mixed model ANOVA and were analyzed as such with treatment (octopamine or control), age of treatment, or the interaction between treatment and treatment age as fixed effects and cage as a random effect. Data were analyzed for this and subsequent data using SAS software Version 9.4 for Windows © 2002–2012 SAS Institute Inc., Cary, NC, USA. Specific, planned contrasts were made between the octopamine-treated and control bees separately for each age that they were treated.

Abdominal lipid measurements

Stressed bees have higher octopamine levels (Harris and Woodring, 1992) and lower abdominal lipids (Corby-Harris et al., 2019; Keller et al., 2005) and, in other insects, octopamine causes fat body lipid depletion (Orchard et al., 1982, 1993). We therefore hypothesized that bees treated with the stress hormone octopamine would have reduced abdominal lipids. To test this, we measured the abdominal lipid levels of bees treated with the hormone or the negative control. Ten octopamine-treated and 10 control bees that were also used for the above HG measurements were selected from each of the three replicate cages treated at 5, 6 or 7 days and sampled at 8 days of age. This yielded 30 bees per treatment by treatment age combination, 10 sampled from each of the three replicate cages. The abdominal carcass of each frozen bee was dissected by removing the thorax from the abdomen, cutting the abdomen open using sharp forceps, and removing the digestive tract, sting apparatus and ovary tissue. The residual carcasses were dried (Human et al., 2013) but a dry weight could not be obtained for each individual carcass. Reliable weights can usually only be obtained from pooled tissues, as in Corby-Harris et al. (2019) and the HG lipid content experiments described above. Lipids were extracted and quantified as described above for HGs. The data were analyzed using a generalized linear mixed model (GLMM) and a gamma link function. The denominator degrees of freedom were estimated using the Kenward and Roger method (Schaalje et al., 2002). We tested whether individual abdominal lipid levels (µg) were influenced by octopamine treatment, age of treatment, or an interaction between treatment and age. Cage was a random effect in the model. Planned, specific contrasts were performed to test the effect of octopamine in bees treated at the same age.

Hemolymph lipid measurements

If the nutrients stored in the abdomen or HGs were liberated upon octopamine treatment, we hypothesized that they would be released rapidly into the hemolymph. To test this, we assayed hemolymph lipids in bees treated at either 6 days or 7 days of age at 1 h and 2 h post-treatment. On separate days, bees aged 6 days or 7 days were treated on the thorax with octopamine or the negative control as described above. On each treatment day, bees were treated at two different instances, one group for studying the effects of octopamine at 1 h post-treatment (60 treated and 60 control) and one group for studying the effects of octopamine at 2 h post-treatment (60 treated and 60 control). After treatment, the bees recovered in a cage with water only and no access to sugar or pollen. At 1 h and 2 h post-treatment, the bees were immobilized at −20°C for ∼3–5 min. Hemolymph was drawn by dissecting the abdomen from the thorax, applying gentle pressure to the thorax, and using a 10 µl pipette to draw 2 µl hemolymph from the posterior end of the thorax. Individual hemolymph samples were snap frozen in 1.5 ml centrifuge tubes and maintained at −80°C until the lipid assay was performed. Hemolymph was drawn from 96 of the 120 bees (48 treated and 48 control) at 1 h or 2 h post-treatment. A team of 6 people was used to minimize the time needed to treat and collect hemolymph, treating 120 bees in ∼5 min and drawing hemolymph from 96 bees in ∼10 min. Half (48 total; 24 treated and 24 control) of the hemolymph samples were selected randomly for analysis of lipid analysis at each collection time point (1 h or 2 h post-treatment). Each sample was subjected to one round of a Folsch extraction and the hemolymph lipids were measured as described above. The 24 samples that were collected for each combination of time post-treatment (1 h or 2 h) and age treated (6 days or 7 days) were run on two different assay plates, with 12 treated and 12 control samples present on each plate. The data were then analyzed in two ways. Plate was a random effect in both analyses. First, we used a GLMM with a lognormal distribution to analyze the data from only the samples above the limit of detection of the assay (Table 2 details the number of samples above this cut-off). The model included the fixed effects of age treated (6 days or 7 days), time post-treatment (1 h or 2 h), treatment, and all two- and three-way interaction terms. The denominator degrees of freedom were estimated using the Kenward and Roger method (Schaalje et al., 2002). Planned contrasts were performed to compare the effect of octopamine treatment for samples collected at the same time post-treatment (1 h or 2 h) and treatment age (6 days or 7 days). Second, a logistic regression was used to determine whether the age of the bees when treated (6 days or 7 days), time post-treatment (1 h or 2 h), treatment, and all two- and three-way interaction terms influenced the likelihood that lipids were detected in the hemolymph (yes=sample had detectable lipids; no=sample did not have detectable lipids). The denominator degrees of freedom were estimated using the between-within method (Li and Redden, 2015). Planned contrasts were again performed to compare the effect of octopamine treatment on likelihood that hemolymph lipid was detected for samples collected at the same time post-treatment (1 h or 2 h) and treatment age (6 days or 7 days).

Pollen consumption

HG size and abdominal lipid content are sensitive to dietary pollen (Corby-Harris et al., 2014, 2016, 2019). In addition, consumption and dietary responsiveness increases with octopamine treatment in bees and other invertebrates (Li et al., 2016; Scheiner et al., 2017a; Tao et al., 2016). Because consumption could explain the differences in HG size and abdominal lipids, we tested whether bees treated with octopamine consumed more food. Honey bees were topically treated on the thorax with octopamine or the negative control at 5 days and 6 days of age as described above, except the bees were separated into cages by treatment. We did not test consumption for bees 7 days of age. Twenty cages of bees treated with either octopamine (N=10 cages) or the control (N=10 cages) were constructed. Each cage contained 100 bees. Bees were provided with fresh pollen patty, water and 30% sucrose solution *ad libitum*. Daily consumption of the pollen patty was measured until the bees were 8 days of age. Liquid sucrose was not measured because some liquid was lost when the bottles were removed for weighing. However, we reasoned that the high sugar content of the patty would induce increased consumption if octopamine resulted in elevated sucrose responsiveness. The values for pollen consumption

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Table 1. Primer sequences

Gene	GenBank accession	Forward primer (5′→3′)	Reverse primer (5′→3′)	Probe
atg6	XM_006564732	TTGCGTGTCAACGATGTCTA	TGGATGGGAACTACGTGAAAG	
atg9	XM_395581	CGGGAGTTGGAGATGTTTGTAG	CCCGATCTTGGACAGGAATTT	*FAM*-TGCTGTCTG-*Z*-CCACATTGGATTTCC-*IBFQ*
actin	AB023025.1	TGCCAACACTGTCCTTTCTG	AGAATTGACCCACCAATCCA	

per cage were log transformed to fit the assumptions of a linear model. We then analyzed the transformed data with a repeated measures MANOVA where 24 h, 48 h, and, for bees treated at 6 days of age, 72 h consumption were the response variables. Treatment (octopamine or the control), consumption prior to the treatment, and the interaction between these factors were the predictor variables. Owing to the short half-life of this hormone and its ability to activate an immediate response (Adamo et al., 1995; Harris and Woodring, 1992; Hiripi et al., 1994; Woodring et al., 1993), we also analyzed the 24 h consumption data alone. For this 24 h consumption data, we analyzed an ANOVA where treatment (octopamine or the control), consumption prior to the treatment, and the interaction between these factors were the predictor variables. No bees died during the length of the assay.

Hypopharyngeal gland autophagy gene expression

Autophagy releases nutrients from cellular stores (Singh and Cuervo, 2011; Singh et al., 2009) and is increased in the HGs of bees deprived of pollen (Corby-Harris et al., 2016; Corby-Harris et al., 2019), so we hypothesized that autophagy was involved in the release of lipids from the HGs in response to octopamine. We tested this by measuring the expression of two autophagy genes (*atg6* and *atg9*) in the HGs. Honey bees treated on the thorax with octopamine or the control as part of the hemolymph lipid measurements were assayed for gene expression. Forty bees that were treated with octopamine or the control at 6 days or 7 days of age and collected at 1 h or 2 h post-treatment were tested (N=10 per age×time post-treatment×treatment combination). We focused on these two time points because hemolymph lipid levels were higher by 2 h post-treatment time point (see Results). If HG autophagy led to increased hemolymph lipids, then autophagy gene expression might be elevated in the octopamine-treated bees by 2 h post-treatment. Following the hemolymph draw, the bees were immediately flash frozen in liquid nitrogen and were maintained at −80°C until their HGs were dissected. The HGs of the individually-treated bees were dissected quickly and placed into chilled 2 ml bead beating tubes that contained 0.55 mm diameter silica beads and buffer RLT/β-mercaptoethanol. Each sample contained the HGs from one bee. RNA was extracted from each sample using the RNeasy Mini kit (Qiagen). The samples were treated with DNase (Ambion) in order to remove any remaining genomic DNA. The DNA-free RNA was subjected to a cDNA synthesis reaction (ThermoFisher RevertAid) and the resulting cDNA was used in quantitative RT-PCR reactions. Primer sequences are listed in Table 1. The reaction to detect *atg9* expression used a FAM fluorophore connected to an internal DNA probe (iTaq Universal Probes Supermix, BioRad), while the *atg6* and *actin* reactions used primers alone and the SYBR green flurophore (SsoAdvanced Universal SYBR Green Supermix, BioRad). *C*q values were averaged across the three technical replicates that were run for each sample by gene combination. Relative gene expression estimates were obtained using the $2^{-\Delta\Delta Ct}$ (Livak) method. These values were analyzed using a Wilcoxon/Kruskal–Wallis test (Yuan et al., 2006), where expression was the dependent variable and treatment (control or octopamine-treated) was the independent variable. Data were analyzed separately for the *atg6* and *atg9* genes.

RESULTS

The hypopharyngeal glands contain lipids

In total, the head capsule samples contained an average of 608.6±197.7 µg (mean±s.e.m.) of lipids and the HG samples contained 127.5±13.6 µg lipids. Normalizing for tissue weight, the head capsule contained 7.1±4.0 µg lipids per mg of tissue and the HGs contained 35.5±7.0 µg of lipids per mg of tissue (Fig. 1).

HGs are smaller in octopamine-treated bees

Hypopharyngeal gland size was influenced by the octopamine treatment ($F_{1,169}$=18.59, P<0.0001); bees treated with octopamine had smaller HGs (Fig. 2). HG size was significantly influenced by octopamine treatment for bees treated at 6 days ($F_{1,169}$=13.14, P=0.0004) and 7 days ($F_{1,169}$=9.51, P=0.002), but not for bees treated at 5 days ($F_{1,169}$=0.60, P=0.440) of age.

Abdominal lipid levels were not altered by octopamine

The age of the bee when treated ($F_{2,170}$=4.12, P=0.018) was the only factor influencing abdominal lipid levels (Fig. 3). Octopamine did not influence abdominal lipid levels when the treatments were applied to bees aged 5, 6 or 7 days of age.

Hemolymph lipids increased with octopamine treatment

Focusing only on the samples that were within the limit of detection for the test, both hours post-treatment ($F_{1,51}$=6.75, P=0.0103) and hours post-treatment×age treated ($F_{1,151}$=9.29, P=0.0027) influenced hemolymph lipids (Fig. 4). Treatment was marginally non-significant ($F_{1,151}$=3.81, P=0.0528). In planned contrasts, hemolymph lipids increased due to the octopamine treatment only in bees treated at 7 days of age at 2 h post-treatment ($F_{1,152}$=5.99, P=0.0156). In considering all of the octopamine or control treated samples, even those below the limit of detection for the test, a greater number of octopamine-treated bees than control bees had detectable levels of lipid in their hemolymph (Table 2). Under the logistic model, treatment ($F_{1,183}$=5.79, P=0.0171) significantly influenced the likelihood that lipids would be detected in the hemolymph. Planned contrasts verified more lipid detection events in the octopamine-treated bees for bees treated at 7 days of age and

Table 2. Number of hemolymph lipid detection events for bees treated with octopamine or the negative control (*N*=24 for each age×hours post-treatment×treatment combination)

Age treated	Hours post-treatment	Control	Octopamine	*P**
6 days	1	22	22	NS
	2	14	21	**0.0316**
7 days	1	15	23	**0.0186**
	2	21	22	NS
Total		72	88	

*Significance of planned contrast comparing the effect of octopamine on the likelihood of a sample being above the limit of detection for each age×hours post-treatment combination. NS, not significant.

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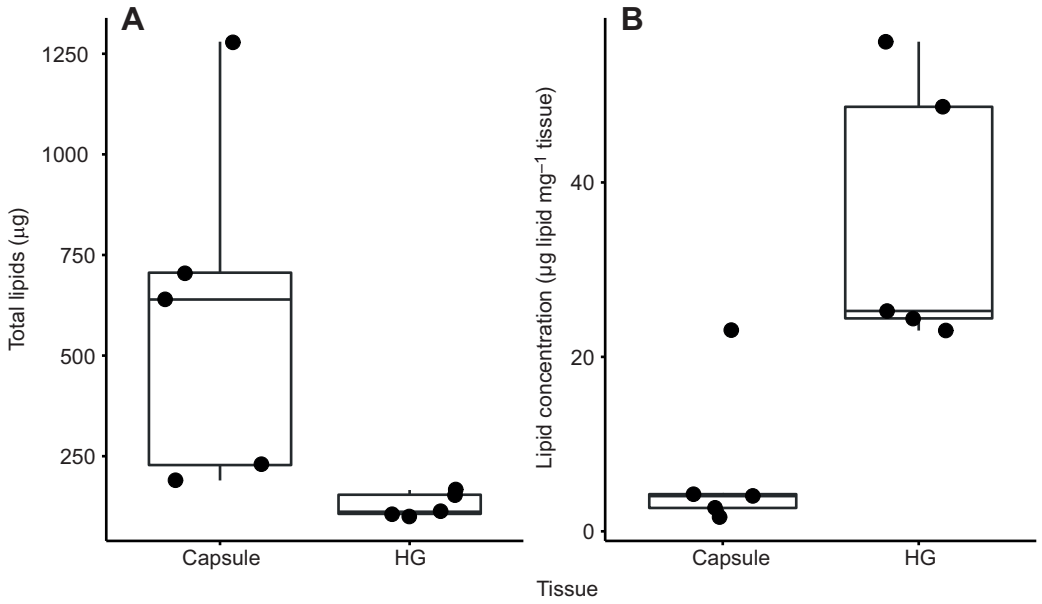

Fig. 1. Lipid concentrations in the head capsule and hypopharyngeal glands of honey bees (*Apis mellifera*). Total lipids (A, µg) and (B) µg lipids per mg of tissue present in the head capsule or HGs of bees obtained from the brood area of five colonies (*N*=5). Tissue from 20 bees per hive was pooled. The boxplot shows the mean (middle line of box) and interquartile range (box boundaries) as well as the minimum and maximum values of the distribution. Outliers are points outside of the maximum and minimum of the distribution.

measured at 1 h post-treatment ($F_{1,183}$=5.64, P=0.0186; Table 2) and for bees treated at 6 days of age and measured 2 h post-treatment ($F_{1,183}$=4.69, P=0.0316; Table 2).

Pollen consumption was not influenced by octopamine

Bees were treated with octopamine or the negative control at 5 or 6 days post-emergence. Pollen patty consumption was measured daily through 8 days of age. Pollen patty consumption over 3 days (for the 5 days treated bees) and over 2 days (for the 6 days treated bees) was equal between the octopamine-treated bees and the bees treated with the control (Fig. 5). Twenty-four-hour pollen consumption was also not influenced by octopamine treatment for bees treated at 5 days and 6 days of age (Fig. 5).

Octopamine-induced autophagy gene expression varied

Bees treated at 6 days of age with octopamine had higher expression of the autophagy gene *atg9* at 1 h post-treatment (χ^2_1 =3.86, P=0.0494; Fig. 6). At 1 h, there were no differences in *atg6* expression due to octopamine treatment. By 2 h post-treatment, both *atg6* and *atg9* were more highly expressed in the treated bees compared with the control (*atg6*: χ^2_1=8.25, P=0.0041; *atg9*: χ^2_1=8.25, P=0.0041; Fig. 6). Bees treated at 7 days of age showed no difference in *atg6* expression at either 1 h or 2 h post-treatment. For the *atg9* gene, expression was higher as a result of octopamine treatment at 1 h (χ^2_1=5.23, P=0.0222). *Atg9* expression did not differ due to octopamine treatment in bees sampled at 2 h post-treatment.

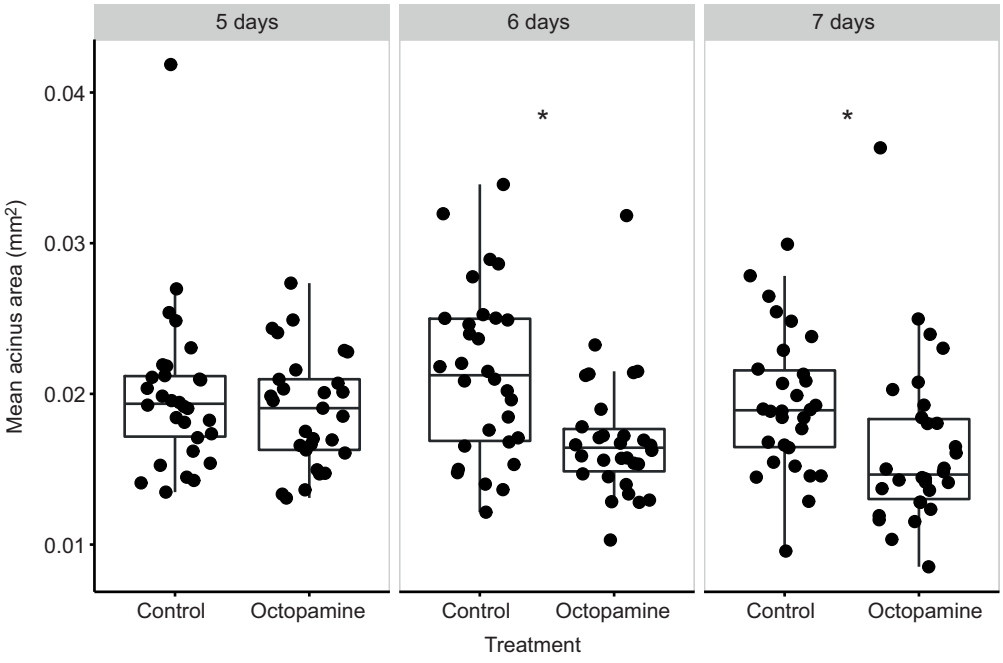

Fig. 2. Effect of octopamine treatment on hypopharyngeal gland acinus size in honey bees. Bees were treated at 5, 6 or 7 days post-emergence and their HGs were measured at 8 days. Each point represents the average acinus area for one bee (*N*=30 per age×treatment combination). Asterisks represent significant differences (P<0.05) between bees treated with octopamine versus the control. The boxplot shows the mean (middle line of box) and interquartile range (box boundaries) as well as the minimum and maximum values of the distribution. Outliers are points outside of the maximum and minimum of the distribution.

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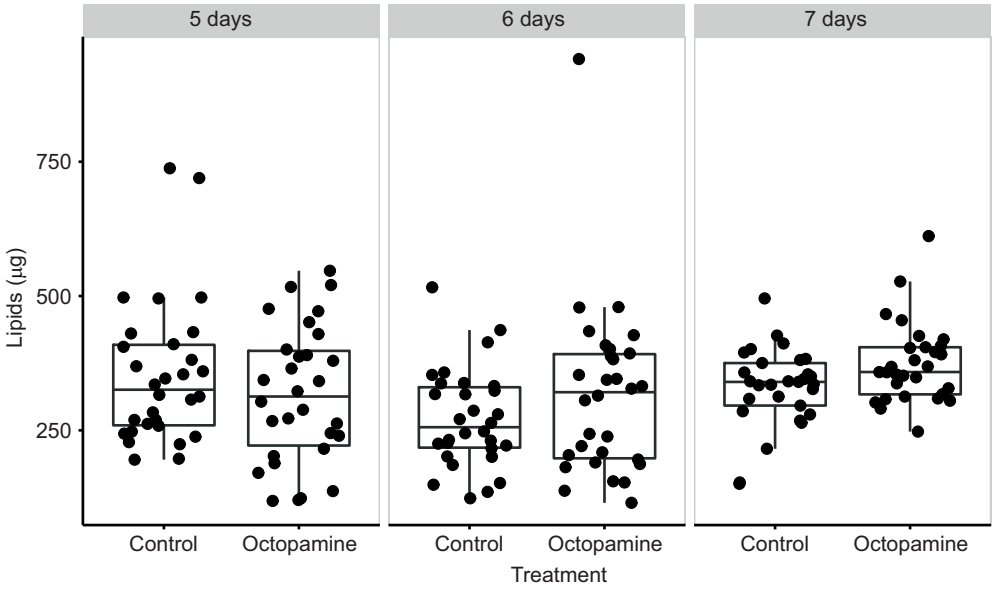

Fig. 3. Effect of octopamine treatment on abdominal lipids in honey bees. Bees treated at 5, 6 or 7 days post-emergence were assayed for abdominal lipids at 8 days of age. Each point represents the average abdominal carcass lipid content for one bee (N=30 bees per age×treatment combination). The boxplot shows the mean (middle line of box) and interquartile range (box boundaries) as well as the minimum and maximum values of the distribution. Outliers are points outside the maximum and minimum of the distribution.

DISCUSSION

Octopamine plays a crucial role in coordinating the invertebrate's physiological and behavioral responses to environmental stimuli. In the context of stress, octopamine liberates stored nutrients to fuel the organism's behavioral response (Adamo et al., 1995; Arrese and Soulages, 2010; Fields and Woodring, 1991; Gage et al., 2018; Harris and Woodring, 1992; Hiripi et al., 1994; Johnson, 2017; Li et al., 2016; Orchard et al., 1982; Tao et al., 2016; Woodring et al., 1993). In honey bees, octopamine also induces the nurse to forager transition (Barron and Robinson, 2005; Barron et al., 2002; Mayack et al., 2019; Scheiner et al., 2002; Schulz et al., 2002a). Bees transitioning from nurse to forager typically show a reduction in abdominal lipid stores and HG size (Crailsheim and Stolberg, 1989; Toth and Robinson, 2005). This behavioral transition is accelerated when the colony is stressed (Goblirsch et al., 2013; Schulz et al., 2002b). Taking into account the role of octopamine in honey bees and other insects, we hypothesized that octopamine would induce the degradation of abdominal and HG tissues, releasing lipids into the hemolymph. The present study partially supports this hypothesis: octopamine induces the degradation of the HGs, but not abdominal lipid stores, releasing energy-rich lipids into the hemolymph. Our key findings relating HG size, abdominal and hemolymph lipids, and autophagy gene expression are presented in Table 3.

In contrast to the abundant evidence that the abdomens of young bees contain lipids (Corby-Harris et al., 2019; Keller et al., 2005; Toth et al., 2005), we found no examples in the literature that HGs contained significant amounts of lipid. However, because the

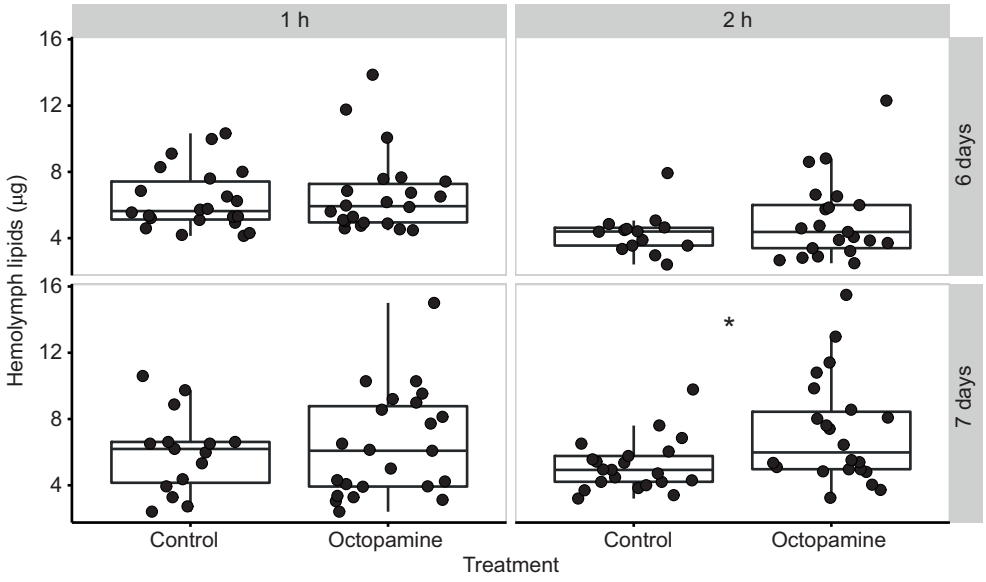

Fig. 4. Hemolymph lipids in bees treated with octopamine or the negative control at 6 days or 7 days post-emergence. Hemolymph was sampled at 1 h and 2 h post-treatment. Asterisks represent a significant difference (P<0.05) in hemolymph lipids between bees treated with octopamine versus the control for the relevant age×time post-treatment combination. Each point represents the lipids in 2 µl of hemolymph collected from one bee. Only samples above the limit of detection for the test are shown, and so N<24 for each combination of age treated, hours post-treatment and treatment (Table 2 details the number of samples above the limit of detection). The boxplot shows the mean (middle line of box) and interquartile range (box boundaries) as well as the minimum and maximum values of the distribution. Outliers are points outside of the maximum and minimum of the distribution.

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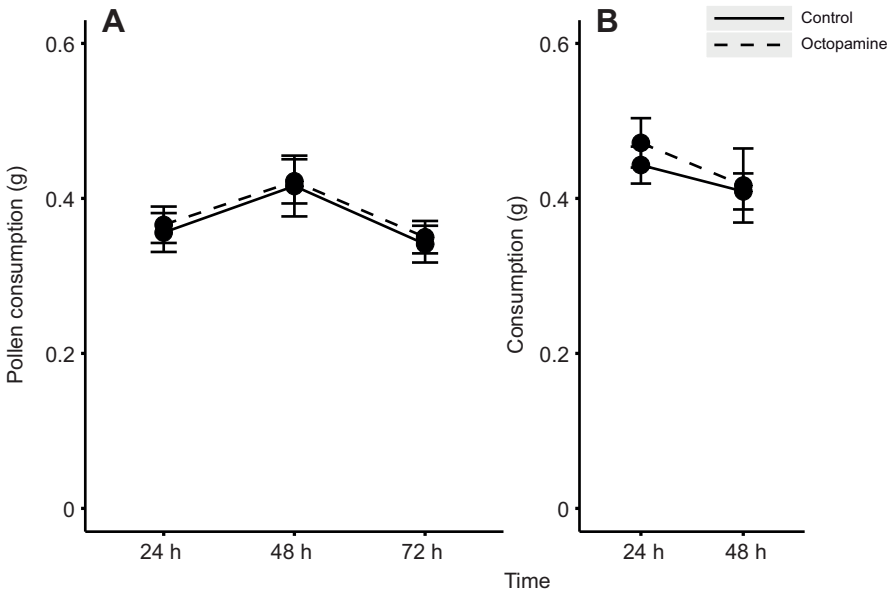

Fig. 5. Average daily pollen consumption of bees treated with octopamine or the negative control at 5 days or 6 days of age. Topical treatment of octopamine or the control was carried out at (A) 5 days or (B) 6 days. Consumption was measured every 24 h for 72 h (A) or 48 h (B) post-treatment. Error bars represent the mean±s.e.m. consumption for *N*=10 cages per treatment type.

lipoprotein vitellogenin is found in the HGs and fat body cells in the head (Seehuus et al., 2007), we assumed that the head and HGs contained lipids, generally speaking. Our experiments confirm our hypothesis that the HGs of young bees are a rich source of lipids. The total amount of lipid in the head capsule was greater than the total amount of lipid in the HGs. However, per unit of tissue mass, the HGs had a higher lipid content than the head capsule. Therefore, per unit of enzymatic activity, the organism could potentially extract more energy-rich lipids from the HGs than other head tissues. It is unclear how localized these octopamine-induced degradation pathways are. One question that we did not investigate here is whether the brain, a lipid-rich structure (Arien et al., 2015), degrades or is otherwise influenced with stress or octopamine treatment. Further experiments could test whether octopamine or stress influences lipids in the honey bee brain. If so, brain lipidome remodeling could explain why octopamine induces foraging behavior.

The HGs were smaller in octopamine-treated bees and hemolymph lipids increased with hormonal treatment. Together

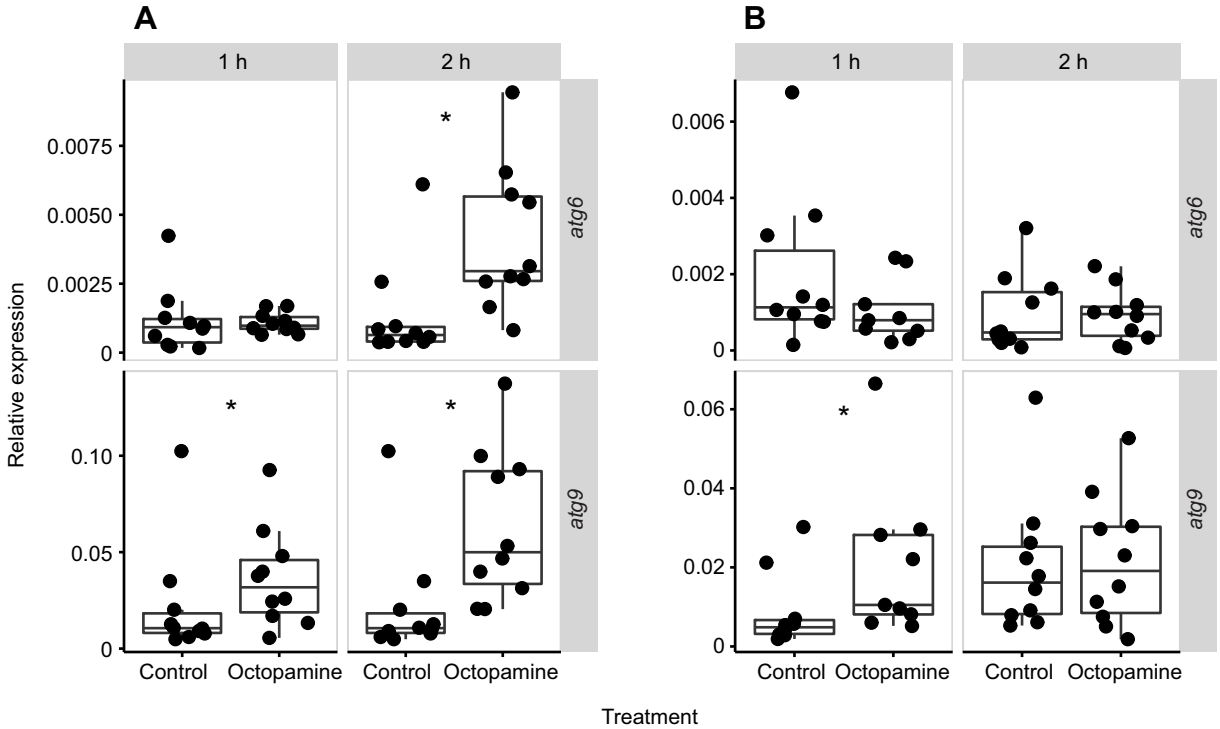

Fig. 6. Expression of autophagy genes *atg6* and *atg9* in the HGs of bees treated with octopamine. Bees were treated with octopamine at (A) 6 days or (B) 7 days of age. Each point represents HG gene expression for one individual bee collected at 1 h or 2 h post-treatment. *N*=10 bees per combination of age treated, hours post-treatment and treatment. Asterisks represent significant differences ($P<0.05$) in expression due to treatment. The boxplots show the mean (middle line of box) and interquartile range (box boundaries) as well as the minimum and maximum values of the distribution. Outliers are points outside of the maximum and minimum of the distribution. Note the different *y*-axis range on each panel.

Table 3. Summary of experiments testing whether octopamine treatment resulted in decreased HG size, increased hemolymph lipids, decreased abdominal lipids or increased autophagy gene expression

Time treated	HG size	Sampling time post-treatment	Hemolymph lipids	Abdominal lipids	*atg6* expression	*atg9* expression
6 days	↓	1 h	–	–	–	↑
		2 h	↑	–	↑	↑
7 days	↓	1 h	↑	–	–	↑
		2 h	↑	–	–	–

↑ and ↓ indicate an increase or decrease, respectively, relative to control bees; – indicates no significant change.

with the data showing that HGs contain lipids, this suggests that the HGs can be used to fuel the stress response. Past work shows that these autophagy genes are upregulated in the HGs during periods of sustained pollen deprivation (Corby-Harris et al., 2016; 2019) and are important in macroautophagy (Koga et al., 2010; Singh and Cuervo, 2011; Singh et al., 2009). We tested whether the increased hemolymph lipid levels and reduced HG size that we observed in the octopamine-treated bees were due to HG autophagy. While the results for the bees treated at 6 days supported the idea that autophagy acts in the HGs to liberate lipids, the data were less clear for bees treated at 7 days. In these bees, increased autophagy gene expression was only evident for the *atg9* gene at 1 h post-treatment. This variable response may be due to age-specific sensitivity of the organism to the hormone or day-to-day differences in our ability to pinpoint a potentially quick 'pulse' of autophagy gene expression following acute octopamine exposure. More data are needed to definitively say whether octopamine causes HG depletion via autophagy, perhaps using samples collected at more frequent intervals post-treatment.

In contrast to the HGs, abdominal lipids were not influenced by octopamine treatment. This contrasts with previous work in other insects showing that octopamine causes lipids to be mobilized from the fat body (Orchard et al., 1982, 1993; Roeder, 1999; Zeng et al., 1996). More work is needed to say definitively whether octopamine acts on the HGs and not the fat body. For example, octopamine may only act on the fat body at certain ages or after a more prolonged exposure. It would also be helpful to test whether abdominal versus thoracic application of octopamine influenced our results, as thoracic application may not raise abdominal octopamine levels to a physiologically relevant level (Barron et al., 2007). However, with regard to the question of whether acute octopamine exposure early in adulthood depletes abdominal lipid stores to accelerate the nurse-to-forager transition, our results to date do not support that hypothesis.

We measured pollen patty consumption because HG and abdominal lipid content is sensitive to dietary pollen (Corby-Harris et al., 2016; Corby-Harris et al., 2019). In addition, consumption and dietary responsiveness increases with octopamine treatment in bees and other invertebrates (Li et al., 2016; Scheiner et al., 2017a; Tao et al., 2016). In our experiments, pollen patty consumption was not influenced by octopamine treatment. This could be because young bees are not yet responsive to sucrose (Scheiner et al., 2017a). Nonetheless, our experiments show that compensatory pollen consumption does not explain the equivalent abdominal lipid levels seen in octopamine-treated and control bees.

Octopamine induced HG degradation and the release of lipids into the hemolymph. As in other invertebrates, rising octopamine levels in honey bees may facilitate the release of lipids from storage tissues in order to fuel an energetically expensive stress response. Our data suggest that the HGs are an accessible source of lipids and that octopamine may induce autophagic HG degradation to liberate these lipids. Finding ways to keep octopamine levels low in young bees may help hives to tolerate stress and maintain a healthy colony size.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: V.C.-H.; Methodology: V.C.-H.; Formal analysis: V.C.-H.; Investigation: V.C.-H., M.E.D., L.S., C.M., A.C.W., A.H., B.T.O.; Resources: V.C.-H.; Data curation: V.C.-H.; Writing - original draft: V.C.-H., M.E.D.; Writing - review & editing: V.C.-H., M.E.D.; Supervision: V.C.-H., L.S.; Project administration: V.C.-H., L.S.

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References

Adamo, S. A., Linn, C. E. and Hoy, R. R. (1995). The role of neurohormonal octopamine during 'fight or flight' behaviour in the field cricket *Gryllus bimaculatus*. *J. Exp. Biol.* **198**, 1691-1700.

Arien, Y., Dag, A., Zarchin, S., Masci, T. and Shafir, S. (2015). Omega-3 deficiency impairs honey bee learning. *Proc. Natl. Acad. Sci. USA* **112**, 15761-15766. doi:10.1073/pnas.1517375112

Arrese, E. L. and Soulages, J. L. (2010). Insect fat body: energy, metabolism, and regulation. *Annu. Rev. Entomol.* **55**, 207-225. doi:10.1146/annurev-ento-112408-085356

Barron, A. B. and Robinson, G. E. (2005). Selective modulation of task performance by octopamine in honey bee (*Apis mellifera*) division of labour. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **191**, 659-668. doi:10.1007/s00359-005-0619-7

Barron, A. B., Schulz, D. J. and Robinson, G. E. (2002). Octopamine modulates responsiveness to foraging-related stimuli in honey bees (*Apis mellifera*). *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **188**, 603-610. doi:10.1007/s00359-002-0335-5

Barron, A. B., Maleszka, J., Vander Meer, R. K., Robinson, G. E. and Maleszka, R. (2007). Comparing injection, feeding and topical application methods for treatment of honeybees with octopamine. *J. Insect Physiol.* **53**, 187-194. doi:10.1016/j.jinsphys.2006.11.009

Corby-Harris, V. and Snyder, L. A. (2018). Measuring hypopharyngeal gland acinus size in honey bee (*Apis mellifera*) workers. *JoVE* **139**, e58261. doi:10.3791/58261

Corby-Harris, V., Jones, B. M., Walton, A., Schwan, M. R. and Anderson, K. E. (2014). Transcriptional markers of sub-optimal nutrition in developing *Apis mellifera* nurse workers. *BMC Genomics* **15**, 134. doi:10.1186/1471-2164-15-134

Corby-Harris, V., Meador, C. A., Snyder, L. A., Schwan, M. R., Maes, P., Jones, B. M., Walton, A. and Anderson, K. E. (2016). Transcriptional, translational, and physiological signatures of undernourished honey bees (*Apis mellifera*) suggest a role for hormonal factors in hypopharyngeal gland degradation. *J. Insect Physiol.* **85**, 65-75. doi:10.1016/j.jinsphys.2015.11.016

Corby-Harris, V., Snyder, L. and Meador, C. (2019). Fat body lipolysis connects poor nutrition to hypopharyngeal gland degradation in *Apis mellifera*. *J. Insect Physiol.* **116**, 1-9. doi:10.1016/j.jinsphys.2019.04.001

Crailsheim, K. and Stolberg, E. (1989). Influence of diet, age and colony condition upon intestinal proteolytic activity and size of the hypopharyngeal glands in the honeybee (*Apis mellifera* L.). *J. Insect Physiol.* **35**, 595-602. doi:10.1016/0022-1910(89)90121-2

Cruz-Landim, C. (1967). Estudo comparativo de algumas glândulas das abelhas (Hymenoptera, Apoidea) e respectivas implicações evolutivas. *Arquivos de Zoologia* **15**, 177-290. doi:10.11606/issn.2176-7793.v15i3p177-290

De Smet, L., Hatjina, F., Ioannidis, P., Hamamtzoglou, A., Schoonvaere, K., Francis, F., Meeus, I., Smagghe, G. and de Graaf, D. C. (2017). Stress indicator gene expression profiles, colony dynamics and tissue development of honey bees

exposed to sub-lethal doses of imidacloprid in laboratory and field experiments. *PLoS ONE* **12**, e0171529. doi:10.1371/journal.pone.0171529

DeGrandi-Hoffman, G., Gage, S. L., Corby-Harris, V., Carroll, M., Chambers, M., Graham, H., Watkins DeJong, E., Hidalgo, G., Calle, S., Azzouz-Olden, F. et al. (2018). Connecting the nutrient composition of seasonal pollens with changing nutritional needs of honey bee (*Apis mellifera* L.) colonies. *J. Insect Physiol.* **109**, 114-124. doi:10.1016/j.jinsphys.2018.07.002

Downer, R. G. H., Orr, G. L., Gole, J. W. D. and Orchard, I. (1984). The role of octopamine and cyclic AMP in regulating hormone release from *corpora cardiaca* of the American cockroach. *J. Insect Physiol.* **30**, 457-462. doi:10.1016/0022-1910(84)90025-8

Even, N., Devaud, J.-M. and Barron, A. B. (2012). General stress responses in the honey bee. *Insects* **3**, 1271-1298. doi:10.3390/insects3041271

Fields, P. E. and Woodring, J. P. (1991). Octopamine mobilization of lipids and carbohydrates in the house cricket, *Acheta domesticus*. *J. Insect Physiol.* **37**, 193-199. doi:10.1016/0022-1910(91)90069-C

Gage, S. L., Kramer, C., Calle, S., Carroll, M., Heien, M. and DeGrandi-Hoffman, G. (2018). *Nosema ceranae* parasitism impacts olfactory learning and memory and neurochemistry in honey bees (*Apis mellifera*). *J. Exp. Biol.* 221, jeb161489. doi:10.1242/jeb.161489

Goblirsch, M., Huang, Z. Y. and Spivak, M. (2013). Physiological and behavioral changes in honey bees (*Apis mellifera*) induced by *Nosema ceranae* infection. *PLoS ONE* **8**, e58165. doi:10.1371/journal.pone.0058165

Goosey, M. W. and Candy, D. J. (1982). The release and removal of octopamine by tissues of the locust *Schistocerca americana gregaria*. *Insect Biochem.* **12**, 681-685. doi:10.1016/0020-1790(82)90057-9

Harris, J. W. and Woodring, J. (1992). Effects of stress, age, season, and source colony on levels of octopamine, dopamine and serotonin in the honey bee (*Apis mellifera* L.) brain. *J. Insect Physiol.* **38**, 29-35. doi:10.1016/0022-1910(92)90019-A

Heylen, K., Gobin, B., Arckens, L., Huybrechts, R. and Billen, J. (2011). The effects of four crop protection products on the morphology and ultrastructure of the hypopharyngeal gland of the European honeybee, *Apis mellifera*. *Apidologie* **42**, 103-116. doi:10.1051/apido/2010043

Hiripi, L., Juhos, S. and Downer, R. G. H. (1994). Characterization of tyramine and octopamine receptors in the insect (*Locusta migratoria migratorioides*) brain. *Brain Res.* **633**, 119-126. doi:10.1016/0006-8993(94)91530-X

Human, H., Brodschneider, R., Dietemann, V., Dively, G., Ellis, J. D., Forsgren, E., Fries, I., Hatjina, F., Hu, F.-L., Jaffé, R. et al. (2013). Miscellaneous standard methods for *Apis mellifera* research. *J. Apic. Res.* **52**, 1-53. doi:10.3896/IBRA.1.52.4.10

Johnson, E. C. (2017). 2.21 - Stressed Out Insects II. Physiology, Behavior, and Neuroendocrine Circuits Mediating Stress Responses. In *Hormones, Brain and Behavior*, 3rd edn (ed. D. W. Pfaff and M. Joëls), pp. 465-481. Oxford: Academic Press.

Keller, I., Fluri, P. and Imdorf, A. (2005). Pollen nutrition and colony development in honey bees: part I. *Bee World* **86**, 3-10. doi:10.1080/0005772X.2005.11099641

Knecht, D. and Kaatz, H. H. (1990). Patterns of larval food production by hypopharyngeal glands in adult worker honey bees. *Apidologie* **21**, 457-468. doi:10.1051/apido:19900507

Koga, H., Kaushik, S. and Cuervo, A. M. (2010). Altered lipid content inhibits autophagic vesicular fusion. *FASEB* **24**, 3052-3065. doi:10.1096/fj.09-144519

Li, P. and Redden, D. T. (2015). Comparing denominator degrees of freedom approximations for the generalized linear mixed model in analyzing binary outcome in small sample cluster-randomized trials. *BMC Med. Res. Methodol.* **15**, 38. doi:10.1186/s12874-015-0026-x

Li, Y., Hoffmann, J., Li, Y., Stephano, F., Bruchhaus, I., Fink, C. and Roeder, T. (2016). Octopamine controls starvation resistance, life span and metabolic traits in *Drosophila*. *Sci. Rep.* **6**, 35359. doi:10.1038/srep35359

Mayack, C., Natsopoulou, M. E. and McMahon, D. P. (2015). *Nosema ceranae* alters a highly conserved hormonal stress pathway in honeybees. *Ins. Mol. Biol.* 24, 662-670. doi:10.1111/imb.12190

Mayack, C., Phalen, N., Carmichael, K., White, H. K., Hirche, F., Wang, Y., Stangl, G. I. and Amdam, G. V. (2019). Appetite is correlated with octopamine and hemolymph sugar levels in forager honeybees. *J. Comp. Phys. A* **205**, 609-617. doi:10.1007/s00359-019-01352-2

Orchard, I., Carlisle, J. A., Loughton, B. G., Gole, J. W. and Downer, R. G. (1982). *In vitro* studies on the effects of octopamine on locust fat body. *Gen. Comp. Endocrinol.* 48, 7-13. doi:10.1016/0016-6480(82)90031-4

Orchard, I., Ramirez, J.-M. and Lange, A. B. (1993). A multifunctional role for octopamine in locust flight. *Annu. Rev. Entomol.* **38**, 227-249. doi:10.1146/annurev.en.38.010193.001303

Perry, C. J., Sovik, E., Myerscough, M. R. and Barron, A. B. (2015). Rapid behavioral maturation accelerates failure of stressed honey bee colonies. *Proc. Natl. Acad. Sci. USA* **112**, 3427-3432. doi:10.1073/pnas.1422089112

Rittschof, C. C., Vekaria, H. J., Palmer, J. H. and Sullivan, P. G. (2019). Biogenic amines and activity levels alter the neural energetic response to aggressive social cues in the honey bee *Apis mellifera*. *J. Neurosci. Res.* **97**, 991-1003. doi:10.1002/jnr.24443

Roeder, T. (1999). Octopamine in invertebrates. *Prog. Neurobiol.* **59**, 533-561. doi:10.1016/S0301-0082(99)00016-7

Roeder, T. (2005). Tyramine and octopamine: ruling behavior and metabolism. *Annu. Rev. Entomol.* **50**, 447-477. doi:10.1146/annurev.ento.50.071803.130404

Schaalje, G. B., McBride, J. B. and Fellingham, G. W. (2002). Adequacy of approximations to distributions of test statistics in complex mixed linear models. *J. Ag. Biol. Envir. Stat.* **7**, 512. doi:10.1198/108571102726

Scheiner, R., Plückhahn, S., Öney, B., Blenau, W. and Erber, J. (2002). Behavioural pharmacology of octopamine, tyramine and dopamine in honey bees. *Behav. Brain Res.* **136**, 545-553. doi:10.1016/S0166-4328(02)00205-X

Scheiner, R., Entler, B. V., Barron, A. B., Scholl, C. and Thamm, M. (2017a). The effects of fat body tyramine level on gustatory responsiveness of honeybees (*Apis mellifera*) differ between behavioral castes. *Front. Syst. Neurosci.* **11**, 55-55. doi:10.3389/fnsys.2017.00055

Scheiner, R., Reim, T., Sovik, E., Entler, B. V., Barron, A. B. and Thamm, M. (2017b). Learning, gustatory responsiveness and tyramine differences across nurse and forager honeybees. *J. Exp. Biol.* **220**, 1443-1450. doi:10.1242/jeb.152496

Schulz, D. J. and Robinson, G. E. (1999). Biogenic amines and division of labor in honey bee colonies: behaviorally related changes in the antennal lobes and age-related changes in the mushroom bodies. *J. Comp. Physiol. A* **184**, 481-488. doi:10.1007/s003590050348

Schulz, D. J., Huang, Z.-Y. and Robinson, G. E. (1998). Effects of colony food shortage on behavioral development in honey bees. *Behav. Ecol. Sociobiology* **42**, 295-303. doi:10.1007/s002650050442

Schulz, D. J., Barron, A. B. and Robinson, G. E. (2002a). A role for octopamine in honey bee division of labor. *Brain Behav. Evol.* **60**, 350-359. doi:10.1159/000067788

Schulz, D. J., Vermiglio, M. J., Huang, Z. Y. and Robinson, G. E. (2002b). Effects of colony food shortage on social interactions in honey bee colonies. *Insectes Soc.* **49**, 50-55. doi:10.1007/s00040-002-8279-x

Seehuus, S. C., Norberg, K., Krekling, T., Fondrk, K. and Amdam, G. V. (2007). Immunogold localization of vitellogenin in the ovaries, hypopharyngeal glands and head fat bodies of honeybee workers, *Apis mellifera*. *J. Insect Sci.* **7**, 1-14. doi:10.1673/031.007.5201

Sherer, L. M. and Certel, S. J. (2019). The fight to understand fighting: neurogenetic approaches to the study of aggression in insects. *Curr. Opin. Insect Sci.* **36**, 18-24. doi:10.1016/j.cois.2019.06.004

Singh, R. and Cuervo, A. M. (2011). Autophagy in the cellular energetic balance. *Cell Metab.* **13**, 495-504. doi:10.1016/j.cmet.2011.04.004

Singh, R., Kaushik, S., Wang, Y., Xiang, Y., Novak, I., Komatsu, M., Tanaka, K., Cuervo, A. M. and Czaja, M. J. (2009). Autophagy regulates lipid metabolism. *Nature* **458**, 1131-1135. doi:10.1038/nature07976

Spivak, M., Masterman, R., Ross, R. and Mesce, K. A. (2003). Hygienic behavior in the honey bee (*Apis mellifera* L.) and the modulatory role of octopamine. *J. Neurobiol.* **55**, 341-354. doi:10.1002/neu.10219

Tao, J., Ma, Y.-C., Yang, Z.-S., Zou, C.-G. and Zhang, K.-Q. (2016). Octopamine connects nutrient cues to lipid metabolism upon nutrient deprivation. *Sci. Adv.* **2**, e1501372. doi:10.1126/sciadv.1501372

Toth, A. L. and Robinson, G. E. (2005). Worker nutrition and division of labour in honeybees. *Anim. Behav.* **69**, 427-435. doi:10.1016/j.anbehav.2004.03.017

Toth, A. L., Kantarovich, S., Meisel, A. F. and Robinson, G. E. (2005). Nutritional status influences socially regulated foraging ontogeny in honey bees. *J. Exp. Biol.* **208**, 4641-4649. doi:10.1242/jeb.01956

Ushitani, T., Perry, C. J., Cheng, K. and Barron, A. B. (2016). Accelerated behavioural development changes fine-scale search behaviour and spatial memory in honey bees (*Apis mellifera* L.). *J. Exp. Biol.* **219**, 412-418. doi:10.1242/jeb.126920

Van Handel, E. (1985). Rapid determination of total lipids in mosquitoes. *J. Am. Mosq. Control Assoc.* **1**, 302-304.

Wagener-Hulme, C., Kuehn, J. C., Schulz, D. J. and Robinson, G. E. (1999). Biogenic amines and division of labor in honey bee colonies. *J. Comp. Physiol. A* **184**, 471-479. doi:10.1007/s003590050347

Winston, M. L. (1987). *The Biology of the Honey Bee*. Cambridge, Massachusetts: Harvard University Press.

Woodring, J., Boulden, M., Das, S. and Gäde, G. (1993). Studies on blood sugar homeostasis in the honeybee (*Apis mellifera*, L.). *J. Insect Physiol.* **39**, 89-97. doi:10.1016/0022-1910(93)90022-J

Yang, Z., Yu, Y., Zhang, V., Tian, Y., Qi, W. and Wang, L. (2015). Octopamine mediates starvation-induced hyperactivity in adult *Drosophila*. *Proc. Natl. Acad. Sci. USA* **112**, 5219-5224. doi:10.1073/pnas.1417838112

Yuan, J. S., Reed, A., Chen, F. and Stewart, C. N. (2006). Statistical analysis of real-time PCR data. *BMC Bioinformatics* **7**, 85.

Zeng, H., Loughton, B. G. and Jennings, K. R. (1996). Tissue specific transduction systems for octopamine in the locust (*Locusta migratoria*). *J. Insect Physiol.* **42**, 765-769. doi:10.1016/0022-1910(96)00013-3