The neural basis of associative reward learning in honeybees

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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.

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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 extrinsic 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[1–4]. 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[5,6]. 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 flowers[7,8] and even abstract features such as the symmetry of visual patterns[9] 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[7,10]. 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 odors[11] 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[8]. 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 conditioning[10], and preconditioning of one odorant blocks the subsequent conditioning of

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Box 1. Error-correcting learning rules

The Rescorla and Wagner rule (R&W rule)[a], an influential behavioral learning rule, makes the change in associative strength ΔV_i of stimulus i in a conditioning trial dependent on a predictive error between actual and 'expected' strength of reinforcement. V_i represents the strength of an associative connection between a stimulus and a reinforcer and determines how well that stimulus predicts reinforcement. The R&W rule can be formalized as:

$$\Delta V_i = \alpha_i \beta (\lambda - \Sigma V_i)$$

where λ is the maximal associative strength a given reinforcer supports and ΣV_i the combined associative strength of all stimuli present at a trial. ΣV_i can be interpreted as expectancy of reinforcement. α_i and β are conditioned stimulus (CS) and unconditioned stimulus (US) related learning-rate parameters. The R&W rule predicts inhibitory learning about i, if i occurs without US ($\lambda = 0$) in the context of other stimuli that predict the US, since then the reinforcement term $\lambda - \Sigma V_i$ becomes negative. Similarly, blocking of learning of i results from compound presentations with a CS that already predicts the US ($\Sigma V_i = \lambda$), since then $\lambda - \Sigma V_i = 0$. Other trial-based rules make learning dependent on alterations in CS processing, assuming that α_i changes with experience[b]. As non-substitutional rules, these rules commonly fail to explain second-order conditioning, in which a learned CS serves as reinforcer, and as trial-based rules they cannot account for time-dependent conditioning phenomena. Real-time models[c,d] overcome both problems as their reinforcement term includes a positive expectancy-related variable (the CS can substitute for the US in driving reinforcement), and some form of temporal derivative of the expectancy of reinforcement (see Refs c,d for further details).

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another odorant when a compound of both is rewarded[17]. 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, Smith[18] 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 memories[19,20], both PER conditioning and learning of flower-related signals appear to follow the same learning rule.

The neural basis of reward and reinforcement processing

In honeybees, the biogenic amine octopamine (OA) enhances olfactory reward conditioning and memory retrieval[3,21] and mediates a transient form of food arousal[22]. 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 the reinforcing function of rewards during olfactory conditioning[23], 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 probosis 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 glomeruli, the calyces of the mushroom bodies (MBs), and the lateral protocerebral lobe (LPL) as potential sites of olfactory reward learning (see Box 2). What is the experimental evidence for the involvement of these networks?

In bees, local-cooling experiments after single-trial olfactory conditioning that induce retrograde amnesia[24] implicate the MBs. The effects of local cooling depend on application time. Resistance to amnesia develops with the same time course for cooling the MB calyces and the whole animal; that of cooling the α-lobe and even the antennal lobe is faster. Thus, a certain period of undisturbed 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

this produces behavioral learning in multiple-trial olfactory conditioning[25].

Physiological evidence that associative plasticity occurs in, or upstream from, the MBs comes from an extensively studied MB-extrinsic neuron, the PE1 neuron[26]. 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 formation 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[24] and substituting the reward in multiple-trial conditioning by injections of OA into the antennal lobe also results in learning[25]. 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.

Comparison with Drosophila *olfactory learning*

In *Drosophila*, the use of structural mutants[27] and chemical-lesioning experiments[28] also implicate the MBs, and octopaminergic drugs influence learning[29]. 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[31], and dopaminergic neurons innervate these MB substructures[30]. This pattern of expression of the DAMB-receptor resembles that of the gene of the learning and memory mutant *rut*, which has a defective adenylate cyclase[30]. From this Davis and colleagues[30,31] suggest that dopaminergic neurons convey reinforcement to the axons of the MB-intrinsic KC. The mismatch between the honeybee and the *Drosophila* model for processing of reinforcement might simply be related to species-specific differences. Alternatively, it could reflect a functional separation of the two aminergic systems. In bees, dopamine does not interfere with memory formation, as does OA (Ref. 3), but selectively with retrieval[32] (possibly indicating dopamine in the modulation of motor aspects of learning and memory). Dopamine-immunoreactive neurons innervate consistently the calyces, and the α-lobe and the peduncles[33] and brain-injected OA, but not dopamine, repairs olfactory conditioning in bees that have been depleted of amines by reserpine treatment[34]. On the other hand, OA and other amines might represent different reinforcing systems. Whereas OA mediates appetitive reinforcement, dopamine might be linked to aversive reinforcement. Interestingly, in *Macaca fascicularis* monkeys midbrain

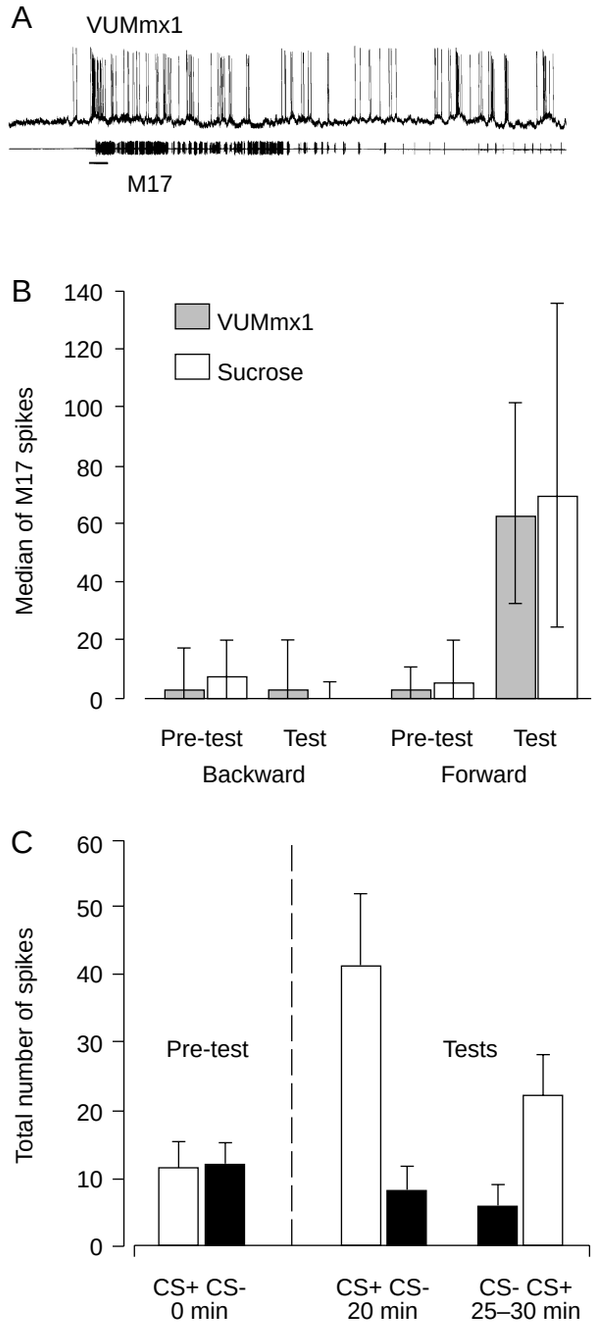

Fig. 1. ***Behavioral learning produced by substitution of reward in proboscis-extension response (PER) conditioning by depolarizing VUMmx1, and experience-dependent response plasticity of VUMmx1 (Ref. 23).*** **(A)** *Excitation of VUMmx1 following sucrose stimulation of the antennae and proboscis. Lower trace: corresponding PER recorded as electromyogram from muscle M17. Stimulus duration 1 s.* **(B)** *Number of odor-evoked M17 spikes (median with interquartile ranges) 5 min before (pre-test) and 10 min after (test) a single forward or backward pairing of an odor with either a depolarization of VUMmx1 or sucrose as reward (see Ref. 23 for details). During pairing, odor onset preceded VUMmx1 depolarization (duration 30 s) or the sucrose stimulus (duration 1 s) by 2 s (forward) or followed onset of depolarization or sucrose stimulation by 5 s (backward).* **(C)** *Odor-evoked activity of VUMmx1 before and after differential conditioning. Total spike number (± SEM) of VUMmx1 in response to two different odors (CS+: rewarded with sucrose, CS–: unrewarded) recorded intracellularly before and after differential conditioning with five CS+ and CS– trials. Spikes were counted during an interval of 15 s after odor onset. Pre-test: response to either odor 5 min prior to differential conditioning. Tests: responses 20 and 25–30 min after pre-test. Two tests were performed with reversed order of odors to control for extinction-like effects.* Adapted, with permission, from Ref. 10.

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[a,b]. Multiglomerular projection neurons connect glomeruli with the lateral protocerebral lobe (LPL) and other parts of the protocerebrum, uniglomerular projection neurons (about 800) with the LPL and the calyces of the mushroom bodies (MBs) via two fiber tracts (see Fig. A) (Refs b,c). 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[d]. Dendrites of individual KCs are restricted to these compartments[d,e] and each KC probably receives convergent input from several projection neurons. The 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[f]. Groups of MB-extrinsic output neurons and large individual neurons[e,g] 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 the subesophageal ganglion[h].

In honeybees (Ref. i; M. Hammer, unpublished) and locust[j], ascending bilaterally symmetric dorsal and ventral unpaired median interneurons (DUM and VUM neurons) of the SOG innervate most principal brain neuropiles with their enormous axo–dendritic arborizations. In bees, these neurons stain with an antibody against the biogenic amine octopamine (OA) (Ref. k), a well-known neuromodulator in insects[l,m]. 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[i].

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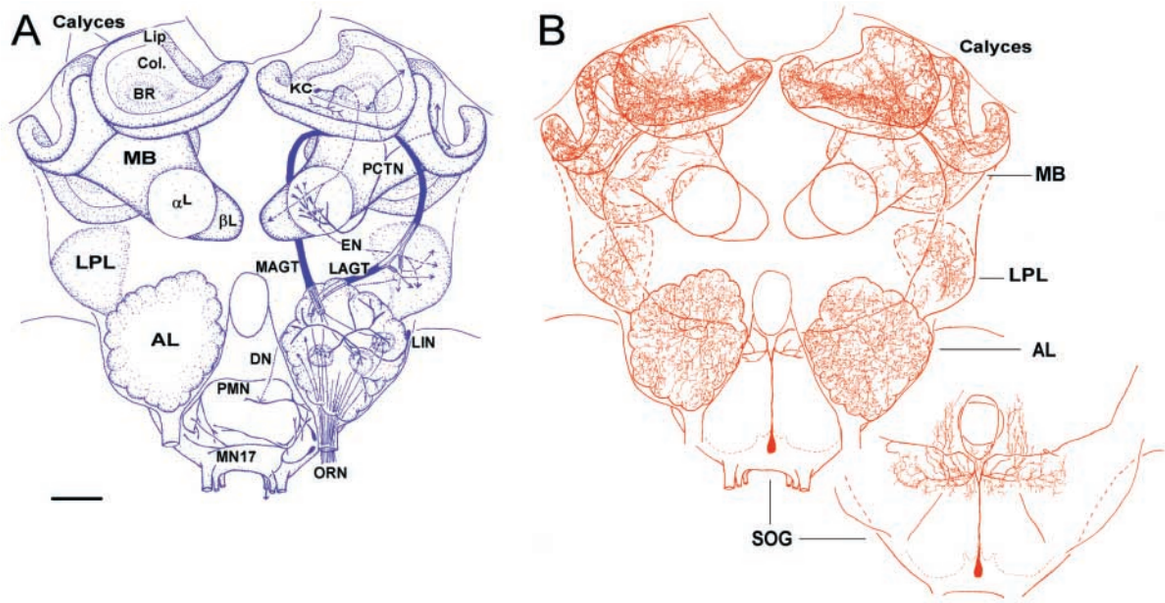

Fig. *Summary of circuit that underlies olfactory PER conditioning.* **(A)** *Schematic diagram of the central honeybee brain. Representatives of the different neuronal groups involved in PER conditioning drawn on the background of neuropile borders. Axonal arborizations of projection neurons in the MB calyces are not shown. Abbreviations: AL, antennal lobe; αL, α-lobe; βL, β-lobe; BR, basal ring; Col., collar; DN, descending neuron; EN, extrinsic MB-output neuron; KC, Kenyon cell; LAGT, MAGT, lateral and median antenno-glomerularis tract, respectively; LIN, local interneurons; LPL, lateral protocerebral lobe; MB, mushroom body; MN17, motor neuron 17; ORN, olfactory receptor neurons; PCTN, protocerebral-calycal tract neuron; PMN, premotor neuron.* **(B)** *Morphology of the VUMmx1 neuron with soma in the ventral subesophageal ganglion (SOG), dendritic arbor in the dorsal SOG, and axonal arborizations in the brain. VUMmx1 was stained intracellularly with Lucifer Yellow and drawn from photomicrographs from whole mounts and serial sections. Scale bar, 100 μm for* (A) *and* (B). Adapted, with permission, from Ref. n.

dopaminergic neurons respond selectively to rewards but not to aversive stimuli[35], 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 not identified (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. Kenyon cells express an ACh-mediated Ca^{2+} current[36] (ACh is a putative transmitter of olfactory projection neurons[37]) and several voltage-dependent inward and outward currents[38], which could be modulated by OA via the activation of second-messenger pathways. An OA-receptor subclass in the insect brain stimulates adenylate cyclase[39]. Therefore, a primary candidate for a molecular substrate of learning is, as in other systems[30,40–42], 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, Müller[45] 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. Müller[45] 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 allows 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.

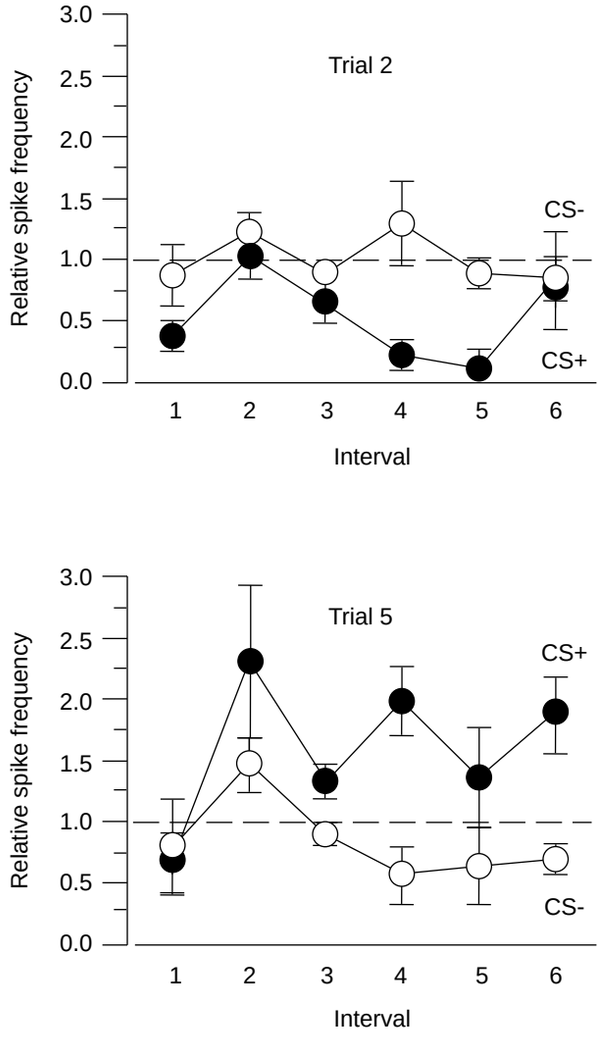

Fig. 2. ***Response plasticity of the PE1 neuron, an mb-output neuron, during differential conditioning[26].*** *Responses of the PE1 neuron to two different odors (CS+ and CS–) during trials 2 and 5 of a differential conditioning protocol. CS+ was paired five times with sucrose (interstimulus interval, 2 s), CS– was delivered unpaired between CS+ trials (intertrial interval 1 min). Spike frequencies of the PE1 neuron are shown during the first 600 ms after odor onset divided into 6 (numbers on abscissae) consecutive 100 ms intervals. Spike frequencies (relative spike frequency; mean ± SEM) in each of the 100 ms intervals are normalized to that of the respective intervals during a control odor stimulus applied 5 min before differential conditioning (dashed line represents the control response level).* Adapted, with permission, from Ref. 10.

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 timing[49]. Moreover, stimulation of ascending neuronal systems, which promotes stimulus-induced synchronization in cortical networks[50], and neuromodulators influence synaptic plasticity in cortical networks[51]. 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 permit coincident-dependent plasticity, or both. This, in turn, could result in neural assemblies that encode

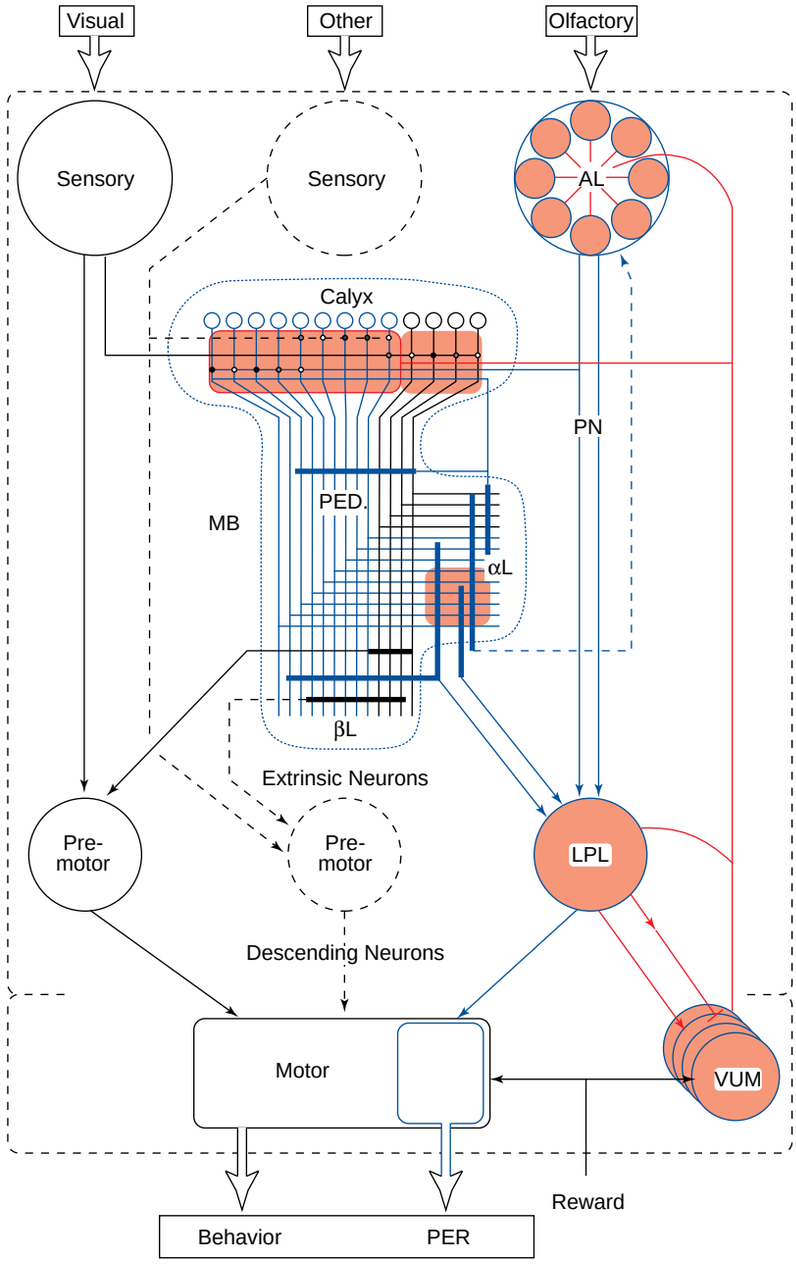

Fig. 3. ***Schematic model of the functional organization of the bee brain that underlies learning, memory, and experience-dependent control of behavior (see text).*** *Blue lines indicate neuropiles and neurons involved in olfaction and odor-driven behaviors. Solid and broken black lines show the putative organization of systems other than the olfactory system. The VUMmx1 neuron, other VUM neurons, and potential sites of odor–reward convergence are shown in red. Light red shading indicates sites in the mushroom body (MB) that exhibit octopamine (OA) immunoreactivity[47] that is not related to the VUMmx1 neuron. Red arrows indicate potential convergence of – memory-processed – excitatory and inhibitory olfactory input onto VUMmx1. Abbreviations: αL, βL, α- and β-lobe, respectively; AL, antennal lobe; LPL, lateral protocerebral lobe; PED., pedunculus; PER, proboscis-extension response; PN, projection neurons.*

behaviorally relevant odors. In addition, NO, which alters oscillations in the olfactory systems in the mollusc *Limax maximus*[52], could mediate the transition to lasting representations.

In natural environments, odors (normally blends of several components) must be learned and detected against fluctuating backgrounds. Reward-related information in the antennal lobe could, on a functional level, serve to enhance the processing of a particular odor during repeated experiences. This, in turn, could facilitate the formation of permanent odor memories 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[53] 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[54,55]. However, output neurons of the MB are frequently multimodal[56], presumably because their dendrites are not restricted to modality-specific sites[26,54,55] and one KC subgroup violates the overall MB topography[55]. Other output neurons of the MB are probably modality specific, for example, olfactory[55]. 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[47], 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 α-lobe with probably all the glomeruli of the antennal lobe[55], 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[26], 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. 3, 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 it activates a neuron or neurons with a putative arousing function. Since VUM neurons, other than VUMmx1, with a different morphology might also be activated by reward-predicting odors this might bias several appetitive behavioral components. Apparently the plasticity of the VUMmx1 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 VUMmx1, dopaminergic neurons of the basal ganglia in *Macaca fascicularis* have experience-dependent properties[57]. They are activated by rewards and reward-predicting stimuli. Moreover, they stop responding to predicted rewards and might, therefore, process a predictive error[6,57,58]. Montague *et al.*[20] proposed a model for bee foraging based on some of the properties of VUMmx1. During flower visits, VUMmx1 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 VUMmx1 by reward-predicting signals. In contrast, reward-predicting odors fire VUMmx1. One possibility that would allow VUMmx1 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 VUMmx1 neuron (red lines in Fig. 3). The inhibitory input should be delayed as proposed by real-time models of associative learning[14,15] to allow for both activation of VUMmx1 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 VUMmx1 neuron during stimulus protocols that lead to inhibitory learning and blocking.

Finally, plasticity of VUMmx1 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 VUMmx1 to these ensembles could allow the whole sequence of the odor representation to be learned. Because odor-evoked activity that follows the onset of VUMmx1 activity does not result in learning, this transfer could also protect learning against 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 this enterprise for the following 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 substrate 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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NF-κB: a crucial transcription factor for glial and neuronal cell function

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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 is involved in 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.

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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[3]. These are summarized in Table 1. The commonest complex that is activated in mammalian cells appears to involve IκBα, bound to the p50/RelA 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

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