Neural Mechanisms of Reward in Insects

Clint J. Perry and Andrew B. Barron*

Department of Biological Sciences, Macquarie University, Sydney, NSW 2109 Australia; email: Andrew.Barron@mq.edu.au

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*Corresponding author

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Abstract

Reward seeking is a major motivator and organizer of behavior, and animals readily learn to modify their behavior to more easily obtain reward, or to respond to stimuli that are predictive of reward. Here, we compare what is known of reward processing mechanisms in insects with the well-studied vertebrate reward systems. In insects almost all of what is known of reward processing is derived from studies of reward learning. This is localized to the mushroom bodies and antennal lobes and organized by a network of hierarchically arranged modulatory circuits, especially those involving octopamine and dopamine. Neurogenetic studies with *Drosophila* have identified distinct circuit elements for reward learning, "wanting," and possibly "liking" in *Drosophila*, suggesting a modular structure to the insect reward processing system, which broadly parallels that of the mammals in terms of functional organization.

INTRODUCTION

Liking: the unconscious hedonic value of or pleasure gained from a cue or reward

Wanting: the unconscious motivational attribution of a cue or reward

Rewards are actively sought and are motivating and reinforcing of behavior. Actions that result in obtaining rewards, or stimuli that are reliably predictive of rewards, can be learned and may take on rewarding properties themselves. Because rewards are so pivotal to the operation of behavior, it is hardly surprising that the study of how rewards are processed by the brain has become a prominent neuroscience discipline. Most research has been performed with vertebrate model systems, but insect behavior is no less organized and driven by reward. Our aim in this review is to examine what is known of insect reward processing systems.

Here, we discuss insect reward processing systems in contrast with mammalian reward systems to place the insect research in a comparative context. We first address the concept of reward as applied to the behavioral sciences, which has evolved alongside advances in neuroscience. A contemporary understanding of reward recognizes that pleasure (hedonia) is a valid physiological concept, and the capacity to elicit a pleasurable response is a property of rewards. Progress in vertebrate neuroscience has identified reward learning, liking, and wanting as separate but interacting modules within the complete reward processing system. There are also extremely close interactions between the neural systems for pain and pleasure facilitating assessment of a balance of positive and negative stimuli.

The insect reward processing system is less well understood, but the emerging picture reveals an organizational structure that parallels in many ways that of vertebrates. The insect reward processing system is dispersed across brain regions and organized by a network of interacting circuits involving particularly octopaminergic and dopaminergic neurons. Within this network functional modules for reward learning, "wanting," and perhaps "liking" are beginning to be understood. Different dopaminergic signals represent rewarding and aversive stimuli, suggesting mechanisms for calculation of the balance of opposing stimuli. Below, we discuss the insect and mammalian systems in detail, beginning with experimental approaches to assess reward responses.

DEVELOPMENT OF CONCEPTS OF REWARD IN THE BEHAVIORAL SCIENCES

Experimental concepts of reward and punishment began with Loeb (41), who defined rewards as stimuli that elicit approach and punishments as stimuli that elicit avoidance. These are operational definitions, because it is the behavior that a stimulus induces within a certain context that defines it as either reward or punishment.

That rewards can shape and direct behavior was first studied experimentally by Thorndike (83) in the context of learning stimulus–response associations. He observed that cats placed in puzzle boxes learned to ever more rapidly escape the box, and he proposed that responses followed by satisfaction were more likely to recur in a similar context, whereas responses followed by annoyance were less likely to recur (83). Following the influential research of Skinner (74), by the 1930s, there was a relative consensus within experimental psychology that rewards affected behavior by strengthening the association between response and stimuli (74, 84).

Note that we avoid the term reinforcement in this review due to its many different meanings and often synonymous use with reward (95). Pavlov used reinforcement to refer to the strengthening of stimulus–stimulus associations; Thorndike defined it as the association between stimulus and response; and Skinner defined reinforcement as the strengthening of the response. Currently reinforcement and reward are often used interchangeably, and therefore we choose to avoid any complications around the term reinforcement by using reward only.

Considering rewards as the strengthening of stimulus–response associations was simple and appealing, but it did not explain how rewarded behavior was strengthened. Hull (31) proposed drive reduction theory to explain motivational systems underlying reward. This essentially stated that an organism had physiological needs that induced drives to obtain rewards. Rewards then reduced the drives by satisfying the needs. Although appealing and parsimonious, it did not explain why people would eat something tasty even after being satiated nor odd behaviors such as treating predictive stimuli as reward (e.g., gnawing on a food dish as if it were food).

CS: conditioned stimulus/stimuli

US: unconditioned stimulus/stimuli

Drive reduction theories lost their appeal after Olds & Milner (53) observed that rats could be trained using mild electrical stimulation to areas of the lateral hypothalamus. Rats could be taught to lever-press for brain stimulation, and even preferred this over food or water rewards (63). These experiments showed that there need not be a drive for reward responses to take place and that hedonia was a physiologically valid concept that was itself motivational.

This led to the incentive theory of reward, which recognizes that reward stimuli have hedonic value, which is the degree of subjective pleasure elicited by a stimulus. This hedonic value of a reward stimulus elicits an incentive value (or degree of wanting) that along with hedonic value can be transferred through associative learning to a conditioned stimulus (CS) or even an animal's own behavioral responses. These stimuli or actions become predictors of reward and in some sense types of rewards themselves (10). Toates (87) extended incentive theory by postulating that hedonic and incentive values were modulated by drive states (e.g., greater hunger makes food better tasting and more sought after) (14) and inversely that the CS could increase the drive for a reward stimulus (e.g., a cue for food can increase hunger).

Modern incentive theory states that an unconditioned stimulus (US) activates the pleasure networks of the brain through the senses. The brain then assigns, through association with the rewarding stimulus, a hedonic value to the neutral stimulus, which becomes conditioned. The CS subsequently activates the pleasure networks of the brain on its own and gains its own incentive salience, which in turn motivates approach behavior (**Figure 1**). Incentive theory recognizes that the degree of liking (hedonic value) and the degree of wanting (incentive salience) interact but are distinct elements of the reward system (7). Note that here we follow Berridge's (7) terminology, using quotes to designate the unconscious rather than conscious aspects of what we would normally think of as a conscious liking and wanting of reward.

Objective reactions to rewards have been used to study the liking and wanting components of responses to reward separately. In mammals this involves observing orofacial reactions to a sweet-tasting reward compared with the approach or operant behavior to obtain the reward. In most mammalian study systems, sweet tastes elicit tongue protrusions and lip licking, whereas unpleasant (e.g., bitter) tastes elicit mouth gapes and head shaking (4, 6, 7). Liking and wanting components of reward can be assessed independently by comparing the intensity of reactions to the taste of food reward, with approach and consummatory behavior assessed in instrumental learning paradigms.

INSECT STUDIES OF REWARD PROCESSING

In mammals the study of reward processing systems has grown into an entire neuroscience field in its own right. However, for insects almost everything we know of reward processing has been gained from studies examining learning using rewarding US. Our perspective of insect reward learning is further limited in that research has focused almost exclusively on learning in honey bees and *Drosophila*, and in the context of appetitive reward using sucrose. In these studies learning is considered to have taken place if there is a post-training appetitive response to the CS, such as extension of the proboscis or other mouthparts.

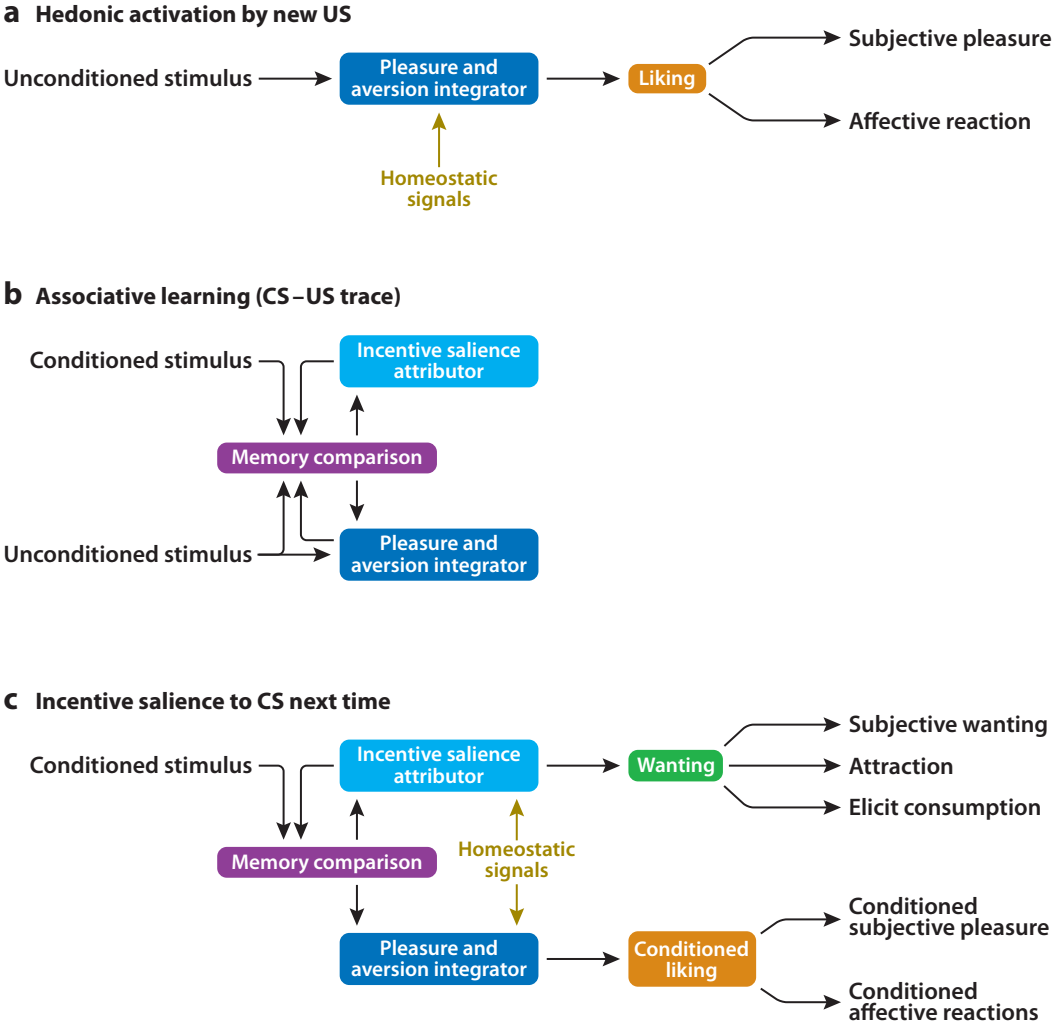

Figure 1

Schematic illustrating processing in the acquisition of reward learning according to the incentive salience model. (*a*) The first time an unconditioned stimulus (US) is experienced, the brain, being modulated by homeostatic signals (e.g., hunger, thirst), assigns a hedonic value to it. (*b*) Associative learning systems correlate the memory trace of conditioned stimuli (CS) with the hedonic event (reward), sometimes causing CS to become types of rewards themselves. (*c*) When CS are experienced again, incentive salience ("wanting") is attributed to them by activation of dopamine-related systems. The CS can then act as an incentive motivating future behavior. The CS may also activate conditioned "liking." Figure adapted from Reference 7 with permission.

Proboscis extension response (PER) conditioning is certainly the most historically important reward learning paradigm for insects (38, 44, 80). For PER conditioning, animals are harnessed such that they can move only their antennae and mouthparts. Sucrose can then be presented to the gustatory organs temporally paired with the presentation of conditioned stimuli: most often odor but also tactile or visual stimuli (43, 45, 64). PER has been successfully adapted for *Drosophila* (21), but the most commonly used learning assay for *Drosophila* remains an olfactory T-maze (88). This typically assesses learning in a group of flies, which can distribute themselves between two

PER: proboscis extension response

odor fields, one of which has previously been associated with sucrose (or electric shock in the case of aversive conditioning). If there is an increase in the proportion of the group of flies that orients toward the sucrose-associated odor post-training, flies are considered to have learned an odor preference (70).

Both of these assays are classical conditioning paradigms in that the presentation of the CS and US is controlled by the experimenter and independent of the actions of the animal (11, 60). This contrasts with mammalian reward learning bioassays, which are mostly forms of operant conditioning (e.g., lever pressing for reward in a rat Skinner box). Although there has been tremendous progress exploring mechanisms of reward processing in bees and flies, it is worth remembering that currently the generality of the mechanisms uncovered has not been widely tested, either across paradigms or across insect species.

Another difficulty is that currently there is no good bioassay to explore liking of reward independently from wanting of reward in insects. Mammalian model systems have relied on judgments of orofacial reactions to assess liking (6, 7), but that obviously has no direct parallel for insects with their expressionless head capsules. Finding ways to assay liking separately from wanting could be difficult because any orientation response could be interpreted as indicating liking, wanting, or both. Some natural behavioral responses could be interpreted as indicative of reward liking. For example, the honey bee dance language is a signal performed by foragers to communicate the value of floral resources to the colony, or how much foragers liked the rewards they just collected (1), but this is hardly a generalizable bioassay.

Progress in exploring the structure of the insect reward system is forthcoming from careful behavioral analyses in combination with genetic manipulation of discrete, identified cell populations. In the sections below we discuss what is known of the anatomy, chemistry, and circuitry of this system.

DA: dopamine

NAc: nucleus accumbens

VP: ventral pallidum

AL: antennal lobes

MB: mushroom body/bodies

NEURAL SUBSTRATES OF REWARD

Neuroanatomy of the Mammalian Reward System

Direct brain stimulation, functional neuroimaging, neural recording, and tracing studies have identified a number of overlapping areas in the mammalian brain that respond to reward (both CS and US components), including areas within the brain stem and midbrain up into the frontal cortex, with the strongest response to reward occurring along the medial forebrain bundle (39, 99). Histochemical work has revealed dopaminergic neurons in many of the rewarding areas of the brain. One of the major dopamine (DA) pathways, known as the mesocorticolimbic (dopamine) pathway, connects dopaminergic neurons in the ventral tegmentum to the nucleus accumbens (NAc) and separately to the frontal cortex. Two areas in this pathway, the ventral pallidum (VP) and NAc, have been found to be major foci for reward learning and pleasure (76, 78, 97).

Neuroanatomy of Reward Learning in Insects

Neuroanatomical analyses of olfactory reward learning in insects have focused on the points of convergence of the pathways for processing of the CS (odor) and US (sucrose) (**Figure 2**). In honey bees, the olfactory (CS) pathway begins with axons of chemoreceptors that project to the antennal lobe (AL), where they synapse with local interneurons within the glomerular structure of the AL (29). Projection neurons from the AL synapse with the mushroom bodies (MB) and the lateral protocerebral lobe (some synapse with both). The MB contains approximately 170,000 local interneurons (Kenyon cells) that receive olfactory input through dendritic connections in

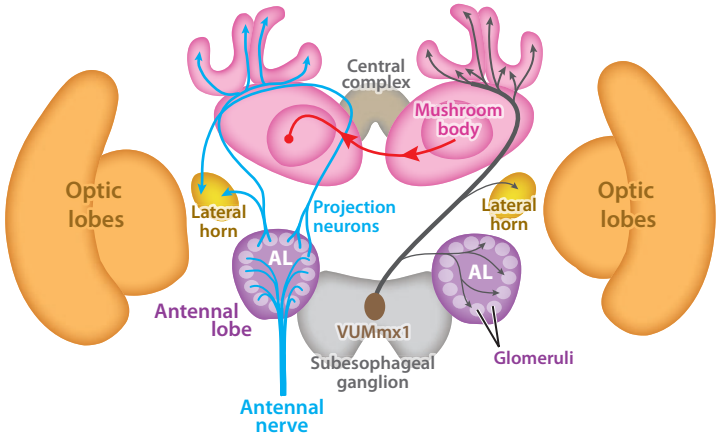

Figure 2

The olfactory and gustatory circuits of the honey bee brain. Schematic frontal view of a cross-section of the central bee brain (carapace and eyes removed). The olfactory (CS) pathway is depicted in light blue. Olfactory neurons send information to the brain via the antennal nerve. These neurons form synapses within the glomeruli of the antennal lobes (ALs) onto local interneurons (not shown) and projection neurons conveying olfactory information to the lateral horn (LH) and the mushroom bodies (MB). MBs are interconnected through commissural tracts (*red*). The gustatory (US) pathway involves the VUMmx1 neuron (*brown*), which projects from the subesophageal ganglion to the LH, AL, and MB. VUMmx1 is bilaterally symmetrical, but in this figure only the right side is shown. Abbreviations: CS, conditioned stimulus; US, unconditioned stimulus; VUMmx1, ventral unpaired median neuron 1 of the maxillary neuromere. Figure adapted from Reference 24 with permission.

the cup-shaped calyx and output through long bunched axons that form the peduncle and lobes of the MB (17). Output neurons from the MB project to the lateral protocerebral lobe, feed back to the MB, or reach other regions of the ipsilateral and contralateral brain hemispheres (26, 48).

The pathway mediating a sucrose US consists of contact chemoreceptors on the antenna and proboscis that project to the subesophageal ganglion (SOG) close to a group of ventral unpaired median (VUM) neurons. VUM neurons project to higher brain areas, especially the AL and MB (26, 48, 65) (**Figure 2**). As both of these regions are sites of convergence of the CS and US pathways, they were both seen as potential foci for associative reward learning.

Although scales and specifics differ, the basic organization of the CS and US pathways is similar in most insects. The organization of the *Drosophila* brain is similar to that of honey bees, to the degree that *Drosophila* also has a population of large VUM neurons with comparable morphology projecting from the SOG and connecting to AL, MB, and the lateral protocerebrum (12).

PREDICTIVE SIGNALS OF REWARD

Dopamine Signals in Mammals

In the mammalian system, DA neurons that respond strongly to reward show phasic activation not only to the presentation of reward stimuli such as food and drink but also to sensory stimuli that predict reward (67, 96). Schultz (67) suggests that the phasic responses of these DA neurons reflect a prediction error of reward, meaning that the value difference between predicted and actual reward is indicated in the change of dopaminergic neural activity. Phasic activity increases with unexpected reward and is depressed with reward omission. Earlier models of conditioning and learning have viewed reward prediction error as vital to the learning process (67, 68); therefore

it is believed that DA may act as a teaching signal for reward response learning. However, there remains debate about the exact roles of phasic DA signals in learning. Some phasic DA signals may not be the immediate cause of reward learning but rather a consequence of learning elsewhere in the brain (62, 76, 86), possibly the prefrontal cortex and hippocampus, which influence bursting rates of DA neurons (5, 52).

VUMmx1: ventral unpaired median neuron 1 of the maxillary neuromere

Predictive Signals of Reward in Insects: The Role of VUMmx1

The honey bee ventral unpaired median neuron 1 of the maxillary neuromere (VUMmx1) (**Figure 2**) in particular has remarkable anatomical and response properties that have clearly demonstrated a role for this neuron in honey bee reward processing. The cell body of VUMmx1 is located deep in the SOG, but the neuron projects to the dorsal SOG, the glomeruli of the AL, the lateral protocerebrum, and the calyces of the MB (25, 65). VUMmx1 responds strongly to sucrose stimulation (25, 65). To enable lengthy intracellular recording, it was critical that the animal's head did not move at all. For this reason Hammer (25) removed the mouthparts (proboscis extension causes small head movements) and recorded firing from the muscle controlling proboscis extension (M17) as the appetitive response, rather than observing proboscis extension as is more common in PER conditioning.

Hammer (25) recorded prolonged firing from both VUMmx1 and muscle M17 in response to sucrose stimulation of the antennae and proboscis. But after training in which an odor stimulus predicted sucrose presentation, an odor alone without sucrose was able to evoke a response from muscle M17. In training it was possible to substitute conditioning with sucrose reward for intracellular stimulation of VUMmx1 and obtain the same learned change in performance of M17 (25, 26). Firing of VUMmx1, therefore, is a neural correlate of sucrose reward in this classical conditioning paradigm.

The response of VUMmx1 to the CS was also experience dependent. In a differential conditioning paradigm with one odor paired with the US sucrose (CS+) and one not (CS-), over the course of training VUMmx1 responded to the CS+ before sucrose presentation. After training the response of VUMmx1 to the CS+ was indistinguishable from the response to the US (25), suggesting that VUMmx1 can manifest learned changes and respond to stimuli that are learned to be predictive of reward, as well as primary rewards. Menzel (43) draws a parallel between this property of VUMmx1 and the dynamic reward-predictive firing properties of the DA neurons in the mammalian midbrain that can change their properties to respond to stimuli that are predictive of reward (67, 69). Following the identification of VUMmx1, attention then focused on the neurochemistry of the system and how VUMmx1 might be affecting downstream circuits.

NEUROMODULATORS OF REWARD

Neuromodulators of Reward Processing in Mammals

DA has been recognized as the main neurotransmitter/neuromodulator of the vertebrate reward system (54, 98). Electrophysiology, microdialysis, and voltammetry techniques have shown that DA neurons are activated and DA is released by presentation of food, drink, sex, drugs of abuse, social reward, and electrical stimulation to key areas of the brain (33, 96). DA receptor antagonists introduced to numerous regions along the mesocorticolimbic pathway reduce the rewarding effect of food and other hedonic stimuli (59).

DA is not the only neurochemical in the reward system. Opioids, serotonin, endocannabinoids, and gamma-aminobutyric acid (GABA) also play a role in modulating various aspects of reward

behavior (23, 35, 91, 93). Although many of these do so by interacting with DA levels within reward centers of the brain, lesions to dopaminergic neurons do not entirely eliminate their effects on reward, suggesting DA-independent mechanisms of reward processing (35, 42).

OA: octopamine

Octopamine and Reward Processing in Insects

Of the biogenic amines in insects, octopamine (OA) rather than DA has always been most strongly linked with reward responses (46, 61). In the honey bee PER paradigm, injection of OA into the region of the thick ocellar neurons just behind the MB increased spontaneous proboscis extension to both conditioned and unconditioned odor stimuli and even to water vapor, suggesting a general excitation of appetitive responses (46). OA injected into the AL stimulated feeding behavior and mimicked the sensitizing action of a food stimulus, suggesting a role for OA in perception of sucrose reward (9). Injection of OA into the peduncle of the MB close to the calyx enhanced both memory formation and recall of sucrose-associated stimuli, suggesting a role in sucrose reward learning (9, 16).

The VUM neurons stain for OA immunoreactivity in honey bees (37, 72) and *Drosophila* (49, 50, 58). Therefore, it was proposed (27) that VUMmx1 is octopaminergic, and OA release from this neuron might substitute for sucrose in associative learning (27). Injection of OA into either the calyces or the AL (projection zones of VUMmx1) paired with presentation of the odor CS in the PER learning paradigm was sufficient to substitute for the sucrose US in proboscis conditioning (27). This suggests that VUMmx1 may affect reward learning by OA release and that both the AL and MB are anatomical foci for olfactory learning.

NEUROCHEMICAL CODING OF REWARD AND PUNISHMENT

Reward Pleasure and Pain in Mammals

In mammals functional imaging and electrophysiology studies show a striking overlap between the neural substrates of pain and pleasure (39). The NAc and VP release endogenous opioids during painful stimulation in humans, and both areas contain neurons receiving input from nociceptors (pain receptors). Analgesia studies have shown that pain can be reduced by pleasure and pleasure can be reduced by pain. The opioid system plays a part in both of these; opioid receptor agonists and antagonists can reduce the effect pain has on pleasure and the effect pleasure has on pain (39). Both pleasant and painful events are associated with the release of endogenous opioids within the NAc (79, 102). Whereas bursts of firing from dopaminergic neurons signal pleasant events, basal firing of DA neurons is inhibited by aversive events (69). Further, tonic DA activity is associated with both increased pain and decreased pleasure (39). Therefore, in mammals the functions of the DA and opioid systems are not limited to reward; rather, they play a complex role in a broader integrated motivational system that balances both reward and punishment and pleasure and pain.

Early Evidence for a Neurochemical Code in Insects: Octopamine Good, Dopamine Bad

For insects, early pharmacological and neurogenetic studies with bees and flies suggested a distinct neurochemical code; reward learning was strongly influenced by OA signals and punishment learning by DA signals. But (as we discuss below) research using new selective neurogenetic tools has yielded findings that are changing this view. Investigation of individual neurochemical circuits

in insects is revealing a modular but integrated system of circuits within which OA and DA signals interact.

Schwaerzel et al. (70) used neurogenetic tools to manipulate OA or DA signals in *Drosophila* and examined the effects on learning odor paired with rewards or punishments in a T-maze assay. Flies in which the tyramine-β-hydroxylase gene had been knocked out could not synthesize OA (51, 61). These flies performed normally in a learning task associating odor with electric shock but did not learn to associate sugar reward with odor (70). This defect could be rescued by a transgene containing the wild-type tyramine-β-hydroxylase gene downstream of a heat shock promoter, such that after heat shock to activate the promoter and restore OA synthesis, flies performed normally in both paradigms (70).

To examine the role of DA signaling in the two learning assays, Schwaerzel et al. (70) used a temperature-sensitive *shibire* gene construct that blocked neurotransmitter release from dopaminergic neurons when flies were maintained at an elevated temperature. At the elevated temperature, flies performed poorly in the aversive learning paradigm but normally in an appetitive learning paradigm (70). Schwaerzel et al. therefore proposed a model of DA as a mediator of punishment and OA as a mediator of reward.

The model was reinforced by Schroll et al.'s (66) studies of *Drosophila* larvae. They used channelrhodopsin gene constructs that allowed different neuronal populations in the translucent larvae to be activated by pulses of blue light (66). Larvae learned to avoid an odor that had been paired with light activation of dopaminergic neurons, but they became attracted to odors paired with light activation of octopaminergic and tyraminergic neurons (66). Using different gene constructs and conditioning paradigms, Honjo & Furukubo-Tokunaga (30) showed similar results: Manipulation of OA circuits affected learning of sucrose reward, and DA circuits affected learning of quinine punishment in fly larvae (30).

Learning of both punishment and reward required functioning cAMP signaling in the Kenyon cells of the MB in both adult (70) and larval flies (30). Schwaerzel et al. (70) argue that it is most likely that cAMP signaling is operating at the presynaptic terminals connecting the Kenyon cells to MB output neurons to cause long-term changes in synaptic connectivity at this junction. Building on this evidence, Heisenberg (28) presented a circuit model for olfactory associative memory in *Drosophila* that proposed a distinct neurochemical coding of rewarding and punishing US, with OA signals modulating learning of rewarding stimuli and DA signals modulating learning of aversive stimuli, with the memory engram being cAMP-mediated changes in synaptic connectivity at the connection between the Kenyon cells and the MB output neurons (**Figure 3**).

This model of a discrete neurochemical code of punishment and reward by DA and OA signals, respectively, has been broadly supported by pharmacological studies with honey bees (92) and crickets (*Gryllus bimaculatus*) (47, 89, 90). But DA is not the only neurochemical system for punishment in honey bees: Wright et al. (100) have found that pharmacological blocking of either DA or serotonin receptors inhibits learned aversion to odors paired with distasteful foods. The cricket studies (47, 89, 90) are noteworthy in that the animals were trained with water as a reward (water-deprived crickets are very strongly attracted to water) and concentrated saline as a punishing stimulus; they are among the few examples of reward learning that did not use sucrose.

All these studies reinforced the perception that octopaminergic signals conveyed reward and dopaminergic signals conveyed punishing signals in the insect brain, but this view is now changing. New evidence from *Drosophila* using neurogenetic tools that selectively manipulate distinct neural populations is revealing a far more complex structure to the insect reward system. Different DA signals perform different functions, and OA and DA signals interact. The contemporary view of mammalian reward systems recognizes a functionally modular (sensu 2) structure involving distinct elements for liking, wanting, and learning of reward (6, 7). Below, we discuss the modular

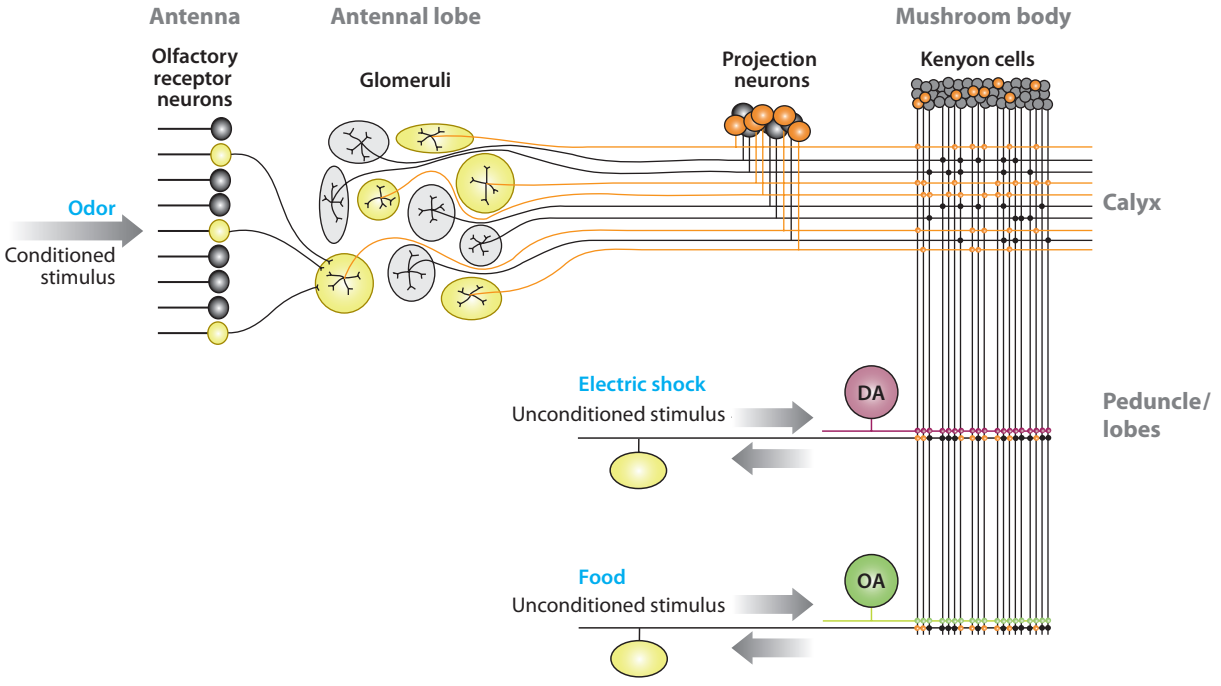

Figure 3

Circuit model of odor memory. Odor stimuli activate different sets of glomeruli within the antennal lobe. Odor information is conveyed to the MB by projection neurons that synapse with the MB calyx. Odors are represented in the MB as distinct patterns of activity across sets of Kenyon cells. Extrinsic MB output neurons are connected to the Kenyon cells by latent synapses. Modulatory octopaminergic or dopaminergic input neurons convey the US (electroshock or food) to the Kenyon cells. Simultaneous arrival of the CS and US coupled with activation of specific output neurons controlling the conditioned response results in strengthened synaptic connections from Kenyon cells to output neurons. Abbreviations: CS, conditioned stimulus; DA, dopamine; MB, mushroom body; OA, octopamine; US, unconditioned stimulus. Figure adapted from Reference 28 with permission.

nature of the mammalian reward system, and how new findings from *Drosophila* suggest a parallel organization for the insect reward system.

MODULES OF THE REWARD SYSTEM

Modules of Liking and Wanting in the Mammalian Brain

The NAc and VP are important for generating pleasurable reactions to reward stimuli. Within these regions are small hedonic hot spots, so called because stimulation of these areas can increase liking reactions to reward stimuli. Opioids, endocannabinoids, and GABA have been shown to enhance liking in the hot spots (7, 42, 55, 77).

Increases in wanting responses have been produced through microinjection of DA receptor agonists directly into the NAc, or by increases in extracellular DA through genetic manipulations; however, liking for stimuli was not changed (56, 101). The inverse has also been shown: Suppression of endogenous DA in these regions through either pharmacological means or extensive damage to DA systems reduced wanting but left liking unchanged (5, 6, 8).

Endogenous opioid signals modulate both wanting and liking in different regions. Microinjections of opioid receptor agonists within the NAc or VP but outside of the hot spots increase

wanting without increasing liking reactions and in other regions can even reduce liking (55, 57), indicating that the areas responsible for liking and wanting are anatomically separated.

The mammalian reward system therefore includes distinct areas for reward liking, wanting, and learning that operate as an integrated system to organize reward responses. These modules share common neurochemical elements but involve different circuits.

PAM: paired anterior medial (cluster of dopamine neurons in *Drosophila*)

Revising the Insect Neurochemical Code: Multiple Interacting Signals in a Modular System

A new generation of *Drosophila* neurogenetic tools has allowed very precise targeting of specific cell populations. This level of resolution is revealing different roles for distinct DA signals in both punishment and reward and that OA and DA signaling pathways interact (94). Further, an ability to focus on specific cell groups is revealing different functional modules of the insect reward processing system for liking, wanting, learning, and drive that parallel the organization of the mammalian reward system.

Waddell (94) found that flies could still learn an odor/sugar association in a PER paradigm even when synaptic transmission from octopaminergic neurons was transiently blocked by a temperature-sensitive *shibire* construct driven in neurons expressing tyrosine decarboxylase (the enzyme converting tyrosine to tyramine, the immediate precursor of OA) (94). Despite lacking OA signaling, these flies still performed well in a reward-learning task, but only if during training flies both tasted and ingested a nutritious sugar. Flies could not learn if octopaminergic signaling was blocked while training with sweet but nonnutritious sugar (94). Waddell (94) proposes, therefore, that in flies OA neurons signal sweet taste as a reward but nutrition as a reward is signaled by other means.

Waddell's (94) study appears to contradict Schwaerzel et al. (70), but the genetic manipulations used in the two studies are very different. Waddell used a construct to transiently inhibit neurotransmitter release from OA neurons in flies that otherwise had a functional OA system (94). Schwaerzel et al. used a construct to transiently activate OA synthesis in flies that were otherwise lacking OA (70). These manipulations clearly yield different behavioral results, but why this is so is presently unclear.

Waddell's (94) finding changes the imagined role of OA in the reward system. Rather than responding to general rewards, Waddell suggests that OA might most strongly signal the perception of sugar reward. This questions whether OA signals reward generally or more specifically to sweet taste (or perhaps reward liking), but this hypothesis certainly needs further experimental investigation.

Multiple Roles for Dopamine in the Insect Reward System

Rather than separate roles for OA signaling rewarding and DA signaling punishing stimuli, it is now clear that different DA circuits in *Drosophila* modulate learning of either rewarding or punishing stimuli (40, 94). Mutant *Drosophila* lacking the dDA1 receptor (a D1-like DA receptor that activates adenylate cyclase) in the MB and central complex failed to learn to associate odor with electric shock and also showed partial impairment of learning of odor associated with sucrose (34). Both of these defects could be rescued by restoring dDA1 expression in the MB (34), demonstrating that for flies DA signaling via the dDA1 receptor in the MB is involved in learning of both rewarding and punishing stimuli. Similar findings have been reported for larval *Drosophila* (71).

DA neurons in the paired anterior medial (PAM) cluster close to the calyces of the MB are involved in reward learning (40, 94). Presentation of an odor paired with artificial activation of these

CR neurons: efferent neurons of the lobes of the mushroom bodies of insects involved in the conditioned response

neurons (via transgenic temperature-sensitive ion channels) triggered approach and appetitive responses to the odor similar to those responses seen when training with an odor/sugar association (40, 94). Clearly, the dopaminergic PAM neurons contribute to signaling of sugar reward in *Drosophila*. Further, it was possible to implant an appetitive memory in *Drosophila* by direct stimulation of OA neurons paired with an olfactory stimulus, but only if there was a functional DA system (94). This would indicate that OA and DA signals interact in memory formation with DA downstream of OA (40, 94).

Circuits in *Drosophila* have also been identified that control the degree of wanting of reward (36). Animals perform better in a learning task rewarded with food when hungry, because they want to gain the reward more. Krashes et al. (36) identified part of the neural mechanism by which satiation state regulates learning performance. In fed flies, appetitive memory performance is low because MB neurons are inhibited by tonic DA release from the MB-MP neurons [a population of neurons innervating the medial lobe and medial peduncle of the MB (the MB-MP, part of the PAM cluster) (36)]. These neurons were themselves inhibited by *Drosophila* neuropeptide F (dNPF), an ortholog of mammalian neuropeptide Y that regulates food seeking (32, 82). dNPF is released when flies are hungry. One of its actions is to suppress the inhibitory MB-MP neurons, which then enables the expression of food-associated conditioned responses (36). The MB-MP neurons are also modulated by OA acting through the *octβ2R* OA receptor (94). Waddell (94) has identified how an appetitive state interacts with the reward processing system with a structure of layered hierarchical control involving interacting neuromodulatory elements, including OA and DA.

In summary, these new findings are revealing the complexity of neurochemical coding of rewarding and punishing stimuli. Different DA signals mediate punishment and reward. OA seems to mediate learning of sweet taste rather than nutrition as a reward, but some OA signals modulate general satiation state and thereby the level of wanting of reward (40, 94). Other neuromodulators, such as serotonin (100) and dNPF (36) (and likely many others), are also involved in a complex decision-making system whose output depends on the balance of interacting and stratified elements.

DEVELOPING A CIRCUIT MODEL OF THE INSECT REWARD PROCESSING SYSTEM

VUMmx1 provided the first circuit understanding of insect reward processing. Direct stimulation of VUMmx1 could substitute for sucrose in conditioning of the PER response (25). The neuron is assumed to operate by release of OA into either the AL or MB calyces (27) to act on downstream neurons to effect a learned behavioral change. The foci of these learning circuits are assumed to be the AL and/or the MB calyces.

Heisenberg's (28) model of reward and punishment learning in *Drosophila* was the next synthesis of odor learning circuits. His model (**Figure 3**) assumes that each perceptually different odor activates a different pattern of Kenyon cells. US (rewarding or punishing) are assumed to be separate modulatory inputs that reach all Kenyon cells. The conditioned response is controlled by output neurons that synapse with the Kenyon cells at the peduncles and lobes and carry output from the MB. Synapses between Kenyon cells and output neurons are initially thought to be latent, but during conditioning, simultaneous activity of a US representation at the Kenyon cells, along with activity of a distinct set of Kenyon cells representing the CS odor and activity in the specific output neuron, results in strengthened synaptic contact between the set of Kenyon cells and the output neuron. Consequently, after training, the CR neuron can be activated by CS odor alone. In effect, the output comes to act as an odor-specific neuron responding to odors predictive of the

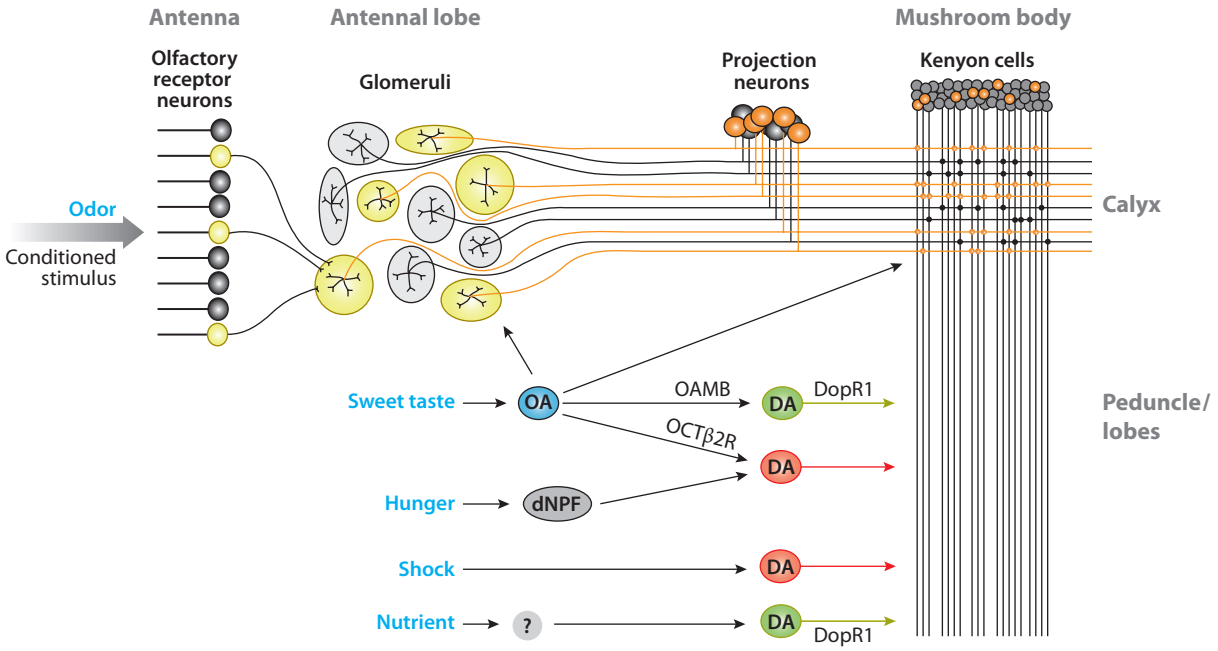

Figure 4

Model of the modulatory systems representing sugar unconditioned stimulus in the *Drosophila* appetitive learning system adapted from Reference 16 with permission. OA signals the sweet taste of sugar, which delivers a rewarding signal to the MB possibly via the OAMB receptor acting on DA neurons in PAM. OA also reduces the inhibitory effects of the dopaminergic MB-MP1 neurons (a specific subset of mushroom body neurons innervating the mushroom body heel in *Drosophila*) via the Octβ2R receptor. The MB-MP1 neurons are also inhibited by dNPF released when flies are hungry. This module regulates reward learning according to satiation state. The nutritive value of sugar activates other DA neurons in the PAM cluster that convey parallel modulatory signals to the MB representing the nutritive aspect of sugar reward. DA signals are registered at the MB by the DopR1 receptor. Abbreviations: DA, dopamine; dNPF, *Drosophila* neuropeptide F; MB, mushroom body; OA, octopamine; OAMB, octopamine receptor expressed in mushroom bodies; PAM, paired anterior medial cluster of dopamine neurons in *Drosophila*.

US. As initially presented (28), the model assumed that punishing US were represented at the MB by DA signals and rewarding US by OA signals (28). The essence of this model is not invalidated by proposing a more complex neurochemical coding of punishment and reward.

Waddell (94) proposes an updated model of odor learning in flies that incorporates new information on the representation of US at the MB (**Figure 4**). OA represents sweet taste as a US that may then act via DA neurons in PAM to influence learning. Nutrient value in parallel activates other DA PAM neurons directly. DA signals are registered in the MB by the DopR1 receptor. OA also reduces the dopaminergic inhibitory signals from the MB-MP neurons. It is the balance of modulatory inputs to the Kenyon cells from different OA and DA neuron populations that determines the form of the US representation at the Kenyon cells, but it is still assumed that coincident activation of the CS and US representation at the Kenyon cells with activation of the specific output neurons during training is required to change the strength of synaptic connectivity between the output neurons and the Kenyon cells representing the specific CS odor.

Both models assume that the locus of this learned change in synaptic connectivity is the presynaptic terminals of the Kenyon cells where they contact the downstream output CR neurons, because most studies have found synaptic plasticity to occur in the axon of the presynaptic cell and olfactory memory formation requires cAMP signaling within the Kenyon cells (28, 70). Until

recently it was assumed that the presynaptic output terminals of the Kenyon cells were located at the lobes of the peduncles (28). This made it difficult to integrate VUMmx1 into Heisenberg's model of reward learning. VUMmx1 is supposed to mediate the reward signal by releasing OA, but in both bees and flies VUMmx1 releases OA mostly into the MB calyces, not the lobes (12, 13, 50, 65, 72). This is removed from the presumed point of synaptic modification at the lobes of the MB. It seemed unfeasible for a cAMP signal generated by octopaminergic input at the dendrites of the Kenyon cells in the calyces to diffuse the entire length of the neuron to affect presynapses at the axon terminals in the lobes (28). Therefore, the site of octopaminergic input from VUMmx1 seemed to be too remote from the locus of synaptic change to easily fit with Heisenberg's model (28).

But new evidence has shown that the MB calyces are not exclusively postsynaptic (15). There are also presynapses along the dendrites that are capable of releasing synaptic vesicles (15). Although the function of these calycal presynapses is currently unknown, they would allow for microcircuitry to occur within the calyx. The presynapses might synapse onto projection neurons carrying olfactory information to the calyx, the GABAergic anterior lateral neuron, or other Kenyon cells. This is all within the zone of octopaminergic and dopamergic input to the MB. We propose that calycal presynapses could be a vital locus of synaptic modification during reward learning and that reward learning could involve Kenyon cell communication within this zone, as well as postsynaptic modification of the projection neurons and feedback to the AL.

REWARD PROCESSING IN THE ANTENNAL LOBES

The insect reward processing system does not just involve the MB; the AL are also a locus of reward learning. Memory formation of a sucrose/odor association in honey bees can be disrupted by selective cooling of the AL (44). OA injection into the AL could substitute for sucrose in appetitive conditioning (27), and blocking OA receptors in the AL either pharmacologically or by knockdown of the OA receptor AMOAR with double-stranded RNA inhibited acquisition and recall of reward memory (18, 19).

Similar organization is seen in *Drosophila*. In flies the projection neurons from the AL to the MB can themselves accommodate an olfactory reward memory trace that is adenylate cyclase–dependent (85), because rescue of adenylate cyclase in *rutabaga* mutant *Drosophila* in the projection neurons only is sufficient to rescue learning (85). Therefore, in *Drosophila* there are two memory traces for olfactory reward learning located within a connected pathway: within the projection neurons to the MB and within the Kenyon cells (85).

In bees and flies VUMmx1 arborizes within the dendritic fields of both of these neuron populations and likely releases OA into both regions. OA may be involved in reward learning in both locations (27). The internal circuitry of both the glomeruli of the AL and the MB calyces is quite similar. Both dendritic fields contain transmissive and receptive synapses capable of interacting with each other and with inhibitory GABAergic local interneurons. Within both neuropil regions there is the potential for local computation (15).

Processing within the AL also sharpens the neural representation of reward-associated odors (22, 75). In honey bees differential conditioning of two odors (one with reward and one with punishment) causes the patterns of glomerular activation for each odor in the AL to diverge (75). Presumably this increases the ease with which a bee can discriminate the two odors (75). Therefore, the reward processing pathway in insects involves serially arranged but interacting loci for reward learning.

SUMMARIZING INSECT REWARD PROCESSING SYSTEMS

In insects the reward processing system is a distributed system with reward learning of olfactory stimuli occurring within two steps of the odor processing pathway: the MB and AL. It seems likely that OA signals modulate reward learning at both of these loci. The broader reward system involves modular and hierarchical elements for the processing of sugar taste (perhaps analogous to liking), nutrition and hunger (which together influence reward wanting), and learning. There is also a close interaction of neurochemical systems conveying punishment and reward.

In all these aspects the operational organization of the insect system parallels that of the mammalian reward system, which invites the question of whether reward systems are conserved across these two highly divergent groups. We would argue that despite their similarities this is not an example of true evolutionary conservation. The circuit substrates of the two systems are not conserved (20). The neurochemical elements involved are similar but not the same. The involvement of biogenic amines in both mammalian and insect reward systems more likely reflects an ancient role for the biogenic amines in regulation and initiation of movement rather than conserved functions in reward per se (3).

Modular processing of liking and wanting may be an essential element of a functional reward system. Liking is a hedonic property that can be attached to other stimuli via learning, which enables animals to alter their behavior to achieve reward or learn predictors of reward. Independent of this process, the degree of wanting must be modulated on physiological timescales according to nutritional and other physiological demands. Because liking and wanting are modulated in different ways and across different timescales, they have to be processed independently.

The Next Steps for Exploring Insect Reward Systems

VUMmx1 has always been front and center in the insect reward learning literature, but there is still far too much we do not know about this neuron. After almost 20 years, only one study has succeeded in long-term recording from this neuron during the learning process (25). VUMmx1 can change its properties to fire in response to stimuli predictive of sucrose reward (25), and understanding how it does this would be a major advance in understanding the total insect reward processing system. Local circuits within the dendritic fields of the MB and AL may be involved, but given that electrophysiological analysis of this neuron in bees is challenging, how do we proceed? VUM neuron morphology is largely conserved across bees, cockroaches, and flies (12, 13, 50, 65, 72). Perhaps it will be possible to use the new light-activated neurogenetic constructs now available to *Drosophila* researchers to manipulate the VUM neurons in learning flies as a new strategy to explore their function in a different system.

If Waddell (94) is correct in that octopaminergic neurons respond most strongly to sweet taste as a reinforcer rather than to nutrition, to what extent is OA involved in the learning of nonappetitive rewards? A key feature of the mammalian reward system is its generality. Midbrain DA neurons are activated by rewards in many different modes (e.g., sex, food, monetary gain) (69). Is the insect reward system similarly general? The only way to assess this is to study rewards other than sucrose. Sitaraman et al. (73) were able to train heat-stressed flies using access to a cool place as a reinforcer: This learning was not dependent on OA. Tanimoto et al. (81) were able to train flies to learn that a stimulus immediately preceding cessation of electric shock was rewarding, which could be a brilliant system for examining the interactions of reward and punishment.

CONCLUSION

The insect reward processing system is composed of a network of interacting neurochemical elements with a functionally modular structure that parallels the organization of vertebrates. Appreciating the similarities of the reward processing systems of mammals and insects only reinforces once again the utility of insects as a comparative neuroscience model. Thus far the study of reward processing systems in insects has been almost entirely a by-product of the study of insect learning. We hope that the study of insect reward systems will now emerge as an endeavor in its own right.

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