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Chapter 8

**MODULATION OF OLFACTORY AND GUSTATORY
SENSITIVITY MEDIATED BY SENSORY EXPERIENCE
IN MOTHS: A SIMPLE FORM OF SOCIAL LEARNING?**

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ABSTRACT

As do vertebrates, insects show a high degree of phenotypic plasticity, which enables them to adapt their behavioural responses to their surrounding world. For example, social insects such as honeybees are able to perform complex learning tasks, similar to vertebrates. Non-social insects such as flies or moths can also associate sensory information with rewards or punishment.

Non-associative forms of learning include sensitization and habituation to sensory cues originating from conspecifics or the biotic or abiotic environment. Often sensitization and habituation are the result of multiple exposures to sensory stimuli, but we have evidence in moths that a single exposure can also elicit long-lasting sensitization. The capacity to use sensory experience to adapt responses to social contexts by developing increased sensitivity to salient sensory cues from conspecifics and heterospecifics can be interpreted as a simple form of social learning.

We summarize here intra- and cross-modal effects of brief pre-exposure to different stimuli on the behaviour and underlying neural mechanisms in male moths. Moths are an ideal model for this type of approach because different sensory stimuli are well known to trigger a number of well-defined behaviours in different contexts and their nervous system is relatively simple, well-described and accessible.

Adult males respond to low doses of highly specific female-produced sex pheromones to find a mating partner and to flower odours to find food sources. Host plant odours can also complement the sex pheromone for a better localization of conspecific females. For feeding, the quality of a food source is evaluated upon contact, where sucrose concentration indicates nutritional value and bitter substances indicate non-

palatability or toxicity. Male moths also detect ultrasound emitted by bats, their natural enemies. Behavioural analyses have shown that a brief exposure to any of the mentioned sensory stimuli increases responses to the sex pheromone after 24 hr. In addition, responses to gustatory stimuli are increased by brief pre-exposure to gustatory and pheromone stimuli. These reciprocal effects indicate that the described phenomena represent a form of general sensitization or maturation of the sensory systems, rather than selective attention.

Attempts to localize modifications within the nervous system after brief pre-exposure indicate no or rather small changes in peripheral sensory systems caused by intra-modal exposure. In the primary olfactory centre, the antennal lobe, response thresholds of projection neurons to the sex pheromone and plant odours decreased after pre-exposure to the sex pheromone, to a bat-mimicking sound, but not to sucrose. In addition, the part of the antennal lobe processing information on the major sex pheromone compound increased in size after pre-exposure to the sex pheromone, probably indicating an increase in synaptic connections. These neurobiological approaches indicate that intra- and cross-modal effects of sensory pre-exposure might originate from modality-dependent changes at multiple levels within the nervous system.

Keywords: Noctuid moth, behavioural plasticity, sensitization, brief pre-exposure, olfaction, taste, bat sound, sex pheromone, receptor neuron, antennal lobe

INTRODUCTION

The world around us shows both spatial and temporal variability. Plasticity is an adaptive strategy to cope with a variable environment and to increase fitness [1]. It allows individuals to adapt to variation in local conditions and to respond to changes in intra- and interspecific interactions. Phenotypic changes depend on responses to environmental stimuli, and their detection and processing is therefore crucial. One strategy to adapt to the environment and increase animal fitness is to take advantage of earlier sensory experiences, i.e. learning [2]. In animals, learning is thus a way to use earlier sensory experiences in order to adapt their behaviour to biotic and abiotic changes in the environment. Sensory signals can elicit innate reactions or be neutral in a first place.

During single or repeated exposure, associated or not to an innately meaningful signal, the behavioural reaction to a sensory signal can be modified. In arthropods, non-associative learning such as habituation and sensitization caused by repeated exposure to sensory stimuli has been shown in many species [3, 4]. Evidence for more complex forms of learning, such as associative learning and even configural learning exists predominantly in social insects, e.g. honeybees, but also in fruitflies and moths [5-7].

Social learning in insects is a phenomenon, which has been ignored for a long time, but several cases of learning by observation have been described recently, not only in social but also in non-social insects.

In social insects such as ants, collective performance is improved with experience, as shown in the species *Leptothorax albipennis*, where collective memory improves performance during the emigration to new nest sites [8]. Social learning in social hymenoptera has been otherwise described in the context of food search (for review see [9]). In tandem-running ants, a leader seems to "teach" the follower how to reach a food source [10], reminding of the waggle dance of honeybees [11]. In honeybees, odours of food sources

are also taught to nestmates [12]. Learning of suitable food sources by observation has also been found in bumblebees and social wasps [13, 14] and, in stingless bees, eavesdropping of scent trails of other bees is used to find food sources [15]. In non-social arthropods, learning by observations has been described first in the context of mate choice.

For example in wolf spiders, subadult females exposed to courting males of a specific phenotype (experimentally manipulated brown or black foreleg colour), prefer during adulthood to mate with the familiar male phenotype [16]. In damselflies, males raised in the presence of a female colour pattern morph, later prefer females with the same morph over females of different colour morphs [17].

More recently, social learning also has been shown in the context of mate choice in fruitflies, where females prefer a type of male which has been observed to previously mate with other females [18]. Another example of social learning has been described in the predator-prey context: wood crickets hide in the presence of their major predators, wolf spiders, and remain hidden for some time after the spiders depart. Non-spider-experienced crickets kept with other crickets that had been previously in an environment with spiders copied hiding behaviour. Chemical cues persisting in the environment such as alarm pheromones have been excluded by control experiments [19].

In these examples, observational learning has been described in its "original" sense; observation is essentially achieved by using visual cues to "spy" on conspecifics. One essential question is how to transpose observational learning to other sensory modalities. Can an animal deduce from what it has, for example, heard, smelled, or tasted in reference to another animal's experience to copy behaviours and learn consequences perhaps encoded in such signals? There are indications that this might indeed be the case as shown for mechanosensory and acoustic cues.

In field crickets, female preference for a male calling song changes as a function of the previously experienced song pattern; depending on the attractiveness of the initially experienced calling pattern, females adjust their attraction to later encountered males in a predictable manner.

For example, when they listen to a highly attractive male, they respond less to a less-attractive male than naïve females [20].

So far, no case of simple social learning has been described involving well-defined chemical signals in non-social insects to our knowledge, although they often cannot be excluded in the complex experiments described before. Independent of the sensory modality, in the above described cases, insects would thus be able to use "public information" to adapt their individual behaviour as many vertebrate species can [21].

In this chapter we will present data on how experience to chemical and other sensory cues can affect subsequent behaviour in our model species, the Egyptian cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae).

EXPERIENCE-INDUCED BEHAVIOURAL PLASTICITY MODULATED BY DIFFERENT SENSORY INPUTS IN A MOTH MODEL

Moths, such as the cotton leafworm *S. littoralis*, are not a well-known model for complex learning tasks. However, they are, similar to the classical insect model for associative

learning, the honeybee [22], able to associate a sucrose reward with olfactory signals, such as plant-related compounds or even individual sex pheromone compounds [6, 7]. Moreover, they also have been recently shown to change their behaviour long-lastingly upon brief pre-exposure to sensory stimuli.

As night-active animals, noctuid moths rely on different sensory cues, such as chemosensory, auditory and tactile cues, rather than on vision. The advantage of the moth model to study behaviour and neuronal modifications caused by experience is that different behavioural paradigms are easy to apply and behaviour is highly reproducible. Physically and chemically well-defined sensory stimuli of different modalities have been shown to influence moth behaviour, and the behaviours these signals elicit with high reproducibility can be tested with different paradigms that are easy to apply (see [23]). In addition, the moth peripheral and central nervous systems are relatively well described, and numerous studies have especially examined the structure and function of the olfactory and gustatory system.

In many insect species, olfactory cues are very important when searching for a sexual partner. In moths, the female produces a sex pheromone attracting males. Species specificity is achieved by using unique blends of compounds and, even in some cases, unique ratios of compounds to solely attract males from their own species. Furthermore, in some moth species, such as *S. littoralis*, the female can also detect its own pheromone similar to detection by males through antennal receptor neurons [24]. The function of this auto-detection of the pheromone is still not clear, but it is probably involved in guiding reproductive behaviours. Sex pheromones are released over a background of plant odours emitted from both host and non-host plants. For males, plant odours indicate the presence of food, but also could be used as additional cues for locating females. Synergistic interactions between plant odours and female pheromones have been found in moths, resulting in increased male attraction [25-27]. Thus, the mating behaviour of males might be also influenced by host plant use of females and influenced by social interactions [28, 29]. In moths, receptor neurons detecting sex pheromones and plant odours are situated in cuticular sensilla situated on the antennae [30] and olfactory information is subsequently transmitted to the primary olfactory centre of the brain, the antennal lobe (AL) [31]. The AL is composed of globular subunits, the glomeruli, shown to play a role in spatio-temporal odour coding [32].

Gustatory stimuli are used by insects to locate food resources and evaluate their quality. Direct contact with a food resource is vital for the final assessment before accepting or rejecting a host plant [33]. In addition to the mouthparts, the moth's antennae carry gustatory sensilla with receptor neurons detecting appetitive signals, such as sucrose, indicating high food quality and neurons detecting aversive compounds, such as quinine, indicating toxicity or non-palatability [34]. Contacting antennae with sucrose elicits the proboscis extension reflex (PER) in moths [6, 7], like in other insects [22, 35-37].

Interactions between taste and olfactory stimuli have been mainly analysed in associative learning paradigms, where odours can be associated to an attractive gustatory stimulus such as sucrose [6, 22].

During odour-guided search for host plants or a mating partner, moths are exposed to predators, such as bats. To detect their major predators, insectivorous bats, moths use a simple ear with two receptor neurons attached to a tympanic membrane situated on the thorax [38].

These neurons can detect the echo-location sounds bats emit to locate their victims, and the moths respond by conducting evasive manoeuvres, such as loops or dropping to the ground in flying moths or "freezing" in moths walking on vegetation [39, 40]. In a trade-off

situation, where males simultaneously detect the sex pheromone emitted by a female and a bat-emitted sound, the behavioural response depends on the distance to the predator. In the presence of pheromone, the predator is allowed to come closer before an escape behaviour occurs [41].

We below describe changes in *S. littoralis* behaviour elicited by olfactory and gustatory stimuli as a consequence of intra- and cross-modal experience and then describe some first indications of the underlying neural mechanisms in the cotton leafworm moth, *S. littoralis*.

BEHAVIOURAL SENSITIZATION CAUSED BY BRIEF PRE-EXPOSURE

Modulation of Olfactory-Guided Behaviour

Female *S. littoralis* produce two sex pheromone compounds, which are essential for male attraction in the field (99% Z,E-9,11-tetradecadienyl acetate and 1% Z,E-9,12 tetradecadienyl acetate) [42]. Male moths also use plant odours to find food sources or host plants, where potential mating partners might be located [43-46]. Plant odour compounds such as geraniol and linalool have been previously shown to modulate detection and behavioural responses to the sex pheromone, when provided as an odour background in *S. littoralis* [47, 48]. We summarize below data that show that male responses to the female sex pheromone can be modulated by prior experience with stimuli of the same or other sensory modalities.

Intra-modal effects on male responses to pheromone. Behavioural responses of male *S. littoralis* to the female-emitted sex pheromone, after brief pre-exposure to either the sex pheromone or individual plant-related odours, have been studied using different types of bioassays: a walking olfactometer, a wind tunnel and a locomotion compensator.

Although the wind tunnel best reflects the natural situation for flying insects, the walking olfactometer allows fast acquisition of large datasets and the locomotion compensator allows detailed analysis of orientation behaviour. Male *S. littoralis* briefly pre-exposed to 1 female equivalent of the sex pheromone showed improved orientation towards a low dose pheromone stimulus after different time lags (15 min, 24 hr) independent of the used assay [23, 49, 50]. In addition, analyses of walking trajectories on a locomotion compensator showed that the walked distance and the mean speed of male *S. littoralis* were higher after pre-exposure to the sex pheromone than in naïve males [23].

A similar effect on responses to a low dose pheromone stimulus occurred when males were pre-exposed to the plant-related compounds linalool or geraniol 24 hr earlier [23]. Whereas the presence of the sex pheromone provides direct information about the presence of a potential mating partner, plant-related odours might provide additional information that there is an enhanced chance to encounter females associated with suitable host-plants or food sources. The ecological/evolutionary significance of these effects has, however, not yet been studied. Will pre-exposure to a sex pheromone or a plant odour influence future mate choice in a male, thus representing a primitive form of social learning, where the signal without the actual presence of the female influences male behaviour? Will combined pre-exposure to sex pheromone and plant-related odours have an even stronger effect than either signal on its own?

Cross-Modal Effects on Male Responses to Pheromone. Curiously, not only do brief olfactory signals improve pheromone-guided behaviour in male *S. littoralis*, but other sensory signals have a similar effect. The sound of a bat, a natural predator of moths, elicits improved pheromone responses 24 hr after pre-exposure. However, a behaviourally non-relevant tone with the same frequency as the bat sound, but without frequency modulation, does not change male behaviour [51].

Both aversive and appetitive gustatory signals (sugar and quinine respectively) lead to an increase in pheromone responses in male *S. littoralis*, as shown on a locomotion compensator [23].

As for the effect of olfactory pre-exposure, the ecological/evolutionary significance of cross-modal pre-exposure is not known to date. The improved response to the sex pheromone after exposure to a bat sound could be a way to privilege reproductive behaviour in an environment where the risk of predation is enhanced. Males, in this case, might be able to find a female over a larger distance. An interesting question to investigate is to see if a male would be also less "choosy" and fly towards and accept a prospective mate even when such females produce a less optimal pheromone blend that increases risk of attraction by another species. Up to date, we do not know if the presence of predators in the environment leads to a change in mating strategy, which could indicate a form of environmental influence on social learning.

It would be also interesting to investigate whether experience with either appetitive or aversive potential food sources would have an influence on mate choice. We have found in *S. littoralis* that reproductive behaviours are modulated by plant quality. For example, female calling started earlier and was more intense on a host plant than on a non-host plant [52]. Mating was delayed on non-host compared to host plants as well [52]. Similar to the effects of exposure to a predator sound, aversive gustatory signals might increase the sensitivity for the pheromone, pushing the male to "mate before it is too late".

Intra-Sexual Modulation of Female Pheromone Emission. It has been earlier shown that *S. littoralis* females can detect their own pheromone, but the function of this auto-detection is still unknown [53].

We have, however, recently shown that females sensing their conspecific pheromone change their calling behaviour [54]. The proportion of females calling increased after pre-exposure compared to unexposed females. Furthermore, the rate and the duration of calling increased in the pre-exposed females. It is thus likely that females adjust their calling behaviour after eavesdropping (*i.e.* using "public" information, [21]) on competing females to increase their chance of attracting a male.

Modulation of the PER Response

The presence of sucrose in plants is considered for phytophagous insects as a sign of high food quality, whereas the presence of secondary compounds, such as quinine, indicates toxicity or non-palatability [34].

In *S. littoralis* males, a PER is elicited when the antennae are touched with a sucrose solution [7, 23]. However, males never show a PER when antennae are touched with a quinine solution [23]. We summarize below how the sucrose-elicited PER is modulated by prior experience with the same or other sensory stimuli.

Intra-Modal Effects on PER Responses to Sucrose. Similar to oriented sex pheromone responses, proboscis extension responses to sucrose solutions were improved after brief pre-exposure with an appetitive (sucrose) or an aversive (quinine) stimulus as compared to water pre-exposed males [23]. We hypothesize that the increased responses to sucrose after sucrose pre-exposure might express familiarity with the stimulus and, therefore, enable recognition of lower doses than before. On the other hand, an aversive experience seems to result in a comparative evaluation of the subsequent stimulus. Males pre-exposed to quinine apparently judged the sucrose solutions to have an hedonic “more attractive” nutritional value than water pre-exposed males. Moreover, when pre-exposed males were subsequently tested with mixtures of a constant dose of sucrose and increasing concentrations of quinine, the same pattern was observed; males always responded better to the mixture when they had been pre-exposed to quinine. Males pre-exposed to sucrose responded less to sucrose solutions with increasing amounts of quinine added, as compared to males pre-exposed with water only, indicating a higher sensitivity to the aversive substance (Minoli, Colson, personal observations). In other words, males pre-exposed to sucrose evaluated the mixture less attractive than sucrose alone. Insects might then “compare” the taste between two feeding events and decide according to an increase or decrease of attractiveness.

These observed phenomena are so far only described within the same individuals.

Here again, pre-exposure to sensory signals provides information about the environment conditioning subsequent behaviour. It is, however, unlikely that “knowledge” of attractive or aversive gustatory signals can be transmitted in any way between individual moths.

Cross-modal Effects on Responses to Sucrose. Not only does pre-exposure to gustatory stimuli elicit changes in sensitivity to gustatory stimuli, but males pre-exposed to the sex pheromone were also more sensitive to sucrose than males pre-exposed to the solvent hexane [23]. We can only speculate about the biological meaning of this effect. It is possible that males need food to gather more energy when searching for a female.

NEURAL MECHANISMS UNDERLYING BEHAVIOURAL PLASTICITY CAUSED BY BRIEF PRE-EXPOSURE

Modifications in the Peripheral Sensory System

Behavioural plasticity elicited by brief pre-exposure to the different sensory signals is associated with changes in the neural mechanisms at one or more levels of the nervous system.

Changes in the detection of chemosensory cues were analysed by electrophysiological recordings. In addition, the expression of genes involved in the first steps of pheromone detection was also compared before and after pre-exposure to the sex pheromone.

Intra-Modal Effects on Pheromone-Responding Sensilla. Odorant reception occurs in olfactory receptor neurons (ORNs) situated in cuticular sensilla on the antennae. Hydrophobic odour molecules penetrate pores in the cuticle and then bind to odorant binding proteins, which transport the molecules across the aqueous sensillum lymph to the ORN membrane. At the ORN membrane, an interaction with membrane receptors occurs, leading directly or indirectly to the opening of ion channels [55]. The resulting ion flows lead to a depolarisation

of the ORN membrane, transformed into action potentials [55]. When investigating responses of ORNs to the sex pheromone by using single sensillum recordings, a small but significant increase in sensitivity was observed between antennae of naïve and pre-exposed males, although earlier electroantennographic recordings had not revealed significant differences [50, 56]. When analysing the expression of genes, previously identified as involved in the first steps of pheromone detection [57], a significant up-regulation was caused by brief pheromone-exposure only for a gene coding for one pheromone binding protein [56]. Genes coding for putative membrane pheromone receptors, the ubiquitous co-receptor ORco and for a candidate pheromone-degrading enzyme, did not show significant differences in expression levels after pre-exposure to the sex pheromone [56]. The expression analyses of a larger panel of olfaction-related genes and biochemical analysis of the associated proteins might, in the future, give more insight on the involvement of the peripheral olfactory system in the observed behavioural effects of intra-modal pre-exposure.

Intra-Modal Effects on Gustatory Receptor Neurons. Single sensillum recordings from gustatory receptor neurons (GRNs) on the male antennae did not reveal any significant difference in the sensitivity to sucrose between naïve males and males pre-exposed to sucrose or quinine [23]. Although technical limitations do not allow sorting action potentials within a gustatory sensillum, meaning that recordings cumulate action potentials from several neurons, it is unlikely that the activity of individual neurons changes significantly after pre-exposure. We hypothesize therefore that the behavioural sensitization effects induced by sucrose and quinine originate from modifications within higher levels of the central nervous system.

Unfortunately, integration centres for gustatory information are much less circumscribed in the brain than the ALs [58, 59]. However, it would be interesting to search for modulation of sensitivity in the tritocerebrum and the suboesophageal ganglion.

Modifications in the Primary Olfactory Centre, the AL

The ALs of insects are the first processing centres of olfactory information coming from the antennae. We summarize results of anatomical and electrophysiological analyses which indicate that structure and function of the AL are modified by pre-exposure to different sensory signals.

Intra-Modal Effects on AL Structure and Pheromone-Processing. Information from the axons of ORNs enters the AL via the antennal nerve [31]. Sex-pheromone responding receptor neurons send their axons to the male-specific macroglomerular complex, whereas axons from other receptor neurons innervate the so-called ordinary glomeruli of the AL [31]. After local processing, odour information is transmitted to higher brain centres [31].

Intracellular recordings from neurons within the macroglomerular complex of the AL revealed a statistically significant increase in sensitivity of the neuron population to the sex pheromone one day after pheromone pre-exposure. Although neurons with a broad spectrum of response thresholds are found in both naïve and pre-exposed males, pre-exposure to 1 female-equivalent of the pheromone shifts the mean response threshold of the neuron population to the major sex pheromone component over several orders of magnitude [50].

As differences in the sensitivity of antennal pheromone receptor neurons are less pronounced after pheromone pre-exposure, it is possible that the high sensitivity of AL neurons is reached by an increase of synaptic contacts between receptor neurons and central

neurons. A first indication that this might indeed be true is the finding that the glomerulus processing the major pheromone component, the cumulus of the macroglomerular complex, increases significantly in size 24 hr after pheromone pre-exposure [56]. Transmission electron microscopic studies now need to be done to confirm if the increased size of the glomerulus is really caused by an increase in synaptic connections.

Another supplementary or alternative origin of increased sensitivity of AL neurons could be modulation of the neuronal network, for example, under the influence of biogenic amines, such as octopamine, serotonin and dopamine, or neuropeptides [60-62].

Cross-Modal Effects on Central Pheromone Processing. Intracellular recordings from AL neurons stimulated with the sex pheromone revealed cross-modal changes in sensitivity for one of the behaviourally effective stimuli, but not for another. Bat-sound pre-exposure elicited a strong increase of sensitivity of AL neurons to the sex pheromone. However, it was significantly lower than pre-exposure with the sex pheromone itself. A tone, by comparison, without frequency modulation did not have any effect [51]. Interestingly, pre-exposure to the bat sound also increased the sensitivity of neurons within "ordinary glomeruli" to flower odours [51]. On the other hand, pre-exposure to sucrose did not elicit changes in AL neuron sensitivity to the sex pheromone [23], indicating that the behavioural effect observed must originate from changes at higher integration levels within the nervous system.

Different hypotheses can be put forward to explain the differences in neuronal levels affected by cross-modal pre-exposure. On one hand, sensory input is received on different body parts; the ears of the moth lie on the thorax, whereas the gustatory receptor neurons are situated on the antennae, as are ORNs. Intra-modal effects within the AL might seem easy to understand because the sensory information on the pre-exposure- and the test-stimulus enter into the same brain area.

Although olfactory and gustatory receptor neurons are both situated on the antennae, axons from gustatory neurons target different areas within the tritocerebrum and sub-oesophageal ganglion and the antennal mechanosensory and motor centre, whereas olfactory axons project to the AL. For the auditory system, it is known that one axon originating from a receptor neuron of the ear ends within the thoracic ganglia [38]. The second neuron projects to the brain, but it is not known to which area [38]. Therefore different circuits receiving sensory information might lead to effects at different levels of the concerned sensory pathways to finally lead to similar changes in olfactory-guided behaviour. On the other hand, we have only investigated cross-modal central nervous effects of one aversive and one attractive stimulus. Depending on the significance of the used stimuli, affected neural levels may differ. For a stimulus, which is highly relevant for survival, such as a bat sound, it might indeed be more important to influence responses to the pheromone at a low integration level.

Whereas an attractive stimulus such as sucrose might have a more subtle influence on pheromone-guided behaviour and eventually mate choice by being integrated at higher brain levels.

Our data indicate that modulation through pre-exposure to different sensory cues involves neural changes at multiple levels and allow us to expect higher processing levels to be involved.

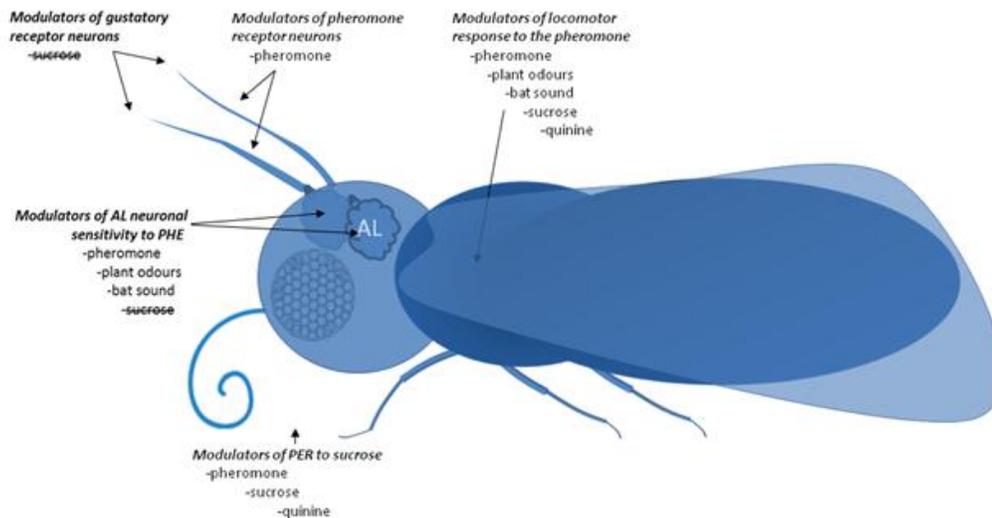


Figure 1. Schematic overview of effects of brief sensory pre-exposure with different stimuli on behaviour and neural processing of pheromone and sucrose in the male noctuid moth *Spodoptera littoralis*. Stimuli that elicit a modulation of behaviours and different neuronal levels are indicated. Crossed out stimuli do not affect the respective neurons. AL: antennal lobe; PER: proboscis extension reflex; PHE: pheromone.

To achieve a more global picture of the neurobiological background explaining intra- and especially cross-modal pre-exposure effects on moth behaviour, we also should investigate other brain centres which have been previously shown to be involved in higher olfactory processing and/or multimodal integration, such as the mushroom bodies, the lateral protocerebrum and the suboesophageal ganglion (e.g. [63, 64]). Even brain centres connected to motor-output centres, such as the lateral accessory lobe, might be involved in this behavioural plasticity and need to be investigated [65].

CONCLUSION

In the present chapter, we have included data we have so far accumulated on the effects of intra- and cross-modal brief pre-exposure on the behaviour of a male moth and some first results on the underlying neuronal mechanisms. We propose that the observed effects (behaviourally relevant sensory information switching the male from a "low" to a "high" sensitivity mode) represent a form of non-specific general sensitization. This general sensitization might be part of a maturation process allowing the moth to adapt mate finding (and eventually mate choice), to exploit food sources depending on environmental conditions, and thus to "improve" behaviour in a kind of social context. This process is initiated by sensory cues from con- and heterospecifics in the environment of the individual. It allows behavioural plasticity and an adaption to local conditions depending on the presence and activity of other individuals.

The presence of a brief sensory signal seems to be enough to prepare a moth at a longer time scale for future encounters with the same or a different behaviourally relevant stimulus.

Cross-modal effects confirm that what we observe is not a case of selective attention, where an animal is sensitized to a particular type of stimulus in a trade-off for sensitivity to other stimuli, which might be less important in the actual situation [66, 67].

In the case of a male *S. littoralis*, in which female pheromone, plant odours, food sources (plants) and bat sounds can be parts of the same ecological scenario, the presence of one of these cues can act as a trigger to a higher sensitivity level, increasing the probability of a quick response, if needed to the same or even to other related stimuli.

A study in field crickets has explored the effects of exposure to different sensory signals originating from a mating partner. The exposure of female field crickets to cuticular hydrocarbons produced by males (a signal indicating high population density) increases the attractiveness of simultaneously proposed male calling songs.

However, differently from the cases reported in moths, pre-exposure to chemical signals did not influence mating decisions to later proposed calling songs [68]. The degree to which some signalling modalities are prioritized over others during social interactions and decision-making processes remains thus still a central question in animal communication [69].

The observed effects of brief pre-exposure are different from other types of experience-dependent plasticity described in the literature. Most forms of experience depend on repeated signals, especially when regarding sensitization or habituation processes. Moreover, sensitization is mostly described as a form of short-term memory.

Although associative learning in vertebrates as well as in invertebrates can be very fast, that is, an association can be learned within a single trial, long-term memory in the range of days is generally only formed with multiple trials [70]. Thus, the long lasting effects of brief pre-exposure we describe here are rather unique and might open new perspectives in understanding behavioural plasticity.

We presented in this chapter what is known so far about brief pre-exposure effects in *S. littoralis* and some of its neurobiological bases. Our studies show that biotic signals from conspecifics and heterospecifics, as well as abiotic environmental signals, do modify moth sexual and feeding behaviour.

Yet, how much these modifications influence social learning (*i.e.*, if there is transmission of experience among individuals) remains unknown so far. Adjusting attraction to pheromone or calling behaviour after exposure to pheromone might indeed be a primitive case of social learning, because the signal of a conspecific modifies subsequent behaviour.

Other sensory cues may also provide information on environmental conditions, in turn influencing intraspecific communication and, thus, indirectly affecting social learning.

We hope to draw the attention of scientists with the required competences to these interesting phenomena and look forward to future tests of our ecological and evolutionary hypotheses to get insight into the potential social learning phenomena of our moth model.

REFERENCES

- [1] Auld, H. L., Punzalan, D., Godin, J. G. and Rundle, H. D. (2009). Do female fruit flies (*Drosophila serrata*) copy the mate choice of others? *Behavioural Processes*, 82(1), 78-80.

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- [2] Agrawal, A. A. (2001). Ecology - Phenotypic plasticity in the interactions and evolution of species. *Science*, 294(5541), 321-326.
- [3] Walters, E. T., Illich, P. A., Weeks, J. C. and Lewin, M. R. (2001). Defensive responses of larval *Manduca sexta* and their sensitization by noxious stimuli in the laboratory and field. *Journal of Experimental Biology*, 204(3), 457-469.
- [4] Rakitin, A., Tomsic, D. and Maldonado, H. (1991). Habituation and sensitization to an electrical shock in the crab *Chasmagnathus*. Effect of background illumination. *Physiology and Behavior*, 50(3), 477-487.
- [5] DeBelle, J. S. and Heisenberg, M. (1994). Associative odor learning in *Drosophila* abolished by chemical ablation of mushroom bodies. *Science*, 263(5147), 692-695.
- [6] Fan, R. J., Anderson, P. and Hansson, B. S. (1997). Behavioural analysis of olfactory conditioning in the moth *Spodoptera littoralis* (Boisd.) (Lepidoptera : Noctuidae). *Journal of Experimental Biology*, 200(23), 2969-2976.
- [7] Hartlieb, E., Hansson, B. S. and Anderson, P. (1999). Sex or food? Appetitive learning of sex odors in a male moth. *Naturwissenschaften*, 86(8), 396-399.
- [8] Langridge, E. A., Franks, N. R. and Sendova-Franks, A. B. (2004). Improvement in collective performance with experience in ants. *Behavioral Ecology and Sociobiology*, 56(6), 523-529.
- [9] Chittka, L. and Leadbeater, E. (2005). Social learning: Public information in insects. *Current Biology*, 15(21), R869-R871.
- [10] Franks, N. R. and Richardson, T. (2006). Teaching in tandem-running ants. *Nature*, 439(7073), 153-153.
- [11] Von Frisch, K. (1974). Decoding the language of the bee. *Science*, 185 (4152), 663-668.
- [12] Farina, W. M., Grüter, C. and Díaz, P. C. (2005). Social learning of floral odours inside the honeybee hive. *Proceedings Royal Society B*, 272 (1575), 1923-1928.
- [13] Leadbeater, E. and Chittka, L. (2005). A new mode of information transfer in foraging bumblebees? *Current Biology*, 15(12), R447-R448.
- [14] Worden, B. D. and Papaj, D. R. (2005). Flower choice copying in bumblebees. *Biology Letters*, 1(4), 504-507.
- [15] Nieh, J. C., Barreto, L. S., Contrera, F. A. L. and Imperatriz-Fonseca, V. L. (2004). Olfactory eavesdropping by a competitively foraging stingless bee, *Trigona spinipes*. *Proceedings Royal Society B*, 271(1548), 1633-1640.
- [16] Hebets, E. A. (2003). Subadult experience influences adult mate choice in an arthropod: Exposed female wolf spiders prefer males of a familiar phenotype. *Proceedings of the National Academy of Sciences USA*, 100 (23), 13390-13395.
- [17] Fincke, O. M., Fargevieille, A. and Schultz, T. D. (2007). Lack of innate preference for morph and species identity in mate-searching *Enallagma* damselflies. *Behavioral Ecology and Sociobiology*, 61(7), 1121-1131.
- [18] Mery, F., Varela, S. A. M., Danchin, E., Blanchet, S., Parejo, D., Coolen, I. and Wagner, R. H. (2009). Public versus personal information for mate copying in an invertebrate. *Current Biology*, 19(9), 730-734.
- [19] Coolen, I., Dangles, O. and Casas, J. (2005). Social learning in noncolonial insects? *Current Biology*, 15(21), 1931-1935.
- [20] Bailey, N. W. and Zuk, M. (2009). Field crickets change mating preferences using remembered social information. *Biology Letters*, 5(4), 449-451.

-
- [21] Danchin, E., Giraldeau, L. A., Valone, T. J. and Wagner, R. H. (2004). Public information: From nosy neighbors to cultural evolution. *Science*, 305(5683), 487-491.
- [22] Bittermann, M. E., Menzel, R., Fietz, A. and Schäfer, S. (1983). Classical conditioning of proboscis extension in honeybee (*Apis mellifera*). *Journal of Comparative Physiology A*, 97(2), 107-119.
- [23] Minoli, S., Kauer, I., Colson, V., Party, V., Renou, M., Anderson, P., Gadenne, C., Marion-Poll, F. and Anton, S. (2012). Brief exposure to sensory cues elicits stimulus-nonspecific general sensitization in an insect. *PLoS ONE*, 7(3), e34141.
- [24] Ljungberg, H., Anderson, P. and Hansson, B. S. (1993). Physiology and morphology of pheromone-specific sensilla on the antennae of male and female *Spodoptera-littoralis* (Lepidoptera, Noctuidae). *Journal of Insect Physiology*, 39(3), 253-260.
- [25] Schmidt-Büsser, D., von Arx, M. and Guerin, P. M. (2009). Host plant volatiles serve to increase the response of male European grape berry moths, *Eupoecilia ambiguella*, to their sex pheromone. *Journal of Comparative Physiology A*, 195(9), 853-64.
- [26] Landolt, P. J. and Phillips, T. W. (1997). Host plant influences on sex pheromone behavior of phytophagous insects. *Annual Review of Entomology*, 42, 371-391.
- [27] Varela, N., Avilla, J., Anton, S. and Gemeno, C. (2011). Synergism of pheromone and host-plant volatile blends in the attraction of *Grapholita molesta* males. *Entomol. Exp. Appl.*, 141(2), 114-122.
- [28] Duckworth, R. A. (2009). The role of behavior in evolution: a search for mechanism. *Evolutionary Ecology*, 23(4), 513-531.
- [29] Turlure, C. and Van Dyck, H. (2009). On the consequences of aggressive male mate-locating behaviour and micro-climate for female host plant use in the butterfly *Lycaena hippothoe*. *Behavioral Ecology and Sociobiology*, 63(11), 1581-1591.
- [30] Keil, T. A. (1999). Morphology and development of the peripheral olfactory organs. In: Hansson, B. S. (Ed.), *Insect Olfaction* (pp. 5-48). Berlin: Springer.
- [31] Anton, S. and Homberg, U. (1999). Antennal lobe structure. In: Hansson, B. S. (Ed.), *Insect Olfaction* (pp. 98-125). Berlin: Springer.
- [32] Hansson, B. S. and Anton, S. (2000). Function and morphology of the antennal lobe: new developments. *Annual Review of Entomology*, 45, 203-231.
- [33] Renwick, J. A. A. and Chew, F. S. (1994). Oviposition behavior in lepidoptera. *Annual Review of Entomology*, 39, 377-400.
- [34] Chapman R. (1974). Chemical Inhibition of Feeding by Phytophagous Insects. *Bulletin of Entomological Research*, 64(3), 339-363.
- [35] Amakawa, T. (2001). Effects of age and blood sugar levels on the proboscis extension of the blow fly *Phormia regina*. *Journal of Insect Physiology*, 47(2), 195-203.
- [36] Dethier, V. G. and Bowdan, E. (1989). The effect of alkaloids on sugar receptors and the feeding-behavior of the blowfly. *Physiological Entomology*, 14(2), 127-136.
- [37] Le Bourg, E. (1996). Hypergravity and aging in *Drosophila melanogaster*. 8. Proboscis-extension-response threshold to sucrose. *Gerontology*, 42(4), 235-240.
- [38] Surlykke, A. and Miller, L. A. (1982). Central branching of three sensory axons from a moth ear (*Agrotis-segetum*, Noctuidae). *Journal of Insect Physiology*, 28(4), 357-364.
- [39] Werner, T. K. (1981). Responses of nonflying moths to ultrasound: the threat of gleaning bats. *Canadian Journal of Zoology*, 59(3), 525-529.
- [40] Miller, L. A. and Surlykke, A. (2001). How some insects detect and avoid being eaten by bats: Tactics and countertactics of prey and predator. *Bioscience*, 51(7), 570-581.

- [41] Skals, N., Anderson, P., Kannevorff, M., Löfstedt, C. and Surlykke, A. (2005). Her odours make him deaf: Crossmodal modulation of olfaction and hearing in a male moth. *Journal of Experimental Biology*, 208(4), 595-601.
- [42] Kehat, M., Greenberg, S. and Tamaki, Y. (1976). Field evaluation of the synthetic sex pheromone, as an attractant for males of the cotton leafworm, *Spodoptera littoralis* (BOISD.), in Israel. *Applied Entomology and Zoology*, 11(1), 45-52.
- [43] Barrozo, R. B., Gadenne, C. and Anton, S. (2010). Switching attraction to inhibition: Mating-induced reversed role of sex pheromone in an insect. *Journal of Experimental Biology*, 213(17), 2933-2939.
- [44] Gabel, B. (1992). Tansy Flowers Attract European Grapevine Moth Females, *Lobesia Botrana* Den and Schiff (Lep, Tortricidae). *Journal of Applied Entomology*, 113(2), 153-158.
- [45] Haynes, K. F., Zhao, J. Z. and Latif, A. (1991). Identification of floral compounds from *Abelia grandiflora* that stimulate upwind flight in cabbage looper moths. *Journal of Chemical Ecology*, 17(3), 637-646.
- [46] Anderson, P., Sadek, M. M., Larsson, M., Hansson, B. S. and Thöming, G. (2013). Larval host plant experience modulates both mate finding and oviposition choice in a moth. *Animal Behavior* (accepted for publication).
- [47] Party, V., Hanot, C., Said, I., Rochat, D. and Renou, M. (2009). Plant terpenes affect intensity and temporal parameters of pheromone detection in a moth. *Chemical Senses*, 34(9), 763-774.
- [48] Rouyar, A., Party, V., Prešern, J., Blejcek, A. and Renou, M. (2011). A general odorant background affects the coding of pheromone stimulus intermittency in specialist olfactory receptor neurones. *PLoS ONE*, 6 (10), e26443.
- [49] Anderson, P., Sadek, M. M. and Hansson, B. S. (2013). Pre-exposure modulates attraction to sex pheromone in a moth. *Chemical Senses*, 38 (4), 285-291.
- [50] Anderson, P., Hansson, B., Nilsson, U., Han, Q., Sjöholm, M., Skals, N. and Anton, S. (2007). Increased behavioral and neuronal sensitivity to sex pheromone after brief odor experience in a moth. *Chemical Senses*, 32(5), 483-491.
- [51] Anton, S., Evengaard, K., Barrozo, R. B., Anderson, P. and Skals, N. (2011). Brief predator sound exposure elicits behavioral and neuronal long-term sensitization in the olfactory system of an insect. *Proceedings of the National Academy of Sciences US*, 108(8), 3401-3405.
- [52] Sadek, M. and Anderson, P. (2007). Modulation of reproductive behaviour of *Spodoptera littoralis* by host and non-host plant leaves. *Basic and Applied Ecology*, 8, 444-452.
- [53] Anderson, P., Hansson, B. S. and Löfqvist, J. (1995). Plant-odor-specific receptor neurons on the antennae of female and male *Spodoptera littoralis*. *Physiological Entomology*, 20(3), 189-198.
- [54] Sadek, M. M., Wowern, G. V., Löfstedt, C., Rosén, W. and Anderson, P. (2012). Modulation of the temporal pattern of calling behavior of female *Spodoptera littoralis* by exposure to sex pheromone. *Journal of Insect Physiology*, 58(1), 61-66.
- [55] Jacquin-Joly, E. and Lucas, P. (2005). Pheromone reception and transduction: mammals and insects illustrate converging mechanisms across phyla. *Current Topics in Neurochemistry*, 4, 75-105.

-
- [56] Guerrieri, F., Gemeno, C., Monsempes, C., Anton, S., Jacquin-Joly, E., Lucas, P. and Devaud, J. M. (2012). Experience-dependent modulation of antennal sensitivity and input to antennal lobes in male moths (*Spodoptera littoralis*) pre-exposed to sex pheromone. *Journal of Experimental Biology*, 215(13), 2334-2341.
- [57] Legeai, F., Malpel, S., Montagne, N., Monsempes, C., Cousserans, F., Merlin, C., Francois, M. C., Maibeche-Coisne, M., Gavory, F., Poulain, J. and Jacquin-Joly, E. (2011). An Expressed Sequence Tag collection from the male antennae of the noctuid moth *Spodoptera littoralis*: A resource for olfactory and pheromone detection research. *BMC Genomics*, 12(1), 86.
- [58] Jørgensen, K., Kvello, P., Almaas, T. J. and Mustaparta, H. (2006). Two closely located areas in the suboesophageal ganglion and the tritocerebrum receive projections of gustatory receptor neurons located on the antennae and the proboscis in the moth *Heliothis virescens*. *Journal of Comparative Neurology*, 496(1), 121-34.
- [59] Kvello, P., Jorgensen, K. and Mustaparta, H. (2008). Integration of taste information in the CNS of the moth *Heliothis Virescens*. *Chemical Senses*, 33(8), S45-S45.
- [60] Barrozo, R. B., Jarriault, D., Simeone, X., Gaertner, C., Gadenne, C. and Anton, S. (2010). Mating-induced transient inhibition of responses to sex pheromone in a male moth is not mediated by octopamine or serotonin. *Journal of Experimental Biology*, 213(7), 1100-1106.
- [61] Dacks, A., Christensen, T. A. and Hildebrand, J. G. (2008). Modulation of olfactory information processing in the antennal lobe of *Manduca sexta* by serotonin. *Journal of Neurophysiology*, 99(5), 2077-2085.
- [62] Ignell, R., Root, C. M., Birse, R. T., Wang, J. W., Nässel, D. R. and Winther, A. M. E. (2009). Presynaptic peptidergic modulation of olfactory receptor neurons in *Drosophila*. *Proceedings of the National Academy of Sciences USA*, 106(31), 13070-13075.
- [63] Balkenius, A., Bisch-Knaden, S. and Hansson, B. S. (2009). Interaction of visual and odour cues in the mushroom body of the hawkmoth *Manduca sexta*. *Journal of Experimental Biology*, 212(4), 535-541.
- [64] Nishino, H., Nishikawa, M., Yokohari, F. and Mizunami, M. (2005). Dual, multilayered somatosensory maps formed by antennal tactile and contact chemosensory afferents in an insect brain. *Journal of Comparative Neurology*, 493(2), 291-308.
- [65] Martin, J. P., Beyerlein, A., Dacks, A. M., Reisenman, C. E., Riffell, J. A., Lei, H. and Hildebrand, J. G. (2011). The neurobiology of insect olfaction: Sensory processing in a comparative context. *Progress in Neurobiology*, 95(3), 427-447.
- [66] Van Swinderen, B. and Greenspan, R. (2003). Saliency modulates 20–30 Hz brain activity in *Drosophila*. *Nature Neuroscience*, 6(6), 579-586.
- [67] Wolf, R. and Heisenberg, M. (1980). On the fine structure of yaw torque in visual flight orientation of *Drosophila melanogaster*. *Journal of Comparative Physiology A*, 140(1), 69-80.
- [68] Bailey, N. W. (2011). Mate choice plasticity in the field cricket *Teleogryllus oceanicus*: effects of social experience in multiple modalities. *Behavioral Ecology and Sociobiology*, 65(12), 2269-2278.
- [69] Rutledge, J. M., Miller, A. and Uetz, G. W. (2010). Exposure to multiple sensory cues as a juvenile affects adult female mate preferences in wolf spiders. *Animal Behaviour*, 80(3), 419-426.

- [70] Hammer, M. and Menzel, R. (1995). Learning and memory in the honeybee. *Journal of Neuroscience*, 15(3), 1617-1630.