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A Role for Octopamine in Honey Bee Division of Labor

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Octopamine · Division of labor · Neuromodulation · Response thresholds · Biogenic amines · Behavioral development · Honey bees · *Apis mellifera* · Juvenile hormone

Abstract
Efficient division of labor is one of the main reasons for the success of the social insects. In honey bees the division of labor is principally achieved by workers changing tasks as they age. Typically, young adult bees perform a series of tasks within the colony before ultimately making the transition to foraging outside the hive for resources. This lifelong behavioral development is a well-characterized example of naturally occurring behavioral plasticity, but its neural bases are not well understood. Two techniques were used to assess the role of biogenic amines in the transition from in-hive work to foraging, which is the most dramatic and obvious transition in honey bee behavioral development. First, associations between amines and tasks were determined by measuring the levels of amines in dissected regions of individual bee brains using HPLC analysis. Second, colonies were orally treated with biogenic amines and effects on the onset of foraging were observed. Octopamine concentration in the antennal lobes of the bee brain was most reliably associated with task: high in foragers and low in nurses regardless of age. In contrast, octopamine in the mushroom bodies, a neighboring neuropil, was associated with age and not behavior, indicating independent modulation of octopamine in these two brain regions. Treating colonies with octopamine resulted in an earlier onset of foraging in young bees. In addition, octopamine levels were not elevated by non-foraging flight, but were already high on return from the first successful foraging trip and subsequently remained high, showing no further change with foraging experience. This observation suggests that octopamine becomes elevated in the antennal lobes in anticipation of foraging and is involved in the release and maintenance of the foraging state. Foraging itself, however, does not modulate octopamine levels. Behaviorally related changes in octopamine are modulated by juvenile hormone, which has also been implicated in the control of honey bee division of labor. Treatment with the juvenile hormone analog methoprene elevated octopamine and octopamine treatment 'rescued' the delay in behavioral development caused by experimentally depleting juvenile hormone in bees. Al-

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though the pathways linking juvenile hormone and octopamine are presently unknown, it is clear that octopamine acts 'downstream' of juvenile hormone to influence behavior and that juvenile hormone modulates brain octopamine levels. A working hypothesis is that octopamine acts as an activator of foraging by modulating responsiveness to foraging-related stimuli. This is supported by the finding that octopamine treatment increased the response of bees to brood pheromone, a stimulator of foraging activity. Establishing a role for octopamine in honey bee behavioral development is a first step in understanding the neural bases of this example of naturally occurring, socially mediated, behavioral plasticity. The next level of analysis will be to determine precisely where and how octopamine acts in the nervous system to coordinate this complex social behavior.

Introduction

Honey bees *(Apis mellifera)* are obligate social animals. They depend on the efficient functioning of their colony, which relies on a division of labor among the worker bees. Living in societies that exhibit an advanced level of eusociality, worker honey bees engage in little, if any, direct reproduction [Ratnieks, 1993; Visscher, 1996] and instead contribute to their inclusive fitness by performing all tasks necessary for colony growth and reproduction. Even though all workers share a common social environment, they respond in different ways to the suite of task-related stimuli in the hive such that different bees specialize on different tasks. Individual bees also change their role as they age performing different tasks in a predictable sequence [Winston, 1987]. This form of behavioral development, known as 'temporal polyethism,' is extremely flexible and adapts to colony needs. Typically, a worker bee spends the first two to three weeks of adult life performing brood care and food processing tasks in the hive and then moves to a phase of life dominated by working outside the hive foraging for resources (pollen, nectar, water and propolis). Honey bee behavioral development, however, can be accelerated, delayed or even reversed depending on the needs of the colony [Robinson, 1992]. Thus, the honey bee colony relies on the behavioral plasticity of individual workers to adapt to changes in conditions.

The neural bases of honey bee behavioral development are not well understood. The biogenic amines, with established roles as neuromodulators and widespread distribution in the bee brain [Bicker, 1999], have been among the first neurochemicals studied to elucidate underlying neural mechanisms related to the division of labor in honeybees. This paper reviews our current understanding of such mechanisms, focusing on the role of octopamine in controlling the transition from working in the hive to foraging. We also discuss the factors that change biogenic amine levels in the honey bee brain.

Honey Bee Behavioral Plasticity

The most dramatic change in honey bee behavioral state is the change from in-hive work to foraging outside. There is a temporal pattern to the various in-hive tasks performed by young bees [Winston, 1987] and transitions from one task to another are detectable [Robinson, 1987a; Seeley and Kolmes, 1991], but the sequence of task performance is highly variable and there is considerable overlap of in-hive tasks [Winston, 1987]. In contrast, once bees have begun foraging they very rarely participate in in-hive tasks [Ribbands, 1953; Bloch and Robinson, 2001]. This is the case even when foraging is not possible [Moore et al., 1998; Bloch and Robinson, 2001]. The shift from in-hive tasks to foraging is accompanied by dramatic changes within the CNS and in non-neural tissues. The onset of foraging is associated with changes in: circulating hormones [Robinson, 1992; Robinson and Vargo, 1997; Bloch et al., 2002]; brain structure [Withers et al., 1993; Fahrbach and Robinson, 1996; Winnington et al., 1996; Sigg et al., 1997]; brain gene expression [Ohashi et al., 1997; Toma et al., 2000; Shapira et al., 2001; Kucharski and Maleszka, 2002; Ben-Shahar et al., 2002]; metabolic activity [Harrison, 1986]; and the development of endocrine and exocrine glands [summarized in Winston, 1987]. These factors together with the stability and exclusivity of foraging behavior identify foraging as a behavioral state [sensu Oster and Wilson, 1978; Adamo et al., 1995].

Biogenic Amines and Behavioral Plasticity

The biogenic amines are recognized as modulators of nervous function [Evans, 1980; Erber et al., 1993] and have been documented as modulators of behavior in diverse animal groups. These substances have been found to be involved in many different aspects of behavior. Amines modulate responsiveness to stimuli by changing

the stimulus threshold to which an animal will respond, for example, octopamine modulation of the flight behavior of male moths in response to female sex pheromone [Linn and Roelofs 1986; Linn et al., 1992]. Amines also are involved in establishing novel stimulus-response relations by associative learning processes. The octopaminergic neuron VUMmx1 mediates the pairing of conditioned and unconditioned stimuli in an olfactory associative learning paradigm [Hammer, 1993; Hammer and Menzel, 1995]. Changes in arousal or behavioral states also are mediated by biogenic amines. Arousal state is defined by the level of sensory alertness, motor activity and reactivity [sensu Tinbergen, 1951; Pfaff et al., 2002]. Changes in behavioral state involve coordinated changes in responsiveness to stimuli and the adoption of a new, stable, behavioral repertoire. Some changes in behavioral state will involve a change in arousal [Jing and Gillette, 2000], and biogenic amines are involved in changes in both. In molluscs, serotonin is considered a general arousal factor [Kupfermann and Weiss, 1981; Satterlie and Norekian, 1996; Katz et al., 1994; Jing and Gillette, 2000; Gillette and Jing, 2001]. Results from *Pleurobranchaea* have revealed a hierarchy of serotonergic modulatory neurons that coordinate changes in activity and responsiveness [Jing and Gillette, 2000]. Serotonin releases a dominant behavior, escape, that always overrides feeding. In crustaceans the modulation of retreat behavior by amines is not so absolute. It is not clear whether the three types of biogenic amine effects on behavioral responsiveness, learning, and arousal represent distinct functional categories or elements of a common mechanism.

Honey bees of all ages are exposed to stimuli associated with in-hive tasks and foraging, yet young bees respond to the stimuli associated with the former and older bees respond to the latter. The onset of the foraging state must therefore involve profound alterations in the responsiveness to task-related stimuli. It is unlikely that the transitions in task performance are mediated entirely by modifications of the peripheral nervous system. Rather, we suspect that there is broad modulation of circuits within the CNS to affect behavioral plasticity.

Honey Bee Division of Labor and Levels of Biogenic Amines in the Brain

The biogenic amines dopamine, serotonin, and octopamine are all found in the brain of the honey bee [Mercer and Erber, 1983]. Early studies [Fuchs et al., 1989; Harris and Woodring, 1992; Taylor et al., 1992] first suggested a role for biogenic amines in honey bee behavioral development. Harris and Woodring [1992] determined that brain levels of dopamine, serotonin, and octopamine increased with age, and were highest during the summer when foraging activity was greatest. Taylor et al. [1992] showed that foragers had significantly elevated levels of dopamine in their brains as compared to younger bees performing in-hive tasks such as nursing or food storing. These studies suggested that foraging is associated with higher levels of biogenic amines in the brain, but it was not clear whether these increases were associated with the bee's occupation or age. Given the normal pattern of temporal polyethism, foragers are usually one or two weeks older than nurse bees and so the variables of age and task are confounded.

Taking advantage of social manipulations that modify the normal pattern of temporal polyethism [Rösch, 1930; Lindauer, 1954; Robinson, 1992], the effects of age and task on amine levels in the bee brain were evaluated separately [Schulz and Robinson, 1999; Wagener-Hulme et al., 1999]. By changing the social environment it is possible to induce bees to forage precociously or to nurse for an extended period. Age-matched samples of nurses and foragers can be made at both young and old ages in 'single-cohort colonies,' initially comprised of all one-day-old bees [Robinson et al., 1989; Wagener-Hulme et al., 1999]. Foragers can even be induced to revert to nursing behavior [Page et al., 1992; Robinson et al., 1992; Huang and Robinson, 1996; Bloch and Robinson, 2001].

Wagener-Hulme et al. [1999] analyzed the relationships among amine levels, task and age by collecting foragers and nurses from colonies with typical age demography as well as age-matched nurses and precocious foragers, age-matched overage nurses and foragers, and age-matched reverted nurses and foragers from experimental colonies. All bees were collected from the field and placed directly into liquid nitrogen to minimize any effect of handling on amine levels. HPLC analysis was used to measure amines in individual brains (minus optic lobes and subesophageal ganglion). There was a relationship between amines and age; older bees, especially foragers, had higher amounts of all three amines than younger bees working in the hive. There were also associations between amines and behavior that were independent of age. Of the three biogenic amines examined, octopamine was most strongly associated with behavior and was greater in the brains of foragers than nurses [Wagener-Hulme et al., 1999]. Serotonin level was also behavior-related but the association was less compelling [Schulz and Robinson, 1999].

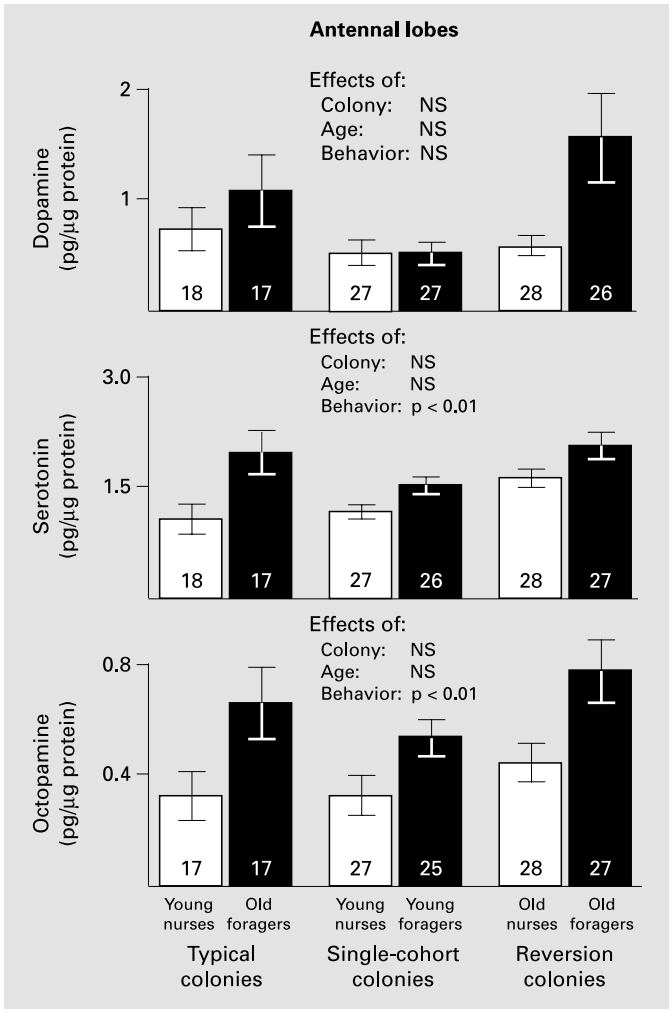

Fig. 1. Mean (± SE) concentrations of dopamine, serotonin and octopamine in the antennal lobes of honey bees from typical colonies (control nurses and foragers), single-cohort colonies (normal-age nurses and precocious foragers), and reversion colonies (reverted nurses and normal-age foragers). Sample size is indicated in each bar. Summary results of ANOVA testing the effect of colony, age and behavior on amine concentration are shown. [From Schulz and Robinson, 1999.]

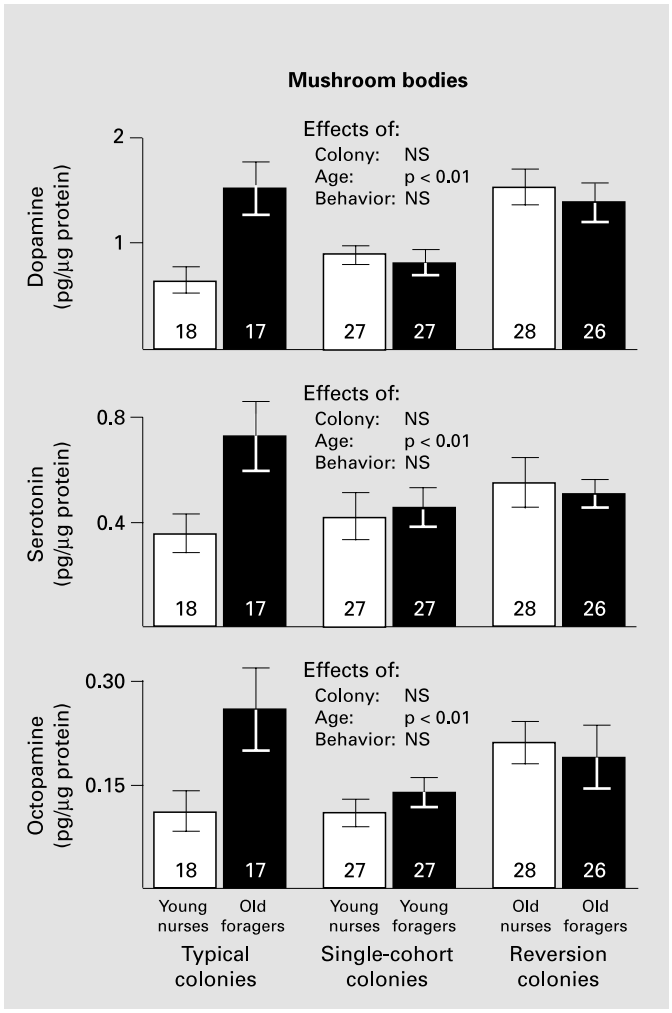

Fig. 2. Mean (± SE) concentrations of dopamine, serotonin and octopamine in mushroom bodies (calyces and Kenyon cell somata) of honey bees. Behavioral groups and statistical analyses as in figure 1. [From Schulz and Robinson, 1999.]

Schulz and Robinson [1999] demonstrated that amine profiles differ in different regions of the brain. Two brain regions were studied, the antennal lobes and the mushroom bodies. The HPLC analyses were sensitive enough to allow for analysis of each brain region from within an individual brain. The antennal lobes were selected because chemical communication is crucial to the functioning of the honey bee colony and many task-related stimuli are olfactory [Robinson, 1987b; Barron et al., 2002]. The mushroom bodies were selected because of their roles in both multi-modal integration of sensory stimuli and cognition [Menzel and Müller, 1996]. Figure 1 shows that antennal lobe levels of octopamine and serotonin, but not dopamine, were consistently associated with worker task [Schulz and Robinson, 1999]. Foragers (both normal-age and precocious) had elevated octopamine and serotonin in the antennal lobes, whereas nurses (normal-age, overage, and reverted) did not. Octopamine showed the most robust relationship; differences between nurses and foragers in the antennal lobes were twice as large for octopamine relative to serotonin. In contrast, amines in the mushroom bodies were more closely associated with age rather than task, and were higher in older bees regardless of

behavioral status (fig. 2). The antennal lobes are connected to the mushroom bodies via the antennoglomerular tract, yet the foraging-related changes in the antennal lobes are not seen in the mushroom bodies. This suggests that the foraging-related change in octopamine is well localized. Consistent with this idea, we found that differences between nurse bees and foragers were larger and more consistent in antennal lobes rather than whole brains.

Octopamine Influences Honey Bee Division of Labor

Honey bee foraging is associated with high concentrations of both octopamine and serotonin in the antennal lobes. Amine treatment experiments [Schulz and Robinson, 2001] have demonstrated that octopamine, and not serotonin, plays a role in the transition from hive work to foraging. Colonies of bees in the field were treated orally by dissolving a quantity of an amine in a 50% sucrose solution and presenting the solution in empty honey combs as the sole food source [Schulz and Robinson, 2001]. This provided a simple and non-invasive method of chronically treating bees with amines in the field. Treatment with octopamine resulted in elevated brain levels of octopamine (and not serotonin or dopamine), whereas treatment with serotonin or its immediate precursor 5-hydroxytryptophan resulted in elevated brain levels of serotonin (and not octopamine or dopamine) [Schulz and Robinson, 2001; Schulz et al., 2002a]. Injections of octopamine directly into the bee brain have been used successfully for experiments on short-term changes in behavior [Hammer and Menzel, 1998], but a single injection elevates octopamine for only a few hours [Linn et al., 1994]. Given that the onset of foraging is measured along a time scale of days, we assumed that chronic treatment was necessary and repeat injections were not practical because of handling stresses and the large number of bees involved in a study of division of labor. The difficulty with oral treatment is that it is likely that other tissues in addition to the central and peripheral nervous systems were affected by the amines, making it difficult to attribute subsequent effects to a particular location.

Octopamine-treated colonies consistently produced between two and eight times more precocious foragers than did control colonies matched for size, age demography, genotypic composition, and microenvironment [Schulz and Robinson, 2001]. Octopamine treatment appears to have an activational effect on behavior. Bees initiated foraging shortly after treatment [Schulz and Robinson, 2001] and the effect waned quickly after treatment stopped [Schulz et al., 2002b]. Furthermore, only bees that were physiologically competent to forage (greater than three days old) responded to treatment [Schulz and Robinson, 2001]. The timing of the octopamine effect contrasts sharply with the effect of juvenile hormone (JH) analog treatment, which also causes precocious foraging [Bloch et al., 2002]. Hormone treatment has a much longer latency, an issue that will be discussed more fully later in this paper when exploring the links between octopamine and JH.

The effect of octopamine on honey bee foraging was dose-dependent, highly consistent (replicated numerous times over different field seasons) and was specific to octopamine. Treatment with the octopamine precursor tyramine did not cause an increase in the number of foragers, nor did treatment with serotonin or 5-hydroxytryptophan. Cocktails of serotonin and octopamine led to a forager increase, but not beyond what was seen with octopamine alone [Schulz and Robinson, 2001; Schulz et al., 2002a]. Given that treatment experiments with biogenic amines can sometimes produce variable results depending on the state of experimental animals [Pribbenow and Erber, 1996], the effects of octopamine treatment on foraging behavior were robust.

Modulation of Biogenic Amines in the Brain

Age- and task-related changes in biogenic amine levels reflect pathways of modulation that affect the antennal lobes and mushroom bodies in distinct ways. The rate of honey bee behavioral development is sensitive to the demography of the colony; old bees inhibit the development of younger bees, delaying the age at onset of foraging. In an experimental colony devoid of old bees, young adult workers experience a greatly accelerated behavioral development and become foragers precociously [Rösch, 1930; Lindauer, 1954; Robinson, 1992]. The socially mediated acceleration of behavioral development is mirrored by acceleration in the development of brain octopamine and serotonin profiles [Wagener-Hulme et al., 1999], especially in the antennal lobes [Schulz and Robinson, 1999].

Given the strong task-related modulation in the antennal lobes, it was of interest to determine whether this was due to the effects of flight. Attention was directed to octopamine because of the results of the treatment experiments described above. In addition, flight activity is posi-

tively correlated with circulating OA titers in *Periplaneta americana* [King et al., 1986] and Erber [1993] posits an association between octopamine and stress in honey bees. However, Schulz et al. [2002a] found no association between the amount of foraging or flight activity (both potential physiological stressors) and octopamine in the brain. Using detailed observations of individually tagged bees in colonies confined to a large, outdoor, screen enclosure, it was found that antennal lobe levels of octopamine were already high as the bee returned from its very first foraging flight and did not vary with the amount of subsequent foraging experience. These results indicate that the increase in octopamine occurs prior to the onset of foraging [Schulz et al., 2002a]. This is consistent with findings showing that octopamine also increased in the antennal lobes of pre-forager bees when they were confined to the hive and were unable to forage [Schulz et al., 2002b]. Octopamine levels in forager antennal lobes also were high at a 4:00 AM sampling point, hours before the onset of foraging for that day [Schulz et al., 2002a]. Conversely, octopamine in antennal lobes was low in younger bees sampled just as they returned from a pre-foraging orientation flight [Schulz et al., 2002a]. These results indicate that the increase in octopamine in the antennal lobes is not the result of flight activity, but rather is involved in the initiation and maintenance of the foraging state.

One factor that regulates octopamine in the bee brain is juvenile hormone (JH) [Schulz et al., 2002b]. This hormone, a sesquiterpenoid product of the corpora allata, is involved in the regulation of diverse aspects of development and maturation [Nijhout,1994], including honey bee behavioral development [Bloch et al., 2002]. Blood levels of JH are elevated in foragers compared to nurses (regardless of age), and treatment with JH or JH analogs results in precocious foraging [Bloch et al., 2002]. Juvenile hormone is involved in controlling the pace at which bees develop into foragers [Sullivan et al., 2000]; bees devoid of circulating JH (by 'allatectomy,' surgical removal of the corpora allata) still become foragers, but at significantly delayed ages. This delay was eliminated by hormone treatment [Sullivan et al., 2000].

Juvenile hormone analog treatment resulted in high, forager-like, levels of octopamine in the antennal lobes of 6- and 12-day-old bees sampled before they foraged [Schulz et al., 2002b]. These results suggest that one way that JH influences foraging is to cause an increase in octopamine. This idea is consistent with findings that allatectomized bees devoid of circulating JH were still able to respond to octopamine treatment and initiate foraging precociously (fig. 3). This effect was reversible; when octopamine treatment was discontinued, the number of allatectomized bees becoming foragers was significantly lower than bees with intact corpora allata, which is consistent with the allatectomy effect reported by Sullivan et al. [2000]. The possibility that octopamine acts downstream of JH also is consistent with the differences in the timing of octopamine and JH analog treatment effects. Octopamine influences foraging more rapidly than does JH [Robinson, 1987a; Sullivan et al., 2000; Schulz and Robinson, 2001].

Octopamine was previously shown to be able to affect the production of JH by the corpora allata [Kaatz et al., 1994], but our findings suggest a reciprocal relationship also exists with respect to octopamine levels in the antennal lobes. It appears that JH acts earlier in the process of forager development, upstream of octopamine, with octopamine acting more proximally as a releaser of foraging. Priorities for future research are to elucidate the pathway(s) that link these two factors, and perhaps those related to the age and task effects, as well as to determine how JH controls octopamine in the antennal lobes.

How Does Octopamine Influence Honey Bee Foraging Behavior?

Octopamine in the antennal lobe is most closely associated with foraging behavior. Because the antennal lobes are the primary olfactory processing centers of the insect brain [Winnington et al., 1996; Joerges et al., 1997; Galizia and Menzel, 2001], it is possible that the switch to foraging behavior may be due, in part, to a change in the way task-related olfactory stimuli are processed by the antennal lobes. Barron et al. [2002] studied the effect of octopamine treatment on responsiveness to brood pheromone, an olfactory stimulus involved in foraging behavior [Pankiw et al., 1998]. Brood pheromone is a mixture of cuticular waxes produced by honey bee larvae that is involved in several different aspects of honey bee behavior including foraging activity [Le Conte et al., 1990, 1995, 2001; Pankiw et al., 1998]. Application of synthetic brood pheromone to colonies results in a rapid dose-dependent increase in foraging activity for most of the pheromone doses tested [Pankiw et al., 1998; Barron et al., 2002]. Barron et al. compared how much brood pheromone increased foraging activity in octopamine-treated and control colonies. A pheromone dose that elicited a minor response from control colonies evoked a strong positive response from octopamine-treated colonies (fig. 4), demonstrating that octopamine modulated the

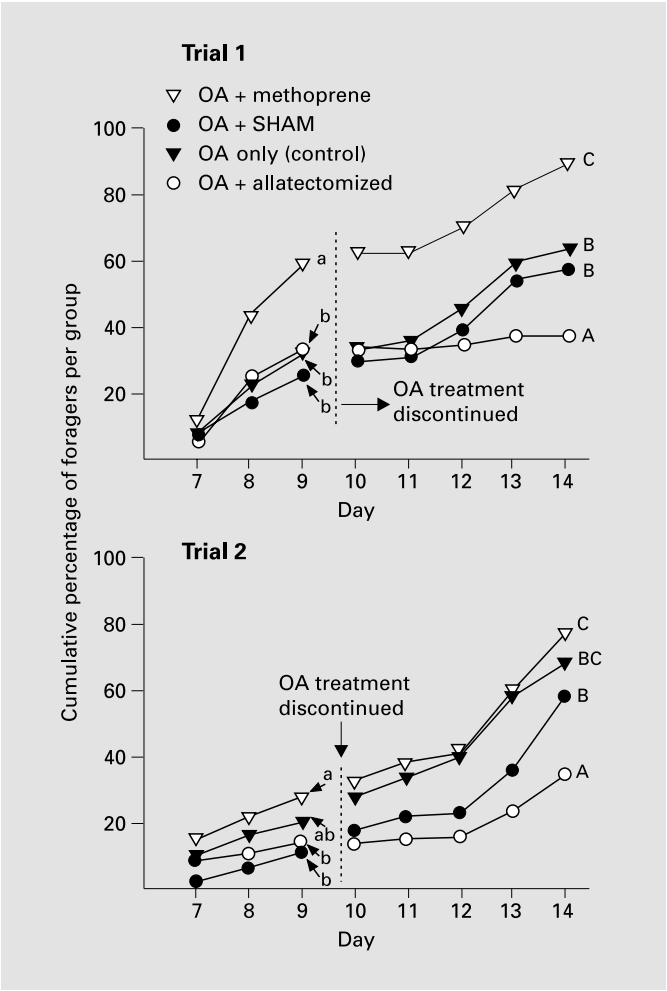

Fig. 3. Effects of allatectomy on responsiveness of honey bees to octopamine treatment. Cumulative percentage of bees foraging from each of four focal groups within an octopamine-treated colony. Different letters represent significant differences ($p < 0.05$; pairwise 2 × 2 G tests) in the distribution of foragers and non-foragers when bees were 9 and 14 days old. Dashed lines represent the point at which the octopamine treatment was stopped. Octopamine treatment 'rescued' the allatectomy effect; the allatectomy effect is then seen when octopamine treatment was stopped. [From Schulz et al., 2002b]

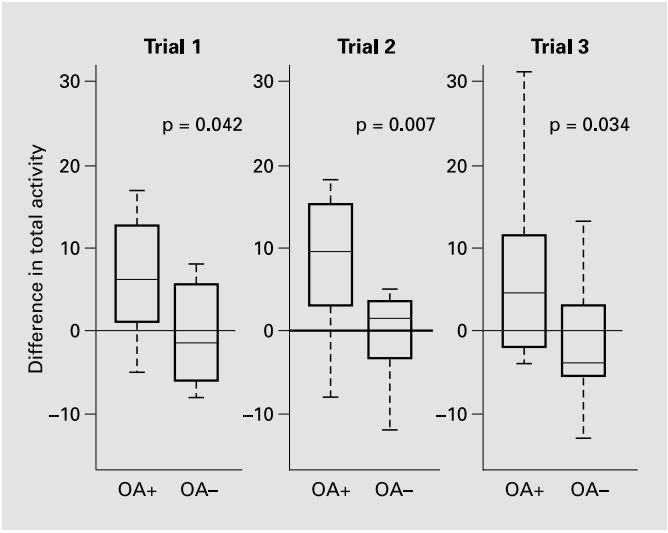

Fig. 4. Effects of octopamine treatment (OA) on responsiveness of honey bees to brood pheromone. Each box shows the difference in total foraging activity between a colony exposed to brood pheromone (BP) and a matched colony that was not. The line within each boxplot marks the median, boxes extend to the upper and lower quartiles and whiskers extend to 1.5 × interquartile distance. Data from three independent trials shown. p values refer to Wilcoxon rank sum tests of the null hypothesis that the response to brood pheromone did not differ between octopamine-treated (OA+) and control (OA–) colonies. The effect of brood pheromone treatment on activity was significantly greater in OA+ colonies than OA– colonies. [From Barron et al., 2002.]

response to brood pheromone. This experiment showed that one mode of action of octopamine is to modulate responsiveness to foraging stimuli. Whether octopamine modulates responsiveness to other foraging stimuli, especially non-olfactory stimuli, or indeed stimuli related to other tasks, has yet to be determined.

Octopamine and the Organization of Honey Bee Behavior

The studies reviewed here demonstrate that octopamine modulates the division of labor in honey bee colonies, in particular, the likelihood of initiating foraging. However, octopamine has been recognized previously as a modulator of bee behavior in other contexts. Octopamine is involved in associative learning [Hammer and Menzel, 1995], sensitization [Mercer and Menzel, 1982], feeding arousal [Braun and Bicker, 1992; Pribbenow and Erber, 1996], stinging [Burrell and Smith, 1995], and nestmate recognition [Robinson et al., 1999]. The action of the octopaminergic neuron VUMmx1 [Hammer, 1993] demonstrates that octopamine can modulate higher order pathways as well as sensory processing [Kloppenburg et al., 1999; Pophof, 2000]. The challenge is to understand how a single neurochemical can be involved in so many different behavioral systems [Seigel et al., 1999]. Lacking direct information that bears on this question, in this sec-

tion we speculate on a few possibilities based on insights at the cellular, circuit, and colony levels.

At the cellular level, the different actions of octopamine could be mediated by different octopamine receptor subtypes, expressed by different populations of neurons, that perhaps are part of different signaling pathways. Currently there have been relatively few studies to identify the pharmacological properties of the honey bee octopamine receptors [Blenau and Baumann, 2001] and so far only one honey bee octopamine receptor subtype has been characterized [W. Blenau, personal communication]. Given extensive serotonin and dopamine receptor subtype diversity in other species [Roeder, 1994], it seems likely that there is more than one type of octopamine receptor in honey bees. Four functional categories of octopamine receptors have been found in locusts [Blenau and Baumann, 2001]. In addition, octopamine receptors in the mushroom bodies may differ from those in the rest of the bee brain [Erber et al., 1993; Blenau and Baumann, 2001].

The different actions of octopamine may also reflect organizational diversity at the circuit level, i.e., where octopamine is released, which cells are receptive and which circuits are modulated. Octopamine can change responsiveness to olfactory stimuli either by modulating olfactory receptors directly [Pophof, 2000], or it can act within the mushroom bodies to modulate circuits involved in the processing of the olfactory stimulus [Mercer and Erber, 1983; Kloppenburg et al., 1999]. The octopaminergic neuron VUMmx1 participates in circuits in the mushroom bodies responsible for olfactory appetitive conditioning [Hammer, 1997], mediating the reinforcing functions of the reward [Hammer, 1993]. This effect occurs most likely by modulating neurons within the mushroom bodies and antennal lobes [Hammer, 1993]. Octopamine can also modulate specific motor patterns [Evans and Siegler, 1982; Sombati and Hoyle, 1984; Malamud et al., 1988; Burrell and Smith, 1995].

At the colony level the effects of octopamine on foraging behavior might be influenced by the colony environment. Octopamine is known to increase general arousal in multiple systems and organisms [Corbet, 1991]. In honey bees, octopamine may modulate sensitivity to foraging-related stimuli [Barron et al., 2002]. If so, to reconcile this situation is to posit that octopamine affects the division of labor via a general arousal mechanism, but that this effect is influenced by aspects of the bees' social environment. For example, it is thought that the probability of performing a task in a beehive is determined both by responsiveness to the task-related stimulus and the probability of encountering the stimulus [Robinson, 1987b]. If so, octopamine may influence foraging more than other tasks if bees with higher levels of octopamine are more likely to encounter foraging-related stimuli. Because of the bee's complex social life, there may be interactions between its physiological state (including octopamine levels), social milieu, and physical position in the hive that ultimately determine its behavioral state.

Despite the strong association between antennal lobe octopamine and foraging, at this point we can only speculate on where in the brain octopamine is acting to influence foraging behavior. The responsiveness to stimuli possibly involves modulation at multiple sites, for example, in both sensory and motor pathways. The high concentration of octopamine in the antennal lobes of foraging bees could reflect greater octopamine release in this region from projection neurons originating in the ventro, deuto- and tritocerebral areas [Kreissl et al., 1994] or the subesophageal ganglion [Hammer, 1993]. Octopamine in the antennal lobes could also originate in the somata of two groups of cells with octopamine-like immunoreactivity close to the antennal lobes on the anterioventral midline of the protocerebrum [Kreissl et al., 1994]. The projections of these cells are not known. Whether octopamine influences foraging by modulating circuits within the antennal lobes and/or other regions of the brain is also not yet known. A better understanding of the effects of octopamine on foraging behavior at the cellular and circuit levels is required to understand how octopamine influences diverse behaviors in honey bees.

Conclusions

Current evidence suggests that the role of octopamine in honey bee division of labor is to modulate the switch from working in the hive to becoming a forager. We propose that the role of octopamine in honey bee division of labor is to modulate responsiveness to task-related stimuli. We suggest that the increase in octopamine in the brain increases the likelihood of responding to foraging-related stimuli, but does not make bees completely unresponsive to nursing or other task stimuli [Schulz and Robinson, 2001; Barron et al., 2002].

Division of labor is a complex socially mediated phenotype. Individual worker bees receive no instruction as to which task to perform, neither do they have a global overview of the colony's requirements. Differences in the responsiveness to task stimuli, however, within the lifetime of an individual bee and between bees in a hive, have

generated an efficient division of labor at the colony level. The experiments summarized in this review have established a role for octopamine in the division of labor. The next level of analysis will be to determine precisely where and how octopamine acts in the nervous system to coordinate this complex social behavior.

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References

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

Barron, A.B., D.J. Schulz, and G.E. Robinson (2002) Octopamine modulates responsiveness to foraging-related stimuli in honey bees *(Apis mellifera)*. J. Comp. Physiol. A., *188:* 603–610.

Ben-Shahar, Y., A. Robichon, M.B. Sokolowski, and G.E. Robinson (2002) Influence of gene action across different time scales on behavior. Science, *296:* 741–744.

Bicker, G. (1999) Biogenic amines in the brain of the honeybee: cellular distribution, development and behavioural functions. Micro. Res. Tech., *44:* 166–178.

Blenau, W., and A. Baumann (2001) Molecular and pharmacological properties of insect biogenic amine receptors: lessons from *Drosophila melanogaster* and *Apis mellifera*. Arch. Insect Biochem. Physiol., *48:* 13–38.

Bloch, G., and G.E. Robinson (2001) Reversal of honeybee behavioural rhythms. Nature, *410:* 1048.

Bloch, G., D.L. Wheeler, and G.E. Robinson (2002) Endocrine influences on the organization of insect societies. *In* Hormones, Brain and Behavior (ed. by D. Pfaff). Academic Press, San Diego, California., vol. 3, 195–235.

Braun, G., and G. Bicker (1992) Habituation of an appetitive reflex in the honeybee. J. Neurophysiol., *67:* 588–598.

Burrell, B.D., and B.H. Smith (1995) Modulation of the honey bee *(Apis mellifera)* sting response by octopamine. J. Insect Physiol., *41:* 671–680.

Corbet, S.A. (1991) A fresh look at the arousal syndrome of insects. Adv. Invert. Physiol., *23:* 81–116.

Erber, J., P. Kloppenburg, and A. Scheidler (1993) Neuromodulation by serotonin and octopamine in the honeybee*:* behaviour, neuroanatomy and electrophysiology. Experientia, *49:* 1073–1083.

Evans, P.D. (1980) Biogenic amines in the insect nervous system. Adv. Insect Physiol., *15:* 317–473.

Evans, P.D., and M.V.S. Siegler (1982) Octopamine mediated relaxation of maintained and catch tension in locust skeletal muscle. J. Physiol., *324:* 93–112.

Fahrbach, S.E., and G.E. Robinson (1996) Juvenile hormone, behavioral maturation and brain structure in the honey bee. Dev. Neurosci., *18:* 102–114.

Fuchs, E., J.H. Dustmann, H. Stadler, and F.W. Schürmann (1989) Neuroactive compounds in the brain of the honeybee during imaginal life. Comp. Biochem. Physiol. C, *92:* 337–342.

Galizia, C.G., and R. Menzel (2001) The role of glomeruli in the neural representation of odors: results from optical recording studies. J. Insect Physiol., *47:* 115–130.

Gillette, R., and J. Jing (2001) The role of escape swim motor network in the organization of behavioral hierarchy and arousal in *Pleurobranchaea*. Am. Zool., *41:* 983–992.

Hammer, M. (1993) An identified neuron mediates the unconditioned stimulus in associative olfactory learning in honeybees. Nature, *366:* 59–63.

Hammer, M. (1997) The neural basis of associative reward learning in honeybees. Trends Neurosci, *20:* 245–252.

Hammer, M., and R. Menzel (1995) Learning and memory in the honeybee. J. Neurosci., *15:* 1617–1630.

Hammer, M., and R. Menzel (1998) Multiple sites of associative odor learning as revealed by local brain microinjections of octopamine in honeybees. Learn. Mem., *5:* 146–156.

Harris, J.W., and J. Woodring (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.

Harrison, J.M. (1986) Caste-specific changes in honeybee flight capacity. Physiol. Zool., *59:* 175–187.

Huang, Z.Y., and G.E. Robinson (1996) Regulation of honey bee division of labor by colony age demography. Behav. Ecol. Sociobiol., *39:* 147–158.

Jing, J., and R. Gillette (2000) Escape swim network interneurons have diverse roles in behavioral switching and putative arousal in *Pleurobranchaea*. J. Neurophysiol., *83:* 1346–1355.

Joerges, J., A. Küttner, C.G. Galizia, and R. Menzel (1997) Representations of odours and odour mixtures visualized in the honeybee brain. Nature, *387:* 285–288.

Kaatz, H., S. Eichmüller, and S. Kreissl (1994) Stimulatory effect of octopamine on juvenile hormone biosynthesis in honey bees *(Apis mellifera)*: physiological and immunocytochemical evidence. J. Insect Physiol., *40:* 865–872.

Katz, P.S., P.A. Getting, and W.N. Frost (1994) Dynamic neuromodulation of synaptic strength intrinsic to a central pattern generator circuit. Nature, *367:* 729–731.

King, L.E., J.E. Steele, and S.W. Bajura (1986) The effect of flight on the composition of haemolymph in the cockroach, *Periplaneta americana.* J. Insect Physiol., *32:* 649–655.

Kloppenburg, P., D. Ferns, and A.R. Mercer (1999) Serotonin enhances central olfactory neuron responses to female sex pheromones in the male sphinx moth *Manduca sexta*. J. Neurosci., *19:* 8172–8181.

Kreissl, S., S. Eichmüller, G. Bicker, J. Rapus, and M. Eckert (1994) Octopamine-like immunoreactivity in the brain and subesophageal ganglion of the honeybee. J. Comp. Neurol., *348:* 583–595.

Kucharski, R., and R. Maleszka (2002) Molecular profiling of behavioural development: differential expression of mRNAs for inositol 1,4,5-trisphosphate 3-kinase isoforms in naive and experienced honeybees *(Apis mellifera)*. Mol. Brain Res., *99:* 92–101.

Kupfermann, I., and K.R. Weiss (1981) The role of serotonin in arousal of feeding behavior of *Aplysia*. *In* Serotonin Neurotransmission and Behavior, M.I.T. Press, Cambridge, MA, pp. 255–287.

Le Conte, Y., G. Arnold, J. Trouiller, and C. Masson (1990) Identification of a brood pheromone in honeybees. Naturwissenschaften, *77:* 334–336.

Le Conte, Y., A. Mohammedi, and G.E. Robinson (2001) Primer effects of a brood pheromone on honeybee behavioural development. Proc. Roy. Soc. Lond. B., *268:* 163–168.

Le Conte, Y., L. Sreng, and S.H. Poitout (1995) Brood pheromone can modulate the feeding behavior of *Apis mellifera* workers (Hymenoptera: Apidae). J. Econ. Entomol., *88:* 798–804.

Lindauer, M. (1954) Temperaturregulierung und Wasserhaushalt im Bienenstaat. Z. Vgl. Physiol. *36:* 391–432.

Linn, C.E., and W.L. Roelofs (1986) Modulatory effects of octopamine and serotonin on male sensitivity and periodicity of response to sex pheromones in the cabbage looper moth, *Trichoplusia ni*. Arch. Insect Biochem. Physiol., *3:* 161–171.

Linn, C.E., M.G. Campbell, and W.L. Roelofs (1992) Photoperiod cues and the modulatory action of octopamine and 5-hydroxytryptamine on locomotor and pheromone response in male gypsy moths, *Lymantria dispar*. Arch. Insect Biochem. Physiol., *20:* 265–284.

Linn, C.E., K.R. Poole, and W.L. Roelofs (1994) Studies on biogenic amines and their metabolites in nervous tissue and hemolymph of male cabbage looper moths – III. fate of injected octopamine, 5-hydroxytryptamine and dopamine. Comp. Biochem. Physiol. C, *108:* 99–106.

Malamud, J.G., A.P. Mizisin, and R.K. Josephson (1988) The effects of octopamine on contraction kinetics and power output of a locust flight muscle. J. Comp. Physiol. A, *162:* 827–835.

Menzel, R., and U. Müller (1996) Learning and memory in honeybees: from behavior to neural substrates. Ann. Rev. Neurosci., *19:* 379–404.

Mercer, A.R., and J. Erber (1983) The effects of amines on evoked action potentials recorded in the mushroom bodies of the bee brain. J. Comp. Physiol. A, *151:* 469–476.

Mercer, A.R., and R. Menzel (1982) The effects of biogenic amines on conditioned and unconditioned responses to olfactory stimuli in the honeybee *Apis mellifera*. J. Comp. Physiol. A, *145:* 363–368.

Moore, D., J.E. Angel, I.M. Cheeseman, S.E. Fahrbach, and G.E. Robinson (1998) Timekeeping in the honey bee colony: integration of circadian rhythms and division of labor. Behav. Ecol. Sociobiol., *43:* 147–160.

Nijhout, H.F. (1994) Insect Hormones. Princeton University Press, Princeton, NJ.

Ohashi, K., S. Natori, and T. Kubo (1997) Change in the mode of gene expression of the hypopharyngeal gland cells with an age-dependent role change of the worker honeybee *Apis mellifera*. Eur. J. Biochem., *249:* 797–802.

Oster, G.F., and E.O. Wilson (1978) Caste and Ecology in Social Insects. Princeton University Press, Princeton, NJ.

Page, R.E., G.E. Robinson, D.S. Britton, and M.K. Fondrk (1992) Genotypic variability for rates of behavioral development in worker honeybees (*Apis mellifera* L.). Behav. Ecol., *3:* 173–180.

Pankiw, T., R.E. Page, and M.K. Fondrk (1998) Brood pheromone stimulates pollen foraging in honey bees *(Apis mellifera)*. Behav. Ecol. Sociobiol., *44:* 193–198.

Pfaff, D., J. Frohlich, and M. Morgan (2002) Hormonal and genetic influences on arousal – sexual and otherwise. Trends Neurosci., *25:* 45–50.

Pophof, B. (2000) Octopamine modulates the sensitivity of silkmoth pheromone receptor neurons. J. Comp. Physiol. A, *186:* 307–313.

Pribbenow, B., and J. Erber (1996) Modulation of antennal scanning in the honeybee by sucrose stimuli, serotonin and octopamine: behavior and electrophysiology. Neurobiol. Learn. Mem., *66:* 109–120.

Ratnieks, F.L.W. (1993) Egg-laying, egg-removal, and ovary development by workers in queenright honey bee colonies. Behav. Ecol. Sociobiol., *32:* 191–198.

Ribbands, R. (1953) The Behaviour and Social Life of Honeybees. Bee Research Association Ltd., London.

Robinson, G.E. (1987a) Regulation of honey bee age polyethism by juvenile hormone. Behav. Ecol. Sociobiol., *20:* 329–338.

Robinson, G.E. (1987b) Modulation of alarm pheromone perception in the honey bee: evidence for division of labor based on hormonally regulated response thresholds. J. Comp. Physiol. A, *160:* 613–619.

Robinson, G.E. (1992) Regulation of division of labor in insect societies. Ann. Rev. Entomol., *37:* 637–665.

Robinson, G.E., and E.L. Vargo (1997) Juvenile hormone in adult eusocial Hymenoptera: gonadotropin and behavioural pacemaker. Arch. Insect Biochem. Physiol., *35:* 559–583.

Robinson, G.E., L.M. Heuser, Y. Le Conte, F. Lenquette, and R.M. Hollingworth (1999) Neurochemicals aid bee nestmate recognition. Nature, *399:* 534–535.

Robinson, G.E., R.E. Page, C. Strambi, and A. Strambi (1989) Hormonal and genetic control of behavioural integration in honey bee colonies. Science, *246:* 109–112.

Robinson, G.E., R.E. Page, C. Strambi, and A. Strambi (1992) Colony integration in honey bees: mechanisms of behavioural reversion. Ethology, *90:* 336–350.

Roeder, T. (1994) Biogenic amines and their receptors in insects. Comp. Biochem. Physiol. C, *107:* 1–12.

Rösch, G.A. (1930) Untersuchungen über die Arbeitsteilung im Bienenstaat, II. Die Tätigkeiten der Arbeitsbienen unter experimentell veränderten Bedingungen. Z. Vgl. Physiol., *12:* 1–71.

Satterlie, R.A., and T.P. Norekian (1996) Modulation of swimming speed in the pteropod mollusc *Clione limacina*: role of a compartmental serotonergic system. Invert. Neurosci., *2:* 157–165.

Schulz, D.J., and G.E. Robinson (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.

Schulz, D.J., and G.E. Robinson (2001) Octopamine influences division of labor in honey bee colonies. J. Comp. Physiol. A, *187:* 53–61.

Schulz, D.J., M.M. Elekonich, and G.E. Robinson (2002a) Biogenic amines in the antennal lobes and the initiation and maintenance of foraging behavior in honey bees. J. Neurobiol., in press.

Schulz, D.J., J.P. Sullivan, and G.E. Robinson (2002b) Juvenile hormone and octopamine in the regulation of division of labor in honey bee colonies. Horm. Behav., *42:* 222–231.

Seeley, T.D., and S.A. Kolmes (1991) Age polyethism for hive duties in honey bees – illusion or reality. Ethology, *87:* 287–297.

Seigel, G.J., B.W. Agranoff, R.W. Albers, S.K. Fisher, and M.D. Uhler (1999) Basic Neurochemistry: Molecular, Cellular and Medical Aspects. Lippincott-Raven, Philadelphia, PA.

Shapira, M., C.K. Thompson, H. Soreq, and G.E. Robinson (2001) Changes in neuronal acetylcholinesterase gene expression and division of labor in honey bee colonies. J. Mol. Neurosci., *17:* 1–12.

Sigg, D., C.M. Thompson, and A.R. Mercer (1997) Activity-dependent changes to the brain and behavior of the honey bee, *Apis mellifera*. J. Neurosci., *17:* 7148–7156.

Sombati, S., and G. Hoyle (1984) Central nervous sensitization and dishabituation of reflex action in an insect by the neuromodulator octopamine. J. Neurobiol., *15:* 481–506.

Sullivan, J.P., O. Jassim, S.E. Fahrbach, and G.E. Robinson (2000) Juvenile hormone paces behavioral development in the adult worker honey bee. Horm. Behav., *37:* 1–14.

Taylor, D.J., G.E. Robinson, B.J. Logan, R. Laverty, and A.R. Mercer (1992) Changes in brain amine levels associated with the morphological and behavioural development of the worker honeybee. J. Comp. Physiol. A, *170:* 715–721.

Toma, D.P., Bloch, G., Moore, D., and G.E. Robinson (2000) Changes in *period* expression in the brain and division of labor in honey bee colonies. Proc. Natl. Acad. Sci. USA, *97:* 6914–6919.

Tinbergen, N. (1951) The Study of Instinct. Oxford University Press, New York.

Visscher, P.K. (1996) Reproductive conflict in honey bees: a stalemate of worker egg-laying and policing. Behav. Ecol. Sociobiol., *39:* 237–244.

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

Winnington, A.P., R.M. Napper, and A.R. Mercer (1996) Structural plasticity of identified glomeruli in the antennal lobes of the adult worker honey bee. J. Comp. Neurol., *365:* 479–490.

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

Withers, G.S., S.E. Fahrbach, and G.E. Robinson (1993) Selective neuroanatomical plasticity and division of labor in the honeybee. Nature, *364:* 238–240.