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Concurrent modulation of neuronal and behavioural olfactory responses to sex and host plant cues in a male moth

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Mating has profound effects on animal physiology and behaviour, not only in females but also in males, which we show here for olfactory responses. In cotton leafworm moths, *Spodoptera littoralis*, odour-mediated attraction to sex pheromone and plant volatiles are modulated after mating, producing a behavioural response that matches the physiological condition of the male insect. Unmated males are attracted by upwind flight to sex pheromone released by calling females, as well as to volatiles of lilac flowers and green leaves of the host plant cotton, signalling adult food and mating sites, respectively. Mating temporarily abolishes male attraction to females and host plant odour, but does not diminish attraction to flowers. This behavioural modulation is correlated with a response modulation in the olfactory system, as shown by electro-physiological recordings from antennae and by functional imaging of the antennal lobe, using natural odours and synthetic compounds. An effect of mating on the olfactory responses to pheromone and cotton plant volatiles but not to lilac flowers indicates the presence of functionally independent neural circuits within the olfactory system. Our results indicate that these circuits interconnect and weigh perception of social and habitat odour signals to generate appropriate behavioural responses according to mating state.

1. Introduction

Animals express stereotypic behaviours that are controlled by sensorimotor circuits and modulated by external and internal factors [1]. Reproductive state, for example, modulates sensory processing and thus influences behavioural choices. In females of the cotton leafworm, *Spodoptera littoralis* (Lepidoptera, Noctuidae), mating modulates olfactory sensitivity and switches odour-mediated attraction from floral food to green host plant cues [2]. A similar behavioural switch in preference, from pheromone to host cues, has been observed in female fruit flies [3] and parasitoid wasps [4,5].

By contrast, little is known about how mating affects behavioural responses to olfactory signals in male insects. Mating-induced downregulation of male attraction to sex pheromone has been investigated in the parasitoid wasp, *Spalangia endius*, and the noctuid moth, *Agrotis ipsilon* [6,7]. In *A. ipsilon* males, this behavioural inhibition is correlated with a decreased sensitivity of antennal lobe (AL) neurons to the conspecific sex pheromone [7–9] and an increased response to a plant odour [9,10]. Available data also suggest that mating-induced changes in the olfactory response are distinct for pheromone and non-pheromone coding pathways [9]. In moths and other animals, males often show a post-ejaculatory refractory period of sexual inactivity but the role of the olfactory system in this behavioural modulation is not understood [6,7,11].

Cotton leafworm, *S. littoralis*, is a suitable species to study peripheral and central olfactory modulation. Its behaviour in response to pheromone and plant odours, and the physiology and morphology of the olfactory apparatus, have been extensively studied (e.g. [2,12–17]). We have investigated whether mating-induced olfactory and behavioural modulation to floral and green cues observed in female moths [2] is sex specific, and whether the response to female sex pheromone is modulated in males after mating.

We show here that males show a mating-induced olfactory and behavioural modulation to green host plant cues, albeit the response is distinct from the female switch of preference from floral to green [2]. After mating, *S. littoralis* males show a reversible downregulation in their attraction to green mating site plant odours, and additionally to conspecific female sex pheromone. This behavioural modulation is reflected by changes in sensitivity of the peripheral olfactory system and the responses elicited in the AL. By contrast, the behavioural and olfactory response to floral odour is not significantly affected by mating. Our findings indicate the presence of separate olfactory circuits, each responding differentially to mating-induced modulation.

2. Material and methods

(a) Insects
Spodoptera littoralis were reared under controlled conditions on semi-artificial diet under a 16 L : 8 D photoperiod [2]. Two-to-three day-old unmated males, and males 3 h or 24 h post mating, were used in the analyses. All analyses of virgin and mated males were started after 4 h of the scotophase. For timing the start of experiments with mated males, about 30 males and females were placed together, either at the beginning of the scotophase (for testing them 3 h post mating) or 3 h after beginning of the scotophase (for testing them 24 h post mating). Most moths started mating within a few minutes, which resulted in a sufficiently high number of males that finished mating within the given mating period of 1 h.

(b) Plant material
Cotton plants (*Gossypium hirsutum*) were grown from seeds (produced in-house from a cultivar of DPL 90, Delta and Pine Land Company, Scott, MS, USA) in a greenhouse, and lilac (*Syringa vulgaris*) flowers were field-collected in Alnarp, Sweden [2].

(c) Plant headspace collection and chemical analyses
Headspace collections from both non-flowering cotton plants and lilac flowers were performed using a dynamic headspace apparatus, drawing charcoal-filtered air over the plant materials onto an air filter with Super Q adsorbent (80/100 mesh, Altech, Deerfield, IL, USA) [2]. Trapped plant volatiles were subsequently eluted from the filter and analysed by gas chromatography-mass spectrometry (GC–MS; 6890 GC and 5975 MS; Agilent Technologies, Palo Alto, CA, USA), as described earlier [2].

(d) Female pheromone gland extraction and chemical analysis
Pheromone glands of calling, two-to-three day-old, unmated *S. littoralis* females were dissected from the extruded ovipositors using a pair of fine forceps. Pheromone glands were extracted in redistilled heptane (Labscan, 20 µl/10 glands) as previously described [18]. A subsample of extracts was used for chemical analysis. For bioassays, extracts were pooled and divided into volumes equivalent to 10 female glands. Extracts were stored in sealed glass capillaries at −20°C until analysis.

Chemical analysis of pheromone extracts was performed by GC–MS, equipped with a fused silica DB-Wax capillary column (30 m × 0.25 mm, d.f. = 0.25 µm; J&W Scientific, Folsom, CA, USA). Helium was used as carrier gas at an average linear flow of 35 cm s^{-1}. The initial temperature of the GC was set to 80°C (5 min hold) and then increased 10°C min^{-1} to a final temperature of 225°C (10 min hold). Pheromone components were identified by co-injection of authentic synthetic samples and comparison with a NIST mass spectra library (Agilent) and our own library [18].

(e) Synthetic chemicals
Synthetic compounds used for electroantennogram (EAG) and optical imaging (OI) analyses included plant-related components (benzaldehyde, benzyl methyl ether, (*E*)-β-ocimene, phenylacetaldehyde, acetophenone, benzyl alcohol, (+)-(*S*)-linalool, 1,4-dimethoxy benzene, estragole, β-myrcene, Z3-hexenyl acetate, *p*-cymene, (+)-(*R*)-limonene and nonanal), female-emitted sex pheromone components (Z9,E11-14Ac, Z9,E12-14Ac, Z9-14Ac, E11-14Ac, Z11-14Ac and 14Ac) and the synthetic mimic of the sex pheromone blend (ratio according to [18], 10(Z9,E11-14Ac) : 0.4(Z9,E12-14Ac) : 1(Z11-14Ac) : 2(E11-14Ac) : 3(Z9-14Ac) : 0.3(14Ac); see electronic supplementary material, table S5, for further details on the synthetic chemicals). For single sensillum recordings (SSRs), Z9,E11-14Ac, Z9,E12-14Ac and Z9-14Ac were tested individually. Synthetic compounds were diluted in decadic steps in redistilled *n*-hexane (Labscan) except for benzyl alcohol, which was diluted in diethyl ether (Fluka).

(f) Wind tunnel bioassay
Wind tunnel experiments were performed according to the protocol described earlier with cotton plant, lilac flowers [2] or calling females [18] (approximately five calling females at a time) as attractants. Take-off, upwind-directed flight and landing at the source were recorded. Behavioural inhibition as described in the Results section for males 3 h post mating (§3(a) Wind tunnel) was observed within the first hour after mating. However, the time required for preparation of mated males for OI (see below) set the starting time for experiments to 3 h post mating at the earliest (corresponding to 4 h after onset of the scotophase for virgin and mated moths).

(g) Electro-physiological and optical imaging recordings
Gas chromatography–electroantennodetection (GC–EAD) analysis with simultaneous (1 : 1 split) flame ionization detection (GC–FID) of the plant headspace or pheromone extracts, as well as stationary EAG recordings, were done as previously described [2]. Single sensillum recordings were performed from the antennal long trichoid sensilla known to respond to pheromone components [16].

Optical imaging was performed as described before [2], with the exception of using Calcium Green-1 AM (Invitrogen) instead of Calcium Green-2 AM. In short, the head of a fixed moth was dissected to expose the brain, bathed for 1 h in Calcium Green-1 AM and then rinsed with Ringer solution and transferred to a microscope (Olympus, Tokyo, Japan) for imaging of odour-evoked responses. Glomerular numbering was based on the number assignment of Couton *et al*. [19].

(h) Odour stimulation for electroantennogram, sensillum recording and optical imaging

Odour stimulation for EAG and OI recordings was performed according to earlier described practice [2], with loadings on filter paper (1×1 cm) ranging from 1 ng to 100 µg or 1 mg for individual plant-related and pheromone components, 0.001–100 min equivalents for cotton plant and lilac flower headspace collections, and 0.001–100 female equivalents (FE) for the synthetic pheromone blend. Odour delivery from filter paper and physiological stimulation are dependent on compound-specific evaporation rates. Stimulus loadings of individual plant and pheromone compounds (1 ng–100 µg or 1 mg) are therefore not equivalent to absolute amounts inducing stimulation. The applied loadings, however, cover a suitable range for physiological stimulation and offer the possibility for replication of the described procedure.

Because of identical OI responses to pheromone gland extract and the synthetic mimic of the pheromone blend (based on GC–MS qualification and quantification, see §2e), EAG and OI experiments were carried out using the synthetic mimic. Odour stimulation for SSR experiments was done as described before [16] with doses ranging from 1 ng to 100 µg.

(i) Statistical analysis

Contrast analysis was used to analyse the effect of mating status (all possible comparisons between virgin, 3 h and 24 h post mating) on the behavioural response of males to lilac flowers, cotton plant or calling females, using a generalized linear model (GzLM) with quasi-binomial distribution [20]. Repeated-measurements (RM) ANOVA, followed by Bonferroni post hoc test, were used to compare the EAG and OI responses, respectively, between unmated and mated males to sequential dilutions of the headspace extracts, the sex pheromone blend and the individual synthetic components of both. The non-parametric Kruskal–Wallis test followed by Dunn's *post hoc* test were used to compare SSR spike frequencies induced by sequential dilutions of the pheromone components in unmated and mated males.

To estimate the congruence (magnitude of biological effects by mating among assays) between behavioural (wind tunnel) and physiological (EAG and OI) data, we calculated the effect size, a unitless measure, which scales the differences between a pair of means by their pooled standard deviation (as the conservative, bias corrected Hedges' *g*) [21]. For OI, we added the values of responding glomeruli (#17, 18 and 37 for pheromone; #5, 6, 7, 10, 11 and 15 for cotton, and #8, 10 and 16 for lilac) to get only one data point per assay and stimuli, similar to EAG measurements. Similarly, take-off, as the primary step of odour-mediated upwind flight behaviour, was used to calculate effect size for the wind tunnel assay. The stimuli and dose used for each such effect size corresponded to a 10 min collection of volatiles of plants or 10 FE, respectively.

Analyses were done with R-Studio software v. 0.96.316 (R Development Core Team, Free Software Foundation Boston, MA, USA), Graphpad Prism v. 5.0a, and IBM SPSS.19.

3. Results

(a) Wind tunnel

Changes in odour-mediated upwind flight attraction illustrated a mating-induced olfactory modulation in male *S. littoralis*. Male moths were attracted to social and plant cues depending on odour stimulus, mating status and time post mating (figure 1). Unmated males were attracted to calling females, cotton plants and lilac flowers (figure 1).

Pheromone-mediated upwind flight was followed by landing at the calling female (figure 1*b*), and several unmated males landed also on cotton or lilac flowers (figure 1*a,c*). Shortly after mating, the response to cotton plants and calling females was entirely abolished. However, 24 h after mating, the response to calling females was fully restored and the response to cotton plant was partially restored (figure 1*a,b*). In comparison, males were highly attracted to lilac flowers, independent of mating status (figure 1*c*).

(b) Gas chromatography – electroantennodetection and gas chromatography – mass spectrometry

GC–EAD and GC–MS analyses were performed to identify antennally active compounds for subsequent studies on olfactory modulation by EAG, SSR and OI. Three active components in the female pheromone gland extract elicited a response in male antennae, Z9,E11-14Ac, Z9,E12-14Ac and Z9-14Ac (figure 2*d*).

In headspace collections from cotton plants and lilac flowers, 6 and 14 compounds, respectively, elicited an antennal response (figures 3*d* and 4*d*). Benzaldehyde was the only compound co-occurring in leaf and flower headspace.

(c) Electroantennogram

Olfactory modulation at the antennal level was then studied by EAG, applying a synthetic pheromone blend, plant headspace samples as well as single pheromone or plant components. Three hours after mating, the antennal response of mated males to a synthetic pheromone blend was significantly lower than in unmated males (figure 2*c*). Tested singly, three of the six synthetic components induced a significantly different response in males 3 h post mating compared with unmated ones: Z9,E11-14Ac (figure 2*g*), Z9,E12-14Ac and Z9-14Ac (electronic supplementary material, figure S1). Twenty-four hours after mating, the antennal responses to these components had partially recovered (figure 2*c,g*; electronic supplementary material, figure S1).

In agreement with attraction to cotton (figure 1*a*), EAG responses to cotton headspace were lowest in males 3 h after mating (figure 3*c*). Two of the six cotton volatiles producing an antennal response, β-myrcene and *p*-cymene, elicited significantly different responses in unmated males and males 3 h post mating (electronic supplementary material, figure S2).

Stimulation with lilac flower headspace revealed no significant difference in response of unmated and mated males (figure 4*c*), in accordance with a consistent behavioural response (figure 1*c*). None of the nine lilac flower components tested with EAG showed differences in response amplitudes between unmated males and males 3 h after mating (electronic supplementary material, figure S3).

(d) Sensillum recording

Next, modulation at the sensory neuron level was studied for the three pheromone components that showed modulation in EAG recordings. Single sensillum recordings from long trichoid sensilla confirmed the transient downregulation observed in EAG recordings. Spike frequencies elicited by Z9,E11-14Ac (figure 2*g*) and Z9-14Ac (electronic supplementary material, figure S4*a*) were decreased in males 3 h after mating, compared with unmated males. Responses did not differ
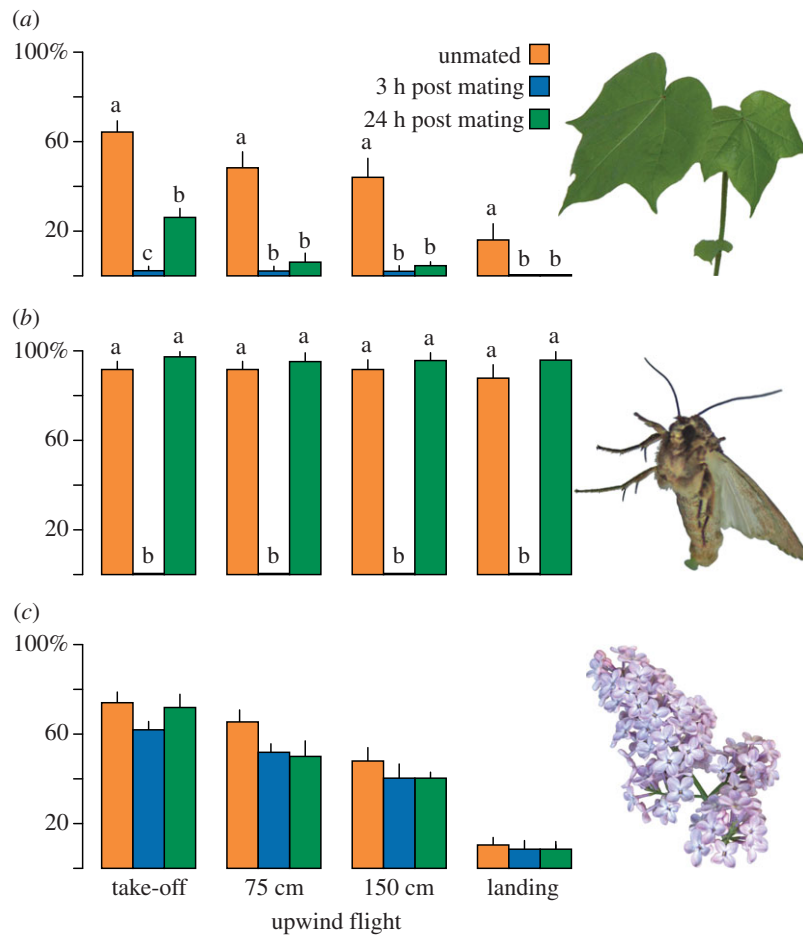
Figure 1. Upwind attraction of unmated and mated (3 or 24 h post mating) male *S. littoralis* to (*a*) cotton plant *G. hirsutum*, (*b*) calling *S. littoralis* females and (*c*) lilac flowers *Sy. vulgaris* in a wind tunnel (mean ± s.e.m., *n* = 50). Different letters indicate significant differences (*p* < 0.05) according to GzLM with quasi-binomial distribution. (Online version in colour.)

between unmated males and males tested 24 h post mating (figure 2*g*; electronic supplementary material, figure S4*a*).

In addition to a change in action potential frequency, temporal patterns of the sensory neuron responses differed according to mating state. Neurons of unmated males and males 24 h after mating showed phasic responses to Z9,E11-14Ac, whereas responses in males 3 h post mating were phasic-tonic (figure 2*f*; electronic supplementary material, figure S4*b*). Similar temporal characteristics were observed for neurons responding to Z9-14Ac and Z9,E12-14Ac (data not shown).

(e) Optical imaging

Mating-dependent modulation of the antennal responses, as measured by EAG and SSR, was corroborated by functional imaging of the AL. The synthetic pheromone blend evoked visible calcium activity in three AL glomeruli (figure 2*a,b*). At the highest dose, the activity of glomerulus 18 was significantly decreased in males 3 h post mating compared with unmated males, and males 24 h post mating. Three pheromone components, Z9,E11-14Ac, Z9,E12-14Ac and Z9-14Ac elicited visible responses in the AL (figure 2*e*; electronic supplementary material, figure S1). The main compound Z9,E11-14Ac (figure 2*g*) and Z9-14Ac (electronic supplementary material, figure S1) elicited a significantly lower response 3 h after mating.

Mating-dependent modulation was also visible in response to cotton volatiles. Cotton plant headspace evoked activity in six

glomeruli in unmated males. Calcium activity in these glomeruli was either completely or partially downregulated 3 h after mating; activity was either partially or fully restored after 24 h (figure 3*a,b*). Of the six cotton volatiles eliciting antennal response, four elicited AL activity: benzaldehyde, Z3-hexenyl acetate, *p*-cymene and nonanal (electronic supplementary material, figure S2). Responses to *p*-cymene and nonanal were significantly lower 3 h after mating; there was no difference in males before and 24 h after mating for any of the cotton volatiles (electronic supplementary material, figure S2).

In contrast to stimulation with pheromone or cotton headspace, no AL modulation was observed in response to lilac odour in three responding glomeruli (figure 4*a,b*). Six out of nine antennally active lilac volatiles (benzaldehyde, benzyl methyl ether, phenyl acetaldehyde, acetophenone, benzyl alcohol and (S)-(+)-linalool) evoked glomerular activity, but no significant difference in unmated and mated males was observed (electronic supplementary material, figure S3).

(f) Neuronal and behavioural responses reflect the concurrent modulation to sex and host plant cues

We finally analysed the congruence of effects induced by mating among assays. Analyses of effect size showed that the behavioural modulation, measured by wind tunnel responses, to three different stimulus types was reflected in neuronal responses measured by EAG and OI. Declines 3 h post mating and the reversed effects 24 h after mating were
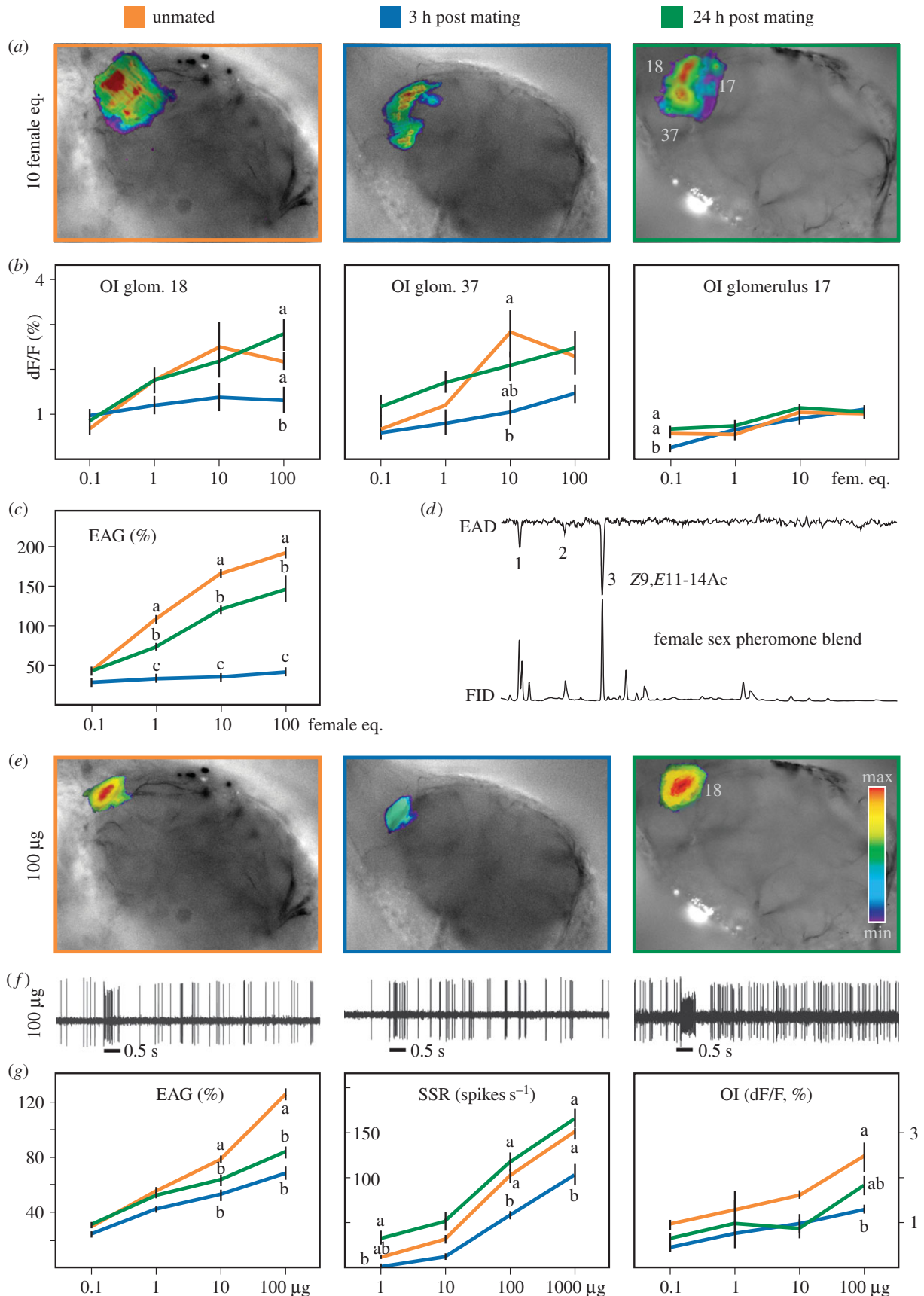
Figure 2. Optical imaging and electro-physiological recordings of unmated and mated (3 or 24 h post mating) male *S. littoralis* olfactory responses to different loadings of (*a−c*) the synthetic pheromone blend and (*e−g*) the main component of the pheromone blend (Z9,E11-14Ac) by OI, EAG and SSR. (*d*) Antennal active components in the pheromone gland extract are (1) Z9-14Ac, (2) Z9,E12-14Ac and (3) Z9,E11-14Ac as identified by synchronous signals from GC−FID and the coupled electroantennodetection (EAD). (*f*) SSR recordings from unmated (left) and mated males, 3 h (middle) or 24 h (right) post mating. (*c,g* left) EAG (%), (*f,g* middle) SSR (spikes s^{-1}) responses and (*a,b,e,g* right) OI intensities ($\Delta F/F$; %) obtained on antennal lobe glomeruli. Stimulus loadings from 0.1 to 100 FE for the synthetic pheromone blend and (0.1 − 100 µg) for Z9,E11-14Ac were applied on filter paper. Insignificant baseline responses at lower loadings are not shown. Different letters within the same dose indicate significant difference ($p < 0.05$, Bonferroni *post hoc* tests following two-way RM ANOVA for EAG and OI; Dunn's multiple comparison following Kruskal−Wallis test for SSR). SSR: horizontal bar represents 0.5 s. (Online version in colour.)
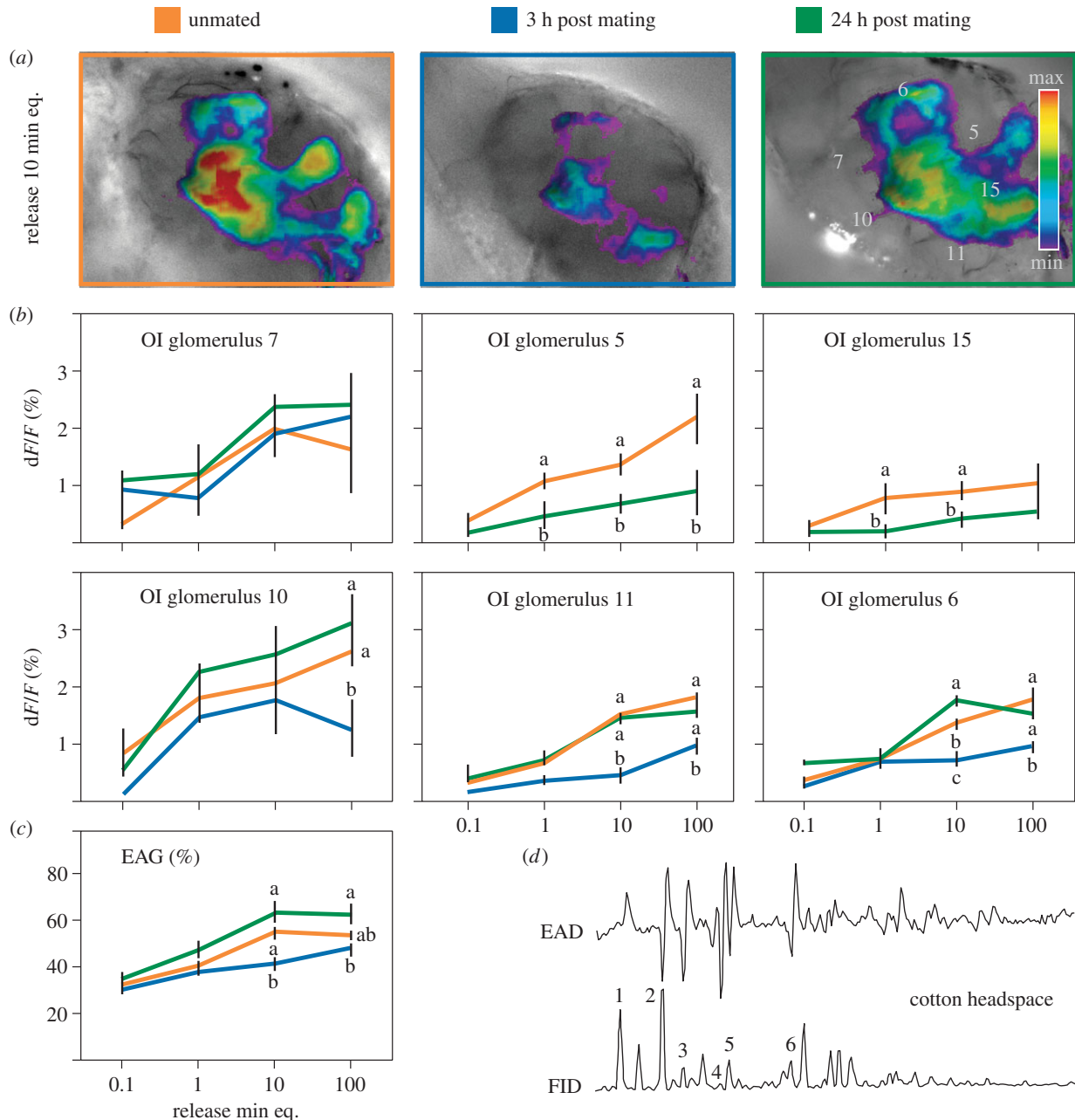
Figure 3. (a,b) The OI of antennal lobe glomeruli and (c) EAG responses of unmated and mated (3 or 24 h post mating) male *S. littoralis* to authentic cotton head space. (d) GC–EAD active components are (1) benzaldehyde, (2) β-myrcene, (3) (Z)-3-hexenyl acetate, (4) *p*-cymene, (5) (+)-(R)-limonene and (6) nonanal. EAG responses (%) and optical imaging intensities (ΔF/F; %) obtained with increasing loadings (applied on filter paper) of cotton plant headspace samples equivalent to 0.1–100 min of sampling. Insignificant baseline responses at lower loadings are not shown. Different letters within the same dose indicate significant differences ($p < 0.05$, Bonferroni *post hoc* tests following two-way RM ANOVA for EAG and OI). (Online version in colour.)

similar in effect size and confirmed olfactory modulation as a result of mating.

The changes measured with different techniques, for each stimulus, mirror each other in the sense that magnitudes of decline and recovery effects are numerically similar. Hence, there is a strong correlation ($r^2 = 0.83$) between electro-physiological, imaging and behavioural experiments, with an intercept of zero and a slope close to unity (figure 5). The magnitude of decline (3 h) and recovery (24 h after mating) differs among stimuli, where pheromone and cotton stimuli induce strong effect sizes ($|g| > 1$ and several were $\gg 1$; figure 5). In response to lilac, none of the techniques revealed a strong modulation ($|g| > 1$). The confidence interval for the effect size shows that six out of six effects (mating and recovery for each of the three techniques) were significant for both phero-mone and cotton. In comparison, only one (recovery EAG) of

six possible effects was significant for lilac (electronic sup-plementary material, table S6). Hence, a strong concurrent modulation of neuronal and behavioural responses was manifest in response to sex and host plant cues only.

4. Discussion

Host-, food- and mate-finding in insects are distinct, exper-imentally tractable types of behaviour, which are largely guided by olfactory cues. We show that modulation of these odour-mediated behaviours enables *S. littoralis* male moths to respond to sensory stimuli in accordance with their physiological condition.

Furthermore, we show that odour-mediated responses to host plants and to females, which are elicited by different
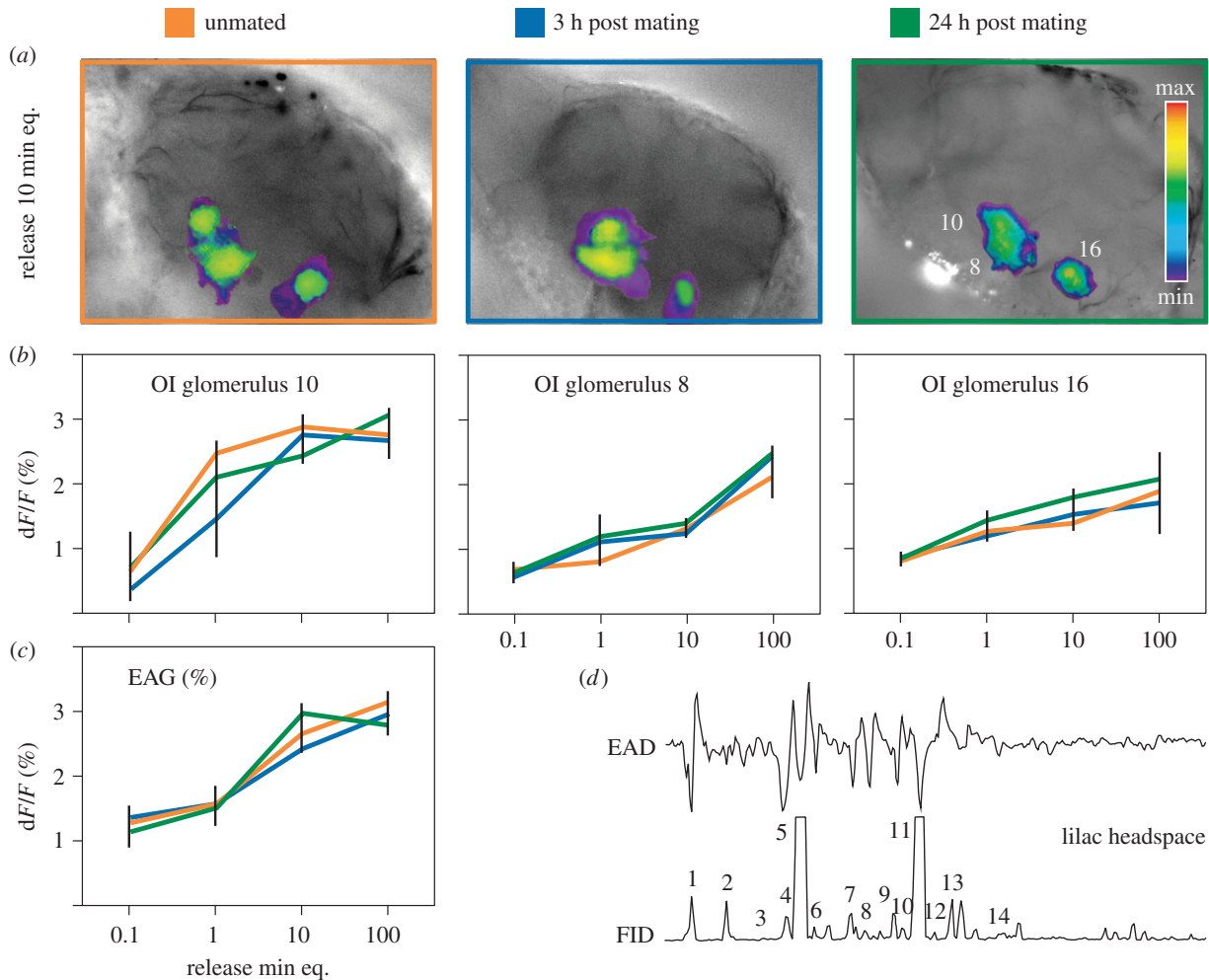
Figure 4. (a,b) The OI of antennal lobe glomeruli and (c) EAG responses of unmated and mated (3 or 24 h post mating) male *S. littoralis* to authentic lilac headspace. (d) GC – EAD active components are (1) benzaldehyde, (2) benzyl methyl ether, (3) 4-methylanisol, (4) (Z)-β-ocimene, (5) (E)-β-ocimene, (6) acetophenone, (7) unknown, (8) (+)-(S)-linalool, (9) lilac aldehyde A, (10) lilac aldehyde B, (11) 1,4-dimethoxy benzene, (12) unknown, (13) estragol and (14) lilac alcohols. EAG responses (%) and optical imaging intensities ($\Delta F/F$; %) obtained with increasing loadings (applied on filter paper) of lilac flower headspace samples equivalent to 0.1 – 100 min of sampling. Insignificant baseline responses at lower loadings are not shown. There were no significant differences between mating status. (Online version in colour.)

olfactory signals, are linked by a concurrent mating-induced modulation. The responses of *S. littoralis* males to sex phero-mone and host plant volatiles are strongly inhibited or downregulated shortly after mating, but are partly or fully restored to pre-mating levels 24 h after mating. The concur-rency of this response modulation is supported by the magnitude of behavioural and physiological effects. The response to floral odours, on the other hand, is not signifi-cantly affected by mating, which clearly demonstrates that male moths distinguish between floral odour and host plant odour, signalling adult food and mating sites, respect-ively. In addition, our comparative physiological analysis of antennae, single sensory neurons and the AL suggests that this modulation occurs already at the peripheral olfactory level.

For male moths, attraction to host plant volatiles has been reported in a few species (e.g. [22,23]), but modulation of flight attraction to host plants has not been described. In *S. littoralis* females, we have previously shown a mating-induced modulation of attraction behaviour to host plant and adult food odour [2].

Attraction to the floral scent of lilac and the green scent of non-flowering cotton in female and male *S. littoralis* suggests that the neural circuitry underpinning the behaviours related

to foraging and host plant attraction is shared between the sexes. However, differential modulation of these circuits in males and females is sex specific and suggests differences in behavioural prioritization [24]. Females engage either in foraging or host-seeking behaviour depending on mating status [2], whereas males display plasticity in their response to host plant volatiles, but not to foraging cues. In addition, males display a drastic mating status-dependent plasticity in their response to sex pheromone.

The concurrent modulation of olfactory response to sex pheromone and host plant volatiles hints at a link between the two distinct, morphologically segregated, olfactory sub-systems dedicated to environmental or social signals [25], and accordingly a common motif of neuromodulation. The fact that sex pheromones and volatiles of cotton, as a host plant and mating site, apparently are compounded into a context of social cues reflects a close ecological relevance of these signals. A similar sensory pathway has been suggested for *Drosophila melanogaster*, in which feeding sites often equate to mating sites and where food odours promote male courtship behaviour [26]. Plant volatiles are also known to synergize attraction to sex pheromone in moths [27–32], reflecting a profound integration of these signals in the AL [33,34]. This emphasizes the idea that sex pheromone
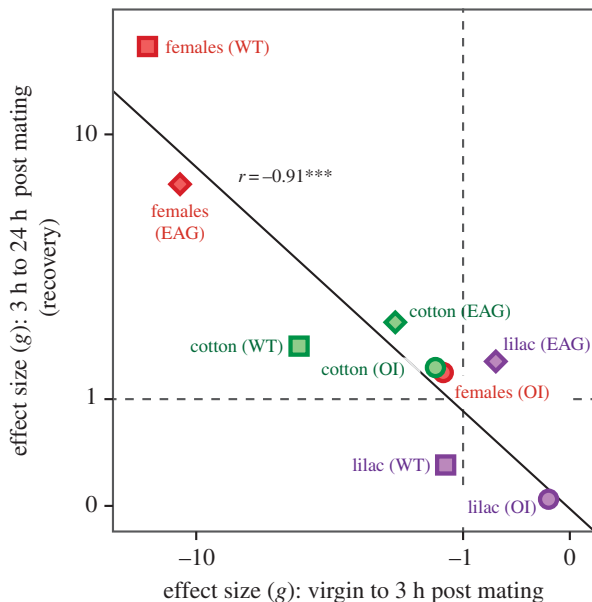
Figure 5. Behavioural and neuronal responses (biological effects determined in behavioural or physiological assays) reflect the concurrent modulation to sex and host plant cues. The observed modulation is demonstrated by a decline in responses 3 h after mating (appear as negative effect sizes on x-axis, 'mating') and a recovery after 24 h (positive effect sizes on y-axis, 'recovery'). Effect sizes are estimates of differences between bioassay means scaled by their pooled standard deviations [21]. The effects that we estimated, for each combination of technique and stimuli, nicely mirror each other as their magnitudes of decline and recovery are numerically similar, giving a regression with an intercept of zero, a slope close to unity and hence a strong correlation ($p = 0.0006$; confirmed also by a non-parametric correlation $r_S = -0.88**$). Squares, behavioural wind tunnel tests (WT); diamonds, physiology, EAG; and circles, physiology, OI. Colour codes used for stimuli: red, female sex pheromone; green, cotton leaf; and lilac, lilac flower adult food source. (Online version in colour.)

is naturally embedded in a host plant volatile background for male moths [27,34,35].

In moths as in other sexually reproducing animals, mating is costly and sperm production in males that mate with several females is generally limited [36–38]. Reproductive investments of male butterflies deplete with multiple mating and male mating-history effects female fitness [39–41]. Accordingly, lepidopteran females generally have a higher reproductive output after mating with a virgin male than after mating with a previously mated male [42].

A refractory period will likely increase male reproductive capacity as it allows replenishment of sperm and accessory gland secretions, and balancing of metabolic energy by food intake and avoiding fruitless arousal and sexual activity. In D. melanogaster, offspring production decreases with successive male matings and females discriminate against recently mated males but not against males that had time to replenish their sperm reserves [38]. Similar mechanisms may exist in S. littoralis. Our data suggest that a strong downregulation of response to both females and host plant rendezvous sites in freshly mated males abolishes subsequent investments in unproductive matings. By contrast, continuous responsiveness to food odour (flowers) likely manifests the requirement of energy for prolonged flight, refilling sex accessory glands and the production of new spermatophores [7,11,36].

Mechanisms of olfactory and behavioural modulation in insects have been studied in D. melanogaster. In female flies, modulation of a number of physiological and behavioural responses is induced by the male sex peptide and other seminal fluid components [43–49]. Neuromodulators mediate behavioural flexibility in response to internal physiological state [1,24] and Drosophila tachykinin or short neuropeptide F were shown to modulate presynaptic activities in olfactory sensory neurons affecting odour-guided behaviour [50,51]. It is plausible that similar mechanisms exist in S. littoralis males, in which mating may regulate the release of neuroactive substances leading to the observed changes in olfactory sensitivity and olfactory-guided behaviour. One such neuroactive substance could be the biogenic amine octopamine, which has been shown to selectively modulate the activity of pheromone sensitive sensilla, but not the sensilla responding to generic plant odours [52]. Likewise, juvenile hormone has been shown to selectively modulate pheromone responses in the AL [53,54].

This study contributes to our understanding of the odour-mediated regulation of value-based decisions as a function of internal physiological condition. Our findings provide an important insight into how behavioural hierarchies can be altered through changes in the olfactory system. Ongoing studies aim at elucidating the neuromodulatory mechanism underlying the observed concurrent modulation of olfactory responses in male S. littoralis.

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