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Switching attraction to inhibition: mating-induced reversed role of sex pheromone in an insect

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SUMMARY

In the moth, *Agrotis ipsilon*, newly mated males cease to be attracted to the female-produced sex pheromone, preventing them from re-mating until the next night, by which time they would have refilled their reproductive glands for a potential new ejaculate. The behavioural plasticity is accompanied by a decrease in neuron sensitivity within the primary olfactory centre, the antennal lobe (AL). However, it was not clear whether the lack of the sexually guided behaviour results from the absence of sex pheromone detection in the ALs, or if they ignore it in spite of detection, or if the sex pheromone itself inhibits attraction behaviour after mating. To test these hypotheses, we performed behavioural tests and intracellular recordings of AL neurons to non-pheromonal odours (flower volatiles), different doses of sex pheromone and their mixtures in virgin and newly mated males. Our results show that, although the behavioural and AL neuron responses to flower volatiles alone were similar between virgin and mated males, the behavioural response of mated males to flower odours was inhibited by adding pheromone doses above the detection threshold of central neurons. Moreover, we show that the sex pheromone becomes inhibitory by differential central processing: below a specific threshold, it is not detected within the AL; above this threshold, it becomes inhibitory, preventing newly mated males from responding even to plant odours. Mated male moths have thus evolved a strategy based on transient odour-selective central processing, which allows them to avoid the risk-taking, energy-consuming search for females and delay re-mating until the next night for a potential new ejaculate.

Key words: moth, olfaction, mating, sex pheromone, plant odour, plasticity, antennal lobe.

INTRODUCTION

In animals, mating involves detection and central nervous processing of relevant sensory cues that lead to appropriate behaviours. In many species, the most prominent use of olfactory signals is sex pheromone communication, with males generally attracted by a female-produced pheromone. Responses to such pheromones depend not only on their chemical properties as signals but also on environmental conditions and the physiological state of the receiver individual (Hofmann, 2003; Kolb and Whishaw, 1998; Meinertzhagen, 2001). In insects, males receive sex pheromone information through receptor neurons on their antennae, and approach behaviour is elicited after central processing of the sex pheromone in the macroglomerular complex (MGC) of the primary olfactory centre, the antennal lobe (AL) (Anton and Homberg, 1999; Hansson, 1995; Koontz and Schneider, 1987).

Male reproductive success depends not only on the ability to locate and copulate with a female but also on the ability to effectively transfer an ejaculate (Dewsbury, 1982). However, males are limited with respect to the number of ejaculates they can deliver, and need time to restore depleted reserves (Dewsbury, 1982). During this period, newly mated males should avoid the risk-taking and energy-consuming search of new sexual partners (Dewsbury, 1982). In contrast to females, for which mating induces drastic behavioural and physiological changes (Gillott, 2003; Serguera et al., 2008; Yang et al., 2009), males can often re-mate after a variable time delay. Although males of many species are known to enter a post-ejaculatory refractory period (Bateman and Ferguson, 2004; Fischer and King, 2008; Lachmann, 2000; Phillips-Farfán and Fernández-Guasti, 2009; Reddy and Guerrero, 2000; Soulairac, 1952; Ureshi and Sakai, 2001), the mechanisms that lead to this sexual satiety are far from being understood in any organism.

In the noctuid moth, *Agrotis ipsilon* (Lepidoptera: Noctuidae), evidence is accumulating that the modulation of behavioural output occurs through neuronal plasticity (Anton et al., 2007). We previously showed that males copulate only once during a single scotophase, and that mating induced a transient inhibition of sex pheromone response in newly mated males (Gadenne et al., 2001). This plasticity is not only seen at the behavioural level but is accompanied by a decrease in the sensitivity of neurons within the AL: most neurons have much higher pheromone response thresholds after mating (Gadenne et al., 2001). Behavioural sex pheromone responses and the AL sensitivity are fully recovered during the next night (Gadenne et al., 2001). This plasticity allows newly mated males to avoid mating attempts while they produce a new ejaculate during the following night (Duportets et al., 1998). However, it was not clear if the absence of behavioural attraction after mating might only result from insufficient sensory input due to the low sensitivity of central neurons. Even if high pheromone doses provide sufficient sensory input, the sex pheromone might in turn be ignored or could even inhibit male attraction to females.

To test these possible scenarios, we chose a strategy in which the sex pheromone was tested in combination with a non-pheromonal type of attractant (flower odour). Males rely on plant volatiles for the detection of food sources. Males and females of *A. ipsilon* were previously observed to be highly attracted to flower volatiles from blooming plants in field experiments (Wynne et al., 1991; Zhu et

al., 1993). Blooming linden (*Tilia americana*) was the most attractive plant to *A. ipsilon* and other moths (Zhu et al., 1993). Heptanal, which has been identified as a component of linden flower extracts, was found to be attractive to *A. ipsilon* in field bioassays, and elicited clear responses both in the antennae (Zhu et al., 1993) and in the neurons within the 'ordinary glomeruli' (OG) of the ALs, which process plant odours (Greiner et al., 2002). We therefore tested the behavioural and central nervous responses of both virgin and newly mated males to the flower-related odour or sex pheromone alone and to their mixture. By using this strategy, we were able to show that the sexual abstinence of newly mated males is the consequence of a differential processing of sex pheromone, which becomes inhibitory after mating.

MATERIALS AND METHODS

Insects

Adult males and females of *Agrotis ipsilon* Hufnagel originating from a laboratory colony in Bordeaux, France, were used in the experiments. Wild insects are introduced into the colony each spring. The animals were reared on an artificial diet (Poitout and Buès, 1974) in individual cups until pupation. Pupae were sexed and males and females were kept separately in an inversed light:dark cycle (16 h:8 h light:dark photoperiod) at 22°C. Newly emerged adults were removed every day and were given access to a 20% sucrose solution *ad libitum*. The day of emergence was considered as day 0.

Pairing experiments were performed as previously described (Barrozo et al., 2010; Gadenne et al., 2001). Briefly, virgin 5-day-old sexually mature males and virgin 3-day-old sexually mature females were individually paired in cylindrical plastic containers before the onset of scotophase in a mating room under a 16 h:8 h light:dark photoperiod and at 22±1°C. Visual observation of matings was done every half an hour during the mating period at mid-scotophase (see below). Once males had mated (copulation lasts between one and two hours), they were quickly removed from the pairing box and submitted within 1 h (newly-mated males) to wind tunnel experiments or electrophysiological tests. Due to the preparation of the insects and the actual recording procedures, the intracellular recordings often outlasted the 1-hour time window following mating but always remained within the scotophase. To be sure that the male introduced a spermatophore during mating, all mated females were checked for the presence of the spermatophore.

Stimuli

The artificial pheromone blend contained (Z)-7-dodecen-1-yl acetate (Z7-12:OAc), (Z)-9-tetradecen-1-yl acetate (Z9-14:OAc) and (Z)-11-hexadecen-1-yl acetate (Z11-16:OAc) (Sigma-Aldrich, Saint-Quentin Fallavier, France) at a ratio 4:1:4 (Gemeno and Haynes, 1998; Picimbon et al., 1997). For the electrophysiological tests, we used the plant odour, heptanal (Sigma-Aldrich). For the behavioural tests, a linden flower extract (*Tilia tormentosa*, 55% purity, Boiron laboratories, Sainte-Foy les Lyon, France) was used at a 50 µl dose, which proved attractive in a dose–response curve experiment performed on virgin males (data not shown). Linden flower extract was used instead of heptanal, because this last one, although we obtained some positive responses with the males, did not give good reproductive results in the wind tunnel tests.

Wind tunnel experiments

The behaviour of virgin and newly mated 5-day-old male moths was observed in a 2 m-long wind tunnel under the same conditions as for the mating observations (inversed 16 h:8 h light:dark photoperiod, 22±1°C) (Barrozo et al., 2010; Gadenne et al., 2001). Newly mated males were transferred into the wind tunnel room in the darkness (both the mating room and wind tunnel room were under inversed light regimes and separated by a dark corridor). Virgin males were transferred before the onset of scotophase from their rearing chamber into the wind tunnel room. Experiments with newly mated or virgin males were run during mid-scotophase (i.e. from 2 h to 6 h after lights off). This period corresponds to the female calling and mating time, during which virgin males show the maximal oriented behavioural response to pheromone (Gadenne, 1993; Gemeno and Haynes, 2000; Swier et al., 1976). Previous experiments showed that virgin males without females, or males that had been exposed to females but did not mate, responded similarly in the wind tunnel to sex pheromone (Gadenne et al., 2001). For stimulation either a synthetic pheromone blend was used at four doses (0.01; 0.1; 1; 10 ng), 50 µl linden flower extract, or mixtures of each pheromone dose with the flower extract were used. Control experiments (no odour) were performed with 10 µl hexane. Stimuli were dispensed on a filter paper and placed in the airflow upwind to the release site in the wind tunnel on a vertical holder. For the mixture, the pheromone blend and the flower extract were applied on two individual filter papers placed on the same holder. Each experimental male was tested only once to one stimulus and at a single dose, and then the animal was discarded. Assays were performed during 3 min, and partial flight, complete flight and landing on the pheromone source were considered as an oriented response (Barrozo et al., 2010; Jarriault et al., 2009a). The proportion of males performing an oriented flight was analysed. Statistical differences ($P<0.05$) were evaluated using a $R\times C$ test of independence using a G-test and applying the Williams' correction (Sokal and Rohlf, 1995). Global comparisons were performed among sex pheromone doses tested either alone or in the mixture, and between the two series of stimuli (i.e. pheromone alone *vs* mixture). In addition, individual *post hoc* comparisons were carried out and the experimental-wise error rate was adjusted by using the Dunn–Šidák method (Sokal and Rohlf, 1995).

Intracellular electrophysiology

Preparation, intracellular recordings and response analysis of AL neurons from virgin and newly mated males were performed as described previously (Gadenne et al., 2001). Briefly, moths were immobilised in a cut disposable pipette tube, the head capsule was opened and the ALs were exposed. Standard intracellular recording techniques were used (Christensen and Hildebrand, 1987). AL interneurons were randomly impaled within the array of both the MGC and the OG. Data were recorded and analysed using Autospike 32 software (Syntech, Kirchzarten, Germany). For stimulation, a synthetic pheromone blend was used at six doses (0.001; 0.01; 0.1; 1; 10; 100 ng) diluted in 10 µl hexane, and the flower odour, heptanal (Sigma-Aldrich, 95% purity), was used at four doses (1; 10; 100; 1000 µg) diluted in 10 µl mineral oil (Sigma-Aldrich, 95% purity). All stimuli were presented after a minimum evaporation time of 30 min. A 500 ms air pulse (5 ml s^{-1}) was blown through a Pasteur pipette, loaded with the odours by means of a stimulation device (CS 55, Syntech, Kirchzarten, Germany). Odours were presented, separated by inter-stimulus intervals of at least 10 s, with lower stimulus loads tested first, and doses around the threshold were tested several times. Both solvents, hexane and mineral oil, were used as controls. Mixtures of heptanal (1; 10; 100 µg) and the pheromone blend (10 ng) were also tested. Both odours were loaded on separate filter papers.

The examination of AL neuron sensitivity to the pheromone was carried out within the array of the MGC and within the OG in the case of heptanal. The response threshold was determined as the lowest dose, which elicited a response from the neuron exceeding the solvent response by at least 20%. The percentage of AL neurons responding at different thresholds was calculated. Statistical analysis (P<0.05) was performed using a $R\times C$ test of independence using a G-test and applying the Williams' correction (Sokal and Rohlf, 1995).

The effect of the mixture (heptanal + pheromone) and its single components on the responses of AL neurons in virgin and mated males was studied only within the array of OG. The instantaneous spike frequency of OG neurons was calculated between 0.2 s from the stimulus onset and 2 s after (Jarriault et al., 2009b). Differences between groups (virgin and mated) were statistically evaluated by means of the Wilcoxon signed rank test (P<0.05).

RESULTS

Mating does not affect responses to plant odour in the absence of pheromone

To be able to evaluate the responses to combinations of non-pheromonal odours and sex pheromone, we first examined behavioural and central nervous responses to the flower-related odour alone in virgin and newly mated males. Males were tested in wind tunnel experiments with a linden flower extract (Fig. 1). There was no statistical difference in the response levels of virgin (grey dot at 0 ng of pheromone in Fig. 1A) and mated (grey dot at 0 ng of pheromone in Fig. 1B) males to flower volatiles (G-test, n.s.).

In accordance with these behavioural data, we did not find any difference in response thresholds of central olfactory neurons to a flower-related odour between virgin and mated males (G-test, n.s.) (Fig. 2A). Here we recorded from neurons within the OG in the AL during stimulation with heptanal. The linden flower extract at the dose used in behavioural experiments also evokes responses of the same neurons responding to heptanal (Fig. 3). Our results indicate that copulation does not alter plant-odour perception.

Mating affects responses to sex pheromone

In a next step, virgin and newly mated males were tested in the wind tunnel to different doses of the sex pheromone blend alone. Although the proportion of responding virgin males increased with increasing pheromone doses (black dots in Fig. 1A) (G-test, P<0.0001), mated males did not orientate to any of the pheromone doses tested (G-test, n.s.) (black dots in Fig. 1B).

By means of intracellular recordings performed in the macroglomerular complex (MGC) of the AL, we confirmed that the sensitivity of MGC neurons from newly mated males was significantly lower than that of virgin males (G-test, P<0.0001) (Fig. 2B) (Gadenne et al., 2001). We considered one ng of pheromone as a clear response threshold in mated males because only 10% of neurons responded to the 0.1 ng dose, and no neuron responded to lower doses (Fig. 2B). Newly mated males thus do not behaviourally respond any longer to the sex pheromone. However, sex pheromone is still detected and processed by mated males above a specific threshold of 1 ng.

Mating inhibits responses to plant odour in the presence of above threshold amounts of sex pheromone

In a last step, we tested the behavioural responses of virgin and mated males to the linden flower extract to which we added increasing doses of the pheromone blend (Fig. 1). Our results show an overall enhanced response to the flower extract with the added pheromone in virgin males as compared with responses to the pheromone alone (G-test, P<0.0001) (grey dots in Fig. 1A). By comparing behavioural responses of males with the individual doses of pheromone alone or the mixture, a significant increase was observed for 1 ng of pheromone (G-test, P=0.0006) (Fig. 1A). Newly mated males did respond to the flower volatile alone and to the mixture but only if the pheromone dose did not exceed 0.1 ng (grey dots in Fig. 1B) (G-test, n.s.). Pheromone doses above 1 ng inhibited upwind flight behaviour to the flower extract (grey dots in Fig. 1B) (G-test, P=0.024).

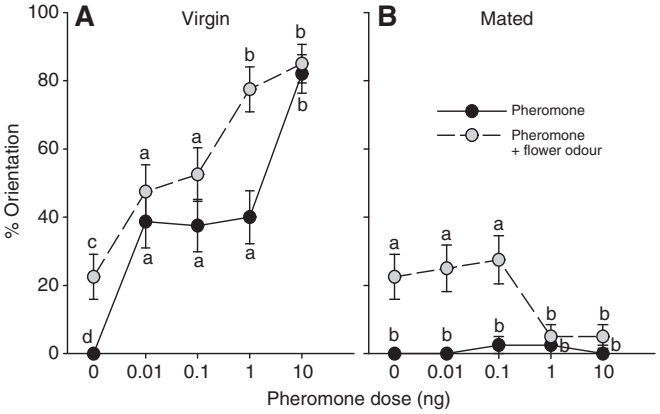

Fig. 1. Mating-induced switch of the behavioural significance of sex pheromone in male moths. Increasing doses of the pheromone blend were tested as single stimulus (black dots) or combined with 50 µl of the linden flower extract (grey dots). Responses to the flower extract alone correspond to the first grey point (0 ng pheromone blend). (A) The addition of the flower extract increased the behavioural responses to sex pheromone in virgin males. (B) Newly mated males oriented to the mixture if the pheromone dose did not exceed 0.1 ng. Pheromone doses above 1 ng added to the flower extract inhibited upwind flight behaviour. Statistical significance was analysed by means of an $R\times C$ test of independence using a G-test and is indicated by different letters (P<0.05). Values are percentages of 40 insects responding to each stimulus ±s.d. [s.d. was calculated as $\sqrt{p(1-p)/N}$; p=proportion of response; N=number of animals tested].

Thus, mated males responded to flower odours only if sub-threshold pheromone doses for MGC neurons (< 1 ng) were present in the mixture (see Fig. 1B, Fig. 2B).

Central neuron responses correlate with behavioural changes in response to odour mixtures

Finally, we investigated if the response characteristics of neurons within OG in the ALs might provide a substrate for the observed behavioural synergistic and inhibitory effects of mixtures (heptanal + sex pheromone) in virgin and mated males, respectively. In virgin males, all recorded OG neurons (N=13) responded to heptanal alone (with different response thresholds); however, none of them responded to the sex pheromone alone. Individual neurons showed a synergistic response to the mixture of heptanal and sex pheromone (Fig. 4A). Seven of these 13 OG neurons exhibited a significantly higher spike frequency in response to the mixture than to heptanal (median=60 spikes s^{-1} for heptanal and median=127 spikes s^{-1} for the mixture; Wilcoxon signed rank test, P=0.02) (Fig. 4C, group 1). The remaining six neurons showed no differences in the spike frequency in response to heptanal compared with the mixture (median=112 spikes s^{-1} for heptanal and median=112 spikes s^{-1} for the mixture; Wilcoxon signed rank test, n.s.) (Fig. 4C, group 2).

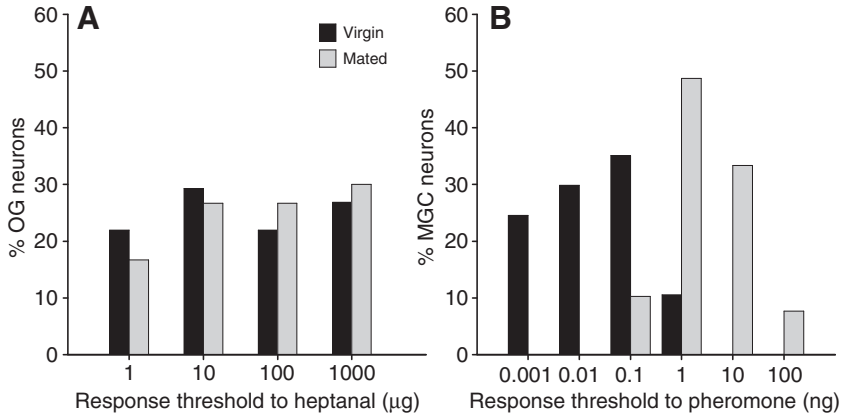

Fig. 2. Differential sensitivity of antennal lobe (AL) neurons in virgin and mated male moths. (A) Response thresholds of ordinary glomeruli (OG) neurons for the plant-associated volatile, heptanal. Both groups of insects (41 and 30 neurons in virgin and mated males, respectively) show no differences in the neuron sensitivity to the plant-related odour. (B) Response thresholds of macroglomerular complex (MGC) neurons for the sex pheromone blend. Neurons in newly mated males (40 neurons) show significantly higher response thresholds (i.e. they are less sensitive) than in virgin males (60 neurons) (P<0.0001). Statistical analysis was performed using an $R\times C$ test of independence using a G-test (P<0.05).

In mated males, all recorded OG neurons (N=13) responded to heptanal, and one of these also responded to pheromone. Individual neurons showed a reduced response to the mixture of heptanal and sex pheromone (Fig. 4B). Five out of the 13 OG neurons showed a significant decrease or total suppression of the action potential response to heptanal when the pheromone was added (median=118 spikes s^{-1} for heptanal and median=39 spikes s^{-1} for the mixture; Wilcoxon signed rank test, P=0.04) (Fig. 4D, group 1). The response to heptanal alone (spike frequency) was thus higher in mated males than in virgin males (median=60 spikes s^{-1}, see paragraph above and Fig. 4). The spike frequency of the other eight neurons in response to the mixture did not significantly differ from the response to heptanal alone (median=84 spikes s^{-1} for heptanal and median=77 spikes s^{-1} for the mixture; Wilcoxon signed rank test, n.s.) (Fig. 4D, group 2).

DISCUSSION

Our results show that mating in *A. ipsilon* males changes the functional interpretation of the sex pheromone from an attractive cue to one that is inhibitory. Before mating, sex pheromone is highly attractive, and the presence of plant odour increases the response of virgin males, thus increasing their chances of finding a calling female located on a host plant. After mating, there is a change in the sensitivity of the AL neurons to sex pheromone, and newly mated males are therefore not attracted to females at long range. However, pheromone processing is not completely shut off as higher doses inhibited male attraction to plant odours. Interestingly, by detailed analysis of MGC neuron responses to sex pheromone, we found a clear pheromone response threshold of 1 ng, indicating that at this level the pheromone starts to elicit a response in the brain of newly mated males. We have recently shown that mating indeed induced drastic changes in the responses of AL neurons to the pheromone in mated males; together with changes in spike frequency, the latency, and durations of excitatory and inhibitory phases were also changed as compared with virgin males (Barrozo et al., 2010). This could explain the observed lack of response of newly mated males in the wind tunnel: below a specific threshold, males do not detect the pheromone and therefore do not respond; above this threshold, males do detect the pheromone, which has become inhibitory. In the female Mediterranean fruit fly, *Ceratitis capitata*, there is a preferential switch as a result of mating. Virgin females cease to be attracted to the male-emitted sex pheromone and instead start to be attracted to fruit odours (Jang, 1995). This behavioural switch originates from a factor in the sex accessory glands of the male (Jang, 1995). Similarly, in mated females of the cricket, *Gryllus bimaculatus*, the phonotactic behaviour is stopped for about an hour, and mated females are no longer attracted by the songs emitted by the males (Loher et al., 1993).

Although studies dealing with males are scarce, there are numerous examples of behavioural responses to plant odours in female insects (Reddy and Guerrero, 2004). Our data, which show a clear behavioural response of *A. ipsilon* males to linden flower extracts, confirm earlier field observations of adults found on linden flowers (Wynne et al., 1991). Behavioural and central nervous responses to plant odours indicate that copulation does not alter plant-odour attraction, as it should be adaptive for newly mated males to still search for food sources. Similar results were found in the responses of virgin *versus* mated males of the diamondback moth, *Plutella xylostella* (Reddy and Guerrero, 2000). Our new data can be compared with our previous results showing that, although the behavioural and central nervous processing of sex pheromone was age- and hormone-dependent (Anton and Gadenne, 1999; Gadenne and Anton, 2000), the processing of plant odour was age-independent (Greiner et al., 2002). These two forms of neuronal plasticity (maturation and mating) seem to be restricted to the MGC.

Our strategy, consisting in testing combinations of pheromone and non-pheromonal odours, allowed us to have a better understanding of the behavioural lack of response of mated males. As expected, virgin males showed an enhanced behavioural response

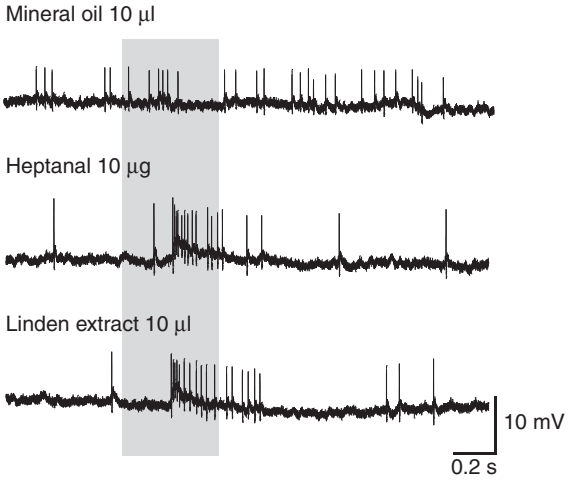

Fig. 3. Typical responses of an ordinary glomerulus (OG) neuron to mineral oil, heptanal and linden flower extract stimulations. The grey bar indicates the stimulus duration (0.5 s). Horizontal scale bar, 0.2 s; vertical scale bar, 10 mV.

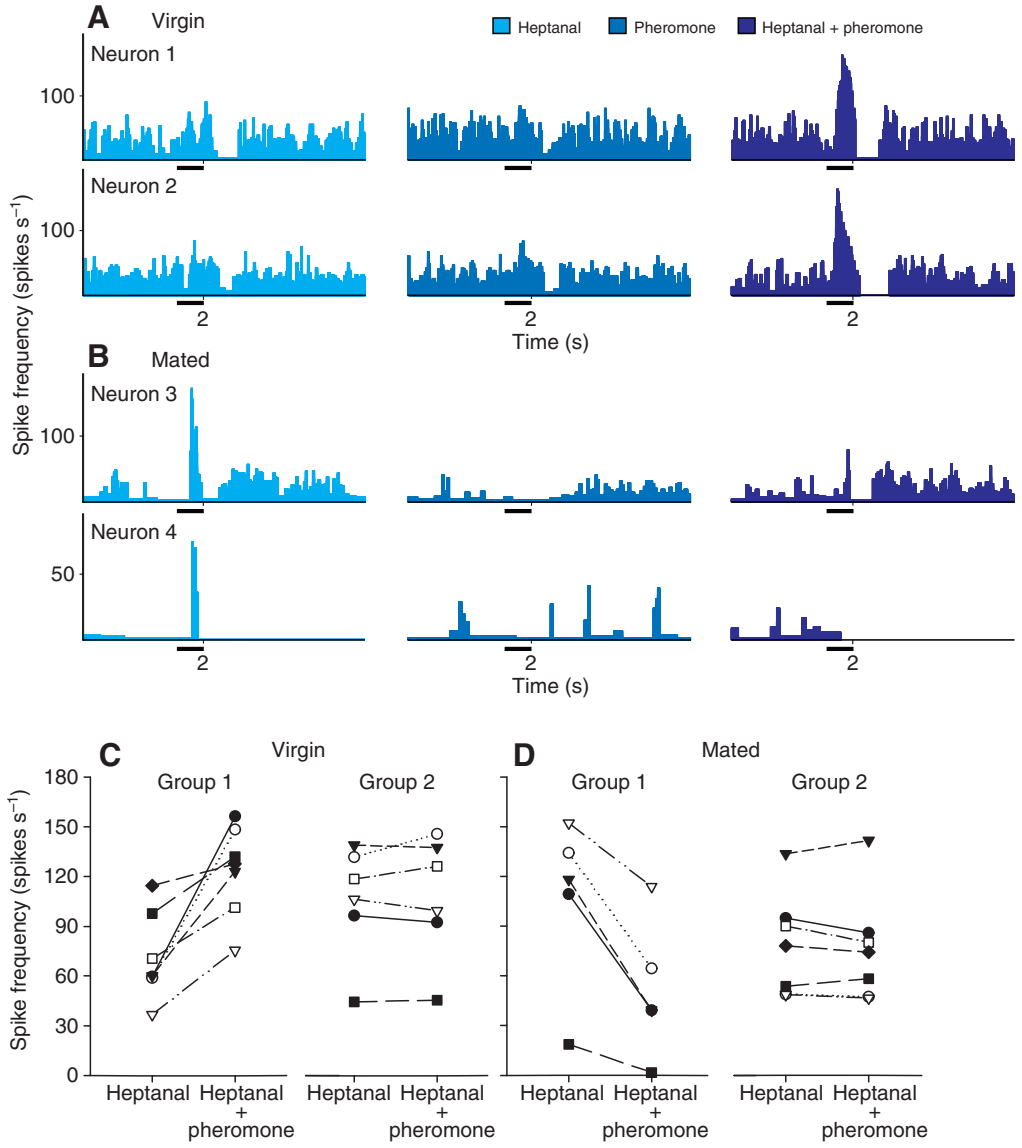

Fig. 4. Synergistic and inhibitory effects of mixtures in individual ordinary glomerulus (OG) neurons in virgin and mated males. (A) Peri-stimulus-time histograms of two OG neurons in virgin males (neurons 1 and 2) responding with a higher action potential frequency to the mixture than to the sum of the responses to the individual components of the mixture, thus displaying a true synergism. (B) Peri-stimulus-time histograms of two OG neurons in mated males showing a response to heptanal alone, and a decrease (neuron 3) or total suppression (neuron 4) of the response to the mixture. Black bars indicate stimulus presentation time (0.5 s). (C) Seven neurons out of 13 analysed OG neurons in virgin males show a significantly higher spike frequency to the mixture of heptanal and pheromone than to heptanal alone (group 1, P=0.02, N=7). The remaining six neurons (group 2) show no differences in the spiking rate to the heptanal and the mixture. (D) In mated males, the firing rate of five out of 13 OG neurons decreased during mixture stimulation in comparison to heptanal alone (group 1, P=0.04, N=5). The other eight OG neurons show no changes in the spike frequency to odours (group 2). For each individual neuron, the same dose of heptanal was used as single compound and in the mixture. Different doses of heptanal (but not the pheromone dose, which was always 10 ng) were used in different neurons, because the occurrence of the observed interaction effects was dose-dependent but never inversed. Statistical analysis was performed using the Wilcoxon signed rank test (P<0.05).

when the flower extract was added to the pheromone. The synergistic effect of plant volatiles and sex pheromones has indeed been previously described in other insect species (Reddy and Guerrero, 2004) and might reflect a strategy to optimise mating and reproduction: a virgin flying male moth may increase its chances of finding a calling female located on a host plant. Surprisingly, the behavioural response of newly mated males to plant odours was not altered only if the added dose of sex pheromone did not exceed 0.1 ng. Above this threshold, there was a complete inhibition of response to the plant odour, thus showing that the sex pheromone was here acting as an inhibitory factor. Moreover, our results show that males are not physically exhausted after mating; in fact, the behavioural response of mated males to flower odours is normal or similar to virgin males. These findings indicate that copulation changes the response to a given dose of sex pheromone (i.e. 1 ng or higher) of the male from attraction to inhibition.

Numerous examples have shown that behavioural responses to odours can be decreased as a function of physiological state (Baker and Cardé, 1979; Takken, 2001). However, these changes might be interpreted as a lack of motivation to respond rather than a case of

true inhibition. A well-established example of reduced odour responses is known in vertebrates in the context of food intake and satiety, where olfactory responses to a food odour decrease after feeding to satiety with a food containing that odour (Rolls, 2006). There are also several known examples in which attractiveness to odours is modified by context. For example, the attractive effects of odours can diminish when other odours are added, although independently of the physiological state. Behavioural responses to sex pheromone can be inhibited when sex pheromones of sympatric moth species are released simultaneously (Baker, 2008; Potting et al., 1999). Moreover, the effect of a sex pheromone has been shown to change from attraction to inhibition at unnaturally high doses (Roelofs, 1978). Our findings in *A. ipsilon* males are clearly different from the other cited cases – here the same naturally occurring dose of sex pheromone switches from attraction to inhibition after mating.

Neurons displaying the same type of mixture interactions, as observed in the behaviour of both virgin and mated males, were specifically found in OG in *A. ipsilon* whereas similar effects of mixtures in virgin males of the silkmoth *Bombyx mori* were only found in MGC neurons (Namiki et al., 2008). The intracellular recordings in *A. ipsilon* thus revealed that response patterns exist among OG neurons within the AL, which might indeed serve as a neural substrate for the synergistic and inhibitory behaviour observed, even though another part of the neuron population does not display these effects. It is however common that parallel processing of sensory information serves different purposes (Hansson and Christensen, 1999) and we suggest that neurons, which do not change their responses to mixtures as compared with single stimuli serve as an additional information channel, which is not involved in the behaviour studied here. In the brain of *B. mori*, it was suggested that the synergistic effect of plant–pheromone interaction on AL neurons sensitivity originated from an excitatory lateral interaction between the MGC and the OG (Namiki et al., 2008). Moreover, lateral interactions seem to play an essential role in the processing of natural odour mixtures by AL neurons of *Manduca sexta* (Lei and Vickers, 2008; Riffell et al., 2009). The observed AL response to mixtures in *A. ipsilon* males could also possibly result from interglomerular interactions. Some mixture interaction might additionally occur already at the antennal level, as shown in other moth species (Ochieng et al., 2002; Party et al., 2009). However, we found no evidence so far that mating-dependent plasticity occurs at the antennal level, because electroantennogram and single-sensillum recordings did not reveal any differences in the sensitivity between virgin and newly mated males to the sex pheromone (R.B.B., personal observation) (Gadenne et al., 2001).

In mated *A. ipsilon males*, as in other moths, the accessory sex glands are depleted of their protein reserves, which are used for the formation of a spermatophore during copulation (Duportets et al., 1998; Gillott, 2003). Therefore, if they would re-mate, they could not produce a new spermatophore, and then the mating would be sterile. During the next night, the sex glands are replenished and males can successfully re-mate (Duportets et al., 1998). Although we have not studied the duration of this post-copulatory refractory period in detail, it probably lasts for the remainder of the scotophase, as no newly mated males were observed to re-mate during the same night, when they were offered new virgin females (Gadenne et al., 2001). In vertebrates, this post-copulatory refractory period lasts from a few seconds in hamsters to minutes in Norway rats and to hours–days in some other mammals (Aversa et al., 2000). In the Queensland fruitfly, males transfer a spermatophore to the female during mating that inhibits the female receptivity. The accessory glands decrease in size after mating, and between 5.5 h and 11 h are needed for a full replenishment and recovery of the initial size of the glands in order to induce an inhibition in the next female (Radhakrishnan and Taylor, 2008).

Mating and courtship, including responding to pheromones, can be energetically costly (Cardé and Haynes, 2004) and should be avoided if successful mating is not physiologically possible. In male fruitflies, sexual activity reduces lifespan (Partridge and Farquhar, 1981) and the courtship period seems to be particularly costly (Cordts and Partridge, 1996). There was indeed a cost of mating for males, which did not transfer sperm during copulation (Chapman, 1992). In addition to saving energy, mated males of *A. ipsilon* reduce the risk of predation by stopping their search for new females. As male moths following a pheromone plume are less responsive to, e.g. the sound emitted by predating bats in a trade-off situation between finding a mate and being caught by a predator (Skals et al., 2005; Svensson et al., 2004) than moths flying without pheromone or sitting still, responding to the pheromone without being able to mate successfully would be disadvantageaous. Indeed, two species of moths, *Pseudaletia unipuncta* and *Ostrinia nubilalis*, reduce their mate-seeking behaviour under high levels of predation risk (Acharya and McNeil, 1998).

Conclusions

In the present paper we show that the significance of an important sensory signal, a sex pheromone, changes from attraction to inhibition in the context of mating in a male moth, where the behavioural switch coincides with the detection threshold for the pheromone in AL neurons in mated moths. Males have thus developed a ‘double’ strategy to avoid encountering new females, and thus unsuccessful matings, during the time they need to refill their sex glands, before being able to re-mate. The central olfactory system reduces its sensitivity to sex pheromone and males are therefore not attracted to females at a long range. However, the sex pheromone detecting system is not completely shut off, and high doses inhibit even an attraction to food plant odours. This neuroethological mechanism induces a post-mating sexual abstinence in male moths, thus allowing them to avoid the risk-taking, energy-consuming search for females.

Work is now in progress to understand the mechanisms underlying the rapid neuronal plasticity in mated males of *A. ipsilon*. Although biogenic amines have been shown to be involved in the modulation of pheromone sensitivity in animals including insects, octopamine and serotonin are probably not involved in the transient post-mating olfactory switch-off in *A. ipsilon* males (Barrozo et al., 2010). As an analogy to what is known for the lack of receptivity and pheromonostasis following mating in females, we will test the hypothesis that there could be either an inhibitory factor present or a stimulatory factor lacking in the sex accessory glands or in some part of the brain in newly mated *A. ipsilon* males.

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