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cGMP-dependent changes in phototaxis: a possible role for the *foraging* gene in honey bee division of labor

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Summary

Division of labor in honey bee colonies is influenced by the *foraging* gene (*Amfor*), which encodes a cGMP-dependent protein kinase (PKG). *Amfor* upregulation in the bee brain is associated with the age-related transition from working in the hive to foraging for food outside, and cGMP treatment (which increases PKG activity) causes precocious foraging. We present two lines of evidence in support of the hypothesis that *Amfor* affects division of labor by modulating phototaxis. We first show that a subset of worker bees involved in the removal of corpses from the hive had forager-like brain levels of *Amfor* brain expression despite being middle aged; age-matched food-handlers, who do not leave the hive to perform their job, had low levels of *Amfor* expression. This finding suggests that occupations that involve working outside the hive are associated with high levels of *Amfor* in brain. Secondly, foragers were much more positively phototactic than hive bees in a laboratory assay, and cGMP treatment caused a precocious onset of positive phototaxis. The cGMP effect was not due to a general increase in behavioral activity; cGMP treatment had no effect on locomotor activity under either constant darkness or a light:dark regime. The cGMP effect also was not due to changes in circadian rhythmicity; cGMP treatment had no effect on age at onset of locomotor circadian rhythmicity or the period of rhythmicity. The effects of *Amfor* on phototaxis are not related to peripheral processing; electroretinogram analysis revealed no effect of cGMP treatment on photoreceptor activity and no differences between untreated hive bees and foragers. The cAMP/PKA pathway does not appear to be playing a similar role to cGMP/PKG in the honey bee; cAMP treatment did not affect phototaxis and gene expression analysis revealed task-related differences only for the gene encoding the regulatory subunit, but not the catalytic subunit, of PKA. Our findings implicate one neural process associated with honey bee division of labor that can be affected by naturally occurring changes in the expression of *Amfor*.

Key words: honey bee, *Apis mellifera*, *foraging* gene (*Amfor*), cGMP-dependent protein kinase, PKG, phototaxis, division of labor, behavioral development.

Introduction

One challenge in the study of genes and behavior is to determine how a gene exerts its influence on neurons and neural systems to influence behavioral plasticity. *Amfor* is the ortholog of the *Drosophila melanogaster foraging* gene (Osborne et al., 1997) in the honey bee *Apis mellifera*, which encodes a cGMP-dependent protein kinase (PKG). In the honey bee, an age-related increase in *Amfor* expression in the brain during the life of a bee is associated with the onset of foraging behavior, and treatment with cGMP causes an increase in PKG activity and precocious foraging (Ben-Shahar et al., 2002).

The onset of foraging in honey bees is the culmination of the process of behavioral development that underlies colony division of labor (Robinson, 1992). A worker bee begins her adult life by progressing through a series of tasks in the beehive and then typically begins to forage at about 3 weeks of age. The timing of a bee's shift from hive to foraging duties is flexible, and depends on the needs of the colony. It is also associated with changes in metabolism, exocrine gland activity, hormone levels, brain structure, brain chemistry and gene expression in the brain (Robinson, 2002).

PKG has numerous roles in a nervous system (Ruth, 1999; Wang and Robinson, 1997), but how it influences the shift from working in the hive to foraging in honey bees is not known. Ben-Shahar et al. (2002) suggested that perhaps the upregulation of PKG activity affects honey bee behavioral

development *via* effects on the visual system, because they found strong expression of *Amfor* in the optic lobe lamina and in a subset of intrinsic cells of the mushroom bodies known to receive visual input (Ehmer and Gronenberg, 2002; Gronenberg, 2001). In addition, cGMP has been shown to have an important role in the development of the visual system in *Drosophila* (Gibbs et al., 2001). Flies carrying a mutation in a subunit of soluble guanylate cyclase (the enzyme that makes cGMP) show reduced photoreceptor response to light stimuli and altered phototactic behavior (Gibbs et al., 2001). Honey bee division of labor is known to involve maturational changes in responsive to olfactory task-related stimuli (e.g. Robinson, 1987a), but the role of vision in the control of honey bee behavioral development has not been studied.

Bees are extremely visual animals, with a large portion of their brain dedicated to visual processing (Gronenberg, 2001). Foragers perform well in different laboratory-based visual learning paradigms, no doubt because they rely considerably on visual abilities when foraging in the field (Zhang et al., 1999). Foragers use optic flow to measure distance (Esch et al., 2001), discriminate easily between different shapes (Horridge, 2000) and have well-developed color vision (Werner et al., 1988).

We tested the hypothesis that the effects of *Amfor* on honey bee behavioral development are due, at least in part, to an increase in positive phototaxis. We focused on this aspect of the visual system because honey bees experience a major change in exposure to light when they shift from working in the dark hive to foraging outside. Menzel and Greggers (1985) have shown that foragers are positively phototactic, but it is not known whether this behavior is developmentally regulated. Young bees do emerge from the hive to take brief defecation and orientation flights prior to the beginning of their foraging phase (Capaldi et al., 2000), but these are transient events. Perhaps more chronic increases in positive phototaxis occur in older pre-foraging bees, which then positions them closer to the hive entrance. There they may be induced to forage by exposure to olfactory and mechanical stimuli, such as communication by successful foragers *via* the dance language (Frisch, 1967). A behaviorally related change in phototaxis has recently been reported for queen harvester ants (*Messor pergandei*); queens are positively phototactic as virgins but became negatively phototactic after mating (Julian and Gronenberg, 2002).

We tested the hypothesis that the effects of *Amfor* on honey bee behavioral development are due, at least in part, to an increase in positive phototaxis by addressing three issues. First, we determined whether the previously reported increase in *Amfor* brain expression in foragers is also detectable in the brains of bees that are not foraging, but are nevertheless engaged in a task that requires leaving the hive. This was accomplished by comparing two groups of middle-aged bees: food handlers and corpse removers (undertakers). Although a majority of bees that are found outside the hive are foragers, other tasks such as undertaking occur outside as well. Undertakers are a subset group of bees that pick up corpses in the hive and then fly out to remove them (Visscher, 1983). Undertakers are younger than foragers, but they have high, forager-like titers of juvenile hormone (JH), which influences the pace of honey bee behavioral development (Huang et al., 1994). Second, we asked whether there is an ontogeny of phototaxis behavior in association with honey bee behavioral development, and if so, whether it can be accelerated by treatment that activates PKG. Third, we studied whether the observed treatment effects of cGMP on phototaxis are due to changes in overall levels of locomotor activity, the timing of the onset of locomotor circadian rhythmicity (Bloch and Robinson, 2001; Moore et al., 1998; Toma et al., 2000), or general photoreceptor sensitivity.

In addition, we explored whether the cAMP/PKA pathway may also be playing a role similar to the cGMP/PKG pathway in the honey bee. These pathways are known to interact in other behavioral systems including in honey bees (Muller and Hildebrandt, 2002). We determined the effects of cAMP treatment on phototaxis and measured the expression of genes encoding the regulatory and catalytic subunits of PKA in the brains of bees performing different behaviors.

Materials and methods

Bees

We used European honey bees *Apis mellifera* L., which in North America are derived from a mix of European subspecies. All bees were maintained according to standard beekeeping techniques at the University of Illinois Bee Research Facility. 1-day-old-bees were used to set up experimental colonies and as subjects for treatment. They were obtained by removing honeycomb frames containing pupae from large field colonies (derived from naturally mated queens) and placing them in an incubator (33°C, 95% humidity). Bees that emerged over a 24 h period were also marked with a spot of paint (Testor's Pla) on the thorax and used as described below.

Bees for mRNA expression analysis were collected from triple-cohort colonies (Ben-Shahar and Robinson, 2001), which were established by sequentially introducing three cohorts of 800–1000 1-day-old bees to a small hive at 1-week intervals. Each colony was also given two frames of honeycomb for food storage and egg laying and an unrelated, naturally mated, queen. The following behavioral groups were collected (Robinson, 1987b): nurses, identified as 1-week-old bees that repeatedly inserted their heads into honeycomb cells containing larvae; food handlers, 2-week-old bees that were found on a honeycomb frame containing honey; undertakers, 2-week-old bees that removed corpses from the hive; and foragers, bees older than 3 weeks of age returning to the hive with either clearly visible pollen loads on their hind legs or distended abdomens (bearing either nectar or water). To induce undertaking behavior, freshly killed bees (50–100) were put in the hive prior to sampling (Visscher, 1983). All bees were collected directly into liquid nitrogen and stored at –80°C until brain dissection (N=8–9 per group). Bees were sampled from three, unrelated colonies.

mRNA expression analysis

We measured *Amfor* mRNA levels for each brain individually using real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) with TaqMan® technology (ABI, Foster City, CA, USA). Analysis was as described (Ben-Shahar et al., 2002). Brains were dissected frozen (Schulz and Robinson, 1999) and RNA extracted with the mini-RNeasy kit according to the manufacturer's instructions with on-column DNase I treatment (Qiagen, Valencia, CA, USA). The RT reaction was performed with random hexamers on 200 ng total RNA according to the TaqMan® RT-PCR kit protocol. The PCR reaction was performed with gene-specific primers and dual-labeled TaqMan® probes. Primers and probe for *Amfor* were as described (Ben-Shahar et al., 2002). PCR conditions were the default settings of the ABI TaqMan® 9700 SDS machine (ABI). We determined the cycle threshold (Ct) during the geometric phase of the PCR amplification plots, as recommended by the manufacturer. Relative differences in *Amfor* transcripts were quantified using the ΔΔCt method (Bloch and Robinson, 2001) with the *A. mellifera* ortholog of *rp49* (GenBank AF441189) mRNA as a 'housekeeping' gene loading control (Ben-Shahar et al., 2002). *rp49* is widely used in this way in *Drosophila* and other organisms (Daborn et al., 2002; Thellin et al., 1999). For graphical presentation we used the $2^{-\Delta\Delta Ct}$ transformation according to ABI user bulletin 20 (see also Bloch and Robinson, 2001). All data were normalized relative to values for nurse bees.

Brains from the first trial were also used to measure mRNA levels for the genes encoding the regulatory and catalytic subunits of protein kinase A (PKAr and PKAc, respectively). Primers and probes for these genes were as follows. PKAr: probe, FAM6-AGCCGAAGCAGCGCGAGGTTTA-TAMRA, forward primer, TTTACTTCGCCCACAGCGT, reverse primer, CGAATTGGCGCTAGTGACAC; PKAc: probe, FAM6-CAAAAGAAAATCGAGGCCCCGTTCA-TAMRA, forward primer, ACCGATTGGATAGCCGTCTT, reverse primer, CCTGGCCCTTTACATTTTGG.

Treatments

We paint-marked groups of 50 1-day-old bees a distinctive color and placed each group in a 6 cm×12 cm×18 cm wooden cage in an incubator (33°C, 95% relative humidity) for 4 days. Bees were treated orally with a 50% sucrose solution containing either 8-Br-cGMP (500 μmol l^{-1}, Sigma-Aldrich, St Louis, MO, USA) or 8-Br-cAMP (1000 μmol l^{-1}; Sigma-Aldrich) while control bees received sucrose alone. These compounds (and doses) were shown to increase PKG and PKA activity, respectively, without significant 'cross reactivity'; cGMP treatment did not cause an increase in PKA activity and cAMP treatment did not cause an increase in PKG activity (Ben-Shahar et al., 2002). Freshly mixed solutions were given daily to each cage of bees. On day 5, all surviving bees from each cage were counted (80–100% survival for each cage) and used in the following experiments.

Positive phototaxis assay

We first compared the performance of nurses (7–10 days old) and foragers (older than 21 days old). Each bee was removed from its colony in a small glass vial and anesthetized on ice. Once immobile, it was introduced to a small wooden cage as above. We placed 10 nurses and 10 foragers together in each cage. Bees were allowed to recover for 2 h at room temperature in the dark, and then were tested as follows. The cage was attached to a wooden tunnel a little taller than a bee (10 mm) covered with Plexiglass (Fig. 1). A narrow light beam from a 150 W quartz white light illuminator (Fisher Scientific, USA) was aimed through the tunnel towards the bottom part of the cage. All bees that moved through the tunnel from the cage towards the light in a period of 3 min were scored as positively phototactic. All bees were counted once at the end of the testing period to prevent repeat counts of the same bees. Comparisons of nurses and foragers were also made in the same experimental apparatus with the light off, to be able to distinguish differences in positive phototaxis from differences in general locomotor activity. Nurses and foragers from two unrelated colonies were compared in two trials of this experiment (under both light and dark conditions). Effects of cGMP, cAMP and a sucrose control were compared in nine trials. Each trial used bees from a different, unrelated, colony.

Electroretinogram analysis

An electroretinogram (ERG) assay was used to test for differences between nurses and foragers in photoreceptor sensitivity, and for effects of cGMP treatment. We looked for differences in both the amplitude and shape of the ERG response. Nurses and foragers and treated bees were obtained as described above.

ERGs were recorded using techniques described for *Drosophila* (Larrivee et al., 1981). Bees were immobilized with wax with their right side down on a glass coverslip, and

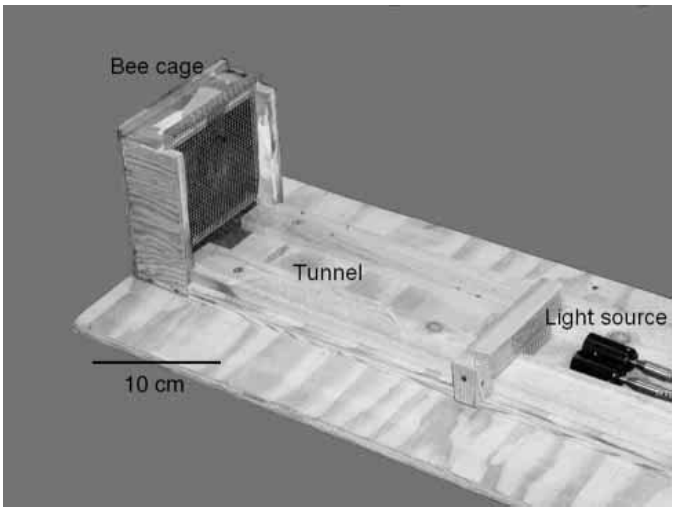

Fig. 1. Phototaxis apparatus. The proportion of bees that walked from the cage to the light in 3 min was used as an index of positive phototaxis.

their left compound eye facing upward. The wax also ensured that the bee could not move any of its legs or antennae. The reference electrode was inserted into the head while the recording electrode was inserted into the compound eye through the cornea. White light produced by a xenon arc lamp (Bausch & Lomb, Rochester, NY, USA) was used with Wratten (Kodak, Rochester, NY, USA) neutral density filtration to achieve the desired light intensity. The unfiltered light intensity (I_0) was 4 mW cm^{-2} at the level of the bee. Recordings were made over a 4-log unit range of stimulus intensities ($\log I/I_0$). The bees were dark-adapted for 1 min before a 3 s light stimulus was given. All recordings were made at 25°C. Voltage signals were recorded and amplified with a high-impedance microprobe amplifier (W-P instruments, Longmount, CO, USA). The signals were then digitized at 2kHz with an analog-to-digital converter (Digi-Data 1200A, Columbia, MD, USA) and the data acquired and analyzed in a computer with Axoscope (Axon Instruments, Foster City, CA, USA). We did not vary either the positioning of electrodes or their depth of penetration into the cornea. Under our recording conditions, we detected no variation in waveforms either within or between behavioral or treatment groups of bees (data not shown). Variation in ERG amplitude was also minor, as indicated by the small standard deviations (Fig. 4).

Measurement of locomotor activity and the ontogeny of circadian rhyhmicity

We studied the effects of cGMP and cAMP on locomotor activity and on the ontogeny of locomotor circadian rhythmicity using a well-established laboratory assay of individual bee behavior (Bloch and Robinson, 2001; Toma et al., 2000). Bees were treated in groups as described above and then transferred on day 5 to individual cages in an environmental chamber (33°C; either 12 h:12 h light:dark 'LD' or 24 h dark 'DD'). Locomotor behavior was monitored with automatic infrared motion sensors (DataCol 3.0 acquisition system; Mini-Mitter Co., OR, USA; Toma et al., 2000); events (crossing infrared beam) were analyzed in 10 min bins to determine overall activity levels. χ^2 periodogram analysis [P<0.01 (Bloch et al., 2001); Tau program, Mini-Mitter Co., OR, USA] was used to determine onset of rhythmicity, and the percentage of bees that showed clear circadian rhythm at each age calculated. We also calculated *tau* (the period of rhythmicity). Data were collected for 4 days. We performed two trials of this experiment, one in each light regime.

Statistical analysis

mRNA data were analyzed using two-way analysis of variance (ANOVA) with trial and task as factors. Pair-wise LSD *post hoc* tests were used to compare the different behavioral groups in each trial. Effects of cGMP and cAMP on phototaxis were analyzed by calculating the proportion of bees in each group showing positive phototaxis in each trial (N=9), followed by the improved Freeman–Tukey arcsine square-root transformation (Freemen and Tukey, 1954), and one-way ANOVA with pair-wise LSD *post-hoc* tests. Differences in the proportion of foragers and nurses showing positive phototaxis were analyzed by 2×2 χ^2 analysis (Fisher's exact test was used when necessary). Effects of cGMP on locomotor activity and *tau* were analyzed by one-way ANOVA and on % rhythmicity by 2×3 χ^2 analysis. ERG data were analyzed with a repeated measure one-way ANOVA. All statistical tests were performed with the SYSTAT 8.0 statistical package (Systat Software Inc., Richmond, CA, USA).

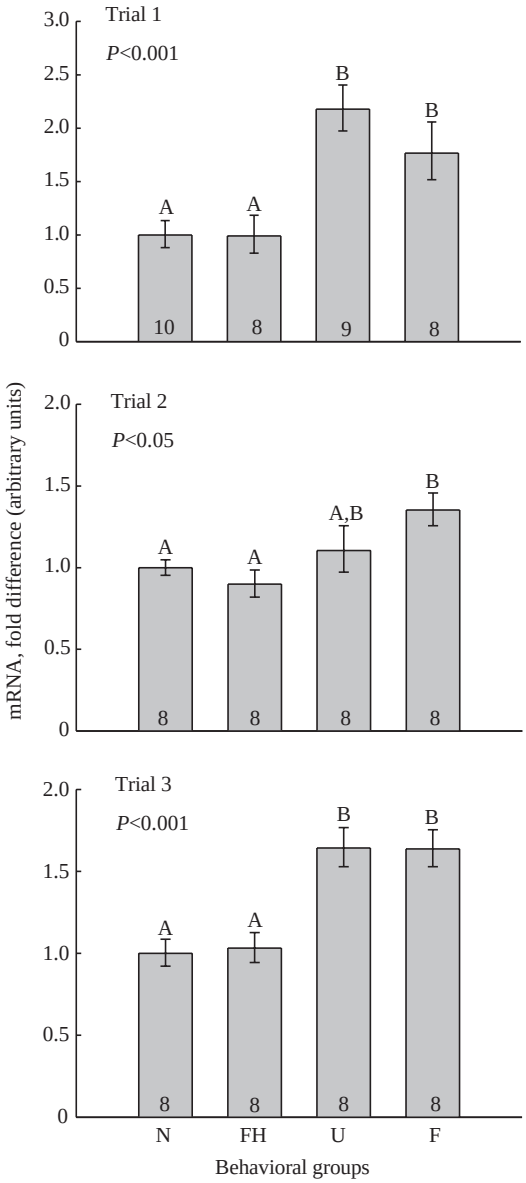

Fig. 2. Behaviorally related differences in *Amfor* brain expression. Bars represent relative mRNA differences, normalized to nurses (N). Error bars represent the standard errors of the ΔCt converted to the same arbitrary scale as the means (see Materials and methods). *P* values on graphs refer to ANOVA; different letters denote statistically significant differences (P<0.05; pair-wise LSD *post hoc* analysis). N=8–10 brains per group (see numbers on bars), each brain was analyzed individually. FH, food handlers; U, undertakers; F, foragers.

Results

Amfor expression as a function of task

Bees that performed activities outside (foragers and undertakers) showed a significant (ANOVA: $P<0.05$) increase in brain *Amfor* transcript levels relative to bees that worked in the hive (nurses and food handlers; Fig. 2). *Amfor* expression also was significantly ($P<0.05$) higher in undertakers relative to food handlers in two out of three trials, despite their similar ages. These results demonstrate a strong association between tasks performed outside the hive and high brain *Amfor* expression, independent of age.

Effect of cGMP treatment on phototaxis

A significantly greater proportion of foragers showed positive phototaxis relative to nurse bees (Fig. 3A; trial 1, $\chi^2_{13.3,1}$, $P<0.01$; trial 2, $\chi^2_{7.2,1}$, $P<0.01$). There were no significant differences between the two behavioral groups under dark conditions (trial 1, $\chi^2_{0,1}$, $P>0.05$; trial 2, $\chi^2_{0,1}$ $P>0.05$). This result indicates that the differences in phototaxis were not due to differences in locomotor behavior.

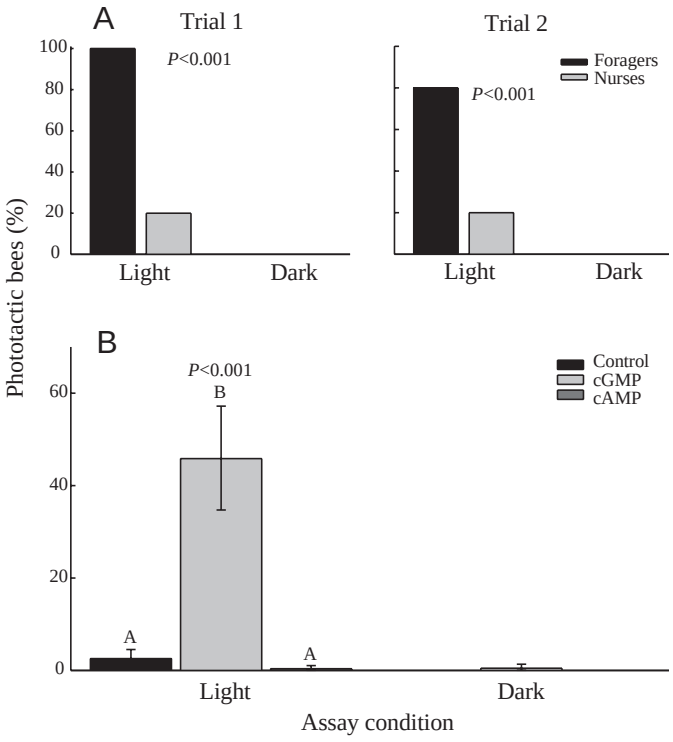

Fig. 3. Phototaxis behavior in honey bees as a function of behavioral state or treatment. (A) Differences in positive phototaxis between nurses and foragers. Group size=20 bees. 2×2 χ^2 tests were conducted for both trials. (B) Effects of 8-Br-cGMP, 8-Br-cAMP on positive phototaxis. Nine trials were conducted; data on the proportion showing positive phototaxis from each trial were arcsine transformed for ANOVA. For graphical purposes, means ± S.E.M. were back-transformed (resulting in asymetrical error bars). For doses, see Materials and methods. 'Light', phototaxis behavior when light source is on; 'Dark', locomotion control when light source is off.

A significantly greater proportion of cGMP-treated bees showed positive phototaxis relative to cAMP-treated or untreated bees (Fig. 3B; $N=9$ cages per treatment; one-way ANOVA; $P<0.001$). There were no significant differences between the groups under dark conditions ($P>0.05$). The proportion of cGMP-treated bees showing positive phototaxis was not as high as for foragers, but the treatment effect was substantial, especially given that these were young bees reared as adults in laboratory cages.

Effect of cGMP treatment on electroretinogram measurements

Repeated-measures ANOVA revealed a highly significant increase in ERG response amplitude with increasing light intensity ($P<0.001$), but no differences between nurses and foragers (Fig. 4). We expected that if cGMP treatment had a direct effect on photoreceptor sensitivity, then treated bees would show an increase in ERG response amplitude relative to control bees, especially at the lower light intensities. However, cGMP treatment did not have a significant effect on ERG amplitude (Fig. 4). These results indicate that the cGMP-treatment effects on positive phototaxis reported above are not due to changes in primary photoreceptor activity.

Effect of cGMP treatment on circadian locomotor activity

The cGMP-treatment effects on positive phototaxis reported above are not due to a general increase in locomotor activity or to changes in circadian rhythms of locomotion. There was no effect of cGMP treatment on activity, under either D:L or D:D light regimes (Fig. 5; ANOVA: D:L, $P>0.05$; D:D, $P>0.05$). Under the DD regime there were also no effects of treatment on *tau* (ANOVA: $P>0.05$; Fig. 5) or the percentage of bees developing a circadian rhythm for locomotor behavior by day 7 ($\chi^2_{0.120,2.000}$, $P=0.057$; Fig. 5). The possibility of some linkage between age at onset of foraging in the field and

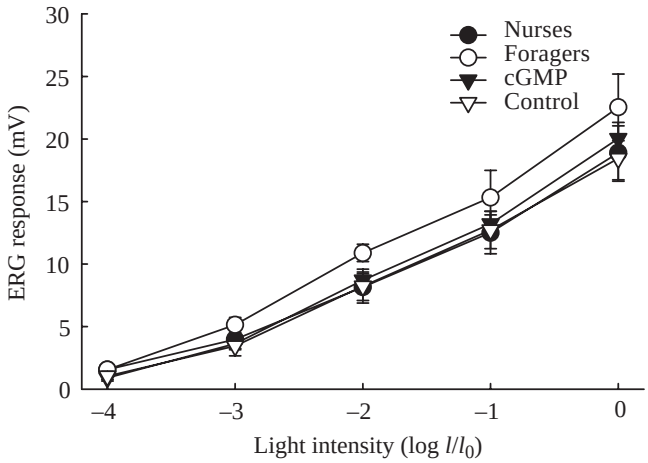

Fig. 4. Electroretinogram (ERG) measurements in honey bees as a function of behavioral state or treatment. $N=6$ bees tested at each light intensity, per group. There were no significant differences between the groups ($P>0.05$; repeated-measures ANOVA).

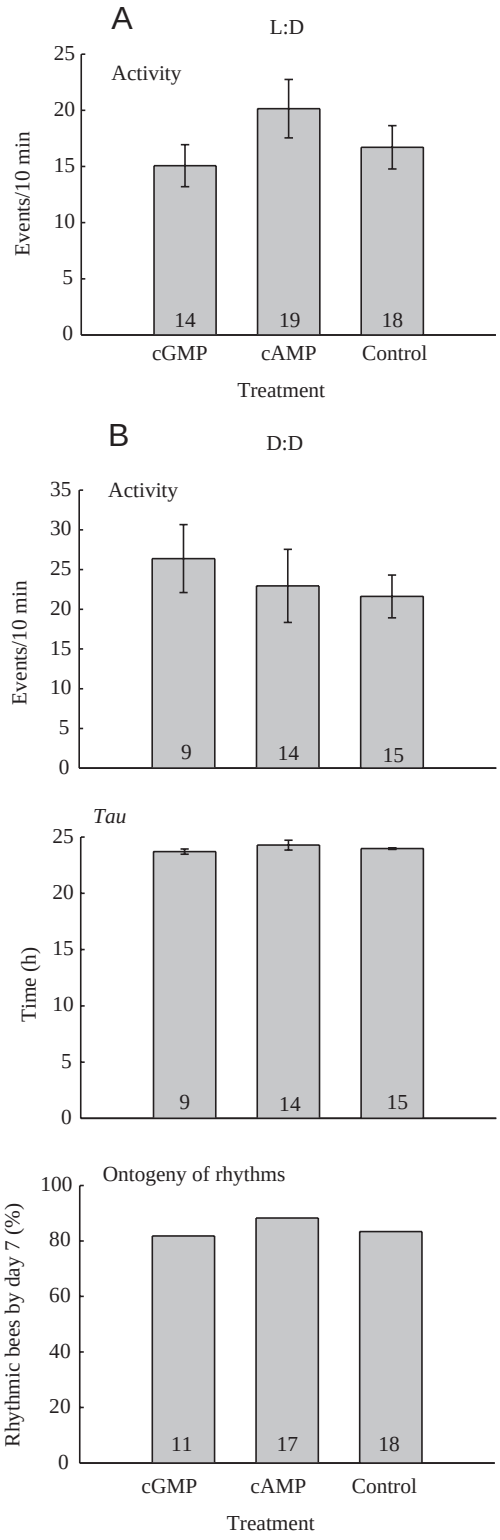

Fig. 5. Effects of 8-Br-cGMP on levels of locomotor activity, age at onset of circadian locomotor rhythms, and *tau* (the period of rhythmicity) under (A) 12 h:12 h light:dark (L:D) and (B) 24 h dark (D:D) regimes. Activity levels were analyzed only under L:D conditions because light entrains activity. There were no significant differences between the different groups in all experiments (activity, tau, ANOVA; % rhythmicity, $P>0.05$; χ^2 tests).

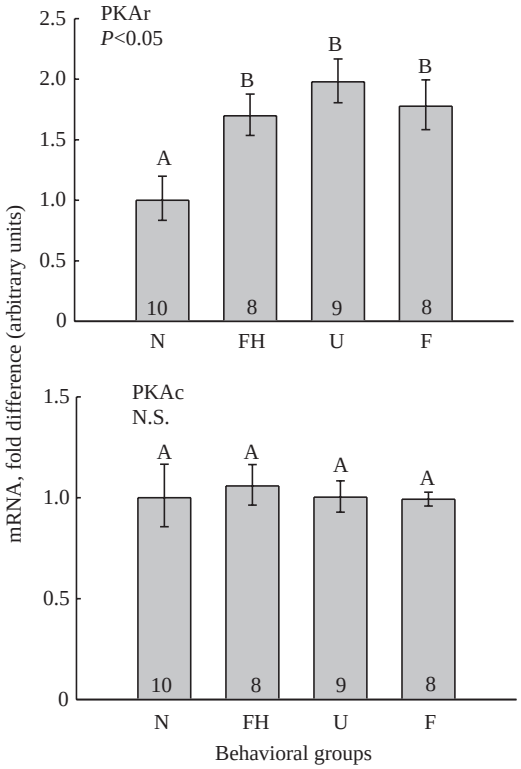

Fig. 6. Brain mRNA levels for genes encoding PKA regulatory (r) and catalytic (c) subunits as a function of behavior. Analysis and graphic representation are as in Fig. 2. cDNA samples were the same as in Fig. 2, trial 1 (with same sample sizes).

the age at onset of circadian rhythmicity in the laboratory was suggested by earlier findings (Moore et al., 1998). The results reported here suggest that there is no obligate connection between these two aspects of behavioral development, at least with respect to the involvement of the cGMP pathway.

PKAc and PKAr expression as a function of task

Expression analysis of genes in the cAMP/PKA pathway revealed no strong association with honey bee division of labor (Fig. 6). Although brain PKAr mRNA levels were significantly ($P<0.01$) lower in nurses relative to food handlers, undertakers and foragers, there were no differences among the four groups for brain PKAc mRNA levels ($P=0.966$). These results are consistent with findings showing no effect of cAMP treatments on either foraging behavior (Ben-Shahar et al., 2002) or positive phototaxis.

Discussion

Our results show that changes in phototaxis occur in association with behavioral development in the honey bee, and this change is influenced by cGMP treatment, just as the onset of foraging (Ben-Shahar et al., 2002). Changes in responses to olfactory and gustatory stimuli have previously been shown to be correlated with honey bee behavioral development

(Robinson, 1987a; Scheiner et al., 1999, 2001). It is now apparent that honey bee behavioral development also involves changes in responsiveness to stimuli in other modalities. Our results also suggest that upregulation of *Amfor* expression in the brain may influence division of labor, at least in part, *via* effects on phototaxis.

Amfor upregulation was previously associated with foraging in honey bees, based on comparisons of nurses and foragers only (Ben-Shahar et al., 2002). Our results indicate that this upregulation is more generally associated with working outside the hive. Undertakers had high, forager-like, brain levels of *Amfor* transcript, despite the fact that they were the same age as food handlers, while food handlers, working inside the hive, had low, nurse-like, levels. It is not known whether the upregulation of *Amfor* occurs as a consequence of exposure to light because both undertakers and foragers could have been out before they were sampled. However, both nurse bees and food handlers probably took orientation flights, which are typical of all younger bees (Capaldi et al., 2000), suggesting that light exposure is not sufficient to trigger the upregulation of this gene. In addition, it appears that exposure to light does not cause a rapid change in *Amfor* expression, because undertakers had high transcript levels even though they were collected just as they were exiting the hive. This suggests that more short-term changes in phototaxis, such as presumably occur when a young bee leaves for orientation flights, are not associated with a short-term increase in *Amfor* expression.

Foragers were known to be strongly positively phototactic (Menzel and Greggers, 1985) and our findings indicate that they are much more so than nurses. This is intriguing in the context of a prominent theory that explains division of labor on the basis of the classical stimulus–response model (Beshers and Fewell, 2001; Beshers et al., 2001, 1999; Manning, 1967; Roeder, 1967). According to the stimulus–response model of division of labor, differences in task performances between individuals occur because of differences both in probability of exposure to certain task-specific stimuli and differences in responsiveness to these stimuli (Beshers and Fewell, 2001; Beshers et al., 2001, 1999). Age-related changes in behavior are thought to be a consequence of developmental changes in these two factors (which are influenced by JH, octopamine, *Amfor*, and no doubt many other agents that influence neural plasticity; see Robinson, 2002). Given that the *Apis mellifera* mostly nests in dark, enclosed cavities, light can serve as a reliable indicator of the location of the nest entrance, which is where much foraging-related activity occurs (Frisch, 1967). A developmental increase in positive phototaxis may thus position bees closer to the hive entrance where they may be induced to forage by exposure to olfactory and mechanical stimuli, such as successful foragers communicating by means of the dance language (Frisch, 1967).

Alternatively, the increased positive phototaxis observed in foragers may relate to a general increased responsiveness to a variety of stimuli associated with the switch from in-hive tasks to foraging. Age-related increases in responsiveness to alarm pheromones (Robinson, 1987a) and sucrose (Pankiw and Page, 1999; Pankiw et al., 2001) have been reported, and octopamine increases responsiveness to brood pheromone (Barron et al., 2002), a multi-functional pheromone that serves as a stimulus for foraging (Pankiw and Page, 2001).

Electroretinogram analysis indicates that the cGMP-induced increase in positive phototaxis was not based on effects of sensitivity to light *per se*. This is in agreement with Menzel and Greggers (1985), who concluded that positive phototaxis in foragers was probably mediated by neural activity in the optic lobe lamina, suggesting regulation by second-order interneurons rather than by the photoreceptor cells themselves. Ben-Shahar et al. (2002) reported strong expression of *Amfor* in the lamina and in a subset of intrinsic cells of the mushroom bodies known to receive visual input (Ehmer and Gronenberg, 2002; Gronenberg, 2001). This is also consistent with findings from Menzel and Greggers (1985), who showed that positive phototaxis in returning foragers is probably due to activity of cells in the eye lamina. Our results are also in agreement with findings from *Drosophila* suggesting that, contrary to vertebrates, insects do not use cGMP signaling as the main phototransduction second messenger (Bloomquist et al., 1988). Perhaps PKG is involved in modifying the function of neuronal circuits in the lamina and/or mushroom bodies *via* phosphorylation of some component molecules, which is similar to the affect of PKG on olfaction in mammals (Kroner et al., 1996).

cGMP/PKG-dependent influences on honey bee behavioral development are not due to effects on locomotor activity or the ontogeny of a circadian locomotor rhythm. Previous work has shown an intriguing association between the onset of circadian behavioral rhythmicity and behavioral development in honey bees (Moore et al., 1998), as well as a major role for PKG signaling in mammalian clock function (Ferreyra and Golombek, 2001; Gillette and Tischkau, 1999). Whether PKG signaling affects other aspects of circadian clock function in bees and other insects awaits further experimentation.

PKG influences phototaxis in honey bees, but our experiments do not rule out effects on other sensory systems as well. As in *Drosophila* (Osborne et al., 1997) and the honey bee (Ben-Shahar et al., 2002), cGMP signaling is involved in the regulation of feeding behavior in molluscs (Della-Fera et al., 1981; Elphick et al., 1995), hydra (Colasanti et al., 1997), and *C. elegans* (Stansberry et al., 2001; Fujiwara et al., 2002; L'Etoile et al., 2002). In most of these cases the influences on feeding are mediated by effects on chemosensation. In *Drosophila*, allelic variation in *pkg* (*for*) causes variation in both spontaneous and evoked neuronal activity (Renger et al., 1999), as well as in habituation of the giant fiber escape circuit (Engel et al., 2000). It is not known whether these effects in flies are related to feeding behavior, but the results demonstrate that PKG can modulate neuronal activity.

There are interactions between the PKA and PKG signaling pathways in other behavioral systems (Centonze et al., 2001; Kroner et al., 1996), and recently it was shown in bees that habituation of the proboscis extension reflex can be affected by cGMP-mediated PKA activation (Muller and Hildebrandt,

2002). We failed to detect evidence for such interactions in the context of phototaxis, which is consistent with earlier findings on the regulation of age at onset of foraging. cAMP analog treatment increased PKA activity in the bee brain but did not cause precocious foraging (Ben-Shahar et al., 2002), and in the present study did not cause precocious phototaxis. In addition, only one of two cAMP-related genes showed consistent changes in association with honey bee behavioral maturation. These results are difficult to interpret because PKA functions as a holoenzyme comprising two regulatory and two catalytic subunits (Johnson et al., 2001), so perhaps increases in mRNA abundance for both are not necessary to increase PKA activity. Nevertheless, our results suggest that upregulation of cGMP signaling is involved in regulating phototaxis and age at the onset of foraging in honey bees, independent of cAMP levels.

We have discovered a role for cGMP signaling in modulating an important sensory process in the honey bee, vision. This process controls a behavioral response – positive phototaxis – that contributes to a complex behavioral transition, the onset of foraging. The transition from working in the hive to foraging plays a major role in colony social organization. Dissection of a complex social trait into behavioral components and identifying underlying mechanisms at the molecular and neural systems levels are the first steps towards understanding how genes influence behavioral plasticity.

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