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Mating-induced transient inhibition of responses to sex pheromone in a male moth is not mediated by octopamine or serotonin

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SUMMARY

In the male moth, *Agrotis ipsilon*, mating induces a transient inhibition of behavioural and central nervous responses to sex pheromone. Newly mated males are not attracted to sex pheromone, and the sensitivity of their antennal lobe (AL) neurons is lower than in virgin males. This rapid transient olfactory inhibition prevents them from re-mating unsuccessfully until they have refilled their sex glands. We hypothesized that this olfactory 'switch off' might be controlled by neuromodulators such as biogenic amines. To test our hypothesis, we studied the effects of octopamine (OA) and serotonin (5-hydroxytryptamine, 5-HT) on the coding properties of pheromone-sensitive AL neurons in virgin and newly mated males. We show that AL neuron sensitivity increased in newly mated males after injection of OA or 5-HT, but only OA treatment affected certain response characteristics of AL neurons in virgin males. Whereas all measured AL neuron response characteristics were different between virgin and newly mated males, amine treatment in newly mated males restored only the latency and spike frequency, but not the duration of excitatory and inhibitory phases, which were initially found in virgin males. Additionally, we investigated the behavioural effects of OA and 5-HT treatments in virgin and mated males. Although OA and 5-HT enhanced the general flight activity of newly mated males, amine treatments did not restore the behavioural pheromone response of mated moths. Altogether, these results show that, although biogenic amines modulate the olfactory system in moths, OA and 5-HT are probably not involved in the post-mating inhibition of responses to sex pheromone in *A. ipsilon* males.

Key words: olfactory coding, moth, antennal lobe, sex pheromone, projection neuron, mating, octopamine, serotonin, plasticity.

INTRODUCTION

Olfaction plays an important role in guiding behaviour of many animals, including insects. The attractiveness of an odorant does not depend only on the nature of the chemical but might also change with the physiological status or environmental conditions of the individual.

In the noctuid moth *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae), evidence is accumulating that the modulation of behavioural output occurs through neuronal plasticity (Anton et al., 2007). In this species, there is an age- and juvenile hormone (JH)-dependent maturation of both behavioural and central nervous responses to the female-produced sex pheromone (Anton and Gadenne, 1999; Gadenne et al., 1993). JH was also shown to control the development of the sex accessory glands (SAGs) in this species. An elevated protein content of the SAGs allows the mature male to produce and transfer a spermatophore into the female (Duportets et al., 1998). Moreover, we previously showed that newly mated males are no longer attracted to sex pheromone and that the response to pheromone is restored during the next night (Gadenne et al., 2001). This plasticity is not only seen at the behavioural level but is also accompanied by a decrease in the sensitivity of neurons within the primary olfactory centre, the antennal lobe (AL); most neurons have much higher pheromone response thresholds after mating (Gadenne et al., 2001). This transient olfactory plasticity allows newly mated males to 'wait' and eventually feed to refill their reproductive glands (Duportets et al., 1998). In newly mated males, the SAG protein content decreases to a low level directly following mating and resumes during the next night without any measurable changes in JH biosynthesis activity (Duportets et al., 1998).

A mechanism explaining the fast change in neuron sensitivity leading to the lack of behavioural pheromone response following mating could involve neuromodulators, such as biogenic amines. In vertebrates, for example, dopamine interacts with steroids to control the behavioural responses to pheromones that activate mating by modulating the central nervous system (Hull et al., 2004). In rats, serotonin (5-hydroxytryptamine, 5-HT) is involved in the sexual satiety following mating by inhibiting the dopamine brain release occurring during copulation (Hull et al., 2004). On the other hand, an increased dopamine level in the olfactory bulb after mating impairs the perception of male urine odours in female mice (Serguera et al., 2008). In male hamsters, maturation of neural processing of female sex pheromone during puberty is dopamine dependent (Hull and Dominguez, 2007; Schulz et al., 2003), and norepinephrine has dose-dependent effects on the display of male rat copulatory behaviour (Hull and Dominguez, 2007).

In insects, biogenic amines participate in the development of male sexual behaviour by acting at different levels of the olfactory pathway (Blenau and Baumann, 2001). Both octopamine (OA) and 5-HT have been shown to act on the behavioural sex pheromone response and on the sensitivity of olfactory receptor neurons (ORNs) and AL neurons (Blenau and Baumann, 2001; Roeder, 2005). OA was shown to play a role in the sexual behaviour of male moths by improving pheromone blend discrimination and orientation towards pheromone sources (Linn et al., 1996; Linn et al., 1992; Linn and Roelofs, 1986; Linn and Roelofs, 1992). It has also been shown to have an effect on ORNs by enhancing the antennal response to pheromone through the modulation of the trans-epithelial potential of olfactory sensilla

(Dolzer et al., 2001; Grosmaitre et al., 2001; Pophof, 2000), but not to plant odours (Pophof, 2002). At the central level, OA has been shown to be the responsible transmitter within the reinforcement pathway during associative learning of the honeybee (Farooqui et al., 2003; Hammer, 1993). 5-HT was shown to enhance the responses of individual AL neurons, and of neuron ensembles as measured by patch-clamp, multi-electrode recordings, and optical imaging experiments, within the central olfactory system in different moth species (for a review, see Dacks et al., 2008; Kloppenburg and Mercer, 2008).

In *A. ipsilon*, OA interacts with JH to control the age-dependent plasticity of pheromone responses (Jarriault et al., 2009a). In particular, OA treatments enhance both the behavioural and the central nervous responses to sex pheromone (Jarriault et al., 2009a). In this species, mating could eventually also trigger modulatory actions of biogenic amines that affect pheromone responses of male moths. We therefore studied the effects of OA and 5-HT injections on the mating-dependent plasticity of sex pheromone responses in *A. ipsilon*.

Results show that biogenic amines do affect the response characteristics of AL neurons to sex pheromone but cannot entirely restore the behavioural and central nervous responses of newly mated *A. ipsilon* males.

MATERIALS AND METHODS

Insects

Adults of *Agrotis ipsilon* Hufnagel, originating from a laboratory colony in Bordeaux, were used in the experiments. The colony originates from field catches in southern France, and wild insects are introduced 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 from the hatching containers every day and were given access to a 20% sucrose solution *ad libitum*. Day of emergence was considered as day-0.

Mating experiments

Virgin 5-day-old fully mature males and 3-day-old mature females were paired in cylindrical plastic containers before the onset of scotophase as described previously (Gadenne et al., 2001). Visual observation of copulations was made every 30 min during the mating period at mid-scotophase (Swier et al., 1976). Once a mating was achieved (mating lasts between 1 and 2 h), the mated male was removed from the box, transferred into a new empty box and then submitted to wind tunnel experiments or electrophysiological tests within 1 h of the end of copulation. To be sure that the male introduced a spermatophore during mating, all mated females were checked for the presence of the spermatophore.

Stimuli

For wind tunnel experiments, sex pheromone gland extracts (three female-equivalents) were prepared as described previously (Jarriault et al., 2009a). In the electrophysiological experiments, hexane dilutions in decadic steps of the artificial pheromone blend from 1 pg to 100 ng were applied to a filter paper and introduced in a Pasteur pipette. 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 of 4:1:4 (Gemeno and Haynes, 1998; Picimbon et al., 1997), as used in field trapping experiments (Causse et al., 1988).

Pharmacology

Male moths (0.2 g body mass; virgin males, and virgin males to be paired with females) received an injection of 1 µl of 145 mmol l^{-1} NaCl containing either 30 µg OA or 10 µg 5-HT (Sigma) in the abdomen (150 µg g^{-1} or 50 µg g^{-1} body mass, respectively) before the onset of the scotophase (Jarriault et al., 2009a). Control experiments were performed by injection of NaCl. A previous study showed that NaCl treatments did not affect the normal behaviour of males (Jarriault et al., 2009a).

Intracellular electrophysiology

Virgin and newly mated males were used for electrophysiological experiments between 4 and 7 h after the beginning of the scotophase. Moths were immobilized in a cut disposable pipette tube, the head capsule was opened, and tissue overlaying the brain removed, as described previously (Gadenne and Anton, 2000). Standard intracellular recording techniques were used (Christensen and Hildebrand, 1987). A KCl-filled glass microelectrode was placed close to the macroglomerular complex (MGC) within the AL of moths. A 500 ms pheromone stimulus was introduced in a constant airstream (5 ml s^{-1}) when intracellular contact had been established. Stimulation started with low intensities of the stimulus, and doses around the threshold were tested several times. A Pasteur pipette containing a filter paper with the solvent (hexane) was used as a control. Data were registered, stored on digital tape and analysed off-line using Autospike 32 software (Syntech, Hilversum, The Netherlands). The response threshold was determined as the lowest dose that elicited a response of the neuron exceeding the solvent response by at least 20% (Gadenne and Anton, 2000).

Responses to pheromone were detected automatically using an algorithm based on the detection of changes in the slope of cumulative action potential (AP) time distribution (modified from Blejec, 2005). The values of this slope were compared with its 95th and 5th percentile calculated during the spontaneous firing activity. The latency (onset of the response) was estimated as the time of the AP for which the slope passed above the 95th percentile. The end of the excitatory phase (and onset of the inhibitory phase) was the time of the AP just before the slope passed under the 5th percentile. The end of the inhibitory phase was the time at which the slope passed above the 5th percentile (searched from the end of the excitatory phase). Neurons with less than five APs in the pre-stimulation period were discarded from the analysis. The duration of excitatory and inhibitory phases was calculated, respectively, as the time between the onset of the response and the end of the excitatory phase, and between this last point and the end of the inhibitory period. The mean maximal spike frequency was calculated measuring the minimum interspike interval (ISI_{min}). We then selected the two ISIs preceding and following the ISI_{min} and calculated the inverse of the mean of these five ISIs (for details see Jarriault et al., 2009b). The four parameters (latency, mean maximal spike frequency, duration of the excitatory phase and duration of the inhibitory phase) were used to analyze the response patterns of AL neurons from virgin and newly mated males.

The response of AL neurons to sex pheromone was evaluated by comparing the proportion of neurons responding at different thresholds from the males of different groups. For statistical treatment, a $R \times C$ test of independence was performed by using a *G*-test and applying the Williams's correction (Sokal and Rohlf, 1995). In addition, *post-hoc* comparisons were carried out and the experimental-wise error rate was corrected by means of the Dunn-Šidák method (Sokal and Rohlf, 1995). For all four measured parameters, differences between mean values for AL neurons of

virgin and mated males were compared by means of the non-parametric Kruskal-Wallis test ($P<0.05$), followed by *post-hoc* multiple comparisons for mean ranks (Sokal and Rohlf, 1995).

Wind tunnel experiments

Experiments were performed using a 2 m-long flight tunnel during the middle of the scotophase (4–7 h after lights off) under red light illumination (Gadenne et al., 2001). Environmental conditions during the bioassay were held constant: 22°C, 50±10% relative humidity, wind speed of 0.3 m s^{-1}. A cage containing a single experimental male was introduced in the wind tunnel. After 30 s, during which the male adjusted to the airflow, a filter paper containing the stimulus was placed 160 cm upwind from the cage. Three female-equivalents of a sex-pheromone gland extract were used for stimulation. The behaviour of the moth was observed for 3 min, and partial flight, complete flight and landing on the pheromone source were considered as an oriented response (Jarriault et al., 2009a). Oriented as well as random flights were counted altogether in order to quantify the general flight activity of insects. Virgin males (which are expected to orient towards the pheromone) and newly mated males were tested in parallel each experimental day.

Statistical differences were evaluated among groups (i.e. NaCl, OA, 5-HT) using a $R\times C$ test of independence by means of a G-test and applying the Williams's correction (Sokal and Rohlf, 1995). In addition, individual *post-hoc* comparisons were carried out, and the experimental-wise error rate was adjusted using the Dunn-Šidák method (Sokal and Rohlf, 1995).

RESULTS

Effects of OA and 5-HT on AL neuron sensitivity to sex pheromone in virgin and mated males

A total of 243 AL neurons from 76 *A. ipsilon* males were tested for their response to sex pheromone. The general response pattern was similar to that observed previously; neurons responded generally with an excitatory response, characterized by a fast increase in AP frequency, followed by an inhibitory period devoid of APs (Anton and Gadenne, 1999; Gadenne and Anton, 2000; Jarriault et al., 2009b).

In virgin males (N=60, 33, and 38 neurons for NaCl-, OA- and 5-HT-injected males, respectively), there was no statistical difference in AL neuron response threshold between NaCl-injected males, and OA- and 5-HT-injected males (G=1.2, $P>0.05$); all males showed a high proportion of very sensitive neurons (with a low response threshold) (Fig. 1).

In newly mated males (N=40, 25 and 47 neurons for NaCl-, OA- and 5-HT-injected males, respectively), the proportion of pheromone-sensitive neurons of NaCl-injected males was significantly lower than in virgin males (G=79.8, $P<0.01$), as shown previously (Gadenne et al., 2001). On the contrary, the proportion of sensitive neurons in OA- and 5-HT-injected mated males was significantly higher than in mated males injected with NaCl (G=22.96, $P<0.001$, and G=31.42, $P<0.001$, respectively), but nevertheless significantly lower than in virgin NaCl-injected males (G=10.89, $P<0.01$ and G=16.89, $P<0.001$, respectively), thus showing intermediate sensitivity (Fig. 1).

Effects of OA and 5-HT on response characteristics of AL neurons in virgin and newly mated males

Four different parameters of the AL response to sex pheromone were analyzed in virgin and newly mated males, i.e. latency, mean maximal spike frequency, duration of excitatory and inhibitory phases (see Materials and methods).

The latency of AL neurons from virgin males was significantly decreased after treatment with both OA and 5-HT ($P<0.05$) (Fig. 2A). Newly mated males showed a longer response latency than virgin males ($P<0.05$) (Fig. 2A). Moreover, the latency of AL neurons in OA- and 5-HT-injected mated males was significantly lower than in NaCl-injected newly mated males ($P<0.01$) and was not different from that of virgin NaCl-injected males ($P>0.05$) (Fig. 2A). Thus, amine injection generally reduced the response latency independent of the mating status, and neurons in amine-injected newly mated males responded with a latency that was not significantly different from that in neurons in NaCl-injected virgin males.

The mean maximal spike frequency of AL neurons in virgin males was significantly increased only after OA treatment, compared with NaCl- and 5-HT-injected males ($P<0.0001$) (Fig. 2B). In NaCl-treated newly mated males, the spike frequency of AL neurons was lower than in virgin males ($P<0.0001$) (Fig. 2B). Moreover, the spike frequency of OA- and 5-HT-injected newly mated males was significantly higher than in mated males injected with NaCl ($P<0.0001$) and not different from that of virgin NaCl-injected males ($P>0.05$) (Fig. 2B).

For NaCl-treated insects, the durations of both the excitatory and the inhibitory phases were lower in newly mated males than in virgin males ($P<0.05$) (Fig. 2C,D). The durations of both the excitatory and inhibitory phase in AL neurons from virgin males were significantly decreased only after treatment with OA ($P<0.01$) (Fig. 2C,D). However, treatments had no effect on newly mated males; the durations of both the excitatory and the inhibitory phase in OA- and 5-HT-injected newly mated males were not different from that of NaCl-injected mated males ($P>0.05$) (Fig. 2C,D).

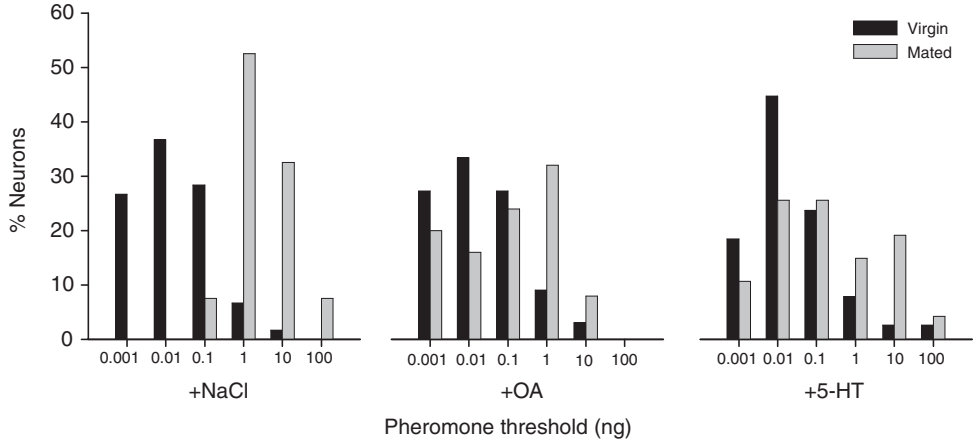

Fig. 1. Effect of octopamine (OA) and serotonin (5-HT) treatments on the response thresholds of antennal lobe (AL) neurons in virgin and newly mated *Agrotis ipsilon* males. Although there was no effect on the AL neuron sensitivity in virgin males after OA and 5-HT injections, the amine treatment partially restored the AL neuron sensitivity in newly mated males. For the number of neurons tested and statistics, see text.

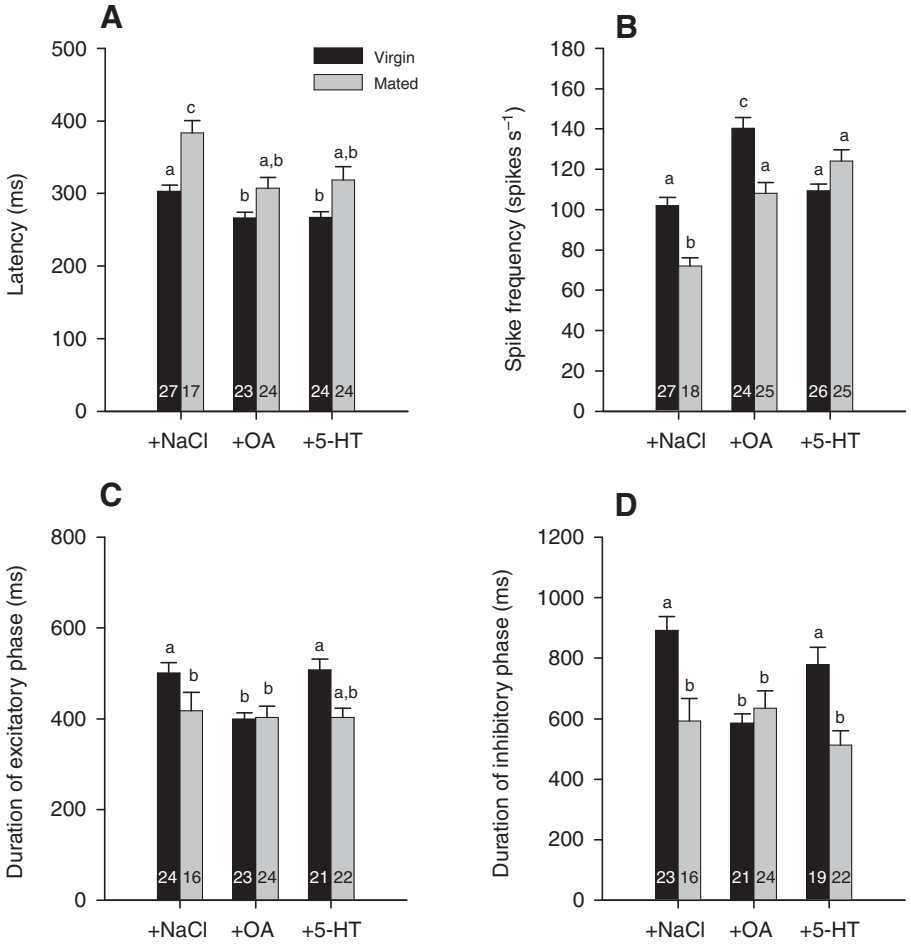

Fig. 2. Effects of octopamine (OA) and serotonin (5-HT) treatments on response characteristics of antennal lobe (AL) neurons in virgin and newly mated *A. ipsilon* males. (A) Response latency. 5-HT and OA decrease the response latency in both virgin and mated males. OA and 5-HT injected in mated males restored the response latency observed in neurons of virgin males. (B) Spike frequency (mean maximal spike frequency). OA but not 5-HT increased the spike frequency in neurons of virgin males. In mated males, OA and 5-HT restored the spike frequency to the levels observed in virgin males. (C) Duration of the excitatory phase. This parameter was only affected by 5-HT in virgin males. (D) Duration of the inhibitory phase. This parameter was only affected by 5-HT in virgin males. Bars represent means ± s.e.m. Numbers in bars indicate the number of neurons tested. Bars with the same letters are not statistically different (Kruskal-Wallis test, $P<0.05$).

Effects of OA and 5-HT on the behavioural response to sex pheromone in virgin and mated males

In virgin males, the general flight activity of NaCl-treated males was high (i.e. more than 90% of all males were highly active in the wind tunnel) and similar to that of OA- and 5-HT-injected virgin males (G=1.58, P>0.05) (Fig. 3A). The oriented flight response of 5-HT-injected virgin males was not different from that of control males (G=0.30, P>0.05) (Fig. 3B). On the contrary, OA-injected virgin males showed a significantly higher oriented response than control males (G=14.8, P<0.0001), which confirmed our previous results (Jarriault et al., 2009a) (Fig. 3B).

In newly mated males, the general flight activity was enhanced in both OA- and 5-HT-injected males as compared with NaCl-injected males (Fig. 3A). However, only NaCl- and 5-HT-treated males showed a significantly different behaviour (G=12.3, P<0.001) whereas OA-treated males showed a behaviour that was neither significantly different from 5-HT-treated males (G=1.73, P>0.05) nor from NaCl-treated males (G=3.12, P>0.05). By contrast, no significant positive oriented response to sex pheromone was observed after injection of OA or 5-HT in newly mated males (Fig. 3B).

DISCUSSION

OA and 5-HT modulate sex pheromone response characteristics of AL neurons but do not seem to be involved in post-mating inhibition

Our results showed that both OA and 5-HT had no effect on the response threshold of AL neurons of virgin males to pheromone, presumably because the AL sensitivity of mature virgin 5-day-old males is already at its maximum, as compared with the low AL sensitivity of immature 1-day-old males or newly mated males, where sensitivity can be increased by amine injection (Anton and Gadenne, 1999; Gadenne et al., 2001; Jarriault et al., 2009a). However, OA induced changes in all four AL neuron response characteristics analyzed here whereas 5-HT only changed the response latency to sex pheromone.

Moreover, we showed that mating induced a strong decrease in AL neuron sensitivity, which confirms our previous results (Gadenne et al., 2001). In addition, we showed here that the four parameters analyzed, which characterized the response of AL neurons, change in correlation with the loss in sensitivity; the response latency increases, the mean maximal spike frequency decreases, and the duration of both excitatory and inhibitory phases decrease. In newly mated males, OA and 5-HT increase the AL neuron sensitivity to pheromone, although not to the same level as in virgin males. The shift in AL neuron sensitivity after amine treatment of newly mated males might be explained by a shortened response latency and an increased firing frequency after both OA and 5-HT treatments, even though the duration of the excitatory and inhibitory phase did not change. Interestingly, the exogenous administration of both OA and 5-HT increased AL neuron sensitivity of mated males. It was previously shown that OA affects the excitability of ORNs (Grosmaitre et al., 2001; Pophof, 2000; Pophof, 2002). Furthermore, indirect evidence shows that OA can increase the sensitivity of central olfactory neurons in the context of olfactory learning; OA has been shown to stimulate protein kinases A and C, which will in turn regulate the olfactory system by phosphorylating different

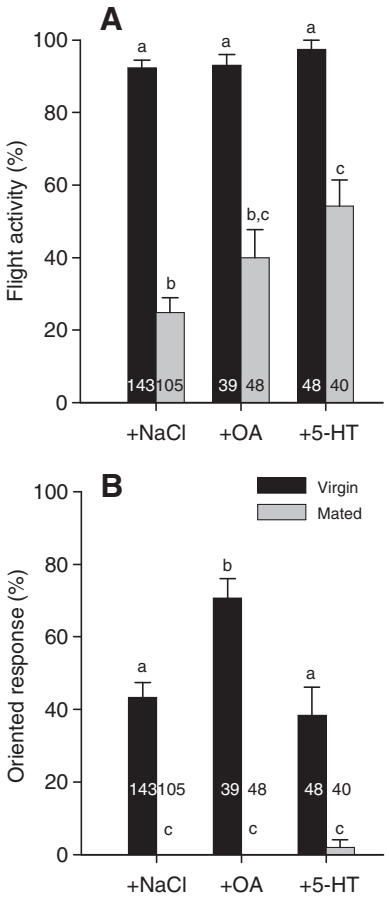

Fig. 3. Effect of octopamine (OA) and serotonin (5-HT) treatments on general flight behaviour (A) and oriented response (B) of virgin and newly mated *A. ipsilon* males towards the conspecific sex pheromone. Although the general flight activity was enhanced, the amine treatments did not restore the oriented response to sex pheromone in newly mated males. Bars represent percentage values ±s.d. [s.d. was calculated as $p(1-p)/n$; p, proportion of response; n, number of animals tested]. Numbers in bars indicate the number of males tested. Bars with the same letter are not statistically different (G-test, $P<0.05$).

target proteins (Farooqui, 2007; Müller and Hildebrandt, 1995). Concerning 5-HT effects, it has been shown that 5-HT enhances AL neuron sensitivity for sex pheromone in the moths *Bombyx mori* (Gatellier et al., 2004) and *Manduca sexta* (e.g. Kloppenburg et al., 1999; Kloppenburg and Heinbockel, 2000) by increasing the excitability of central olfactory neurons (for a review, see Kloppenburg and Mercer, 2008).

The changes in the response parameters of AL neurons between virgin and mated males indicate that modulation of the AL network by centrifugal and/or local interneurons might be at the origin of the previously observed decrease in response threshold of projection neurons (PNs), as responses of ORNs do not change (Gadenne et al., 2001; R. B. Barrozo, manuscript in preparation). Modulation might occur in terms of changes in membrane excitability of PNs and local interneurons within the AL, as shown in *M. sexta* (Kloppenburg and Hildebrand, 1995). Whole-cell patch-clamp recordings from identified PNs in the AL of *M. sexta* have shown that 5-HT modulates two types of voltage-gated potassium channels, which are responsible for the changes in membrane excitability (Kloppenburg et al., 1999). Moreover, 5-HT was shown to increase response duration and firing rate, which in turn increased the amount of spike time cross-correlation and covariance between plant odour-responding units recorded with multichannel extracellular electrodes in *M. sexta* (Dacks et al., 2008). On the contrary, most studies in vertebrates have found that 5-HT attenuates sensory responses (Chen and Regehr, 2003; Hurley et al., 2004). Recently, 5-HT was shown to diminish odour-evoked synaptic input to the olfactory bulb of the mouse, where the activation of brainstem serotonergic neurons seems to reduce the amplitude of olfactory input (Petzold et al., 2009). Apparently, there is, however, not only an attenuating effect of 5-HT in the olfactory system of vertebrates; different subsets of mitral cells within the rat olfactory bulb were either activated (depolarized) or indirectly inhibited (hyperpolarized) *via* GABA-ergic local interneurons affected by serotonin (Hardy et al., 2005). Our results show that, although AL neuron responses in newly mated males are modulated by both OA and 5-HT, the difference in sensitivity cannot be compensated by amine treatments.

OA and 5-HT treatments cannot restore the behavioural response to sex pheromone in newly mated males

Our results show that OA was able to increase the oriented response level of virgin males. This confirms previous results in other moth species (Linn et al., 1996; Linn et al., 1992; Linn and Roelofs, 1986; Linn and Roelofs, 1992) and, more recently, in *A. ipsilon* (Jarriault et al., 2009a). On the contrary, 5-HT treatments had no effect on the oriented response of *A. ipsilon* males, as in the moths *Lymantria dispar* (Linn et al., 1992) or *Trichoplusia ni* (Linn and Roelofs, 1986), although 5-HT could enhance the general locomotor activity of these two moth species. In *A. ipsilon*, this was not the case as the general flight activity in virgin males was already very high. However, although amine treatment could not restore the oriented response to sex pheromone that was observed in the virgin males, 5-HT treatments, and to a lesser extent OA, enhanced the general flight activity occurring during pheromone stimulation in newly mated males. As we previously noticed (Gadenne et al., 2001), newly mated males did not only show a lack of oriented flight to sex pheromone but they were also very often observed to be inactive and reluctant to take flight. Similarly, young immature males are inactive and do not show an oriented flight towards pheromone in the same species, in correlation with a low JH biosynthesis (Duportets et al., 1998; Gadenne et al., 1993). Recently, we showed that injections of OA into these young immature males did not induce a pheromone-guided response, because of the low JH level at this age (Jarriault et al., 2009a). This cannot be the case for the observed lack of OA effect in newly mated males in the present study, because the JH biosynthesis activity was shown to be similar to that in virgin males (Duportets et al., 1998).

In different insect species, such as *Drosophila* and locusts, OA and 5-HT have been shown to affect locomotion behaviour positively through action on different organs that allow coping with high energy demands (e.g. Saraswati et al., 2004; Sombati and Hoyle, 1984; Vierk et al., 2009).

In agreement with these data from other insects, when injected with amines, and especially with 5-HT, newly mated *A. ipsilon* males showed a higher flying activity than the NaCl- injected mated males but were indeed unable to orientate to sex pheromone. Thus, attraction behaviour cannot be restored in newly mated males by biogenic amine treatments. A more drastic effect of 5-HT and OA in the context of mating-dependent behaviour has been observed in crickets; in male *Gryllus bimaculatus*, subcuticular and terminal ganglion injections of 5-HT, OA and most effectively

5-hydroxytryptophan decreased the 1 h refractory period following copulation, characterized by a lack of courtship behaviour, up to 38% of the control (Ureshi et al., 2002).

The post-copulatory inhibition of sexual behaviour, characterized by a lack of oriented response to sex pheromone in *A. ipsilon* males, could be compared with the post-ejaculatory refractory period in males of vertebrates (Soulairac, 1952). Midbrain lesions performed in male rats abolished or significantly reduced this refractory period, and this was attributed to the disruption of biogenic amine pathways (Barfield et al., 1975). Since then, biogenic amines such as 5-HT, dopamine and norepinephrine, the vertebrate counterpart of insect OA, have been shown to play pivotal roles in both sexual behaviour and the control of this post-ejaculatory period (McIntosh and Barfield, 1984a; McIntosh and Barfield, 1984b; McIntosh and Barfield, 1984c). In male rats, 5-HT inhibits, and dopamine induces, sexual behaviour (Hull et al., 2004). 5-HT levels increase during the post-ejaculatory interval and thus inhibit dopamine release, impairing copulation (Lorrain et al., 1997; Lorrain et al., 1999). However, in our male moth model, the mating-dependent change in olfactory-guided behaviour does not seem to be dependent on OA and 5-HT levels.

Conclusions

Our results show that although biogenic amines modulate the sensitivity and certain response characteristics of AL neurons and increase locomotor activity in newly mated male moths, they are most likely not the mediators for the observed mating-induced changes of the olfactory system. The AL neuron sensitivity and its response characteristics, but not the sex pheromone-guided behaviour, were partially restored after amine treatment in newly mated males. The mechanisms we observe are only part of a more complex phenomenon. The strong inhibition of pheromone-guided behaviour following mating might result from modulation at several levels within the nervous system and is probably multicausal. Amine treatment might 'prepare' the newly mated male to respond to a new pheromone stimulation (the next day when males fully recover their sensitivity and behaviour) by increasing the sensitivity of its central nervous olfactory system and by increasing its flying activity. However, other factors, which might probably be induced by the empty SAGs, seem to still inhibit orientation of the male moth towards the pheromone. In females of many insect species (e.g. Diptera, Lepidoptera), a period of non-receptivity following mating was demonstrated. It has been shown that substances derived from the male SAGs, which are transferred with the spermatophore, play a role in switching off female receptivity (Chapman et al., 2003; Wedell, 2005). A sex peptide in *Drosophila* and a pheromonostatic peptide in moths are the responsible agents for the depletion of sexual behaviour in females, both acting through neural pathways (Häsemeyer et al., 2009; Raina and Menn, 1993). It might be possible that the same factors acting in females could have comparable effects in the males themselves. Therefore, it would be interesting to evaluate their role in males by using classical approaches (i.e. ablation of accessory glands of virgin males and observation of their sexual response after mating) or through molecular approaches (i.e. interfering with the expression of the pheromonostatic peptide by means of RNAi in male moths).

LIST OF SYMBOLS AND ABBREVIATIONS

AL	antennal lobe
AP	action potential
5-HT	serotonin
ISI	interspike interval
MGC	macroglomerular complex
OA	octopamine
ORN	olfactory receptor neuron
PN	projection neuron
SAG	sex accessory glands

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REFERENCES

Anton, S. and Gadenne, C. (1999). Effect of juvenile hormone on the central nervous processing of sex pheromone in an insect. *Proc. Natl. Acad. Sci. USA* **96**, 5764-5767.

Anton, S., Dufour, M. C. and Gadenne, C. (2007). Plasticity of olfactory-guided behaviour and its neurobiological basis: lessons from moths and locusts. *Ent. Exp. Appl.* **123**, 1-11.

Barfield, R. J., Wilson, C. and McDonald, P. G. (1975). Sexual behavior: Extreme reduction of postejaculatory refractory period by midbrain lesions in male rats. *Science* **189**, 147-149.

Blejec, A. (2005). Statistical method for detection of firing rate changes in spontaneously active neurons. *Neurocomputing* **65**, 557-563.

Blenau, W. and Baumann, A. (2001). Molecular and pharmacological properties of insect biogenic amine receptors: lessons from *Drosophila melanogaster* and *Apis mellifera*. *Arch. Insect Biochem. Physiol.* **48**, 13-38.

Causse, R., Buès, R., Barthès, J. and Toubon, J. F. (1988). Mise en évidence expérimentale de nouveaux constituants des phéromones sexuelles de *Scotia ipsilon* et *Mamestra suasa*. *Méd. Chim. Comp. Syst. Lép. (Coll. INRA)* **46**, 75-82.

Chapman, T., Bangham, J., Vinti, G., Seifried, B., Lung, O., Wolfner, M. F., Smith, H. K. and Partridge, L. (2003). The sex peptide of Drosophila melanogaster: female post-mating responses analysed by using RNA interference. *Proc. Natl. Acad. Sci. USA* **100**, 9923-9928.

Chen, C. and Regehr, W. G. (2003). Presynaptic modulation of the retinogeniculate synapse. *J. Neurosci.* **23**, 3130-3135.

Christensen, T. A. and Hildebrand, J. G. (1987). Male-specific, sex pheromone-selective projection neurons in the antennal lobes of the moth, *Manduca sexta*. *J. Comp. Physiol. A* **160**, 553-569.

Dacks, A., Christensen, T. and Hildebrand, J. (2008). Modulation of olfactory information processing in the antennal lobe of *Manduca sexta* by serotonin. *J. Neurophysiol.* **99**, 2077-2085.

Dolzer, J., Krannich, S., Fischer, K. and Stengl, M. (2001). Oscillations of the transepithelial potential of moth olfactory sensilla are influenced by octopamine and serotonin. *J. Exp. Biol.* **204**, 2781-2794.

Duportets, L., Dufour, M. C., Couillaud, F. and Gadenne, C. (1998). Biosynthetic activity of corpora allata, growth of sex accessory glands and mating of the male moth *Agrotis ipsilon*. *J. Exp. Biol.* **201**, 2425-2432.

Farooqui, T. (2007). Octopamine-mediated neuromodulation of insect senses. *Neurochem. Res.* **32**, 1511-1529.

Farooqui, T., Robinson, K. O., Vaessin, H. and Smith, B. H. (2003). Modulation of early olfactory processing by an octopaminergic reinforcement pathway in the honeybee. *J. Neurosci.* **23**, 5370-5380.

Gadenne, C. and Anton, S. (2000). Central processing of sex pheromone stimuli is differentially regulated by juvenile hormone in a male moth. *J. Insect Physiol.* **46**, 1195-1206.

Gadenne, C., Renou, M. and Sreng, L. (1993). Hormonal control of sex pheromone responsiveness in the male black cutworm, *Agrotis ipsilon*. *Experientia* **49**, 721-724.

Gadenne, C., Dufour, M. C. and Anton, S. (2001). Transient post-mating inhibition of behavioural and central nervous responses to sex pheromone in an insect. *Proc. R. Soc. Biol. Sci.* **268**, 1631-1635.

Gatellier, L., Nagao, T. and Kanzaki, R. (2004). Serotonin modifies the sensitivity of the male silkmoth to pheromone. *J. Exp. Biol.* **207**, 2487-2496.

Gemeno, C. and Haynes, K. F. (1998). Chemical and behavioral evidence for a third pheromone component in a north american population of the black cutworm moth, *Agrotis ipsilon*. *J. Chem. Ecol.* **24**, 999-1011.

Grosmaitre, X., Marion-Poll, F. and Renou, M. (2001). Biogenic amines modulate olfactory receptor neurons firing activity in *Mamestra brassicae*. *Chem. Senses* **26**, 653-661.

Hammer, M. (1993). An identified neuron mediates the unconditioned stimulus in associative olfactory learning in honeybees. *Nature* **366**, 59-63.

Hardy, A. B., Aïoun, J., Baly, C., Julliard, K. A., Caillol, M., Salesse, R. and Duchamp-Viret, P. (2005). Orexin A modulates mitral cell activity in the rat olfactory bulb: patch-clamp study on slices and immunocytochemical localization of orexin receptors. *Endocrinol.* **146**, 4042-4053.

Häsemeyer, M., Yapici, N., Heberlein, U. and Dickson, B. J. (2009). Sensory neurons in the Drosophila genital tract regulate female reproductive behavior. *Neuron* **61**, 511-518.

Hull, E. M. and Dominguez, J. M. (2007). Sexual behavior in male rodents. *Horm. Behav.* **52**, 45-55.

Hull, E. M., Muschamp, J. W. and Sato, S. (2004). Dopamine and serotonin: influences on male sexual behavior. *Physiol. Behav.* **83**, 291-307.

Hurley, L. M., Devilbiss, D. M. and Waterhouse, B. D. (2004). A matter of focus: monoaminergic modulation of stimulus coding in mammalian sensory networks. *Curr. Opin. Neurobiol.* **14**, 488-495.

Jarriault, D., Barrozo, R. B., de Carvalho Pinto, C. J., Greiner, B., Dufour, M., Masante-Roca, I., Gramsbergen, J. B., Anton, S. and Gadenne, C. (2009a). Age-dependent plasticity of sex pheromone response in the moth, *Agrotis ipsilon*: combined effects of octopamine and juvenile hormone. *Horm. Behav.* **56**, 185-191.

Jarriault, D., Gadenne, C., Rospars, J.-P. and Anton, S. (2009b). Quantitative analysis of sex-pheromone coding in the antennal lobe of the moth *Agrotis ipsilon*: a tool to study network plasticity. *J. Exp. Biol.* **212**, 1191-1201.

Kloppenburg, P. and Hildebrand, J. G. (1995). Neuromodulation by 5-hydroxytryptamine in the antennal lobe of the sphinx moth, *Manduca sexta*. *J. Exp. Biol.* **198**, 603-611.

Kloppenburg, P. and Heinbockel, T. (2000). 5-hydroxytryptamine modulates pheromone-evoked local field potentials in the macroglomerular complex of the sphinx moth *Manduca sexta*. *J. Exp. Biol.* **203**, 1701-1709.

Kloppenburg, P. and Mercer, A. R. (2008). Serotonin modulation of moth central olfactory neurons. *Annu. Rev. Entomol.* **53**, 179-190.

Kloppenburg, P., Ferns, D. and Mercer, A. R. (1999). Serotonin enhances central olfactory neuron responses to female sex pheromone in the male sphinx moth *Manduca sexta*. *J. Neurosci.* **19**, 8172-8181.

Linn, C. E. and Roelofs, W. (1986). Modulatory effects of octopamine and serotonin on male sensitivity and periodicity of response to sex pheromone in the cabbage looper moth *Trichoplusia ni*. *Arch. Insect Biochem. Physiol.* **3**, 161-172.

Linn, C. E. and Roelofs, W. (1992). Role of photoperiod cues in regulating the modulatory action of octopamine on pheromone-response thresholds in the cabbage looper moth. *Arch. Insect Biochem. Physiol.* **20**, 285-302.

Linn, C. E., Campbell, M. G. and Roelofs, W. L. (1992). Photoperiod cues and the modulatory action of octopamine and 5-hydroxytryptamine on locomotor and pheromone in male gypsy moths, *Lymantria dispar*. *Arch. Insect Biochem. Physiol.* **20**, 265-284.

Linn, C. E., Campbell, M. G., Poole, K. R., Wu, W.-Q. and Roelofs, W. L. (1996). Effects of photoperiod on the circadian timing of pheromone response in male *Trichoplusia ni*: relationship to the modulatory action of octopamine. *J. Insect Physiol.* **42**, 881-891.

Lorrain, D. S., Matuszewich, L., Friedman, R. D. and Hull, E. M. (1997). Extracellular serotonin in the lateral hypothalamic area is increased during the postejaculatory interval and impairs copulation in male rats. *J. Neurosci.* **17**, 9361-9366.

Lorrain, D. S., Riolo, J. V., Matuszewich, L. and Hull, E. M. (1999). Lateral hypothalamic serotonin inhibits nucleus accumbens dopamine: implications for sexual satiety. *J. Neurosci.* **19**, 7648-7652.

McIntosh, T. K. and Barfield, R. J. (1984a). Brain monoaminergic control of male reproductive behavior. I. Serotonin and the post-ejaculatory refractory period. *Behav. Brain Res.* **12**, 255-265.

McIntosh, T. K. and Barfield, R. J. (1984b). Brain monoaminergic control of male reproductive behavior. II. Dopamine and the post-ejaculatory refractory period. *Behav. Brain Res.* **12**, 267-273.

McIntosh, T. K. and Barfield, R. J. (1984c). Brain monoaminergic control of male reproductive behavior. III. Norepinephrine and the post-ejaculatory refractory period. *Behav. Brain Res.* **12**, 275-281.

Müller, U. and Hildebrandt, H. (1995). The nitric oxide/cGMP system in the antennal lobe of *Apis mellifera* is implicated in integrative processing of chemosensory stimuli. *Eur. J. Neurosci.* **7**, 2240-2248.

Petzold, G. C., Hagiwara, A. and Murthy, V. (2009). Serotonergic modulation of odor input to the mammalian olfactory bulb. *Nat. Neurosci.* **12**, 784-791.

Picimbon, J. F., Gadenne, C., Bécard, J. M., Clément, J. L. and Sreng, L. (1997). Sex pheromone of the french black cutworm moth, *Agrotis ipsilon* (Lepidoptera: Noctuidae): identification and regulation of a multicomponent blend. *J. Chem. Ecol.* **23**, 211-230.

Poitout, S. and Buès, R. (1974). Elevage de plusieurs espèces de lépidoptères sur milieu artificiel simplifié. *Ann. Zool. Ecol. Anim.* **2**, 79-91.

Pophof, B. (2000). Octopamine modulates the sensitivity of silkmoth pheromone receptor neurons. *J. Comp. Physiol. A* **186**, 307-313.

Pophof, B. (2002). Octopamine enhances moth olfactory responses to pheromones, but not those to general odorants. *J. Comp. Physiol. A* **188**, 659-662.

Raina, A. K. and Menn, J. J. (1993). Pheromone biosynthesis activating neuropeptide: from discovery to current status. *Arch. Insect Biochem. Physiol.* **22**, 141-151.

Roeder, T. (2005). Tyramine and octopamine: ruling behavior and metabolism. *Annu. Rev. Entomol.* **50**, 447-477.

Saraswati, S., Fox, L. E., Soll, D. R. and Wu, C.-F. (2004). Tyramine and octopamine have opposite effects on the locomotion of Drosophila larvae. *J. Neurobiol.* **58**, 425-441.

Schulz, K. M., Richardson, H. N., Romeo, R. D., Morris, J. A., Lookingland, K. J. and Sisk, C. L. (2003). Medial preoptic area dopaminergic responses to female pheromones develop during puberty in the male Syrian hamster. *Brain Res.* **988**, 139-145.

Serguera, C., Triaca, V., Kelly-Barrett, J., Banchaabouchi, A. and Minichiello, L. (2008). Increased dopamine after mating impairs olfaction and prevents odor interference with pregnancy. *Nat. Neurosci.* **11**, 949-956.

Sokal, R. R. and Rohlf, F. J. (1995). *Biometry, The Principles And Practice Of Statistics In Biological Research*. New York: W. H. Freeman & Co.

Sombati, S. and Hoyle, G. (1984). Generation of specific behaviors in a locust by local release into neuropil of the natural neuromodulator octopamine. *J. Neurobiol.* **15**, 481-506.

Soulairac, A. (1952). The physiological significance of the refractory period in the sexual behavior of the male rat. *J. Physiol.* **44**, 99-113.

Swier, S. R., Rings, R. W. and Musick, G. J. (1976). Reproductive behavior of the black cutworm, *Agrotis ipsilon*. *Ann. Entomol. Soc. Am.* **69**, 546-550.

Ureshi, M., Dainobu, M. and Sakai, M. (2002). Serotonin precursor (5-hydroxytryptophan) has a profound effect on the post-copulatory time-fixed sexually refractory stage in the male cricket, *Gryllus bimaculatus* DeGeer. *J. Comp. Physiol. A* **188**, 767-779.

Vierk, R., Pflueger, H. J. and Duch, C. (2009). Differential effects of octopamine and tyramine on the central pattern generator for *Manduca* flight. *J. Comp. Physiol. A* **195**, 265-277.

Wedell, N. (2005). Female receptivity in butterflies and moths. *J. Exp. Biol.* **208**, 3433-3440.