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A bee-line into learning and memory mechanisms

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Introduction

Reliable predictors of danger are as important to honey bees as they are to humans – and yet, honey bee queens produce a pheromone that blocks aversive learning in young worker bees [1]. On the face of it, this would seem difficult to explain. The ability to learn relationships between aversive events and the stimuli that predict them is, after all, an important survival tool. What enables queen bees to regulate the behaviour of their offspring in this way, and what selective advantage might there be in doing so? This review attempts to answer these questions and, in so doing, highlights significant steps that have been made towards understanding the cellular and molecular mechanisms that underlie associative olfactory learning in insects.

Aversive learning in young worker bees is blocked by queen mandibular pheromone (QMP)

Aversive learning can be demonstrated readily in bees using the sting extension reflex, a defensive response that can be elicited by aversive stimuli such as mild electric shock. A bee that is presented several times with an odour paired with an electric shock stimulus begins extending its sting in response to the odour alone, in expectation of the punishment to follow [2]. Young worker bees exposed to their mother's pheromone (QMP) respond to electric shock with sting extension as expected, but they are unable to learn an odour signal that predicts this negative outcome [1]. Their appetitive learning, however, remains intact; young QMP-treated bees can still associate an odour with a sucrose reward [1]. The selectivity of QMP's effects on associative olfactory learning provides a clue to its mode of action.

QMP blocks the negative reinforcing properties of an aversive stimulus by regulating dopamine signalling in the brain

Brain dopamine levels in the young bees that perform tasks within the colony are generally lower than the levels found in foraging bees [3, 4]. If the queen is removed from the colony, however, dopamine in the brain of young workers increases to a level similar to that seen in foragers [5, 6]. The pheromone responsible for lowering brain dopamine levels is QMP and specifically, one of QMP's key components, homovanillyl alcohol [7]. Why QMP's effects on brain dopamine levels are age-dependent remains unclear, but in young workers not only endogenous dopamine levels, but also levels of dopamine receptor gene expression are altered significantly by this pheromone [7].

In a landmark study using the fruit fly, *Drosophila melanogaster*, Schwaerzel et al. [8] blocked synaptic transmission from dopaminergic neurons by expressing in the cells a temperature-sensitive variant of

dynamin (*Shabire*^{ts}, Shi^{ts}), a protein required for neurotransmitter recycling. At permissive temperatures, dynamin functions normally, but at elevated temperatures the dynamin variant malfunctions, inhibiting the recycling of transmitter into vesicles and ultimately blocking synaptic transmission [9]. Transgenic flies conditioned at temperatures that prevent output from dopaminergic neurons are unable to learn to associate an odour stimulus with punishment. Appetitive learning in these flies, however, remains intact [8]. Dopaminergic projections in the fly brain have since been shown to respond weakly to odour, but strongly to electric shock [10], and selective expression of the light-activated cation channel, channelrhodopsin 2, in dopaminergic neurons has enabled Schroll and colleagues [11] to show in *Drosophila* larvae that light-induced activation of dopaminergic neurons paired with an odour stimulus leads to aversive memory formation. These very elegant studies in the fly provide compelling evidence that dopamine signalling conveys the negative reinforcing properties of an aversive stimulus. In light of these findings, it is perhaps no surprise that QMP-induced changes in brain dopamine function compromise aversive learning in young worker bees, but why is there no effect on appetitive learning?

Signalling systems that support appetitive learning remain intact

Searching for neurons that might mediate the reinforcing properties of sucrose, Martin Hammer [12] identified a group of cells in the suboesophageal ganglion (SOG) of the bee (ventral unpaired medial (VUM) neurons) that respond to sucrose stimulation. Recording from a VUM neuron in the maxillary neuromere of the SOG (VUM_{mx1}), Hammer found that if he stimulated the neuron shortly after presenting the bee with an odour stimulus, VUM_{mx1} stimulation mimicked the reinforcing effects of sucrose in one-trial olfactory conditioning; bees began responding to the odour with proboscis extension [12]. The VUM neurons were found to stain with antibody raised against octopamine [13], suggesting that octopamine mediates the reinforcing effects of VUM_{mx1} stimulation, and potentially also those of sucrose reward. To test this hypothesis, Hammer injected octopamine into selected regions of the brain to ascertain whether octopamine treatment, like VUM_{mx1} stimulation, could be used as a substitute for sucrose stimulation during associative olfactory conditioning. Shortly after presenting a bee with an odour stimulus, Hammer injected a small volume of octopamine (10^{-6} M) into the brain. Remarkably, if octopamine was injected into either the antennal lobe (primary olfactory centres of the brain) or adjacent to the mushroom body calyces, bees began responding with proboscis extension to the odour paired with octopamine [14]. Hammer's work provided the first evidence that sucrose reinforcement is mediated by the phenolamine, octopamine. More recent studies have shown that blocking octopamine receptor activity, or down-regulating octopamine receptor gene expression using RNA interference, impairs appetitive learning in bees [15]. Mutant flies deficient in the enzyme used to convert tyramine into octopamine (tyramine-β-hydroxylase), also show impaired associative olfactory learning when sucrose is used as the unconditioned stimulus [8].

Current evidence indicates, therefore, that aversive learning and appetitive learning in insects rely, at least in part, on different modulatory systems; output from dopaminergic neurons is required for shock-reinforced odour memory, whereas sucrose reinforced odour memory requires output from neurons that release octopamine. Differential blockade of aversive and appetitive learning using pharmacological tools supports this view [2, 16, 17] and helps explain the selectivity of QMP's effects on associative olfactory learning behaviour in young worker bees. While QMP has a significant impact on dopamine levels and levels of dopamine receptor gene expression in the brain of young workers [5–7], removing the queen from a honey bee colony has no significant effect on levels of octopamine in the brain [5]. This is consistent with findings at the behavioural level that appetitive learning remains intact in young bees exposed to QMP [1].

G-protein receptors that mediate the effects of dopamine are coupled to the cAMP-signalling pathway

While evidence points to the involvement of dopaminergic neurons in aversive learning and octopaminergic neurons in appetitive learning, recent findings suggest a more complex scenario. Recall of aversive olfactory memories in flies, as well as memories established through appetitive conditioning, requires output from mushroom body neurons [8, 18]. In *dumb* flies, which exhibit abnormal dopamine (dDA1) receptor expression in the mushroom bodies, aversive learning is severely impaired [19]. However, appetitive learning is also compromised in these flies, suggesting that dDA1 may play a role not only in aversive learning, but also appetitive learning in the fly. Interestingly, expression of the honey bee orthologue of dDA1 (AmDOP1, [20]) is down-regulated by

QMP in young workers [7] and may contribute to QMP's effects on aversive learning in bees.

In addition to having been traced to the mushroom bodies of the brain, aversive and appetitive learning share a further property; both rely on cAMP signalling [21–24]. The impact of altering levels of expression of receptors coupled to the cAMP signalling pathway may be complex. Expressed *in vitro*, the honey bee ortholog of dDA1 (AmDOP1) shows constitutive activity, increasing intracellular levels of cAMP even in the absence of the receptor's natural ligand, dopamine [25]. If dDA1 receptors show similar activity in flies, learning in *dumb* flies may be compromised, at least in part, by a shift in basal cAMP levels in cells that normally express dDA1. In addition, most – if not all – intrinsic mushroom body neurons (Kenyon cells) express more than one type of dopamine receptor [26, 27]. Some of these G-protein coupled receptors (GPCRs) increase intracellular levels of cAMP when activated [e.g. 20, 25, 28–31], whilst others reduce levels of this intracellular signalling molecule [27, 32]. Altering receptor ratios is likely to have consequences not only at the cellular level, but also at a behavioural level [33, 34]. Interestingly, not all subpopulations of Kenyon cells express the same complement of dopamine receptor genes [26], supporting the view that different populations of mushroom body neurons subserve different functions [18]. Taken together, these studies highlight the need to reveal, more specifically, the pattern of distribution and possible interaction of GPCRs in neuronal circuits that support aversive and appetitive learning, not only in mushroom bodies, but also in the antennal lobes of the brain. As QMP apparently targets dopamine signalling pathways selectively, and affects aversive but not appetitive learning, the pheromone could serve as a valuable tool to differentiate cellular events essential to the formation of aversive versus appetitive memories.

A strategy for survival?

The queen's ability to selectively block aversive learning in her young offspring is intriguing; what advantage might there be to her in doing so? Queen bees rely on young workers to feed and groom them, and to distribute queen pheromones throughout the colony [35]. As exposure to high levels of QMP can be repellent to bees and elicit aggression [36–38], it may be advantageous to the queen to prevent young bees from being able to associate her with any negative effects of her pheromone. In this sense, the queen's ability to block aversive learning in young workers could be considered a strategy for survival. Consistent with this idea, homovanillyl alcohol, the component of QMP that seems to be primarily responsible for altering dopamine function in the bee brain [7], has been found to enhance survival of honey bee queens [39].

QMP is a complex, multifunctional pheromome [35] that triggers an array of changes in gene expression in the honey bee brain [40]. Its ability to block aversive learning in young bees provides a unique window not only into the biology of the bee, but also into learning and memory mechanisms in the insect brain.

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