The molecular signalling processes underlying olfactory learning and memory formation in honeybees

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Original article

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Abstract – The honeybee *Apis mellifera* provides the opportunity to study molecular signalling processes underlying olfactory learning and memory formation in intact animals. Applying innovative techniques to monitor and manipulate signalling processes *in vivo* during learning led to the identification of dynamic signalling events that contribute to different facets of olfactory learning and memory formation. these techniques opened novel insights into how different training strengths change the dynamics of individual molecular signalling processes, resulting in the induction and maintenance of distinct memory phases. To date, the major contributors were believed to be the mushroom bodies, as shown in *Drosophila*. This *in vivo* work now adds the insight that processes localised in the antennal lobes also contribute considerably to the memory processes. In addition, it shows that the effects of satiation on appetitive learning and memory is most likely mediated by so far unidentified molecular signalling pathways, as the aforementioned evolutionarily conserved and well-known pathways are only partially involved.

learning / memory / second messenger / translation / transcription

1. INTRODUCTION

Within the social context of the hive, individual honeybees *Apis mellifera* perform hierarchic behaviour during brood care, social communication, and foraging. Foraging honeybees are particularly set to optimise their foraging strategies even under highly variable environmental conditions for the benefit of the hive. They solve this task by learning chemical, mechanical, and visual cues associated with the food sources. Studies of honeybee learning under natural conditions provided a large body of detailed knowledge regarding honeybee behaviour (summarised in Collett and Collett 2000; Menzel 2001; Menzel and Giurfa 2006; Menzel and Müller 1996; Srinivasan 2011). Establishing and applying learning paradigms under controlled laboratory conditions was the prerequisite to studying molecular mechanisms of behaviour, such as signalling cascades involved in learning and memory formation. Among the different paradigms (Erber et al. 1997, 1998; Giurfa 2003; Smith et al. 1991; Vergoz et al. 2007) used to analyse behaviour under laboratory conditions, the appetitive olfactory conditioning of the proboscis extension reflex (PER) (Bitterman et al. 1983; Kuwabara 1957) facilitated the most detailed knowledge with regard to the characterisation of learning and memory formation, including the analysis of molecular signalling cascades.

In this appetitive associative learning paradigm, two stimuli are paired: an odour stimulus (conditioned stimulus, CS) predicts a subsequent

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reward (unconditioned stimulus, US). The application to the honeybee reveals characteristics of associative learning very similar to appetitive associative learning in mammals (Menzel and Müller 1996). In honeybees, PER is elicited by an appetitive stimulus (sucrose) to the antennae and/or the proboscis. A single appetitive olfactory conditioning trial consists of an odour stimulus (CS) immediately followed by a sucrose reward to antennae and proboscis (US). This single learning trial takes only a few seconds but already induces an associative memory for the learned odour: a transient memory which remains at a high level for a few hours and decays over the following days (Figure 1) (Menzel 1999; Menzel and Müller 1996). Short interval repetition of the same conditioning trials leads to a stable memory that remains at a high level for many days (Figure 1). Three conditioning trials given within a time window of a few minutes induce a stable long-lasting memory (LTM) that is sensitive to transcription blockers (Grünbaum and Müller 1998; Wüstenberg et al. 1998) and shows all properties of a long-term memory as described in mammals (Davis and Squire 1984; Nguyen et al. 1994).

2. NEURONAL CIRCUITS IMPLICATED IN APPETITIVE OLFACTORY LEARNING

The neuronal circuits that mediate the olfactory and the reward information are well characterised in the honeybee (reviewed in Galizia and Menzel 2000). Chemosensory receptor neurons on the antennae project to the primary olfactory centres, the antennal lobes (ALs), where they terminate in the glomeruli. The 160 glomeruli in each of the ALs are sites of dense synaptic connections between chemosensory neurons (≈60.000), local interneurons (≈4.000), and projection neurons (≈800) (Flanagan and Mercer 1989). Local interneurons connect the glomeruli and build inhibitory networks that modulate the overall activity in the ALs, thus contributing to the sharpening of the odour representation at the level of the projection neurons (Sachse and Galizia 2002). Different types of projection neurons transmit the processed odour information to the lip region of the mushroom body calyces and the lateral horn in the lateral protocerebrum (Arnold et al. 1985). The mushroom bodies (MBs), that are formed by densely packed Kenyon cells, are prominent structures in the insect brain (Mobbs 1982; Witthöft 1967) and play a critical role in olfactory learning and memory formation as demonstrated by extensive studies in *Drosophila* (Davis 2011; Heisenberg et al. 1985). As indicated by the presence of acetylcholinesterase and acetylcholine receptors, the odour input by the projection neurons into the MBs is cholinergic (Kreissl and Bicker 1989). The olfactory input is confined to the lip region of the calyces. Blocking nicotinic acetylcholine receptors (nAChR) at the MB input sites impaired the acquisition of olfactory memory. Detailed pharmacological experiments suggest different functions of at least two nAChRs (summarised in Gauthier 2010). Optical recording techniques *in vivo* demonstrate that odours evoke combinatorial activity patterns in both the glomeruli of the ALs and the lip region of the MBs (Szyszka et al. 2005). Thus, the ALs, the lip region of the MBs, and the lateral horn are activated by odours, and are thus sites involved in the processing of olfactory information.

Gustatory and mechanosensory information is mediated from the tips of the antennae to the dorsal lobes by axons of taste hairs (Haupt 2007). The dorsal lobes are adjacent to the ALs and contain circuits that control antennal muscles and thus movements important for scanning objects with the antennae (Haupt 2004; 2007). Moreover, gustatory information from the tarsae also contributes to appetitive olfactory learning (de Brito Sanchez et al. 2008). So far, however, the neuronal connections of the different gustatory inputs from the antennae, proboscis, and tarsae, as well as their different contributions to appetitive learning, have not been worked out in detail.

Gustatory information is also relayed to the suboesophageal ganglion (Rehder 1989), where motor neurons (control of PER) and the ventral unpaired median neurons (VUM, reward pro-

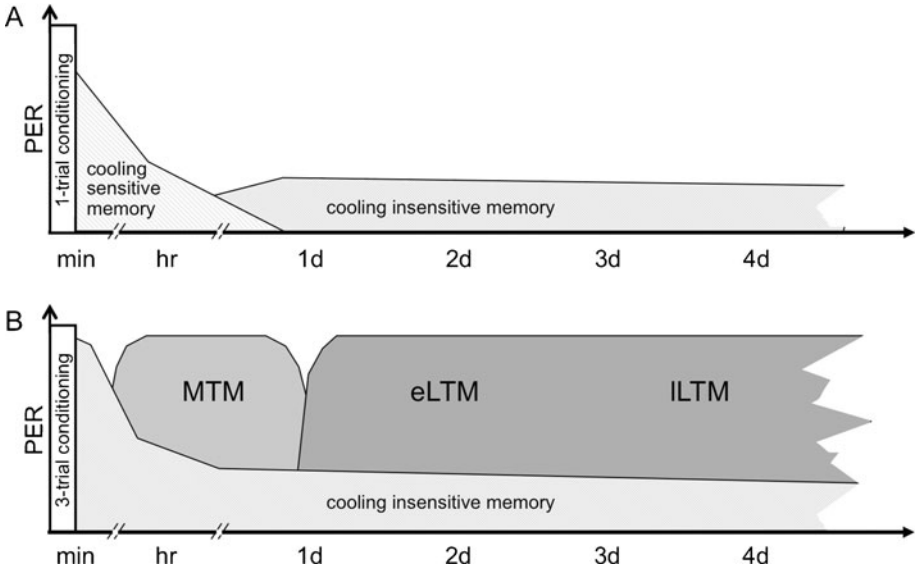

Figure 1. Appetitive olfactory memory in honeybees *Apis mellifera*. **a** A single olfactory conditioning trial induces a memory that is initially at a high level, decays within 1 day, and remains at a low level for several days. Memory performance is evaluated by PER. Cooling of the honeybees immediately after single-trial conditioning erases an early memory phase, defined and indicated as cooling-sensitive memory, while memory from about 1 day is not affected by cooling (cooling insensitive memory). The memory induced by a single-trial conditioning is insensitive to translation and transcription inhibitors. **b** Three conditioning trials applied within a few minutes induce a stable and long-lasting memory that consists of several mechanistically distinguishable phases. No cooling sensitive memory phase is detectable after 3-trial conditioning. An early cooling insensitive memory phase (not so far mechanistically characterised) is followed by a mid-term memory (*MTM*) from about 1 h to 1 day, and a subsequent long-term memory (*LTM*). The LTM can be divided into two phases, an early LTM (*eLTM*) and a late LTM (*lLTM*). eLTM requires only translation processes, lLTM requires both translation and transcription processes. Erasing MTM, eLTM, or lLTM by the appropriate treatments reduces memory performance to the level of the cooling-insensitive memory.

cessing) are located. Especially, the identified neuron VUMmx1 (ventral unpaired median neuron maxillare 1) that substitutes for the US function in associative learning is critically involved in US processing (Hammer 1993; Hammer and Menzel 1995). The octopaminergic VUMmx1 neuron (Kreissl et al. 1994) innervates the ALs, the MBs, and the lateral horn and thus converges exactly with the brain areas that process odour information. Pairing of odour (CS) with an injection of octopamine into the ALs or the MBs leads to a conditioned PER response when tested 20 min after the CS–octopamine pairings (Hammer and Menzel 1995, 1998). However, the PER reaction during the CS–octopamine pairings differs between the groups. Honeybees receiving pairings of CS with octopamine injections into the ALs show a continuous increase in their conditioned response during the successive CS–octopamine pairings. In the case of pairing CS with octopamine injections into the MBs, the honeybees show no conditioned response during the "pairing phase". Moreover, pharmacological blocking and silencing of octopamine receptor expression in the ALs suppressed acquisition and memory recall, but had no effect on odour discrimination (Farooqui et al. 2003). In sum, this points to different roles of the ALs and the MBs in memory formation in agreement with earlier studies, showing that local cooling of either the ALs or the MBs during acquisition has a

different impact on learning (Erber et al. 1980; Menzel et al. 1974).

3. MEMORY FORMATION DEPENDS ON TRAINING PARAMETERS

Training parameters like the number and temporal succession of training trials strongly influence the characteristics of the formed memory. The stronger the training, the longer the memory formed. One of the most basic and conserved rules of memory definition is that formation of long-term memory requires protein synthesis and/or RNA synthesis (Davis and Squire 1984) in all species so far tested. Based on this criterion, at least three distinct memory phases can be identified in honeybees.

A single associative conditioning trial that consists of an odour stimulus (3 s) followed by a partially overlapping sucrose reward (3 s) induces a memory that decays over several days. It is not disturbed in any way by inhibitors of translation and transcription (Figure 1). Exclusively, the early phase in the range of minutes to hours after the single trial conditioning is sensitive to immediate amnestic treatment like cooling (Menzel et al. 1974; Erber et al. 1980; Müller 1996). This, however, does not affect the following slowly decaying memory in the range of days (Müller 1996).

Three successive conditioning trials applied within a time window of a few minutes induce a stable long-lasting memory (Figure 1), defined as LTM (Grünbaum and Müller 1998; Menzel 1999; Menzel and Müller 1996). Blocking translation processes by applying protein synthesis inhibitors during the three-trial associative training phase impairs memory >1 day, while memory performance in the range of minutes to hours after training is unaffected. Actinomycin D blocking of transcription processes leaves memory up to about 2 days unaffected, but impairs memory tested at 3 days or later after training.

Based on the requirement of translation and/or transcription three memory phases can be distinguished after strong training: (1) memory up to 1 day neither requires translation nor transcription; (2) memory in the range of 1–2 days depends on translation (early LTM, eLTM); and (3) memory from 3 days onwards requires both translation and transcription (late LTM, lLTM) (Grünbaum and Müller 1998; Müller 2002; Wüstenberg et al. 1998). Thus, under controlled laboratory conditions, associative olfactory memory in honeybees displays all the different memory phases as observed in other species.

Relying on the ever reproducible behaviour (PER) of the honeybee, the above results set us off on the conceptually new idea of identifying the critical molecular signalling events responsible for triggering the different memory phases during the relatively short associative conditioning.

4. THE MOLECULAR PROCESSES UNDERLYING APPETITIVE OLFACTORY LEARNING AND MEMORY FORMATION

Signalling pathways essential for learning and memory formation have been identified in *Aplysia* and *Drosophila* (reviewed in Kandel 2001). The approach made in the honeybee *in vivo* uncovered the events critical for the induction of distinct memory phases during the aforementioned short time window of associative conditioning. The very short training phase (seconds to minutes) enables a clear separation between the acquisition and the consolidation phases. In accordance with the previous identification of the neuronal networks implicated in processing of olfactory and reward information (Erber et al. 1980; Heisenberg et al. 1985; Hammer 1993; Hammer and Menzel 1995), the analysis of the molecular events was restricted to the ALs and the MBs. Fast-freezing techniques allowed for monitoring learning-induced changes of distinct components of signalling cascades in defined brain areas. At desired time points after conditioning, the *in vivo* induced changes in activity were preserved by freezing the whole honeybee in liquid nitrogen. The tissue of interest was dissected under liquid nitrogen, freeze-dried and subjected to biochemical assays (Hildebrandt and Müller 1995a, b).

4.1. Learning induced activation of cyclicAMP-dependent processes in the antennal lobes contribute to the induction of LTM

Stimulation of one antenna with sucrose results in an immediate and transient increase (<3 s) in the activity of the cAMP-dependent protein kinase (PKA) localised in the ALs. Odour or mechanosensory stimulation has no effects on PKA activity in the ALs (Hildebrandt and Müller 1995a, a). The fact that US-induced PKA activation is mediated by octopamine (Hildebrandt and Müller 1995b) argues for the octopaminergic VUMmx1 neuron (Hammer 1993; Hammer and Menzel 1998) as mediator. Although the VUMmx1 neuron also arborises in the MBs, and although the central role of cAMP-dependent processes in the MBs has been demonstrated in *Drosophila*, no reward-induced changes in PKA activity could be detected in the honeybee MBs.

The temporal pattern of PKA activation triggered by sucrose stimulation of an antenna differs from that induced by the CS–US pairing during conditioning. Compared with the sucrose-induced PKA activation, a single-trial conditioning (CS–US pairing) induces a slightly extended PKA activation that returns to baseline within 60 s (Müller 2000). Repeated conditioning trials that induce LTM trigger an elevation of PKA activity in the ALs that is prolonged up to more than 3 min after the third conditioning trial. The amplitude of PKA activation induced by single- and multiple-trial conditioning is unaffected. This is the first indication that training-induced prolongation of PKA activity in the ALs is implicated in the induction of molecular processes that lead to LTM formation.

Mimicking a locally and temporally defined PKA activation pattern in the ALs, using photolytic release of caged cAMP, was to put this hypothesis to the test. A single-trial conditioning followed by an artificially prolonged PKA activation by photolytic release of caged cAMP is sufficient to induce a long-lasting memory (Figure 2) (Müller 2000). This proves that the learning induced PKA activation that lasts up to a few minutes after conditioning is critical for LTM formation and confirms the initial hypothesis.

The further characterisation of the molecular processes mediating the learning-induced prolonged PKA activation in the ALs identified the unconventional signalling molecule, nitric oxide (NO), as a critical component. In contrast to conventional transmitters that are restricted to single synapses, NO diffuses from its site of production through membranes to act on neighbouring targets. In the honeybee brain, NO producing NO-synthase (NOS) is found at high levels in the ALs and the MB calyces. It plays a role in processing of chemosensory information and learning (Müller and Hildebrandt 1995; Müller 1996, 1997). Inhibition of the NOS during learning impairs both, formation of LTM and learning-induced prolonged PKA activation (Müller 1996, 2000). The same holds for blockers of the soluble guanylyl cyclase (sGC), which produces cGMP upon binding NO (Figure 2). These observations point to a crosstalk between the cGMP and the cAMP systems. In principle, cGMP can interact with the cAMP system via cyclic nucleotide-gated channels, cGMP-dependent protein kinase, or cGMP-regulated phosphodiesterases. Since the honeybee PKA can be synergistically activated by cAMP and cGMP, a direct action of cGMP during the learning-induced prolonged PKA activation in the ALs is possible (Leboulle and Müller 2004).

The latter has been verified using the uncaging technique *in vivo*. As in the case of uncaging cAMP, a single conditioning trial followed by photorelease of cGMP leads to formation of a long-lasting memory (Müller 2000). Thus, in a very narrow time window—during and a few minutes after conditioning—the NO/cGMP system and its action on PKA is critical for the induction of LTM and thus for processes that become evident days later (Figure 2). The molecular and neuronal targets of this early and short-lasting PKA activation in the ALs are as yet unknown. However, due to the central contribution of the MBs to *Drosophila* olfactory learning (Davis 2011), it is very likely that the MBs are the neuronal targets affected by molecular events in the ALs.

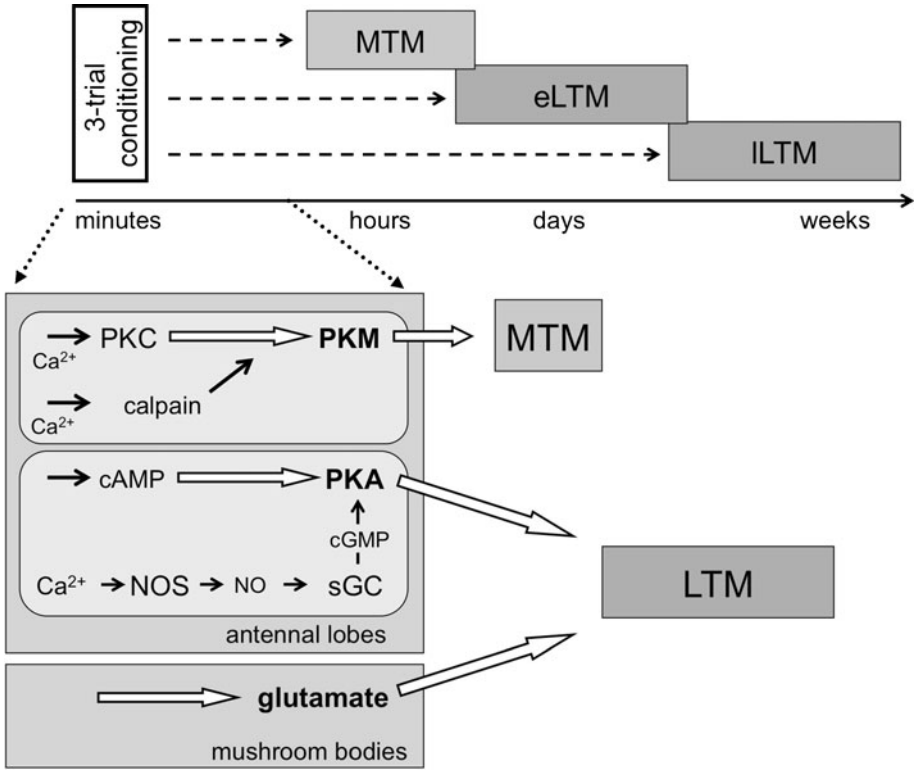

Figure 2. Parallel molecular processes contribute to the formation of different memory phases: *MTM* and *LTM*. In the antennal lobes, at least two independent processes contribute to the formation of two distinguishable memory phases. *MTM* Three-trial conditioning activates both the Ca^{2+}-dependent protease calpain and the Ca^{2+}/phospholipid-dependent protein kinase C (*PKC*). Activated calpain cleaves PKC, which results in the production of constitutively active protein kinase M (*PKM*). PKM, which remains active for hours, is required for mid-term memory (*MTM*). Blocking calpain leads to the specific loss of the MTM phase. *LTM* In an independent process, three-trial conditioning induces a prolonged activation of the protein kinase A (*PKA*) that is essential for the induction of long-term memory (*LTM*). Conditioning activates the formation of nitric oxide (*NO*), which in turn activates the soluble guanylyl cyclase (*sGC*) resulting in cGMP production. The synergistic action of *cAMP* and *cGMP* causes a prolonged PKA activation in the range of minutes, which is required for LTM formation. Blocking NOS, sGC, or PKA activation causes a specific loss of the LTM phase. In addition, glutamate-mediated processes located in the MBs contribute to LTM formation.

It is possible that the cGMP-regulated processes involved in LTM formation are connected to the function of a cGMP-dependent protein kinase also known as the foraging gene (Ben-Shahar et al. 2002). The expression of the foraging gene in honeybees is linked to the transition from hive bee to forager, and has been discussed as an evolutionarily conserved component involved in mediating behavioural changes associated with division of labour in honeybees or foraging variants in other species (summarised in Ben-Shahar 2005).

4.2. Formation and maintenance of the mid-term memory requires Ca^{2+}-regulated processes

Calcium-regulated signalling plays a critical role in numerous physiological processes. Especially, the phosphorylation of target proteins

mediated by the Ca^{2+}/phospholipid-dependent protein kinase C (PKC) is essential for very distinct aspects of synaptic plasticity and memory formation in mammals (Sacktor 2008). In the ALs of honeybees, US and CS stimulation alone, as well as CS–US or US–CS pairings, induce a transient activation of PKC (Grünbaum and Müller 1998). A stimulus-induced PKC activation was not detected in the MBs. Independent of the number of conditioning trials, the conditioning-induced PKC activation in the ALs lasts a few minutes. Since inhibition of PKC during the conditioning phase has no effect on behaviour, the immediate elevation of PKC activation does not contribute to learning and memory formation. In contrast to single-trial conditioning, however, repeated conditioning trials lead to a second increase in PKC activity in the ALs beginning 1 h after conditioning and lasting up to 3 days (Grünbaum and Müller 1998). This long-lasting PKC activation triggered by associative conditioning can be dissected into two different phases, an early and a late phase. The elevated PKC activity between 1 h and about 16 h (early phase) is due to the constitutively active protein kinase M (PKM), a cleavage product of PKC (Figure 2). The cleavage of PKC to PKM is mediated by the Ca^{2+}-dependent protease calpain and only occurs if both PKC and calpain are activated. Blocking calpain activity during conditioning erases PKM production and memory in the time window from 1–16 h after training. Memory tested up to 30 min or 16 h after training is not affected by blocking calpain. Thus, training-induced formation of PKM is required to maintain mid-term memory (MTM) (Figures 1 and 2).

The multiple-trial induced increase in AL-PKC activity in the time window of 1–3 days after conditioning (late phase) is unaffected by calpain blocker, but is erased by translation and transcription inhibitors (Grünbaum and Müller 1998). Up to now, the contribution of the elevated late phase PKC activity (1–3 days) to memory formation is unclear. The early and the late phases of elevated PKC activity and the fast PKA activation in the ALs are based on distinct mechanisms and do not interfere with each other (Figure 2). These findings nicely demonstrate that training triggers several parallel molecular mechanisms that independently contribute to distinct aspects of memory formation. The function of persistent active PKC in memory formation seems to be conserved, since it has later been described in *Drosophila* and mice (Drier et al. 2002; Sacktor 2008)

4.3. Fast glutamate mediated processes in the mushroom bodies contribute to LTM formation

Compared to the enormous knowledge of glutamate transmission in the mammalian brain (Riedel et al. 2003), the contribution of glutamate-mediated transmission in neuronal plasticity in the insect brain is poorly understood. The components required for glutamatergic transmission exist in insects, and several reports show that interference with these components causes learning and memory deficits (Bicker et al. 1988; Barbara et al. 2005; Kucharski et al. 2000; Maleszka et al. 2000; Funada et al. 2004; Si et al. 2004; Zannat et al. 2006). In both honeybee and *Drosophila*, the down-regulation of NMDA-type glutamate receptors lead to the impairment of olfactory learning and long-term memory (Xia et al. 2005; Müssig et al. 2010). Experiments using photolytic uncaging of glutamate *in vivo* offered more specific information concerning the temporal requirement of glutamate during conditioning in honeybees (Locatelli et al. 2005). Only the release of glutamate in the MBs affects memory formation. While release before a single-trial conditioning has no effect, the release immediately (≈3 s) after conditioning improves memory formation. This memory, when tested 2 days after training, is elevated to a level that normally is only observed after multiple-trial conditioning (Figure 2). Thus, in the honeybee, temporally defined glutamate-mediated processes localised in the mushroom body contribute to LTM formation. These findings support the idea that glutamatergic neurotransmission in honeybees is involved in the induction of long-lasting neuronal plasticity

as known from mammals (Riedel et al. 2003; Locatelli et al. 2005; Müssig et al. 2010).

5. INTERNAL AND EXTERNAL PARAMETERS AND THEIR INFLUENCE ON LEARNING PERFORMANCE

With their complex interaction of external, social, and individual factors honeybees are ideally suited to analyse the processes underlying these interactions. Already the probability to elicit the PER, which is used to monitor appetitive learning, depends on sucrose concentration (Braun and Bicker 1992) and is influenced by parameters like age, genotype, social role, motivation and others (Ben-Shahar and Robinson 2001; Page et al. 1998; Scheiner et al. 2001). This and the finding that internal physiological states modulate the sensitivity of olfactory receptor function in the ALs of *Drosophila* (Root et al. 2011) suggests an interference of internal and external processes at the different levels of signal processing involved in learning. Although the impact of circadian rhythm, foraging, social interaction, and navigation on behaviour has been addressed, and some of the involved molecular components have been identified (summarised in Ben-Shahar 2005; Bloch 2010; Rueppell et al. 2004; Srinivasan 2011; Toth and Robinson 2007), their action on the molecular machinery underlying learning and behaviour remains mostly unclear. A few studies show that the effects of internal and external parameters on behaviour are only partially mediated via the already characterised, evolutionarily conserved signalling cascades that contribute to learning and are described above. The identification and characterisation of other contributing signalling cascades is in full progress.

5.1. Nutritional effects on appetitive olfactory learning and memory formation

Appetitive learning and retrieval of appetitive memory in honeybees and in *Drosophila* is promoted by hunger and suppressed by feeding (Ben-Shahar and Robinson 2001; Friedrich et al. 2004; Chabaud et al. 2006; Krashes and Waddell 2008). Thus, feeding animals before appetitive conditioning or memory retrieval results in a suppressed behavioural performance, suggesting a motivational influence on the underlying signalling processes. Studies in *Drosophila* demonstrate that the formation of a stable appetitive memory requires a postingestive reward system that evaluates the nutritional quality of the ingested sugar (Burke and Waddell 2011; Fujita and Tanimura 2011). Honeybees readily learn to associate an odour with an appetitive stimulus to the antenna (Bitterman et al. 1983). The formation of an appetitive LTM, however, also requires the ingestion of the rewarding sucrose (Wright et al. 2007).

Analysing the relationship between the satiation level and the signalling processes underlying appetitive learning in honeybees uncovered a new feature concerning LTM formation (Friedrich et al. 2004). As compared to honeybees starved for 18 h, animals fed 4 h before appetitive olfactory conditioning show a decreased acquisition and memory formation. Interestingly, the basal activity of the cAMP-dependent protein kinase PKA, which is implicated in LTM formation, is correlated with the satiation level. Bees starved for 18 h show a higher basal PKA activity in their brains than bees fed 4 h before (Friedrich et al. 2004). Artificial elevation of the low basal PKA activity in fed animals specifically rescues the transcription-dependent lLTM but not eLTM (Figure 2). Manipulation of basal PKA-activity does not affect the acquisition phase or other memory phases; all are at a low performance level typical for fed animals.

Since PKA activation induced by multiple-trial conditioning is required for both eLTM and lLTM, these findings argue for a more diverse function of the PKA pathway in LTM formation. Strong training induces at least two different PKA-mediated pathways that contribute to LTM formation; the satiation level influences one of these pathways (Figure 2). Identification of the latter signalling pathway is of special importance with regard to the effect

satiation exerts on single-trial learning. Feeding before appetitive conditioning (Friedrich et al. 2004) and cooling immediately after learning (Erber et al. 1980) are presently the only treatments to interfere with memory induced by a single conditioning trial. Thus, the identification of the pathways contributing to the satiation dependent effect of single-trial learning would be a first step to understand the molecular processes underlying memory formation by a single-trial training.

6. CONCLUSION AND OUTLOOK

From early on, the studies on *Drosophila* highlighted the central function of the MBs as the location of molecular processes involved in associative learning (Davis 2011; Heisenberg et al. 1985). While the critical contribution of the cAMP-dependent processes to memory formation was initially identified with the help of mutants, sophisticated techniques meanwhile allow cell specific manipulations with a high temporal resolution (Brand and Perrimon 1993; Lima and Miesenböck 2005; McGuire et al. 2004). Light-triggered activation/inactivation of neurons or temperature-sensitive suppression of synaptic transmission of neurons led to the identification of the different neuronal circuitries mediating distinct features of associative learning and memory formation (Claridge-Chang et al. 2009; reviewed in Davis 2011). These studies demonstrated that the subsets of neurons in the MBs that mediate distinct aspects in *Drosophila* associative learning are quite small. If the same applies for honeybees, this would provide the explanation why the biochemical measurement of large parts of the honeybee MBs failed to detect stimulus- or learning-induced changes in the activity of signalling cascades located in the MBs.

Although the differences regarding the behavioural repertoire and the social interactions between both species are obvious, and the investigations are based on different techniques, the findings in the honeybees with their focus on the ALs and the findings in *Drosophila* mainly focussed on the MBs lead to a consistent picture. Processes in the ALs play a central role in the induction of memory immediately after association and in very early processes of memory formation. Processes located in the MBs are of especial importance for the establishing of long-lasting memories. Presently, cell-specific monitoring and fast manipulation of neuronal processes during learning are restricted to a few tools (turning on and off neuronal activity and synaptic release) established in *Drosophila*. In the honeybee, manipulation of specific signalling processes is possible but lacks cellular specificity. To enable monitoring and cell-specific manipulation of any molecular signalling cascade of interest during the fast learning processes *in vivo*, new tools will have to be developed.

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Les processus de signalisation moléculaire à l'origine de l'apprentissage olfactif et de l'acquisition de la mémoire chez les abeilles.

Apprentissage / mémoire / second messager / translation / transcription

Die molekularen Signalwege des olfaktorischen Lernens und der Gedächtnisbildung von Honigbienen.

Lernen / Gedächtnis / second messenger / Translation / Transkription

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