

CHAPTER 7

VARIATION IN LEARNING OF HERBIVORY-INDUCED PLANT ODOURS BY PARASITIC WASPS

From brain to behaviour

HANS M. SMID

*Laboratory of Entomology, Wageningen University, Binnenhaven 7, 6709 PD
Wageningen, The Netherlands. E-mail: hansm.smid@wur.nl*

Abstract. Two closely related parasitic wasp species, *Cotesia glomerata* and *Cotesia rubecula*, lay their eggs in first-instar caterpillars of *Pieris brassicae* and/or *Pieris rapae* hosts. They find their hosts by responding to secondary plant metabolites, induced by herbivory. Both wasp species have an innate preference for the odours of infested cabbage, common host plants of these *Pieris* caterpillars, but they can also learn to respond to the odours of other host plants, after they have found suitable host caterpillars on that plant. This experience results in an association of the odours of that plant with the presence of suitable hosts. The two wasp species differ profoundly in olfactory learning; *C. glomerata* instantly changes its innate preference for cabbage odours towards the odours of another plant after a single experience, whereas *C. rubecula* never changes its innate preference for cabbage odours. Both wasps show an increase in flight response to a previously unattractive host plant after a single oviposition experience on that plant, but this memory wanes in *C. rubecula* after a day, and remains unchanged for at least 5 days in *C. glomerata*.

In this paper, ultimate factors are discussed that may have contributed to the evolution of the observed differences in learning in these two wasp species. Furthermore, hypotheses on the possible neural mechanisms and genes underlying these differences are given, based on current knowledge on the cellular mechanisms of learning as determined for genetic and neurobiological model species like the fruit fly *Drosophila melanogaster* and the honeybee *Apis mellifera*.

Keywords: learning; memory; olfaction; parasitoid; *Cotesia*; synaptic plasticity; octopamine; CREB; conditioning

INSECTS AND LEARNING

Many people have the idea that insects are little programmed machines, designed to perform a set of simple behaviours in a fixed way, and that they are in no way functionally comparable to higher animals. Current research has shown this idea to be entirely wrong (Collett and Collett 2002; Giurfa 2003; Watanabe et al. 2003). It may feel uncomfortable to man, but inside the head of, e.g., a tiny fruitfly exists a brain of a mere halve millimetre, housing some 200,000 neurons that function in a way that is not different from the 100 billion neurons in our human brains (Figure 1). The networks formed by the fly's neurons result in a functional brain with Marcel Dicke and Willem Takken (eds.), *Chemical ecology: from gene to ecosystem*, 89-103 © 2006 Springer. Printed in the Netherlands.

remarkable capacities, including the ability to learn. It is obvious that the fly's cognitive possibilities are limited, but it is well equipped to respond in a flexible way to its environment, and to gain from its previous experiences. Thus, an experienced insect can display a dramatically different behaviour compared to a naive insect through learning, and this learning effect can last for the rest of the insect's life.

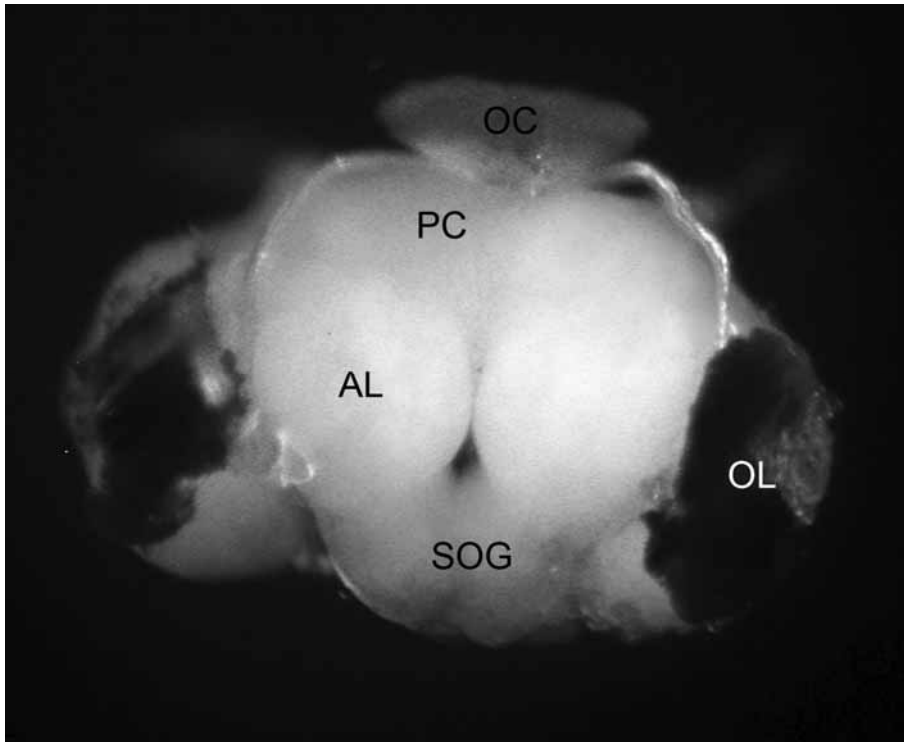


Figure 1. Brain of a parasitic wasp, *Cotesia glomerata*. The size of this brain is approximately 750 μm width. AL, antennal lobe; OL, optic lobe; SOG, suboesophageal ganglion; OC, ocellus; PC, protocerebrum

Insects are well equipped for associative learning. They can learn quickly to search for items by responding to a cue that has previously been rewarded, or they can avoid cues that were sensed within a negative experience. Within the context of the theme of this volume, I will focus on olfactory learning in parasitic wasp species attacking the larval stage of cabbage butterflies (*Pieris* spp.). Having said that, insects are by no means limited to olfactory learning alone.

PARASITIC WASPS AND ASSOCIATIVE LEARNING

Parasitic wasps lay their eggs in or on their insect hosts. There are wasps that parasitize eggs, larvae, pupae or adults. One example is the species *Cotesia glomerata* (Figure 2). This wasp species lays its eggs in young larvae of Pierid butterflies, such as the large and small cabbage white, *Pieris brassicae* and *P. rapae*, resp. The eggs of the wasp hatch inside the body of the caterpillar and both the caterpillar and wasp larvae develop until they reach the final larval instar. At that point, the wasp larvae eat their way out through the cuticle of the caterpillar, spin a



Figure 2. Cotesia glomerata ovipositing in a Pieris brassicae caterpillar

cocoon and moult to the pupal stage, leaving the dying caterpillar behind. The adult wasps that emerge from the cocoons have three different foraging tasks: (1) to find a mate, (2) to find food (nectar or honeydew) and (3) to find host larvae to lay their eggs. The latter foraging task, which is obviously only relevant for females, will be the focus of this paper. The tiny young host caterpillars take care not to spread attractants, they are well camouflaged and do not emit odorants themselves that can be perceived by the wasps from a distance. However, feeding by the caterpillars on their host plants induces the emission of volatiles from their food plant, and these are highly attractive to the wasp. In the case of *Cotesia glomerata*, the odour of cabbage damaged by feeding *P. brassicae* larvae is highly attractive (Geervliet et al. 1994). The response to the odour of damaged cabbage plants is high in naive wasps, and it is not necessary for the wasp to learn to recognize this odour (Geervliet et al. 1996). However, wasps can learn to associate odours from another plant species to the presence of suitable hosts, if they have an oviposition experience on that plant. In this way they can learn to change their foraging behaviour; after the experience on a different plant species, they will specifically search for that plant species (Geervliet et al. 1998). They have learned to associate the odours of that plant with the presence of suitable host caterpillars.

DIFFERENT FORMS OF LEARNING

To understand learning behaviour it is essential to discriminate between the different forms of learning (Rescorla 1988; Krasne and Glanzman 1995). Most important, there is associative learning but there are also simpler, non-associative forms. Sensitization and habituation are examples of non-associative learning. During habituation, an animal is repeatedly stimulated by a stimulus which results in a gradually lower response to that stimulus. Habituation can last minutes to hours, and if many repetitions of the stimulus are given, even much longer. This form of non-associative learning is very important for an animal, because it enables the animal to learn to ignore unimportant stimuli. Habituation is the result of active suppression of the response. It is a process that is different (though often difficult to separate) from sensory adaptation or muscle fatigue. This can be demonstrated when the habituated stimulus is given after a noxious stimulus (e.g. a shock). The response to the habituated stimulus is then completely recovered; this process is called dishabituation (Corfas and Dudai 1989). This phenomenon ensures that an animal can adequately respond to a previously habituated stimulus after an important change in the situation, as perceived by, e.g., a noxious stimulus.

Sensitization occurs when an animal is stimulated by a significant stimulus, such as a shock, a reward, a loud noise or strong odour. This form of non-associative learning is non-specific, and it is characterized by a general increase in response to other stimuli. This effect can, like habituation, last from minutes to hours or even weeks. Sensitization enables an animal to respond better to stimuli when confronted with a significant change in the situation, as signalled by the stimulus that induces sensitization.

Associative learning is different from habituation and sensitization in that it can only occur if two stimuli are presented to the animal; a neutral stimulus, immediately followed by a meaningful, reinforcing stimulus, which can be a reward or a punishment. The animal learns to associate the neutral stimulus with the reinforcing stimulus. This form of learning is called classical conditioning or Pavlovian conditioning, after the famous researcher Pavlov (1927). He trained his dog to respond to a sound, by giving it food as a reward each time it heard the sound. After several pairings of the sound and the reward, the dog started salivating when it heard the sound only. The neutral stimulus that becomes associated with the reward is called the conditioned stimulus (CS), the reinforcer (the reward or punishment) is called the unconditioned stimulus (US). The dog shows the unconditioned response (UR, salivation) to the US. Only after conditioning, it shows the conditioned response (CR, also salivation) to the CS. Only if the CS is directly followed by the US, which is called forward pairing, associative learning will occur. Backward pairing (US followed by CS) does not result in associative learning, but sensitization can occur, resulting in a temporary increase of the response to the CS.

Another form of associative learning is operant conditioning (Thorndike 1901; Skinner 1938). Here, a CS triggers a specific behavioural response, which is followed by the reinforcer only when this appropriate behavioural response is performed. Thus, it is the behavioural response to the CS which is reinforced by the US; an association is formed between the response to the CS and the reinforcer,

rather than between the CS and the US as in classical conditioning. However, elements of classical conditioning are present in operant conditioning as well, and it is sometimes difficult to determine whether a form of learning is purely classical conditioning or operant conditioning (see for a comprehensive overview of both forms of conditioning: Lutz (1994)).

PARASITIC-WASP LEARNING AS CLASSICAL CONDITIONING

How does the theory of associative learning translate into the example of parasitic-wasp olfactory learning? The wasp first smells the odour of a plant on which host caterpillars are feeding, and responds to it by landing on a leaf, where it is rewarded with the presence of suitable host caterpillars. Thus, this is a clear form of operant conditioning. However, in the laboratory set-up, the flight response is usually not incorporated in the learning trial, for reasons of standardization and convenience. The wasp is placed directly on the leaf with caterpillars and stimulated by the taste of host-derived substances such as faeces (frass). The mere perception of host traces on the leaf without any behavioural response is sufficient to learn to recognize the odours of the leaf (Geervliet et al. 1998), which is in line with the CS-US contingency of classical conditioning. The odour of the leaf is the CS and the taste of host-derived substances is the US in this case. Thus, an association between the plant odours and the presence of suitable host caterpillars is already made before oviposition has taken place. However, the increased response to the plant odours lasts longer when the perception of host traces is followed by oviposition (Takasu and Lewis 2003). The test for memory formation is done in a wind tunnel set-up, where it is given a choice to fly towards the naively preferred plant or to the experienced plant. This is actually an operant context; the wasp has learned to adapt its flight response in a classical conditioning learning paradigm. Such a transfer of information has been demonstrated also in another learning paradigm, the proboscis extension reflex of the honeybee (Bitterman et al. 1983). Here, a harnessed honeybee learns to associate an odour with the reward, an application of a droplet of sugar water on its antennae in a purely classical conditioning set-up. Before the learning trial, the bee responds to the US (sugar water) by extension of its proboscis (UR), and after the learning trial, the bee responds to the odour (CS) by extension of the proboscis (CR). Also in this case, the trained bee shows that this experience changes searching behaviour under free flight conditions (Sandoz et al. 2000), thus in an operant context.

DURATION OF MEMORY

Learning results in the formation of memory. Like in higher animals, insects have different forms of memory, ranging from short-term to long-term memories. A number of factors influence what kind of memory is formed; the strengths of both US and CS, the number of repetitions of US-CS pairings, the time interval between the repetitions (the inter-trial interval) and the time interval between the US and CS (the inter-stimulus interval) (Menzel 1999; Menzel et al. 2001). In general, the

longer the inter-stimulus interval, the weaker the association between the stimuli. A single US-CS pairing usually results in memory that lasts no more than hours or one day. If repeated US-CS pairings occur, long-term memory can be formed, but only if there is some time in between each learning trial. This is called spaced learning trials, in contrast to massed learning, which means that the learning trials are in rapid succession following each other. Massed learning is not effective to induce the formation of long-term memory, whereas an identical number of spaced learning trials is (Menzel et al. 2001).

In the case of a parasitic wasp that encounters a gregarious host (e.g. several caterpillars feeding together on a single leaf), the rapid sequence of ovipositions should probably be considered a mass learning experience, although the breaks that wasps often take after several ovipositions before they resume their attacks may interfere with this conclusion. Only after the wasp leaves the plant and lands on another plant of the same species and encounters host frass, an additional conditioning trial occurs that matches the criteria for spaced learning trials.

The mechanism that a long-term, stable memory is only formed after several experiences makes sense. This way, only relevant, reliable information is stored. A single experience results in a less stable memory form that wanes if it is not reinforced by additional experiences. This mechanism of memory formation serves as a filter that ensures that only important and reliable information is stored in long-term memory. It is not efficient to learn too fast, because this way an animal will easily store the wrong information.

DIFFERENCES IN LEARNING BETWEEN CLOSELY RELATED SPECIES.

Closely related species, for example of parasitic wasps, may display large differences in learning (Poolman-Simons et al. 1992; Potting et al. 1997; Geervliet et al. 1998). Such closely related species are ideal subjects for a comparative approach.

The wasp, *C. glomerata* learns to change its innate preference for cabbage plant odours towards the odours of another plant species, *Nasturtium*, which is an alternative host plant of *P. brassicae*. One day after the learning trial (an oviposition experience on a *Nasturtium* leaf) the wasps were released in a wind tunnel where they could choose between a leaf from a cabbage plant and one from a *Nasturtium* plant, both infested with the same number of host caterpillars. The wasps did no longer prefer cabbage but landed on the *Nasturtium* leaves (Geervliet et al. 1998). However, when this experiment was performed with *C. rubecula*, which is a closely related species (Michel-Salzat and Whitfield 2004), the preference shift did not occur. Even after several experiences, the wasps continued to choose the cabbage odours for which they had an innate preference. In a subsequent study, Bleeker et al. (in press-a) investigated this phenomenon in a different way. They did not measure a change in preference using a wind-tunnel set-up with a choice situation (a dual-choice set-up), but measured the flight response to the learned plant odour with a single-choice set-up (also called a no-choice set-up), using controls that distinguished sensitization from associative learning. This way, the increase in response to the *Nasturtium* odours can be determined irrespective of the strength of

the innate preference to cabbage odours. The wasps were released in the wind tunnel and the number of wasps that landed on the *Nasturtium* leaves was determined before and at different time intervals after the oviposition experience. Two remarkable results were obtained in this study. First, *C. glomerata* formed a long-lasting memory of at least 5 days after a single oviposition experience, which is in contrast to the notion that several spaced learning trials are necessary for long lasting memory. Second, it was shown that *C. rubecula* learned to respond to the *Nasturtium* odours by associative learning, even though this memory waned gradually after one day. Thus, *C. rubecula* does learn to associate the odours of *Nasturtium* to the presence of hosts (albeit that this memory lasts much shorter than in *C. glomerata*), but does not change its innate preference for cabbage odours. These two wasp species are closely related, have very similar morphology, including brain morphology (Smid et al. 2003), olfactory sensilla morphology (Bleeker et al. 2004) and olfactory receptive range (Smid et al. 2002). The difference in learning may be an adaptation to the specific differences in host-finding behaviour between the two wasp species.

ULTIMATE FACTORS THAT MAY BE CORRELATED TO VARIATION IN LEARNING

There are a number of ultimate factors commonly associated with variation in learning (see for reviews Shettleworth 1993; Turlings et al. 1993; Vet et al. 1995). What factors drive the evolution of slow learners (i.e. species that need many repeats of experiences to adapt their behaviour), and what factors drive towards fast learning (i.e. species that adapt their behaviour upon a single experience)? What are the limits to the amount of information that can be stored in the tiny brains of parasitic wasps? It is important to realize that learning and memory are costly processes at different levels. There is the energy cost of learning and of the formation and maintenance of memory (Dukas 1999; Mery and Kawecki 2003; 2005). In addition to the energetic costs of memory there is also the ecological cost. Learning trials take time and may constitute a risk (e.g. predation) compared to innate behaviour (see below), and the information that is learned may be wrong, leading to maladaptive behaviour with strong fitness penalties. However, these ecological costs to learning can be different between species (Dukas 1998b).

First, the life span of the animal is important. It is obviously not useful to spend much time on learning for an insect that lives for only one day. Also the number of foraging decisions that an insect makes, determines that a certain subset of these decisions can be spent to learn to optimize the remaining part of the foraging decisions (Roitberg et al. 1993; Dukas and Kamil 2001). Learning takes time, hence there must be a certain optimum of learning trials to spend on learning, and if time is relatively costly, like it is for a short-lived insect, this optimum will be driven towards fewer learning trials.

Another important factor is the reliability of the association that is learned. If the associations that are learned are very variable, more learning trials are necessary before the animal should adapt its behaviour to a supposed relevant new situation.

The strengths of fitness penalties that come with wrong associations are important factors that drive evolution of slower learning. If the information is less variable, fewer learning trials are needed to change behaviour. Thus, the variability of the environment may influence the learning speed of the insect; an insect species living in a less variable environment may evolve towards a fast-learning species, whereas an insect living in a highly variable environment, where there is little predictive value from a single experience, may evolve towards a slow-learning species.

Stephens (1993) distinguished within-generation variability and between-generation variability. In this model, learning is favoured when between-generation predictability is low but within-generation predictability is high. If between-generation predictability is high, innate behaviour is expected to be favoured over learning. Parasitic wasps have a strong innate response to, e.g., the taste of sugars or the taste of host-derived substances, as these substances are invariable between generations. However, the response to plant odours on which the parasitoid's host may occur depends on the predictive value of that odour to the wasp. When the host occurs only on that plant species, the predictive value is high and a strong innate response is favourable (Vet et al. 1990; Vet and Dicke 1992). If the host occurs on several plant species, the innate responses to those plant species are expected to be intermediate, but become stronger after repeated experiences. If the host occurs on several other plant species that are also available in the area, the predictive value is low. The wasp needs to respond to all odours of potential host plants on which its host may be present, and it has to divide its attention over a wide range of plant species. This is disadvantageous for two reasons. First, because herbivory-induced plant odour blends are difficult to detect against a background of non-relevant plant odours, detection becomes less efficient when the wasp has to divide its attention to several different potentially relevant stimuli (the problem of limited attention, Bernays and Wcislo 1994; Bernays 1996; Dukas 1998a; 1998b; Dukas and Kamil 2001). Second, the wasp needs to spend time to visit several host plants that will not be rewarding. Specialization is thought to be an adaptation to this problem. Preference learning can be seen as a way to achieve temporal specialization (Dukas 1998b). Thus, learning is a trait that may be tightly linked to the level of specialization of both the wasp and the host, and especially generalist wasps that parasitize on generalist hosts may benefit from learning by gaining from the advantages of specialization.

In conclusion, there are several factors that may influence learning of a parasitic wasp. How can these ideas help us to understand the difference in learning between *C. glomerata* and *C. rubecula*?

In the case of *C. rubecula*, the wasp remembers the odours of a plant after an experience for a short term, but does not change its preference. Apparently, the cabbage odours remain the most reliable indicators for the presence of hosts. This may be an adaptation to the oviposition behaviour of its host, which is the small cabbage white, *Pieris rapae*. This butterfly lays only a single egg on a plant and does this in a rather unpredictable way (Root and Kareiva 1984; Davies and Gilbert 1985), possibly to avoid parasitization. Thus, the predictive value of a single oviposition experience for *C. rubecula* is low. Apparently, the trait of learning in the brain of this wasp species is adapted to the oviposition behaviour of its host species.

Moreover, *C. rubecula* is a solitary species that lays only one egg into a host, and has to find a large number of hosts on a large number of plants. Thus it makes a large number of foraging decisions. This may also contribute to the slow learning speed.

For *C. glomerata*, the situation is profoundly different. This species is more of a generalist than *C. rubecula*, but our population, which was collected in The Netherlands, strongly prefers the large cabbage white, *Pieris brassicae*. This butterfly species lays clusters of eggs. The caterpillars, after hatching, completely destroy the plant on which they are feeding and subsequently have to migrate to neighbouring plants. Due to their induced dietary specialization, they need to migrate to the same plant species as the one on which they initiated feeding, and therefore the butterfly has to lay its eggs on dense stands of plants of the same species (Le Masurier 1994). Such dense stands are likely to attract more ovipositing butterflies than single plants, and that may be a reason that a single oviposition experience of *C. glomerata* on *P. brassicae* is reliable enough to induce long-term memory formation. Moreover, *C. glomerata* is a gregarious wasp that lays several eggs into a single caterpillar. This means that it can oviposit half of its lifetime fecundity into a single clutch of caterpillars. Thus it needs only a few foraging decisions (see Roitberg et al. 1993), and it may well be the optimal strategy to learn to keep searching on the dense stand of the same plant species because chances are high to discover another rich source of oviposition opportunities.

NEURAL BACKGROUNDS: WHAT HAPPENS IN THE BRAIN DURING LEARNING AND MEMORY FORMATION?

In order to understand how evolution shapes learning, it is crucial to identify the neural mechanisms that are underlying these differences in learning. Which genes and which neurons are involved, and how do they encode for the differences in learning? Only if this information becomes available, will it be feasible to study variations in those genes and neurons in a large number of different species and predict variation in learning ability and correlations with ultimate factors. I will first focus on the level of small neural networks to explain what happens during classical conditioning in the brain of an insect, and then descend to the molecular level to focus on some genes that play a key role in learning.

When an association is made between a CS and a US, it is obviously necessary that the neural responses to these two stimuli are somehow brought together in the brain. Either a reward or a punishment can serve as reinforcement in olfactory conditioning that stimulates formation of odour memory, so that an association is made between the odour and the reward or punishment. How this mechanism works in the case of reward learning was described for the honeybee (Hammer 1993; 1997). The honeybee is a well-known model animal for neurobiological research on classical conditioning. Much research is done using proboscis extension reflex (PER) conditioning. Honeybees extend their mouthparts (proboscis) when stimulated by sugar solution on the taste sensilla on the antenna and mouthparts. This reflex can be conditioned when the sugar stimulus is preceded by an odour

stimulus. The bee learns that the odour predicts the sugar reward, and subsequently extends its proboscis when the odour stimulus is presented alone. Hammer studied the electrical properties of an intriguing neuron that innervates the entire olfactory pathway in the honeybee and releases the neuromodulator octopamine, a substance known to mediate the reward in classical conditioning in insects (Hammer and Menzel 1998; Schwaerzel et al. 2003). This neuron was among a group of neurons lying ventrally along the mid axis of the brain called ventral unpaired median neurons or VUM neurons. The VUM neuron studied by Hammer sends its arborizations bilaterally into the entire olfactory pathway. Hammer succeeded in making electrical recordings of this VUM neuron in the honeybee's brain while performing PER conditioning, and found that this neuron responded strongly when the honeybee was stimulated with the sugar reward. When he applied the odour to the antenna, and subsequently stimulated the VUM neuron artificially (without sugar application but by electrical stimulation of the cell body), he could achieve PER conditioning to the same extent as with a sugar reward. Thus, the sugar reward could be entirely substituted by stimulation of a single neuron. This very simple network gives us a clear idea how learning acts at the neural level and how a reward-sensitive neuron plays a key role in this process. This VUM neuron belongs to a group of other VUM neurons that also express the neuromodulator octopamine (Kreissl et al. 1994), but with different arborization pathways, projecting, e.g., towards the optic lobes or into the antenna (Schröter 2002). Thus, there are most likely more reward-sensitive VUM neurons involved.

Octopaminergic VUM neurons are present in parasitic wasps as well (Smid et al. 2003; Bleeker et al. in press-b, Figure 3), and are candidate neurons that may encode for differences in learning observed in species like *C. glomerata* and *C. rubecula*. For instance, the strength of the response to a reward may be different, or the density of their arborizations into the olfactory pathway, leading to differences in the amount of octopamine released in the olfactory pathway upon a reward stimulus. Another explanation for the observed difference in learning may lie in the sensitivity to the octopamine signal in the olfactory pathway. To understand this, it is necessary to focus on the molecular level of learning.

MOLECULAR BIOLOGY: CANDIDATE GENES ENCODING LEARNING DIFFERENCES

The location where memory is stored at the level of single neurons is the synapse. The cellular equivalent of memory is called synaptic plasticity (Pittenger and Kandel 2003). The properties of a synapse can change after previous activity, and this is the way how the neuron 'remembers' its previous activity. For instance, transmission of a signal may be facilitated by increasing the amount of synaptic vesicles that are released upon electric stimulation, and hence increasing the post-synaptic response levels. The duration of these and other processes that underlie synaptic plasticity, is corresponding with short- or medium-term memory. This is called long-term

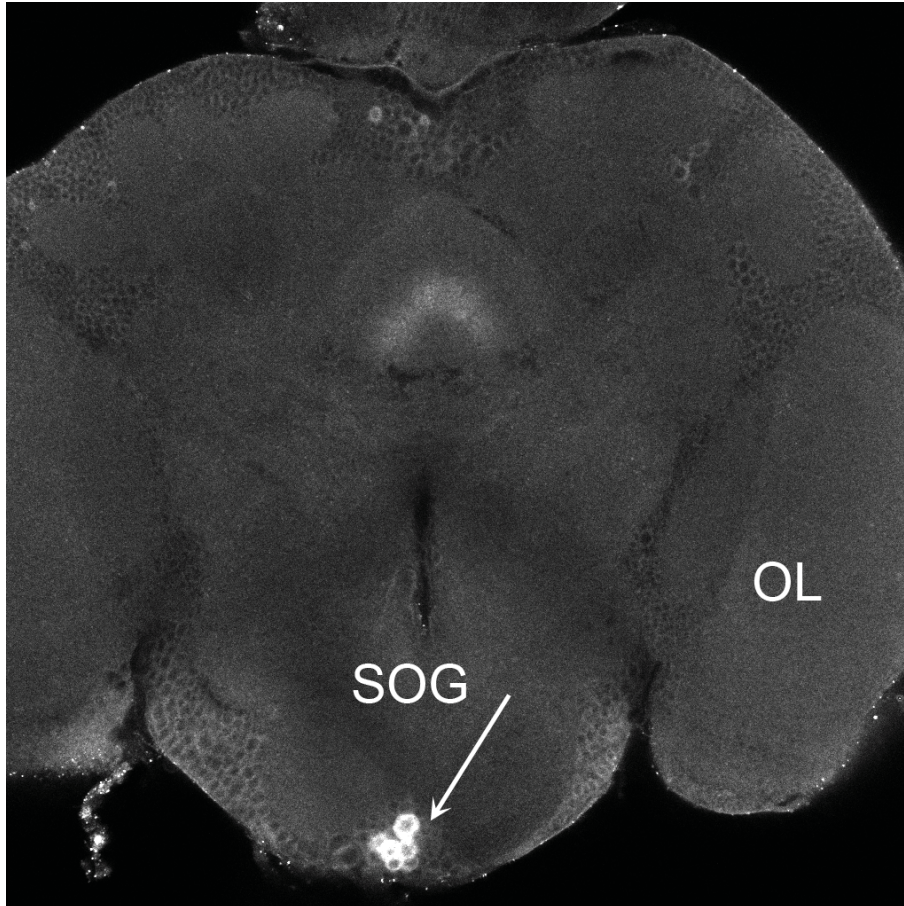


Figure 3. Confocal section of a brain of *C. rubecula*, showing cell bodies of a group of VUM neurons (arrow). These neurons were visualized using a fluorescently labelled antibody against octopamine. OL, optic lobe; SOG, suboesophageal ganglion

potentiation, and the opposite process, long-term depression, can also occur (Huber et al. 2000). There is also an equivalent form of long-term memory, called late long-term potentiation, when synaptic transmission becomes facilitated by the growth of new synaptic contacts. This way, the number of synaptic connections between two neurons is increased, and the increased synaptic strength that is formed by this process results in a more stable form of synaptic potentiation. This mechanism requires gene transcription and the production of new proteins (Nguyen et al. 1994). The learning-induced changes in synapse properties, either long-term or short-term, can occur throughout the brain in various neuropiles that are involved in, e.g., a learned behaviour, rather than at a specific region in the brain dedicated to memory storage. Hence the term 'memory trace' is used to refer to the changes in neural

elements were memory is stored.

The cellular pathways involved in synaptic plasticity are remarkably conserved within the animal kingdom, and it is now well-known that the cAMP – protein kinase A (PKA) signalling pathway plays a central role in species varying from nematodes, snails and insects to mammals (Silva et al. 1998; Eisenhardt in press). Single learning experiences induce a limited elevation of cAMP levels, which activates PKA, acting locally at the site of the synapse. Repeated learning experiences result in the activation of larger amounts of activated PKA, which translocates towards the nucleus where it activates a transcription factor. This transcription factor, called cAMP-responsive element binding protein (CREB) causes the expression of genes that are necessary to produce the proteins required for the formation of stable, long-term memory. There are several different isoforms of CREB resulting from alternative splicing that are different in the way they respond to PKA. Some isoforms are lacking parts of the amino-acid sequences that allow binding and activation by PKA, and therefore inhibit long-term memory formation. The different CREB isoforms represent memory suppressor as well as memory enhancer isoforms, and it is thought that the balance of tissue-specific expression of these isoforms determines the sensitivity of a neuron for the cAMP signal, and thus to the US (Yin et al. 1994; 1995a; Yin and Tully 1996; see however Perazzona et al. 2004; for general reviews on CREB and memory see Pittenger and Kandel 2003).

Memory suppressor or enhancer genes (Abel et al. 1998) like CREB isoforms and others are relevant genes in the light of the evolutionary biology of learning. Possibly, the differences in learning between parasitic wasps like *C. glomerata* and *C. rubecula* are correlated with differences in expression levels of such genes, i.e., a fast learner like *C. glomerata* could have a relatively low level of memory suppressor-gene expression and a slow learner like *C. rubecula* could have relatively high levels of memory suppressor-gene expression. Measuring of gene expression in these non-model organisms, of which the genome is not sequenced, is time-consuming, but feasible since the homologous sequences from a few insect species are now available. Genes that have been linked to a certain phenotype in one organism can be used as so-called candidate genes to investigate the mechanism and evolution of similar phenotypes in another species (Fitzpatrick et al. 2005).

CONCLUSION

The CREB gene has now been sequenced in *C. glomerata* and *C. rubecula* (H.M. Smid et al. unpublished data) and putative enhancer and suppressor isoforms have been found analogous to the isoforms found in the fruit fly and the honeybee (Yin et al. 1995b; Eisenhardt et al. 2003). Together with the characterization of the neuronal networks involved in associative learning of parasitic wasps, such as the VUM neurons and the olfactory pathways in *Cotesia*, this work may resolve mechanisms and genes that are linked to natural differences in learning. This would allow us to raise and test new hypotheses on the evolution of learning of a range of other wasp species that occur in many different ecological contexts. Moreover, since the mechanisms involved in learning are conserved, at least at the cellular level, these

results will be relevant for the understanding of learning in higher animals and man.

ACKNOWLEDGEMENTS

I would like to thank Maartje Bleeker, Joop van Loon and Louise Vet for valuable discussions on parasitoid learning, and the organization committee for their invitation to participate in the Frontis Workshop, *Chemical communication: from gene to ecosystem*.

REFERENCES

- Abel, T., Martin, K.C., Bartsch, D., et al., 1998. Memory suppressor genes: inhibitory constraints on the storage of long-term memory. *Science*, 279 (5349), 338-341.
- Bernays, E.A., 1996. Selective attention and host-plant specialization. *Entomologia Experimentalis et Applicata*, 80 (1), 125-131.
- Bernays, E.A. and Weislo, W.T., 1994. Sensory capabilities, information processing, and resource specialization. *Quarterly Review of Biology*, 69 (2), 187-204.
- Bitterman, M.E., Menzel, R., Fietz, A., et al., 1983. Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). *Journal of Comparative Psychology*, 97 (2), 107-119.
- Bleeker, M.A., Smid, H.M., Van Aelst, A.C., et al., 2004. Antennal sensilla of two parasitoid wasps: a comparative scanning electron microscopy study. *Microscopy Research and Technique*, 63 (5), 266-273.
- Bleeker, M.A.K., Smid, H.M., Steidle, J.L.M., et al., in press-a. Differences in memory dynamics between two closely related parasitoid wasp species. *Animal Behavior*.
- Bleeker, M.A.K., Van der Zee, B. and Smid, H.M., in press-b. Octopamine-like immunoreactivity in the brain and suboesophageal ganglion of two parasitic wasps, *Cotesia glomerata* and *Cotesia rubecula*. *Animal Biology*.
- Collett, T.S. and Collett, M., 2002. Memory use in insect visual navigation. *Nature Reviews Neuroscience*, 3 (7), 542-552.
- Corfas, G. and Dudai, Y., 1989. Habituation and dishabituation of a cleaning reflex in normal and mutant *Drosophila*. *Journal of Neuroscience*, 9 (1), 56-62.
- Davies, C.R. and Gilbert, N., 1985. A comparative study of the egg-laying behavior and larval development of *Pieris rapae* and *Pieris brassicae* on the same host plants. *Oecologia*, 67 (2), 278-281.
- Dukas, R., 1998a. Constraints on information processing and their effects on behavior. In: Dukas, R. ed. *Cognitive ecology: the evolutionary ecology of information processing and decision making*. The University of Chicago Press, Chicago, 89-119.
- Dukas, R., 1998b. Evolutionary ecology of learning. In: Dukas, R. ed. *Cognitive ecology: the evolutionary ecology of information processing and decision making*. The University of Chicago Press, Chicago, 129-164.
- Dukas, R., 1999. Costs of memory: ideas and predictions. *Journal of Theoretical Biology*, 197 (1), 41-50.
- Dukas, R. and Kamil, A.C., 2001. Limited attention: the constraint underlying search image. *Behavioral Ecology*, 12 (2), 192-199.
- Eisenhardt, D., in press. Learning and memory formation in the honeybee (*Apis mellifera*) and its dependency on the cAMP-protein kinase A pathway. *Animal Biology*.
- Eisenhardt, D., Friedrich, A., Stollhoff, N., et al., 2003. The AmCREB gene is an ortholog of the mammalian CREB/CREM family of transcription factors and encodes several splice variants in the honeybee brain. *Insect Molecular Biology*, 12 (4), 373-382.
- Fitzpatrick, M.J., Ben-Shahar, Y., Smid, H.M., et al., 2005. Candidate genes for behavioural ecology. *Trends in Ecology and Evolution*, 20 (2), 96-104.
- Geervliet, J.B.F., Vet, L.E.M. and Dicke, M., 1994. Volatiles from damaged plants as major cues in long-range host-searching by the specialist parasitoid *Cotesia rubecula*. *Entomologia Experimentalis et Applicata*, 73 (3), 289-297.

- Geervliet, J.B.F., Vet, L.E.M. and Dicke, M., 1996. Innate responses of the parasitoids *Cotesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae) to volatiles from different plant-herbivore complexes. *Journal of Insect Behavior*, 9 (4), 525-538.
- Geervliet, J.B.F., Vreugdenhil, A.I., Dicke, M., et al., 1998. Learning to discriminate between infochemicals from different plant-host complexes by the parasitoids *Cotesia glomerata* and *C. rubecula*. *Entomologia Experimentalis et Applicata*, 86 (3), 241-252.
- Giurfa, M., 2003. Cognitive neuroethology: dissecting non-elemental learning in a honeybee brain. *Current Opinion in Neurobiology*, 13 (6), 726-735.
- Hammer, M., 1993. An identified neuron mediates the unconditioned stimulus in associative olfactory learning in honeybees. *Nature*, 366 (6450), 59-63.
- Hammer, M., 1997. The neural basis of associative reward learning in honeybees. *Trends in Neurosciences*, 20 (6), 245-252.
- Hammer, M. and Menzel, R., 1998. Multiple sites of associative odor learning as revealed by local brain microinjections of octopamine in honeybees. *Learning and Memory*, 5 (1/2), 146-156.
- Huber, K.M., Kayser, M.S. and Bear, M.F., 2000. Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science*, 288 (5469), 1254-1257.
- Krasne, F.B. and Glanzman, D.L., 1995. What we can learn from invertebrate learning. *Annual Review of Psychology*, 46, 585-624.
- Kreissl, S., Eichmüller, S., Bicker, G., et al., 1994. Octopamine-like immunoreactivity in the brain and subesophageal ganglion of the honeybee. *Journal of Comparative Neurology*, 348 (4), 583-595.
- Le Masurier, A.D., 1994. Costs and benefits of egg clustering in *Pieris brassicae*. *Journal of Animal Ecology*, 63 (3), 677-685.
- Lutz, J., 1994. *Introduction to learning and memory*. Brooks/Cole Pub. Co., Pacific Grove.
- Menzel, R., 1999. Memory dynamics in the honeybee. *Journal of Comparative Physiology. A. Sensory Neural and Behavioral Physiology*, 185 (4), 323-340.
- Menzel, R., Manz, G., Menzel, R., et al., 2001. Massed and spaced learning in honeybees: the role of CS, US, the intertrial interval, and the test interval. *Learning and Memory*, 8 (4), 198-208.
- Mery, F. and Kawecki, T.J., 2003. A fitness cost of learning ability in *Drosophila melanogaster*. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, 270 (1532), 2465-2469.
- Mery, F. and Kawecki, T.J., 2005. A cost of long-term memory in *Drosophila*. *Science*, 308 (5725), 1148.
- Michel-Salzat, A. and Whitfield, J.B., 2004. Preliminary evolutionary relationships within the parasitoid wasp genus *Cotesia* (Hymenoptera: Braconidae: Microgasterinae): combined analysis of four genes. *Systematic Entomology*, 29 (3), 371-382.
- Nguyen, P.V., Abel, T. and Kandel, E.R., 1994. Requirement of a critical period of transcription for induction of a late phase of LTP. *Science*, 265 (5175), 1104-1107.
- Pavlov, I.P., 1927. *Conditioned reflexes: an investigation of the physiological activity of the cerebral cortex*. Oxford University Press, Oxford.
- Perazzona, B., Isabel, G., Preat, T., et al., 2004. The role of cAMP response element-binding protein in *Drosophila* long-term memory. *Journal of Neuroscience*, 24 (40), 8823-8828.
- Pittenger, C. and Kandel, E.R., 2003. In search of general mechanisms for long-lasting plasticity: Aplysia and the hippocampus. *Philosophical Transactions of the Royal Society of London. B. Biological Sciences*, 358 (1432), 757-763.
- Poolman-Simons, M.T.T., Suverkropp, B.P., Vet, L.E.M., et al., 1992. Comparison of learning in related generalist and specialist eucoilid parasitoids. *Entomologia Experimentalis et Applicata*, 64 (2), 117-124.
- Potting, R.P.J., Otten, H. and Vet, L.E.M., 1997. Absence of odour learning in the stemborer parasitoid *Cotesia flavipes*. *Animal Behaviour*, 53 (6), 1211-1223.
- Rescorla, R.A., 1988. Behavioral studies of Pavlovian conditioning. *Annual Review of Neuroscience*, 11, 329-352.
- Roitberg, B.D., Reid, M.L. and Li, C., 1993. Choosing hosts and mates: the value of learning. In: Papaj, D.R. and Lewis, A.C. eds. *Insect learning: ecological and evolutionary perspectives*. Chapman & Hall, New York, 174-194.
- Root, R.B. and Kareiva, P.M., 1984. The search for resources by cabbage butterflies (*Pieris rapae*): ecological consequences and adaptive significance of markovian movements in a patchy environment. *Ecology*, 65 (1), 147-165.

- Sandoz, J.C., Laloi, D., Odoux, J.F., et al., 2000. Olfactory information transfer in the honeybee: compared efficiency of classical conditioning and early exposure. *Animal Behaviour*, 59 (5), 1025-1034.
- Schröter, U., 2002. *Aufsteigende Neurone des Unterschlundganglions der Biene: Modulatorische Neurone und sensorische Bahnen*. PhD Dissertation, Free University of Berlin.
- Schwaerzel, M., Monastirioti, M., Scholz, H., et al., 2003. Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in *Drosophila*. *Journal of Neuroscience*, 23 (33), 10495-10502.
- Shettleworth, S.J., 1993. Varieties of learning and memory in animals. *Journal of Experimental Psychology Animal Behavior Processes*, 19 (1), 5-14.
- Silva, A.J., Kogan, J.H., Frankland, P.W., et al., 1998. CREB and memory. *Annual Review of Neuroscience*, 21, 127-148.
- Skinner, B.F., 1938. *The behavior of organisms: an experimental analysis*. Appleton-Century, New York.
- Smid, H.M., Bleeker, M.A., Van Loon, J.J., et al., 2003. Three-dimensional organization of the glomeruli in the antennal lobe of the parasitoid wasps *Cotesia glomerata* and *C. rubecula*. *Cell and Tissue Research*, 312 (2), 237-248.
- Smid, H.M., Van Loon, J.J.A., Posthumus, M.A., et al., 2002. GC-EAG-analysis of volatiles from Brussels sprouts plants damaged by two species of *Pieris caterpillars*: olfactory receptive range of a specialist and a generalist parasitoid wasp species. *Chemoecology*, 12 (4), 169-176.
- Stephens, D.W., 1993. Learning and behavioural ecology: incomplete information and environmental predictability. In: Papaj, D.R. and Lewis, A.C. eds. *Insect learning: ecological and evolutionary perspectives*. Chapman & Hall, New York, 195-217.
- Takasu, K. and Lewis, W.J., 2003. Learning of host searching cues by the larval parasitoid *Microplitis croceipes*. *Entomologia Experimentalis et Applicata*, 108 (2), 77-86.
- Thorndike, E.L., 1901. Animal intelligence: an experimental study of the associative processes in animals. *Psychological Review Monograph Supplement*, 2, 1-109.
- Turlings, T.C.J., Wäckers, F.L., Vet, L.E.M., et al., 1993. Learning of host-finding cues by hymenopterous parasitoids. In: Papaj, D.R. and Lewis, A.C. eds. *Insect learning: ecological and evolutionary perspectives*. Chapman & Hall, New York, 51-79.
- Vet, L.E.M. and Dicke, M., 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*, 37 (1), 141-172.
- Vet, L.E.M., Lewis, W.J. and Cardé, R.T., 1995. Parasitoid foraging and learning. In: Cardé, R.T. and Bell, W.J. eds. *Chemical ecology of insects. vol. 2*. Chapman & Hall, New York, 65-101.
- Vet, L.E.M., Lewis, W.J., Papaj, D.R., et al., 1990. A variable-response model for parasitoid foraging behavior. *Journal of Insect Behavior*, 3 (4), 471-490.
- Watanabe, H., Kobayashi, Y., Sakura, M., et al., 2003. Classical olfactory conditioning in the cockroach *Periplaneta americana*. *Zoological Science*, 20 (12), 1447-1454.
- Yin, J.C., Del Vecchio, M., Zhou, H., et al., 1995a. CREB as a memory modulator: induced expression of a dCREB2 activator isoform enhances long-term memory in *Drosophila*. *Cell*, 81 (1), 107-115.
- Yin, J.C. and Tully, T., 1996. CREB and the formation of long-term memory. *Current Opinion in Neurobiology*, 6 (2), 264-268.
- Yin, J.C., Wallach, J.S., Del Vecchio, M., et al., 1994. Induction of a dominant negative CREB transgene specifically blocks long-term memory in *Drosophila*. *Cell*, 79 (1), 49-58.
- Yin, J.C., Wallach, J.S., Wilder, E.L., et al., 1995b. A *Drosophila* CREB/CREM homolog encodes multiple isoforms, including a cyclic AMP-dependent protein kinase-responsive transcriptional activator and antagonist. *Molecular Cell Biology*, 15 (9), 5123-5130.