The neural basis of associative reward learning in honeybees

Martin Hammer

Appetitive learning of food-predicting stimuli, an essential part of foraging behavior in honeybees, follows the rules of associative learning. In the learning of odors as reward-predicting stimuli, an individual neuron, one of a small group of large ascending neurons that serve principal brain neuropiles, mediates the reward and has experience-dependent response properties. This implies that this neuron functions as an integral part of associative memory, might underlie more complex features of learning, and could participate in the implementation of learning rules. Moreover, its structural properties suggest that it organizes the interaction of functionally different neural nets during learning and experience-dependent behavior.

Animals must learn which environmental stimuli or which of their actions predict biological meaningful, rewarding or aversive, reinforcing stimuli. In complex natural environments this requires the integration of different sensory modalities into coherent memories and the coordination of various motor systems. In particular, the brain must learn and store representations of the biological value of stimuli (for example, appetitive or aversive) and recall these representations to control adaptive experience-dependent behavior (for example, approach or retreat). Because behavioral learning can involve a diversity of circuits, evaluative reinforcing information should be globally reported. Possible structural correlates are, therefore, small groups of large ascending neurons that widely innervate the brain. As intrinsic elements these neurons can simultaneously influence local circuits that have different functions, often through the release of neuromodulators. Neuromodulation affects cellular excitability and synaptic transmission and, since this can cause different functional modes of activity in given anatomical circuits, is essential for behavioral plasticity.6,7 Neuromodulators, and hence neurons that release them, are implicated in mediating motivation, arousal, attention and memory processing.2–4 In particular, dopaminergic neurons of the midbrain in mammals have a role in reward and reinforcement processing and behavioral adaptation.2–4 This review investigates the neural basis of reward learning in honeybees and focuses on hypotheses for the functional role of a single identified neuron. This neuron distributes reward-related information simultaneously to several brain structures, making necessary an understanding of how functionally distinctive neural networks interact during behavioral learning.

Appetitive reward learning in honeybees – the conditioning of the proboscis-extension response (PER)

During foraging, honeybees associate several floral parameters such as the location, shape, color and smell of flowers6,7 and even abstract features such as the symmetry of visual patterns6,7 with rewards. Bees evaluate reward conditions (profitability) of different food sources based on experience and build memories that relate floral cues with profitability. With restrained bees features of this reward learning can be analyzed that must be explained ultimately by the performance of the brain.

First, sucrose rewards elicit various appetitive behaviors that are subject to nonassociative and associative learning.2,9 As a strong appetitive stimulus, sucrose stimulation of the antennae and proboscis biases appetitive behavior. For example, it induces short-lived appetitive arousal that enhances the PER to odors10 or tactile stimuli applied subsequently (M. Hammer, unpublished). In particular, a sucrose reward represents the reinforcing or unconditioned stimulus (US) in olfactory conditioning. Bees develop the PER as a conditioned response to an odor after even a single pairing of the odor (conditioned stimulus, CS) with a sucrose reward (PER conditioning). As a form of predictive learning PER conditioning strongly depends on temporal relationships. The odor must shortly precede (forward conditioning) but not follow (backward conditioning) the reward during learning.

Second, behavioral analysis mainly in mammals has shown that during conditioning a CS acquires features (for example, emotional content, perceptual properties, biological value) previously attributed to the US (Ref. 12). Similarly, in PER conditioning an odor acquires an appetitive value. After conditioning it elicits not only appetitive responses but also gains rewarding and arousing properties, since it can serve as a second-order US (Ref. 8) and induces appetitive arousal (M. Hammer, unpublished).

Third, theories derived from associative learning in vertebrates emphasize the notion that experience-dependent factors, such as the unexpected occurrence of the US (Refs 13–15) or the attentional degradation or augmentation in processing of the CS (Ref. 16), govern conditioning. In bees, after differential conditioning with one rewarded and one unrewarded odor, learning of the unrewarded odor is retarded in subsequent forward conditioning16, and preconditioning of one odorant blocks the subsequent conditioning of
The neural basis of reward and reinforcement processing

In honeybees, the biogenic amine octopamine (OA) enhances olfactory reward conditioning and memory retrieval23 and mediates a transient form of food arousal22. Candidate neurons for these modulatory effects of OA on appetitive learning and behavior are octopaminergic VUM neurons that respond to sucrose with long-lasting excitations (Fig. 1A).

One of these neurons, the VUMmx1 neuron (Box 2), has a more specific role. It mediates both PER function of rewards during olfactory conditioning, since its depolarization substitutes the reward in single-trial olfactory conditioning. This substitution effect mimics a basic feature of PER conditioning (Fig. 1B): forward pairing of an odor with a depolarization of VUMmx1, but not odor delivery during depolarization (backward pairing), increases the odor-evoked response of the main proboscis muscle M17 in a later test. Temporal overlap of odor-evoked and VUMmx1 activity is, thus, not sufficient, suggesting neural mechanisms that detect, and change as a consequence of, the temporal sequence of VUMmx1 and odor-evoked activity. The behavioral change in the substitution experiment is the same as in single-trial conditioning experiments with sucrose in the same preparation (Fig. 1B). Other neurons, however, might participate in reinforcement processing, since a slightly higher spike frequency of VUMmx1 was induced by depolarization than by sucrose. However, VUMmx1 activity does not evoke PER, suggesting parallel processing of the response-releasing and reinforcing property of sucrose.

In addition, recordings of VUMmx1 during and after differential conditioning show that reward-predicting odors evoke a long-lasting excitation of VUMmx1 (Ref. 23). This enhanced responsiveness is specific to rewarded odors (Fig. 1C). Moreover, forward, but not backward, pairing of odor-evoked and VUMmx1 activity increases the odor response of VUMmx1 (Ref. 23), showing that VUMmx1 is sufficient to produce this plasticity. Through experience this neuron becomes, therefore, an intrinsic element of the olfactory circuit that generates predictive behavior. Sites of odor–reward learning

The structural properties of VUMmx1 indicate the antennal lobe and even the antennal lobe is faster. Thus, a certain period of unstructured activity in the calyces is necessary for memory formation in, or downstream of, the MB, including putative feedback pathways from the MB to the antennal lobe. Support for an essential role of the MB calyces comes from injections into the calyces of OA, the transmitter of VUM neurons. When substituted for the sucrose reward another odorant when a compound of both is rewarded24. Both, inhibitory learning of an unrewarded CS and the so-called ‘blocking’ phenomenon have been instrumental for theories that relate associative learning to variations in CS attention or in the expectation of reinforcement. Recently, Smith28 has presented evidence for blocking in binary odor mixtures in bees that is compatible with processing a predictive error for the expectation of reinforcement (Box 1).

Since learning that underlies foraging can also be described by variants of predictive error-correcting learning rules that are used to update feeder-specific memories19,20, both PER conditioning and learning of flower-related signals appear to follow the same learning rule.
this produces behavioral learning in multiple-trial olfactory conditioning. Physiological evidence that associative plasticity occurs in, or upstream from, the MBs comes from an extensively studied MB-extrinsic neuron, the PE1 neuron. This neuron presumably collects input from a huge number of Kenyon cells (KCs) and links the MB with the LPL. It undergoes a decrement in odor-evoked excitation shortly after an olfactory conditioning trial and during the second rewarded trial in a differential conditioning protocol. Several differential conditioning trials, however, increase the response of PE1 to the rewarded odor (Fig. 2). This transition in response plasticity suggests an altered contribution of the MBs with trial repetition. Moreover, as PE1 plasticity is transitory (it is expressed during but not after the conditioning procedure), information flow through the MBs may contribute to memory downstream of the MBs (for example, in the LPL).

Cooling of the antennal lobe within 1–2 min after single-trial conditioning disrupts memory formation and substituting the reward in multiple-trial conditioning by injections of OA into the antennal lobe also results in learning. Thus, a direct contribution of the antennal lobe is likely, but odor-OA pairing-specific transient effects in the antennal lobe could also facilitate learning in the MB or LPL. Whereas these results support the involvement of the MB calyces and the antennal lobe in olfactory reward learning and OA as the reinforcement-mediating primary transmitter, the involvement of the LPL has yet to be demonstrated.

Compared with Drosophila olfactory learning In Drosophila, the use of structural mutants and chemical-lesioning experiments also implicate the MBs, and octopaminergic drugs influence learning. However, the learning mutant ddc has defects in the synthesis of dopamine and serotonin (for review, see Ref. 30). Moreover, a novel dopamine receptor, DAMB, that stimulates cAMP synthesis is preferentially expressed in the output lobes and pedunculi but not in the calyces, and dopaminergic neurons innervate these MB substructures. This pattern of expression of the DAMB-receptor resembles that of the gene coding for the primary transmitter, the involvement of the LPL has not been demonstrated. Alternatively, the antennal lobe might simply be related to species-specific differences. The mismatch between the honeybee and the Drosophila model for processing of reinforcement might simply be related to species-specific differences. From this Davis and colleagues’ suggestion that dopaminergic neurons convey reinforcement to the axons of the MB-intrinsic KC.

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Box 2. Structural basis of olfactory proboscis-extension response (PER) conditioning

Olfactory receptor neurons from the antenna (about 60,000) serve the glomeruli (about 160) of the antennal lobe where they converge onto local interneurons (about 4000) and projection neurons (Fig. A). Individual local interneurons connect between 50–100 glomeruli. Multiglomerular projection neurons connect glomeruli with the lateral protocerebral lobe (LPL) and other parts of the protocerebrum, uniglomerular projection neurons (about 900) with the LPL, and the calyces of the mushroom bodies (MBs) via two fiber tracts (see Fig. A) (Ref. b). Each uniglomerular projection neuron widely innervates the calyces, suggesting distribution of olfactory input to a large number of the MB intrinsic neurons, the Kenyon cells (KC). The dendritic processes of the KC (about 170,000 per MB) build the calyces, which receive various modalities separately: the lip, olfactory, the collar, visual, and the basal ring, multimodal, thought to arise partly from collaterals of visual and olfactory fibers. Dendrites of individual KCs are restricted to bifurcating axons of the KC build the MB output lobes (α-lobe and β-lobe). Output lobes are connected via GABAergic feedback neurons through the protocerebral–calycal tract with the calyces. Groups of MB extrinsic output neurons and large individual neurons link the MB with the LPL. The LPL presumably drives premotor neurons in the subesophageal ganglion (SOG) via descending neurons. Motoneurons (for example, MN17, Fig. A) generate the PER in esophageal ganglion (SOG) via descending neurons. Motor neurons stain with an antibody against the biogenic amine octopamine (OA) (Ref. k), a well-known neuromodulator in insects. Therefore, the activity of these neurons might serve as an extrinsic source of neuromodulation that influences large and distributed circuits. One of these neurons in bees, the VUMmx1 neuron (Fig. B), innervates the glomeruli of the antennal lobes, the lip and basal ring of the MB calyces and the LPLs. It represents the reinforcing function of the reward in olfactory PER conditioning.

References
dopaminergic neurons respond selectively to rewards but not to aversive stimuli, suggesting that certain neuronal systems are dedicated for coding a specific biological value.

Possible molecular correlates of odor–reward learning

Cellular and synaptic effects caused by VUMmx1 (OA) that could underlie learning are as yet unidentified (including a mechanism that accounts for the dependence of learning on the sequence of odor-evoked and VUMmx1 activity). Access to these questions might come from cultures of the MB-intrinsic KCs, which express an ACh-mediated Ca2+ current (ACh is a putative transmitter of olfactory projection neurons1) and several voltage-dependent inward and outward currents10, which could be modulated by OA via the activation of second-messenger pathways. An OA-receptor subclass in the insect brain stimulates adenylyl cyclase39. Therefore, a primary candidate for a molecular substrate of learning is, as in other systems10e14, the cAMP cascade. Consistent with this hypothesis OA, cAMP and sucrose rewards, but not dopamine and serotonin, activate a cAMP-dependent protein kinase A (Refs 43,44) in the antennal lobe of bees. (Experimental evidence for a coupling of OA receptors to the cAMP cascade for the MB is as yet lacking.) Recently, however, Müller45 has demonstrated that formation of an appetitive olfactory memory in bees depends on nitric oxide (NO). Injection of inhibitors of NO synthase into the hemolymph prior to conditioning impairs selectively the formation of a long-term memory, which is induced with three conditioning trials, but not a median-term memory, which lasts for several hours and is induced with a single trial. Müller45 suggests that one possibility for the effect of NO is a cGMP-mediated modification of the cAMP-signaling cascade. A major effector of NO is cGMP, and in bees cAMP-dependent protein kinase A is also activated by cGMP (Ref. 46). Thus NO might specifically affect formation of long-term memory as opposed to median-term memory by additional activation of protein kinase A. Interestingly, NO synthase is expressed at high levels in the glomeruli of the antennal lobe, the lip of the MB calyces, and the LPL (Ref. 45), suggesting that these three sites of odor–reward (VUMmx1) convergence are loci for the effect of NO on formation of long-term memory.

Why is reinforcement transmitted to several brain sites?

Independently of the exact nature of the cellular mechanisms that mediate learning, what do the different brain structures contribute to learning, memory, and experience-dependent control of behavior? In natural environments the value of stimuli can depend on both internal and external context and the brain must integrate various sensory-motor systems during both predictive learning and behavior. The fact that different interconnected networks simultaneously receive reward-related input endows their role to be specified. Figure 3 summarizes the underlying general architecture of the central bee brain (blue lines, olfactory system, other sensory-motor systems probably have similar architecture, black lines).

The antennal lobe of insects encodes odors in dynamic, overlapping, across-fiber patterns of activity. In the locust, in response to odor stimulation, sequentially changing ensembles of local interneurons and projection neurons synchronize with 20–30 Hz oscillatory field potentials recorded in the MBs (Ref. 48). However, during certain epochs of processing, individual neurons of the antennal lobe do not synchronize. Synchronization can enhance synaptic transmission through coincident spike timing16. Moreover, stimulation of ascending neuronal systems, which promotes stimulus-induced synchronization in cortical networks17, and neuromodulators influence synaptic plasticity in cortical networks18. In the antennal lobes or its targets, the MBs and the LPLs, activation of the VUMmx1 neuron could, therefore, control synaptic plasticity by favoring synchronization or permitting coincident-dependent plasticity, or both. This, in turn, could result in neural assemblies that encode
in the MB (or the LPL) (Fig. 3). Given that learning also permanently alters olfactory coding in the antennal lobe, odors of behavioral relevance could be processed preferentially.

The LPL, a premotor center, receives olfactory information both directly from the antennal lobe and processed by the MB and is thought to control odor-driven behavior. What type of information does the MB pathway add? It has been suggested that the MB, with its many KCs, forms sparser odor representations than does the antennal lobe that are less susceptible to generalization. The specific neural organization of the MB in bees suggests another (additional) function (Fig. 3). The separation of modality-specific input to the MB calyces (see Box 2) is maintained within the MB by topographic projections of KC axons into the output lobes\(^5,52\). However, output neurons of the MB are frequently multimodal\(^4\), presumably because their dendrites are not restricted to modality-specific sites\(^5,54,55\) and one KC subgroup violates the overall MB topography\(^5\). Other output neurons of the MB are probably modality specific, for example, olfactory\(^5\).

Both types converge onto the LPL (Refs 26,55). Thus, the LPL controls appetitive behavior by an olfactory and a multimodal pathway, in which the MB pathway could provide experience-dependent context information (for example, other floral parameters) if, for instance, VUM neurons, other than VUMmx1, innervate the corresponding calyx compartments. (Immunoreactivity to OA is present throughout the calyx\(^4\), Fig. 3, red shading.) Similarly, convergence of MB-processed information onto other premotor centers (black lines, Fig. 3) could control behaviors, such as flight or landing maneuvers. Since, in bees, a single neuron connects the u-lobes with probably all the glomeruli of the antennal lobe\(^5\), MB-processed information could also facilitate the encoding of odors of behavioral relevance (dashed arrow, Fig. 3).

The MB pathway might, however, not only contribute to retrieval of context-dependent memory but, via extrinsic neurons, to (context-dependent) learning in premotor centers (Fig. 3), allowing for the development of experience-dependent behavioral routines. The slow time course of MB-related memory formation after a single conditioning trial\(^10,24\) and the transition of MB-related associative plasticity in the PE1 neuron with trial repetition\(^10\), suggests that learning in the LPL could be limited shortly after single-trial learning and more extensive when initial experiences are confirmed after multiple-trial learning. In this case, learning in, for example, the LPL would depend on the informational status of the animal. This hypothesis is consistent with the finding that the plasticity of the associative response of the PE1 neuron is transitory, but does not depend on it.

Why does activation of reward-mediating neurons depend on experience?

Despite the evidence for a reinforcing function of VUMmx1, others are not excluded. This or other VUM neurons could also mediate the arousing effects of sucrose and OA. However, both rewards and reward-predicting odors activate VUMmx1. Candidates for the olfactory input are descending neurons (Fig. 1, red lines), since VUM neurons receive input in the subesophageal ganglion (SOG). Thus, VUMmx1 not only induces learning, but participates in the neural substrate...
of appetitive olfactory memory. An odor could, therefore, acquire the value of an appetitive stimulus because of a putative arousing function. Since VUMm1 neurons, other than VUMm1x1, with a different morphology might also be activated by reward-predicting odors this might bias several appetitive behavioral components. Apparently the plasticity of the VUMm1x1 response could also underlie learning phenomena such as second-order conditioning.

Another possibility relates to the idea that reward learning appears to minimize a predictive error for the expectation of reward. Like VUMm1x1, dopaminergic nerve terminal ensembles in Mecaca fuscata Fischer have experience-dependent properties\(^1\). They are activated by rewards and reward-predicting stimuli; however, they do not respond to unpredicted rewards and might, therefore, process a predictive error\(^1,2\). Montague et al.\(^3\) proposed a model for bee foraging based on some of the properties of VUMm1x1. During flower visits, VUMm1x1 would be activated according to a variant of the Rescorla and Wagner rule (see Box 1). If the actual reward was less than expected this would predict inhibition of VUMm1x1 by reward-predicting signals. In contrast, reward-predicting odors fire VUMm1x1. One possibility that would allow VUMm1x1 to participate in processing of a predictive error is that an excitatory and an inhibitory pathway (possibly from different sources upstream), both with increased efficacy after learning, converge onto the VUMm1x1 neuron (red lines in Fig. 3). The inhibitory input would be delayed as proposed by real-time models of associative learning\(^3,4\) to allow for both activation of VUMm1x1 by reward-predicting odors and inhibition of its response to rewards that are predicted. In this case, additional mechanisms must, however, account for inhibitory learning. Experimental access to these problems requires a record to be made of the activity of the VUMm1x1 neuron during stimulus protocols that lead to inhibitory learning and blocking.

Finally, plasticity of VUMm1x1 could be an adaptation to the dynamic neural representation of odor, which consists of a pattern of sequentially activated neurons of the antennal lobe. Access to rewards during foraging can follow the perception of the odor emanated by a food source within seconds. If, initially, only the activity of those ensembles of olfactory neurons whose activity briefly precedes the reward is associated with reinforcement, experience-dependent transfer of the activation of VUMm1x1 to these ensembles could allow the whole sequence of the odor representation to be learned. Because odor-evoked activity that follows the onset of VUMm1x1 activity does not result in learning, this transfer could also protect learning from fluctuating backgrounds and could contribute to the blocking phenomenon in binary odor mixtures.

Concluding remarks

Studies on learning and memory examine problems at different organizational levels, using both experimental and theoretical approaches. Research on the neural basis of behavioral learning in bees might contribute to the formulation of several main reasons. First, the bee provides an excellent case study for associative learning that occurs in a natural behavioral context, since reward learning in bees, evolved as a specific adaptation to the niche of this species, follows the rules of this learning. Second, behavioral studies can be directly combined with physiological and molecular studies of learning and memory. Third, learning depends on lower-level factors, such as synaptic plasticity. Synaptic plasticity acts through the modification of circuits and these eventually determine how experience changes the function of the nervous system and, consequently, behavior. On the other hand, higher-level concepts employed to describe behavioral learning, such as arousal, expectation, attention and value also most likely relate to circuit properties. Reward-mediating neurons influence brain function through the modification of cellular properties in functionally distinct, but interconnected, circuits. Through experience they participate in the neural substrates of associative memory in both vertebrates and invertebrates. In bees, studying the neural basis of predictive learning might represent an opportunity to understand how levels of brain organization are functionally linked and to test some of the theoretical concepts employed to describe experience-dependent adaptations in neural networks.

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36. Acknowledgments

I thank Frank Heiligen, Janna Klin, Randall Mauelshagen, and Thomas J. Carew for valuable comments on the manuscript and Rainer Malaka for fruitful discussions on some of the issues raised here. Work from the author was supported in part by grants from the Deutsche Forschungsgemeinschaft (DFG 372, SFB 315, and 315, and Theory of Neural Networks and the Theory of Neural Networks and the Theory of Neural Networks and the Theory of Neural Networks).
NF-κB: a crucial transcription factor for glial and neuronal cell function


Transcription factors provide the link between early membrane-proximal signalling events and changes in gene expression. NF-κB is one of the best-characterized transcription factors. It is expressed ubiquitously and regulates the expression of many genes, most of which encode proteins that play an important and often determining role in the processes of immunity and inflammation.

Apart from its role in these events, evidence has begun to accumulate that NF-κB in neuronal development is suggested from studies that demonstrate its activation in neurons brain function, particularly following injury and in neurodegenerative conditions such as Alzheimer’s disease. NF-κB might also be important for viral replication in the CNS. An involvement of NF-κB in neuronal development is suggested from studies that demonstrate its activation in neurones in certain regions of the brain during neurogenesis. Brain-specific activators of NF-κB include glutamate (via both AMPA/KA and NMDA receptors) and neurotrophins, pointing to an involvement in synaptic plasticity. NF-κB can therefore be considered as one of the most important transcription factors characterized in brain to date and it might be as crucial for neuronal and glial cell function as it is for immune cells.

Review

One of the major questions in signal transduction concerns how receptor activation by extracellular agents leads to changes in gene expression. Transcription factors such as NF-κB provide the link between early signalling events and such changes. This factor was first described in 1986 as a nuclear factor (hence NF) that, when activated by agents such as bacterial lipopolysaccharide, bound to a 10 bp sequence in the enhancer region of the gene encoding κ light chain (hence κ) of antibody molecules in B cells (hence B) (Ref. 1). The name NF-κB is, with hindsight, clearly a misnomer as this factor is widely expressed and regulates the expression of a variety of genes, the majority of which encode proteins important in immunity and inflammation (for an extensive review on NF-κB see Ref. 2). Recently, a role for NF-κB in neuronal and glial cell function has been proposed. As in the periphery, the target genes for NF-κB in brain encode proteins with immune and inflammatory activities. However, evidence is emerging that NF-κB has roles unique to the CNS, in such processes as neuronal plasticity, neurodegeneration and neuronal development.

NF-κB as a signal transducer

In unstimulated cells NF-κB exists in a latent form, complexed to an inhibitory protein, termed IκB. As shown in Fig. 1, upon activation by a wide range of extracellular agents, IκB is phosphorylated by an, as yet unknown, protein kinase. It is then ubiquitinated and is degraded by the proteasome. This allows NF-κB to translocate to the nucleus where it binds to the κB consensus sequence (the commonest form of which is GGGACTTTCC), generally leading to an increase in the expression of the target gene. Eight proteins in the NF-κB family and seven IκBs have been cloned to date. These are summarized in Table 1. The commonest complex that is activated in mammalian cells appears to involve IκBα, bound to the κB/RetA heterodimer. The process of NF-κB activation has been much studied in such cell types as B and T cells, epithelial cells and fibroblasts. In resting cells, the system is