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ORIGINAL PAPER

Neurophysiological mechanisms underlying sex- and maturation-related variation in pheromone responses in honey bees (*Apis mellifera*)

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Abstract In the honey bee (*Apis mellifera*), social organization is primarily mediated by pheromones. Queen-produced 9-oxo-2-decenoic acid (9-ODA) functions as both a social and sex pheromone, eliciting attraction in both female workers and male drones, but also affecting other critical aspects of worker physiology and behavior. These effects are also maturation related, as younger workers and sexually mature drones are most receptive to 9-ODA. While changes in the peripheral nervous system drive sex-related differences in sensitivity to 9-ODA, the mechanisms driving maturation-related shifts in receptivity to 9-ODA remain unknown. Here, we investigate the hypothesis that changes at the peripheral nervous system may be mediating plastic responses to 9-ODA by characterizing expression levels of *AmOR11* (the olfactory receptor tuned to 9-ODA) and electrophysiological responses to 9-ODA. We find that receptor expression correlates significantly with behavioral receptivity to 9-ODA, with nurses and sexually mature drones exhibiting higher levels of expression than foragers and immature drones, respectively. Electrophysiological responses to 9-ODA were not found to correlate with behavioral receptivity or receptor expression, however. Thus, while receptor expression at the periphery exhibits a level of plasticity that correlates with behavior, the mechanisms driving maturation-dependent responsiveness to 9-ODA appear to function primarily in the central nervous system.

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Introduction

Plasticity in olfactory pathway responsiveness can be an underlying factor that modulates behavioral variation over the course of an individual's life (Davis and Takahashi 1980; Davis 1984; Gadenne and Anton 2000; Gadenne et al. 2001; Anderson et al. 2007; Anton et al. 2007; Barrozo et al. 2010, 2011; George et al. 2011; Guerrieri et al. 2012; Cator et al. 2013). Plasticity in the response to chemosensory cues can ensure appropriate, context-dependent responses, i.e., only responding to sex pheromones at certain times of the day or night or otherwise only when capable of mating (Shorey et al. 1968). At the level of the population, this plasticity may result in reproductive character displacement or reinforcement of speciation-related characteristics involving time-of-day mating activities resulting in temporal isolation (Carde et al. 1977, 1978). Olfactory cues are detected, processed and classified by the olfactory system, which is similarly modularly organized in both vertebrates and invertebrates (Hansson 1999; Wyatt 2014). Modulation of behavioral responses to olfactory cues can be driven by alterations in the sensitivity of olfactory sensory neurons (OSNs) in the peripheral nervous system, as in flies and mosquitoes (Davis and Takahashi 1980; Davis 1984; Crnjar et al. 1990; George et al. 2011; Cator et al. 2013) or noctuid moths (Anderson et al. 2007; Barrozo et al. 2011; Guerrieri et al. 2012). Alterations can also occur in the central nervous system, during the processing and integration of odorant cue information in the antennal lobe and its neurons projecting to protocerebral neuropils, such as occurs in the moth, *Agrotis ipsilon* (Gadenne and Anton 2000; Gadenne et al. 2001).

Honey bees represent a fascinating model system to study the neurophysiological mechanisms underpinning plasticity in chemosensory responses and the evolution of these mechanisms. There are substantial differences in behavioral responses to the same pheromone (9-oxo-2-decenoic acid, 9-ODA) between sexes (female workers and male drones) and also maturation-associated variation in responses to 9-ODA within each sex (see below for further discussion). Here, we investigate whether similar neurophysiological mechanisms mediate sex-associated variation and maturation-associated variation. If common mechanisms are used (i.e., modulation occurs either at the level of the peripheral or central nervous system in both cases), it would suggest that a single mechanism has been co-opted and re-used to regulate distinct types of plasticity within a species. If different mechanisms are used, it would indicate that the olfactory system can readily be modulated through multiple pathways, even with the same species.

The primary pheromone component produced by a honey bee queen, 9-oxo-2-decenoic acid (9-ODA), elicits sexually dimorphic responses in workers and drones. In workers, 9-ODA triggers short-range attraction (over a few centimeters) within the colony (Kaminski et al. 1990; Grozinger et al. 2007), while in drones, 9-ODA stimulates attraction over long distances (up to 100 m) during mating flights (Brockmann et al. 2006). 9-ODA also has primer effects in workers which have not been documented in drones, including reducing hemolymph titers of juvenile hormone and brain RNA levels of the *Kr-h1* transcription factor (Kaatz et al. 1992; Grozinger et al. 2007), both of which are associated with worker behavioral maturation from brood care/nursing to foraging (Sullivan et al. 2000; Grozinger et al. 2003; Grozinger and Robinson 2007). Thus, 9-ODA serves as both a sex pheromone component and a social pheromone component in honey bees. It is important to note that 9-ODA acts synergistically with several other components in the queen pheromone blend to influence drone and worker behavior, and several behavioral, neurophysiological and molecular studies have been performed using "queen mandibular pheromone," or QMP, a five component blend that contains 9-ODA (Slessor et al. 2005; Le Conte and Hefetz 2008; reviewed in Grozinger, in press).

Differences in the sensitivities of drones and workers to 9-ODA related to distances of attraction appear to be driven at least in part by differences in the peripheral detection system. Expression of the olfactory receptor for 9-ODA (*AmOR11)* is tenfold higher in the antennae of drones versus workers (Wanner et al. 2007). Surface electron micrograph studies of the drone and worker antennae demonstrate that drone antennae contain over sixfold greater numbers of poreplate sensilla than workers (~18,000 vs. ~2700), which house *AmOR11* olfactory receptor neurons (Johannes and Kaissling 1976). Finally, physiological studies utilizing electroantennograms have demonstrated that drone antennae are significantly more sensitive to 9-ODA, responding 3–10 times more strongly to it than worker antennae (Vetter and Visscher 1997; Brockmann et al. 1998).

Maturation is a factor known to affect the responses of workers and drones to 9-ODA. Attraction to QMP and live queens is highest in young nurse workers, but decreases as workers mature and become foragers (Pham-Delegue et al. 1993; Grozinger and Robinson 2007). However, the effects of 9-ODA alone have not been tested in both nurses and foragers. Similarly, all the primer effects of 9-ODA, QMP and live queens have been documented in young workers but not in foragers (reviewed in Grozinger, in press). Attraction of drones to 9-ODA presumably increases as they reach sexual maturity, although this has been difficult to test behaviorally since they only respond to 9-ODA during mating flights (Gary 1962; Brockmann et al. 2006).

The mechanisms regulating the hypothesized maturation-related responses to 9-ODA in workers and drones have not been fully characterized. Previous electroantennogram (EAG) studies found that responses of young nurse-age bees to QMP or 9-ODA peaked at 8–12 days and then decreased slightly, but not significantly, in older foraging-age workers (Masson and Arnold 1984; Allan et al. 1987; Pham-Delegue et al. 1993). However, these studies also found an age-related decrease in response to non-behaviorally important general odorants, suggesting an overall senescence in the tissues as a cause of the decline. These studies also did not employ dose–response curves, which can allow for a more precise determination of differences in the absolute lower threshold (highest sensitivity) of response to an odorant. A previous study of EAG responses in drones (Vetter and Visscher 1997) found a significant decrease in response to QMP as drones age, but responses to general control odors and 9-ODA alone were not examined. Thus, there are significant technical limitations associated with these studies which constrain their interpretability. These issues can be addressed by constructing dose–response curves of EAG responses to 9-ODA and standardizing the EAG amplitudes to a general odorant control stimulus to control for possible age-related senescence of the antennal tissue.

Here, we examined whether sex- and maturation-related plasticity in behavioral responses to 9-ODA is driven by common neurophysiological mechanisms in honey bees. Sex-related plasticity appears to be strongly correlated with changes in the peripheral nervous system, in terms of both expression levels of *AmOR11* and electroantennogram (EAG) responses to 9-ODA (Brockmann et al. 1998; Wanner et al. 2007). We tested the effect of maturation in workers and drones on *AmOR11* expression levels and

EAG responses (using dose responses and general odorants as controls) by first examining workers and drones in different behavioral states (nurses, foragers, sexually immature drones, sexually mature drones). In a second set of experiments, we examined workers and drones at ages corresponding to the period immediately before and immediately after the transition from one behavioral state to the next, to determine how rapidly expression and EAG responses changed during the transition. Because behavioral responses to queen pheromone decrease with maturation in workers and are thought to increase with maturation in drones, we hypothesized that *AmOR11* levels and EAG responses decrease with maturation in workers and increase in drones.

Materials and methods

General bee rearing

The following studies were performed using colonies maintained at The Pennsylvania State University (State College, PA, USA). For experiments using colonies headed by a single-drone inseminated (SDI) queen, we obtained queens from Glenn Apiaries (Fallbrook, CA). SDI queens were used to generate offspring with high levels of relatedness. All experimental colonies were maintained using standard management practices.

For experiments using bees reared in colonies, nurses and foragers were collected based on behavioral observations. Nurses were identified as they inspected/fed larvae in honeycomb cells, while foragers were collected as they returned to the colony carrying pollen. Four-day-old (sexually immature) and fourteen-day-old (sexually mature) drones were produced by caging the queen on frames with drone-sized honeycomb; note that queens lay unfertilized drone eggs in honeycomb cells that are slightly larger than the cells in which they lay fertilized, worker eggs (Winston 1987). By caging the same queen twice, 10 days apart, we were able to obtain 4- and 14-day-old drones simultaneously for our studies. Drones reached sexual maturity (initiated mating flights and had mature sperm) approximately 8 days after emerging as adults (Villar, unpublished observation; Vetter and Visscher 1997). The drone frames were housed in their parent colony until a day prior to adult emergence, at which time they were removed and stored in a dark incubator at 34 °C and 50 % relative humidity. Upon emergence, drones were marked on their thorax with enamel paint (Testors) and placed back into their parent colony. When the two drone cohorts reached the ages of 4 and 14 days old, they were collected. The number of colonies used to generate samples is provided in the detailed descriptions of the molecular and EAG studies below.

For experiments using bees reared in cages, groups of (1) 10 newly emerged drones and 20 newly emerged workers or (2) 30 newly emerged workers from the same single-drone inseminated (SDI) colony were established in Plexiglass cages (10 × 10 × 7 cm) and fed with 50 % sucrose, water and crushed pollen ad libitum. SDI workers were used to control for any potential effects of genetic variation. Cages were maintained in a dark incubator at 34 °C and 50 % relative humidity. The presence of the queen was simulated by a daily administration of 0.1 queen equivalents of synthetic queen mandibular pheromone (Contech International, Victoria, BC) dissolved in 1 % water/isopropanol on a glass slide, as in (Grozinger et al. 2003). Cages were set up at different days to supply 4- and 8-day-old workers and 5- and 8-day-old drones on the same collection day. The number of colonies used to generate samples is provided in the detailed descriptions of the experiments below.

Characterization of AmOR11 receptor expression

For the bees reared in colonies, 4- and 14-day-old drones, nurses and foragers were collected on dry ice and stored at −80 °C. Antennal pairs were collected from 64 individuals per treatment and divided evenly to form eight samples. This was replicated using three additional colonies for the worker studies and one additional colony for the drone studies. Antennal samples were homogenized, and RNA was extracted using a Arcturus PicoPure RNA isolation kit (Applied Biosystems, Carlsbad, CA). cDNA was synthesized from 200 µg of extracted RNA using Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA). Expression levels were determined using quantitative real-time PCR (qRT-PCR) with an ABI PRISM 7900 sequence detector and using the SYBR Green detection method (Applied Biosystems). Each sample was tested in triplicate and averaged. A standard curve was generated for each primer using dilutions of genomic DNA, to calculate the relative quantities of RNA levels for each sample. A dissociation curve and negative control (cDNA reaction lacking enzyme) were used to confirm primer specificity and lack of genomic DNA contamination. Quantification was based on the number of PCR cycles (C_T) required to cross a threshold of flourescence intensity as described in (Bloch et al. 2001). Expression levels reported for *AmOR11* (GenBank accession no. NM 001242962; *AmOR11*-F: CTTTTACCGAACAACATGACAG, *AmOR11*-R: TTATCTCGTAATTAGGTGTGG) were normalized to the expression levels of the olfactory receptor co-receptor (ORCO) *AmOR2* (GenBank accession no. NM 001134943; *AmOR2*-F: GGACATGGATCTTCGAGGGAT, *AmOR2*-R: TTGAACGTCATTCCAGCAGTT), which served as the housekeeping gene control in all gene expression experiments. A blast analysis confirmed the specificity of these

primers for *AmOR11* and *AmOR2*; the next best sequence matches differed for the 5′ and 3′ primers, less than a 71 % sequence match and no priming at the 3′ end of the primers. *AmOR2* obligately dimerizes with all honey bee odorant receptors (Sato et al. 2008), and its expression levels should reflect the full complement of olfactory neuron receptors (not just those that express *AmOR11*), and thus, overall *AmOR2* levels are not expected to fluctuate across individuals. Indeed, our results (see below) demonstrate the ability to resolve shifts in receptor expression across, but also, variability within treatments. Significant differences in relative expression levels were statistically analyzed using a two-way ANOVA with colony and caste/age as variables for multiple replicates or using a Student's *t* test for single replicates. Figures represent fold differences in expression, relative to the youngest group tested. All statistical tests were run on JMP 9.0.2 (SAS Institute Inc.).

For the bees reared in cages, drones were collected at 5 and 8 days old, while workers were collected at 4 and 8 days old. As before, paired antennae were collected from eight bees per caste/age group, biologically replicated eight times per colony and technically replicated across two colonies for drones and a single colony for workers. Samples were processed, *AmOR11* expression was characterized, and data was statistically analyzed as previously described.

EAG responses to 9-ODA

In the colony studies, 10–11 individuals from each drone or worker group were collected into cages, housed in an incubator at 34 °C and 50 % relative humidity and tested within an hour of their collection. The experiment was replicated twice using two colony sources. The right antenna of each individual tested was cut at the base and tip and affixed to a quadroprobe electroantennogram system (Park et al. 2002) using Spectra® 360 electro-conductive EKG gel. A constant airflow of charcoal-purified, humidified air was passed across the antenna through a 10 mm i.d. glass tube during the experiments. The odorant was delivered into this constant air stream via the Pasteur pipette odor cartridge (see below) whose tip was inserted through a small hole in the glass airstream tube, 11 cm from the tube's end. A stimulus flow controller (Syntech, Hilversum, Netherlands) pulsed a 40 ml/s air-pulse through the cartridge for 0.05 s, effectively delivering 2 ml of volatiles from the odor cartridge into the airstream and across the antenna. DC responses to the stimulus were amplified 10× using the quadroprobe pre-amp then further amplified 10× and recorded and analyzed on a laptop computer using custom software.

Odor cartridges were created as follows. Synthetic 9-ODA (Contech International, Victoria, BC) was dissolved in 1 % water/isopropanol (Sigma-Aldrich, St. Louis, MO) and diluted in this solvent to create seven serial concentrations (100 pg/µl, 1 ng/µl, 10 ng/µl, 100 ng/µl, 1 µg/µl, 10 µg/µl, and 100 µg/µl). Ten microliters of each dilution of 9-ODA was applied to a piece of Whatman filter paper to achieve doses of 9-ODA of 1 ng to 1 mg, representing the range of concentrations workers and drones may experience under natural conditions (Pankiw et al. 1994) and which include previously tested concentrations (Loper et al. 1996; Brockmann et al. 1998, 2006). The solvent was allowed to evaporate, and the filter paper was placed inside a clean glass pipette. A control odorant was included (2-phenylethanol at a dose of 100 µg) which elicited a moderate EAG amplitude from both drone and worker antennae. A puff of air was passed sequentially through each pipette and onto each antennal preparation in increasing magnitude of 9-ODA concentration, and the amplitude of each EAG depolarization to each concentration was recorded. All puffs were separated by 30 s to allow the antenna to reach its resting potential and began and ended with single administrations of the control odorant. All responses to 9-ODA were quantified relative to the response of each antenna to the averaged response to the control odorant, log-transformed when data was not normally distributed and analyzed using a repeated-measures ANOVA with colony, age and concentration as variables.

For the bees reared in cages, seven to ten 5- and 8-day-old drones or 4- and 8-day-old workers were similarly tested and assessed per colony. Drone data represent two colony replicates, and worker data represent one colony replicate.

Results

Maturation-associated changes in AmOR11 receptor expression in workers and drones

We assayed *AmOR11* expression in the antennae of nurses vs. foragers and sexually immature vs. sexually mature drones. From bees reared in colonies, we found significant differences in *AmOR11* expression associated with age in both workers and drones, and the patterns of expression correspond with behavioral receptivity to 9-ODA (Fig. 1). In workers, *AmOR11* expression levels are significantly higher in nurses than foragers (ANOVA, $F_{7,52} = 10.204$, $p \leq 0.0001$, Fig. 1a). In drones, *AmOR11* expression levels are significantly higher in 14-day-old sexually mature drones than in 4-day-old sexually immature drones (ANOVA, $F_{3,28} = 9.748$, $p = 0.0001$, Fig. 1b). Though expression levels differed significantly with age in both workers and drones, it is important to note that the magnitude of the difference was not very large, especially compared to the tenfold higher expression levels of *AmOR11* in the antennae of drones versus workers (Wanner et al. 2007).

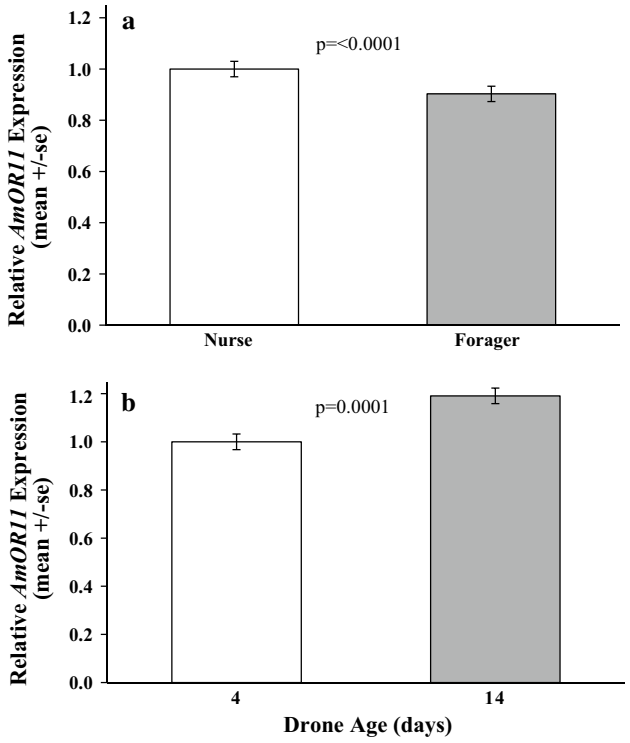

Fig. 1 Colony-reared worker and drone *AmOR11* antennal receptor expression correlates with behavioral attraction to 9-ODA. **a** Nurses show significantly higher expression than foragers. Expression levels of *AmOR11* were measured using qRT-PCR in pooled antennae of nurses and foragers, $N = 7$–8 pools/group/colony, using bees from four colonies. Data were log-transformed and a two-way ANOVA with age and colony was used to assess significant differences in expression. There was a significant effect of age (ANOVA, $F7{,}52 = 10.204$, $p \leq 0.0001$) and colony ($p = 0.0001$) but no colony × age interaction ($p = 0.3889$). **b** Sexually mature 14-day-old drones show significantly higher expression than immature 4-day-old drones. Expression levels of *AmOR11* were measured using qRT-PCR in pooled antennae of sexually immature and mature drones, $N = 8$ pools/group/colony, using bees from two colonies. A two-way ANOVA with age and colony was used to assess significant differences in expression. There was a significant effect of age (ANOVA, $F3{,}28 = 9.748$, $p = 0.0001$) and colony ($p = 0.0021$) but no colony × age interaction ($p = 0.5346$)

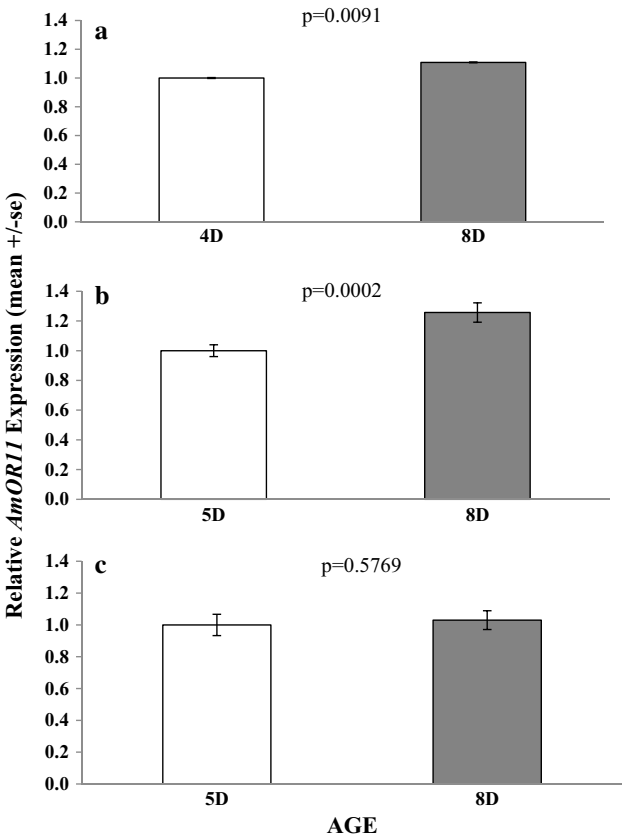

Fig. 2 Cage-reared worker and drone *AmOR11* antennal receptor expression is modulated in the first week post-emergence. Expression levels of *AmOR11* were measured using qRT-PCR in pooled antennae. **a** Young nurses ($N = 8$ pools) exhibit significantly lower *AmOR11* expression than older nurses ($N = 7$ pools), which typically engage in queen rearing tasks, $t = 2.160$, $p = 0.0091$. **b**, **c** A colony × age effect was found ($p = 0.0044$) when analyzing two combined drone cage replicates and were subsequently analyzed separately. In trial 1, younger drones ($N = 8$ pools) exhibited significantly lower *AmOR11* expression than older drones ($N = 8$ pools), $t = 5.438$, $p = 0.0002$. However, in trial 2, there was no difference in expression between younger ($N = 8$ pools) and older ($N = 8$ pools) drones, $t = 0.5714$, $p = 0.5769$

In a second experiment, we reared workers and drones in cages and tested *AmOR11* expression in 4- and 8-day-old workers and 5- and 8-day-old drones. The attraction of young workers to QMP and queen pheromone extracts increases as they age and peaks at 8 days (Pham-Delegue et al. 1993), whereas drones initiate reproductive behavior around 8 days post-emergence [Villar, unpublished observation and (Vetter and Visscher 1997)]. *AmOR11* expression levels are significantly higher in 8-day-old versus 4-day-old workers (Fig. 2a, Student's t test, $p = 0.0091$). An analysis of both of the drone study replicates resulted in a colony × age interaction effect, and each trial was analyzed separately as a result. In trial 1, *AmOR11* expression was found to be significantly higher in 8-day-old drones versus 5-day-old drones (Fig. 2b, Student's t test, $p = 0.0002$); however, no significant differences in expression were found in trial 2 (Fig. 2c, Student's t test, $p = 0.5769$).

Maturation-associated changes in electrophysiological responses of antennae of workers and drones to 9-ODA

We next examined electrophysiological responses of the antennae of nurses, foragers, sexually immature drones and sexually mature drones, to determine whether these responses correlated with variation in *AmOR11* expression, maturation and behavioral responses. We tested the

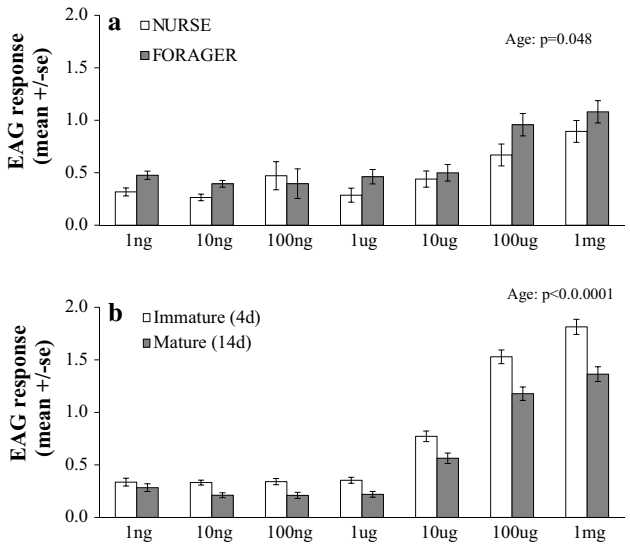

Fig. 3 Colony-reared worker and drone antennal responses to 9-ODA do not correlate with behavior or *AmOR11* expression. **a** A marginally significant effect of age was found in workers (repeated-Measures ANOVA $F1,36 = 4.1915$, $p = 0.048$), with older foragers ($N = 20$, 2 colony replicates) exhibiting stronger overall electrophysiological responses to 9-ODA vs. young nurses ($N = 21$, 2 colony replicates). A colony effect was also present ($p = 0.0428$); however, no colony × age interaction effect was present ($p = 0.0861$). **b** Immature drone antennae ($N = 21$, 2 colony replicates) exhibit a significantly greater response to 9-ODA at most concentrations tested (repeated-measures ANOVA $F1,39 = 21.872$, $p \leq 0.0001$) compared to those of mature drones ($N = 22$, 2 colony replicates). A colony effect was present ($p = 0.001$), but no colony × age effect was found ($p = 0.8135$). A concentration effect was found for both workers and drones, confirming a dose response to increasing 9-ODA concentrations ($p < 0.0001$)

responses of ten to eleven colony-reared individuals per group per colony and tested two colonies. For all electrophysiological assays, responses to the control odorant did not change over time (data not shown), confirming that the antennal preparations remained in good condition for the duration of the experiments. Results are presented relative to the control odorant.

There were marginally significant overall differences between nurse and forager antennal sensitivity to 9-ODA (Fig. 3a, repeated-measures ANOVA, $F_{1,36} = 4.1915$, $p = 0.048$). A significant dose affect was seen (repeated-measures ANOVA, $F_{4,129} = 17.5202$, $p \leq 0.0001$), with responses increasing at higher concentrations of 9-ODA. Interestingly, sexually immature drones showed a significantly stronger overall response to 9-ODA than sexually mature drones at every concentration (Fig. 3b, repeated-measures ANOVA, $F_{1,39} = 21.872$, $p \leq 0.0001$). As with the workers, a significant effect of dose was seen (repeated-measures ANOVA, $F_{3,105} = 445.59$, $p \leq 0.0001$), except with drones, a much higher EAG amplitude relative to the 2-phenylethanol control stimulus was observed.

Studies of bees reared in cages found no effect of maturation on electroantennogram responses in both sexes, as well as the lack of correlation between changes in *AmOR11* receptor expression and antennal response to 9-ODA. Antennal responses did not differ significantly between 4- and 8-day-old workers (data not shown, repeated-measures ANOVA, $F_{1,13} = 0.5258$, $p = 0.4812$) or 5- and 8-day-old drones to 9-ODA (data not shown, repeated-measures ANOVA, $F_{1,31} = 0.3194$, $p = 0.576$).

Discussion

Sexually dimorphic behavioral responses to 9-ODA in honey bee workers and drones have been previously shown to be strongly correlated with differences in antennal expression levels of the 9-ODA receptor (*AmOR11*) and neurophysiological sensitivity of the antennae (Brockmann et al. 1998; Wanner et al. 2007). Here, we investigated whether similar differences in the peripheral nervous system might explain the maturation-related variation in behavioral responses to 9-ODA observed in both workers and drones. Our results demonstrate that there are small but significant differences in *AmOR11* expression associated with maturation in both workers and drones, and these expression differences correlate with behavioral differences. However, neurophysiological responses of the antennae, measured using EAGs, do not correlate with *AmOR11* expression differences or behavioral responses. Surprisingly, EAG responses are significantly lower in sexually mature versus immature drones at all tested concentrations and slightly lower in nurses than in foragers.

What physiological mechanisms regulate this maturation-related increase in *AmOR11* expression levels in drones and maturation-related decrease in *AmOR11* levels in workers? Behavioral maturation in workers and sexual maturation in drones are regulated by common genetic and physiological factors: artificially increasing juvenile hormone titers in both workers and drones accelerates maturation, and worker and drone maturation rates are correlated across genotypes (Giray and Robinson 1996). Thus, it is possible that JH titers may also trigger changes in the sensory system to increase sensitivity or receptivity to relevant cues. In our studies, we found increasing levels of *AmOR11* expression occurring during the first week of adult development for both workers and drones. Previous studies have also shown that an increase of JH titers in both workers and drones begins 4–7 days after adult emergence (Giray and Robinson 1996; Pankiw et al. 1998). However, the effects of JH on transcription events must be modulated by other factors in drones compared to workers, thereby allowing increasing JH titers to be associated with decreased *AmOR11* expression in foragers compared

to nurses and increased expression in older, compared to younger, drones.

Differences in *AmOR11* levels did not correspond to differences in antennal neurophysiological responses to 9-ODA. In colony-reared bees, EAG responses to 9-ODA were significantly higher in sexually immature drones than sexually mature drones, in direct contrast to *AmOR11* levels and the expected behavioral responses. Vetter and Visscher similarly observed decreased EAG responses to QMP as drones aged (Vetter and Visscher 1997). In workers, we found marginally significant differences in response in nurses and foragers, where forager antennae were more responsive at low concentrations and high concentrations, again in direct contrast to *AmOR11* levels and the expected behavioral responses. Though uncommon, a lack of agreement between odor-mediated behavior and EAG responses has been found to occur in other instances (Lemmen 2014). Previous studies of worker EAG responses to QMP found responses peaked at 8–12 days and then remained relatively stable or decreased slightly (Masson and Arnold 1984; Allan et al. 1987; Pham-Delegue et al. 1993). In cage-reared bees, there were no age-associated differences in EAG responses of either drones or workers. The lack of correlation between receptor expression and antennal sensitivity may be due to other associated changes in the olfactory neurons which could change their responsiveness, including changes in concentrations of odorant-binding proteins (Danty et al. 1998; Biessmann et al. 2010; Pelletier et al. 2010), changes in the activity of signal transduction pathways within neurons (Vergoz et al. 2009) or modulation of sensory neuron activity due to biogenic amines (McQuillan et al. 2012) and circulating hormones (Robinson 1987). Another possibility relates to a recent study on the olfactory receptor neuron's ability to handle high levels of molecular flux from incoming plume strands of odorants. Larger diameter dendrites having greater surface areas and expressing more ORs than smaller diameter dendrites do not seem to increase the neurons' sensitivities to their ligands (Baker et al. 2012). It was proposed that a greater expression of ORs on the dendrites of receptor neurons is needed not to increase a neuron's sensitivities but rather to accurately report peak molecular abundances of behaviorally important odorant molecules. With *AmOR11*, therefore, we might not expect to see corresponding increases in EAG amplitudes with increased expression levels, but rather should have expected to see an ability of the antenna to respond with increased EAG amplitudes only to what normally would have been excessively high concentrations of 9-ODA to which a still higher EAG would not have typically been registered. Other studies have demonstrated that olfactory neuron responses to odorants can vary in honey bee workers due to genotype or learning experience. Hygienic strains of honey bees exhibit lower thresholds of sensitivity to diseased brood, characterized by greater olfactory sensitivity to low concentrations of diseased brood odors and faster cleaning of diseased individuals as compared to non-hygienic strains (Masterman et al. 2001). Interestingly, pharmacological manipulation of octopamine (OA) levels modulates physiological responses to diseased brood odors (Spivak et al. 2003). Recently, Claudianos et al. demonstrated that odor conditioning of workers with linalool (a floral odorant) and 9-ODA alike resulted in a significant down-regulation of their respective odor receptors and decreased EAG responses to the odors, both effects contingent on the formation of an odor-associated long-term memory (Claudianos et al. 2014). However, in other studies, the formation of odor memories has also been shown to both increase antennal sensitivity (Wadhams et al. 1994) or have no impact on sensitivity (Sandoz et al. 2001), suggesting that additional factors regulate these processes.

In the honey bee, 9-ODA has evolved to function as a social and sex pheromone in workers and drones, respectively. As such, it is responsible for facilitating critical features of the social environment and its organization. Variation in responsiveness to 9-ODA among drones and workers of different ages is an important factor ensuring that the pheromone elicits the optimal responses in the different contexts. Though the underlying mechanisms responsible for eliciting differential responses to the same signal among young and old workers and sexually immature and mature drones are yet unknown in this system, our results suggest that neuromodulatory factors altering synaptic activities within the antennal lobe or protocerebrum are likely to be responsible. Thus, different aspects of the olfactory system are modulated to mediate sex- and maturation-dependent differences in sensitivity and response to 9-ODA.

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