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Juvenile hormone and ecdysteroids as major regulators of brain and behavior in bees

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The genome sequencing of several bee species, and the development of functional genomics tools, paved the way for understanding the fascinating behaviors of bees in molecular terms. Here we review recent progress in research on the hormonal regulation of bee behavior, with emphasis on two key insect hormones: Juvenile hormones (JH) and ecdysteroids (Ec). We discuss recent progress in deciphering the molecular bases for JH regulation of gene expression in the nervous system and other tissues. The patterns of JH-dependent changes in gene expression show many similarities across tissues, which are associated with the effects of JH on worker task allocation. Ec, which have previously been studied mainly in the context of insect development, now appear to also play imortant roles in the regulation of many molecular processes in the brain that are asociated with bee behavior. Finally, we discuss the possibility that JH-signaling and Ec-signaling pathways interact to shape the complex behavioral repertoire of bees.

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Introduction

Bees provide outstanding model systems with which to study complex behaviors such as learning and memory, foraging, and social behavior, in ecologically relevant contexts [1,2]. Important insights into the evolution of sociality are gained from comparative studies of behavior and its underlying mechanisms in bee species that are taxonomically related but differ in their social lifestyle, from solitary to advanced eusocial [1,2]. The study of bee behavior is also economically important because bees provide essential pollination services to natural and agricultural ecosystems.

The behavior of bees, as well as of other animals, is regulated by many physiological and molecular processes, one of the most important one is the endocrine system. Hormone titers are tightly regulated by multiple internal and external factors and therefore, integrate environmental influences on animal development, physiology and behavior. Another key aspect of hormone action is its simultaneous influence on processes in diverse tissues and cells, enabling the coordination of behavior with relevant morphological and physiological changes. Here, we focus on juvenile hormone (JH) and ecdysteroid hormones (Ec, used hereafter to refer to all types of ecdysteroids), which are the best-studied hormones in bees and in insects in general. These two endocrine systems are the key regulators of insect development and reproduction. However, how exactly they exert their functions in the context of the molecular processes that regulate insect behaviors remains elusive.

Juvenile hormone

Juvenile hormones are *sesquiterpenoids* secreted from the *corpora allata* (CA) of insects. JH is considered as the most versatile animal hormone [3] because it regulates many aspects of insect physiology and development. These include behavior, reproduction, metamorphosis, diapause, polyphenism, stress resistance, and aging [3–7]. Evidence for JH mediated tradeoffs between immunocompetence and sexual attractiveness [8], or between reproduction and lifespan [9] are consistent with the premise that high JH levels have a cost in insects. Several JHs have been characterized in insects; but bees, as well as other insects from the order Hymenoptera, have only JH-III [4,10].

In terms of influence on behavior, JH is best understood for its involvement in the regulation of courtship and mating behavior, which is consistent with its typical gonadotropic function (reviewed in [3,10]). Studies in social insects emphasized the importance of JH for the regulation of physiological (social behavior, reproduction, foraging) and developmental processes (e.g., caste differentiation) that underlie social organization (reviewed in [5,11–14]). JH titers are regulated by many social signals [11,15,16], but are also sensitive to other environmental signals such as light exposure [17]. Interestingly, JH appears to regulate different social behaviors in different social insects, and this seems to be associated with the level of social organization [18•]. As in most insect species, JH coordinates processes related to reproduction in solitary and '*privatively eusocial*' insects (i.e., eusocial insects with relatively small societies and no morphological differences between queens and workers [2]), such as bumblebees [19•,20,21••]. On the other hand, in the advanced eusocial honeybee *Apis mellifera*, JH seems to lose most or all of its gonadotropic functions while acquiring a major role as the '*pacer*' of age-related division of labor [22]. One of the key molecular modifications associated with this change in JH function relates to the regulation of the yolk protein *Vitellogenin* (*Vg*). Whereas in most insects, including bumblebees [21], JH upregulates *Vg* production and accumulation in developing oocytes, in honeybees there is a 'double-repressor' feedback loop between JH and *Vg*. There is evidence suggesting that *Vg* acts as an endocrine signal that together with JH pace the transition from nest activities (mostly brood care) to foraging [23,24]. JH also seems to modulate the ontogeny of flight activity, but not reproduction, in honeybee males (drones)[25•] and queens [26]. The evidence for strong positive selection on JH signaling pathway genes [27•] suggests that the modifications in JH regulation of social behavior are functionally significant. However, it is not yet clear if and how JH regulation of division of labor rather than reproduction relates to the evolution of advanced sociality in honeybees.

Additional functions that are regulated by JH in honeybees include short-term associative memory and the acquisition and recall in an aversive learning paradigm. The influence of a single JH treatment on learning and memory can last for several days and even a week [28,29••]. The influences of JH on behavior in bee species other than *A. mellifera* are less clear. Reproduction in privatively social insects is typically correlated with both high JH titers and high dominance rank, but this does not necessarily imply causation. Whereas in *Polistes* paper wasps, JH analog (JH-A) treatments support the premise that JH influences neuronal and behavioral processes that contribute to dominance [30–32], the situation is more ambiguous in bumblebees. In workers of the bumblebee (*Bombus terrestris*), high dominance rank is positively correlated with JH titers and the rate of *in vitro* JH biosynthesis [33–35]. However, the aggressive establishment of dominance hierarchies in groups of callow orphan workers occurs on the first days, before JH titers and biosynthesis rates are elevated, a finding that is not consistent with the hypothesis that high JH levels are necessary for dominance behavior [34].

The mechanisms of JH influence on behavior

In insects, the major hormones JH and Ec regulate both the development and organization of the nervous system ('*organizational effects*'), and the activation of physiological and behavioral outputs ('*activational effects*') [36]. The organizational effects include, for example, the regulation of caste differentiation, which account for caste-specific behaviors [37]. JH also influences task-related neuroanatomical re-organization of the adult antennal lobes [38]. However, JH does not seem to be necessary for the typical task-related growth of the mushroom body (MB) neuropil [39]. Neurogenesis, another important organizational processes that was shown to be regulated by JH in crickets (*Acheta domesticus*; [40]), does not appear to be important for the development of the nervous system in adult honeybees [41].

Activational effects of JH include the modulation of response threshold. For example, JH influences the responsiveness to alarm pheromone in honeybees, which typically increases with age [42]. JH treatment also reduced the attraction of young workers to the queen mandibular pheromone and this influence has been suggested to be modulated by reducing the antennal levels of a receptor for the biogenic amine *octopamine* (OA). It is notable that the JH treatment also reduced the levels of additional OA receptors, as well as those for *dopamine* (DA) [29••,43]. Thus, one way by which JH can affect social behavior is by modulating the responsiveness to pheromones, which are major communication signals in insects [44]. JH regulation of biogenic amine signaling genes is also important for the regulation of flight onset in both honeybees and the solitary carpenter bee *Xylocopa appendiculata* [38,45]. Although most of the recent progress has been in understanding JH influence on gene expression, earlier studies in *Rhodnius*, *Locusta*, and moths strongly suggest that at least some aspects of JH action are regulated by a transcription-independent process involving a membrane-bound JH receptor (reviewed in [46•]). Non-genomic functions of JH have not been yet explored in bees.

The influence of JH on brain gene expression in bees

The development of Expressed Sequence Tag (EST) libraries [47], and later, the sequencing of the honeybee genome [48], set the stage for high throughput whole genome analyses of gene expression in the brain and other tissues. The recent sequencing of additional bee genomes promises to revolutionize the research on genes and behavior in bee species with diverse levels of sociality [49,50]. Studies that built on these technological advances have established that behavioral states in honeybees are associated with characteristic patterns of brain gene expression [51••,52••]. For example, there are distinctive patterns for workers performing different tasks [53–55]. JH treatment influences the expression of many genes in the honeybee brain (Figure 1). Treatment with the JH analog methoprene altered gene expression toward a forager-like pattern in tissues that change their function in association with worker task, such as the brain [56••], fat body [57••], and hypopharyngeal glands (HPG) [58••]. A forager-like pattern was also found following dsRNA mediated down-regulation (RNA interference, RNAi) of *Vg*. This finding is consistent with evidence that *Vg* down-regulation causes an increase in JH levels [57••,59••,60]. The regulation of state-specific gene expression patterns is regulated by key transcription factors (TFs) that regulate the expression of downstream genes [61]. Analyses