Physiological mismatching between neurons innervating olfactory glomeruli in a moth

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The primary olfactory centres of most vertebrates and most neopteran insects are characterized by the presence of spherical neuropils, glomeruli, where synaptic interactions between olfactory receptor neurons and second-order neurons take place. In the neopteran insect taxa investigated so far, receptor neurons of a specific physiological identity target one glomerulus and thus bestow a functional identity on the glomerulus. In moths, input from pheromone-specific receptor neurons is received in a male-specific structure of the antennal lobe, called the macroglomerular complex (MGC), which consists of a number of specialized glomeruli. Each glomerulus of the complex receives a set of peripheral sensory afferents that encode one of several compounds involved in sexual communication. The complex is also innervated by dendritic branches of antennal lobe output neurons called projection neurons, which transfer information from the antennal lobe to higher centres of the brain. A hypothesis stemming from earlier work on moths claims that the receptor neuron innervation pattern of the MGC should be reflected in the pattern of dendrites of projection neurons invading the different MGC glomeruli. In this study we show that in the noctuid moth *Trichoplusia ni*, as in several other noctuid moth species, this hypothesis does not hold. The degree of matching between axon terminals of receptor neurons and the dendritic branches of identified projection neurons that express similar physiological specificity is very low.

Keywords: olfaction; pheromone; glomerulus; antennal lobe; Lepidoptera

1. INTRODUCTION

The primary olfactory centres of the central nervous system are remarkably similar among neopteran insects and vertebrates despite an evolutionary separation of at least 600 million years (Lowe & Wray 1997). This suggests convergent evolution of a structure serving similar sensory roles in two phyla that are immensely distant in geological time. The main characteristic signifying these centres is their glomerular structure (Shipley & Ennis 1996; Anton & Homberg 1999). The functional significance of this glomerular organization has been one of the major questions occupying researchers in the field of olfaction. Different hypotheses have suggested the glomeruli to be functional units, but not until recently were actual data supporting this notion presented.

One of the strongest arguments that glomeruli are discrete functional units derives from early observations by Bretschneider (1927) showing that male moth antennal lobes possess a set of enlarged glomeruli at the point of entry of the antennal nerve into the brain. Subsequently, Koontz & Schneider (1987) demonstrated that this macroglomerular complex (MGC) was the specific target for the axons of olfactory receptor neurons detecting the female sex pheromone. Christensen & Hildebrand (1987) had also shown that output elements of the antennal lobe, projection neurons, specifically responded to sex pheromone odours, and appeared to restrict their dendritic branches to the MGC. Similarly, Teicher *et al.* (1980)

showed that a modified glomerular complex in rat pups is specifically activated when odours of the mother's nipple pheromone are used as a stimulus. All these studies pointed in the direction of certain glomeruli having a specific functional identity. However, despite these findings, evidence for a functional significance of individual glomeruli was lacking.

To investigate the central nervous projection patterns of individual functionally defined olfactory receptor neurons, Hansson *et al.* (1992) modified a technique originally developed for activity-dependent staining of taste receptor neurons in *Drosophila* (Shanbhag & Singh 1992). Using this technique it could be shown that in several different moth species, olfactory receptor neurons detecting different female sex pheromone components target different glomeruli of the MGC. Mombaerts *et al.* (1996), using molecular recombinant techniques demonstrated a similar phenomenon in the mouse, showing that molecularly identical receptor axons converge to the same subset of glomerular subunits in the olfactory bulb. Thus both insects and mammals demonstrate that innervating olfactory receptor neurons bestow a physiological identity upon a glomerulus.

The receptor neuron axonal branches transfer the peripheral olfactory message to neurons of the insect antennal lobe or the mammalian olfactory bulb. In insects, receptor neurons make synaptic contact both with local, amacrine neurons and output elements, called projection neurons (Distler & Boeckh 1996, 1997). The local neurons of moths show a very wide branching pattern within the antennal lobe, innervating more or less

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Figure 1. Morphology of the antennal lobe in *Trichoplusia ni*. (a) Micrograph of a methylene blue-stained frontal section with MGC glomeruli a–g. The white circle indicates medial cell body group, the white box indicates the lateral group. The boxed area encloses structures summarized in the schematic drawing (b). (b) Schematic of MGC glomeruli (a–g). Frontal view showing which glomeruli receive terminals from characterized receptor neurons (1–7). Scale bar, 50 μ m. Lateral to the right. (c) Outlines of MGC glomeruli as seen in a frontal section of two preparations.

all of the approximately 50 glomeruli (Matsumoto & Hildebrand 1981). Some local neurons invade the MGC, others are restricted to sexually isomorphic glomeruli. The dendritic branches of projection neurons are often constrained to only one or, as in the MGC, a few glomeruli. Projection neurons send their axons out of the antennal lobe to higher centres of the brain (Homborg *et al.* 1988). MGC-innervating projection neurons show diverse dendritic branching patterns, invading anywhere from one to all glomeruli of the MGC.

The first detailed investigation of the projection neuron innervation of MGC glomeruli was performed on the male sphinx moth, *Manduca sexta* (Hansson *et al.* 1991). Neurons responding specifically to the major pheromone component invaded one MGC glomerulus, the toroid, whereas projection neurons responding to the minor pheromone component innervated another MGC glomerulus, the cumulus (Hansson *et al.* 1991). When many olfactory receptor neurons on the male antenna were filled with cobalt, the experiment demonstrated that two pheromone-detecting neurons present in single antennal sensilla each innervated one MGC glomerulus (Christensen *et al.* 1995). A hypothesis arising from these studies was that receptor neuron axon terminals impinged on projection neuron dendritic branches such that both receptor endings and projection neuron dendrites shared identical odour selectivity. The actual projection pattern of physiologically identified receptor neuron types is, however, not known in *M. sexta*.

A recent study in the noctuid moth *Heliothis virescens*, which examined both receptor neuron terminal and projection neuron dendritic arrangements in the male MGC, suggests a tight match between the physiological identity of input to and output from a single MGC glomerulus (Berg *et al.* 1998). This study, however,

focused only a single projection neuron. The dendritic patterns of *Heliothis* projection neurons in glomeruli were also investigated by Vickers *et al.* (1998). If their data are compared with the receptor neuron projection patterns demonstrated by Berg *et al.* (1998), then each projection neuron appears to send some dendrites to the glomerulus predicted from receptor neuron innervation. Many of the projection neurons, however, also sent branches into a large number of glomeruli that would not have been predicted from filling receptor neurons.

We have investigated the correspondence between receptor and projection neurons in a different moth species, *Trichoplusia ni*, where the innervation patterns of male pheromone-specific receptor neurons have been elucidated and where a comparatively elaborate MGC, consisting of seven glomeruli, offers a wide range of possibilities for projection neuron dendritic innervation. Female *T. ni* produce a six component pheromone (see Linn *et al.* 1984). Males possess seven receptor neuron types involved in the sexual communication channels: six types are each tuned to the pheromone components, and one is tuned to a behavioural antagonist (Grant & O'Connell 1986; Todd *et al.* 1992; Mayer 1993). Each of the seven types of uniquely tuned receptor neurons targets a separate glomerulus of the MGC (Todd *et al.* 1995, fig. 1). The aim of the present study was to investigate whether the receptor neuron axonal innervation pattern of the MGC is reflected by the projection neuron dendritic arborizations in MGC glomeruli.

2. MATERIAL AND METHODS

(a) *Insect material*

Male *T. ni* moths were generously supplied from the Department of Entomology, Iowa State University. The moths were obtained

from a laboratory colony started from individuals caught in traps in California, USA. Larvae were reared on a pinto bean diet. After pupation, individuals were sexed, and male pupae were sent to Lund. There they were allowed to emerge in an environmental chamber with a 14L:10D cycle and a temperature of 23 °C. Males were used at an age of one to three days.

(b) Intracellular recording and staining techniques

A male moth was restrained in a 1 ml plastic pipette tip (Finn pipette Oy, Helsinki, Finland) and the brain exposed (Hansson *et al.* 1994). Standard intracellular recording and staining techniques were used (Christensen & Hildebrand 1987; Kanzaki *et al.* 1989). The tip of a microelectrode was filled with Lucifer Yellow CH (4% aqueous solution, Sigma Inc., St Louis, MO, USA) and the remainder of the electrode was filled with a 2 M lithium chloride solution. The electrode was inserted in the antennal lobe close to the entrance of the antennal nerve. After establishing intracellular contact, the activity of the penetrated neuron was monitored before, during, and after stimulation of the ipsilateral antenna. The physiological data were stored on a videotape (Vetter, Rebersburg, PA, USA) and fed into a computer programme (Data Wave, Data Wave Technologies Corporation, Longmont, CO, USA) for visualization and further data analyses.

Physiologically characterized neurons were stained with Lucifer Yellow by injecting 0.5–1.0 nA of constant hyperpolarizing current for 5–10 min. The brains were dissected out, fixed in 2.5% buffered formaldehyde, dehydrated, and embedded in Spurr's resin. Serial sections (10 µm) were analysed directly using a fluorescent microscope (Olympus) or photographed on Fuji Sensia 400 colour slide film. The morphology of the neurons was reconstructed from these slides.

(c) Stimulation

When an intracellular contact was achieved within a neuron, a series of stimuli were presented. The stimulus source was a Pasteur pipette containing a filter paper strip (5 mm × 5 mm) onto which the stimulus substance was applied. A 2 ml air puff of 0.5 s duration was passed through the pipette by using a stimulation device (Syntech, Hilversum, The Netherlands). The air puff, containing the stimulus molecules, was injected into a constant airflow, flushing over the ipsilateral antenna. This airflow was mechanically and charcoal filtered, and subsequently moistened before reaching the antenna. The six sex pheromone components (Z)-7-dodecenyl acetate (Z7-12:OAc), (Z)-7-tetradecenyl acetate (Z7-14:OAc), (Z)-9-tetradecenyl acetate (Z9-14:OAc), dodecyl acetate (12:OAc), Δ-11-dodecenyl acetate (11-12:OAc), (Z)-5-dodecenyl acetate (Z5-12:OAc) and the behavioural antagonist (Z)-7-dodecenol (Z7-12:OH) were purchased from the pheromone library at the Institute for Pesticide Research, Wageningen, The Netherlands. All pheromone compounds were diluted in hexane. All single pheromone components and binary mixtures as well as four- (only some neurons) and six-component mixtures in 1:1 ratios were used at amounts of 1 ng and 10 ng of pheromone on the filter paper. The two plant-related odours (E)-2-hexenal (E2H) and phenylacetaldehyde (PAA) were diluted in paraffin oil and 100 µg were applied in the stimulus pipette. A pipette containing a filter paper with hexane only was used as a control stimulus.

(d) Physiological characterization

Action potentials (spikes) were counted manually from a storage oscilloscope (Tektronix Inc., Wilsonville, OR, USA). The

number of spikes elicited during a 600 ms period after the stimulus had reached the antenna, minus the number of spikes occurring during a 600 ms period before stimulus presentation (representing the spontaneous activity of the neuron) was noted as the number of net spikes. The period duration of 600 ms was chosen because the responses of the majority of the neurons were captured within this time. The net spikes in response to the control (representing the response to mechanical stimulation) were subtracted from the net spikes in response to an odour stimulus to quantify a response to a specific stimulus in one neuron. A response to a stimulus was noted when the activity increased more than 10% from the spontaneous activity.

(e) Morphological characterization and pattern establishment

After reconstruction, the MGC dendritic distribution pattern of single projection neurons was established. The glomeruli targeted by dendritic branches were identified and compared with the glomeruli invaded by receptor neurons expressing the same stimulus response specificity as the projection neuron under investigation. When a projection neuron responded to single components, its expected innervation pattern was the glomerulus or glomeruli targeted by receptor neurons that also responded to those components (thus, if a projection neuron responded to compound A and B, the expected innervation pattern would be the two glomeruli targeted by receptor neurons detecting A and B). If a projection neuron was blend-specific, it was considered to receive input from all the receptor neuron types detecting the components of the blend (e.g. if a projection neuron responded specifically to a blend of components A, B and C but not to any single components, the expected innervation pattern would be projection neuron dendrites invading the three glomeruli targeted by receptor neurons detecting A, B and C).

3. RESULTS

(a) General morphological characteristics

The antennal lobe of male *T. ni* comprises an array of ordinary glomeruli and an MGC consisting of seven glomerular units (figure 1). Two main cell body groups are situated medially and laterally in the antennal lobe. The structure of the MGC and the projection patterns of physiologically identified pheromone-sensitive receptor neurons have been described previously (Todd *et al.* 1995, fig. 1). We attempted to stain 84 physiologically characterized neurons intracellularly with Lucifer Yellow. Five local neurons and 26 projection neurons were stained successfully.

The local neurons all had a cell body in a lateral cell body group, which is typical for local interneurons, and had widespread arborizations throughout the antennal lobe, including all MGC glomeruli. Projection neurons had cell bodies in the lateral or medial cell group and arborized in one ordinary glomerulus (six neurons) or in one to four MGC glomeruli (20) (figures 2, 4 and 5). Completely stained projection neurons were observed to leave the antennal lobe through either the inner antenno-cerebral tract (iACT), through the median antenno-cerebral tract (mACT) (figure 2), or through the outer antenno-cerebral tract (oACT). Projection neuron axons passing through the iACT all branched in the calyces of the mushroom bodies first and then in the inferior lateral protocerebrum or in the lateral horn of the protocerebrum,

Figure 2. Reconstructions of projection neurons from frontal sections through *Trichoplusia ni* brains. (a) Projection neuron projecting through the inner antenno-cerebral tract (iACT) to the calyces (ca) of the mushroom body and to the lateral protocerebrum (lpr). (b) Projection neuron projecting through the median antenno-cerebral tract (mACT) to the lateral protocerebrum. The single branch stretching towards the calyces is situated well in front of these and does not invade the mushroom bodies. Scale bar, 50 µm. Lateral is left.

respectively (figure 2a). The cell bodies of these neurons were situated in the medial cell group. Projection neurons leaving the antennal lobe through the mACT or oACT sent their axons directly to the inferior lateral protocerebrum (figure 2b). Their cell bodies were either in the lateral or the medial cell body group.

(b) General physiological characteristics

Out of the 26 intracellularly stained projection neurons, 23 responded to pheromone stimuli. Seven of the pheromone-responding neurons also responded to plant-related odours. Three neurons responded to plant-related odours only. The spontaneous activity of the neurons varied from ca. 0.5 to 10 Hz. Action potential amplitudes ranged from 10 to 30 mV. Most responses to pheromone and plant-related stimuli were excitatory, followed by an inhibitory period (figure 3). A few neurons were inhibited by some of the pheromone stimuli. All pheromone-responding neurons responded to puffs from 1 ng or 10 ng cartridges (stimulus dissolved in hexane). Plant odour-responding neurons responded to puffs from 100 mg cartridges (stimulus dissolved in paraffin oil). Responses to pheromone odours were either non-integrated responses to single components or so-called blend-specific responses where several types of peripheral input interacted to produce an integrated response. A detailed account of the physiological characteristics of these neurons will be published elsewhere.

(c) Macroglomerular innervation by projection neurons

Comparing the arborization pattern of projection neuron dendrites within the antennal lobe with their

Figure 3. Typical response of a projection neuron to the major pheromone component, Z7-12:OAc, in *Trichoplusia ni*. Horizontal scale bar marks the stimulation (500 ms), vertical scale bar, 10 mV.

response characteristics did not reveal a simple structure–function relationship as expected from studies described in §1. Instead, projection neurons responding to the same pheromone components arborize within different MGC glomeruli. Projection neurons with branches in the same MGC glomeruli express different response characteristics. The overlap between projection neuron dendritic arborizations and receptor neuron terminals within the MGC glomeruli in the case of receptor neurons and projection neurons sharing the same response characteristics is surprisingly rare (figure 4). Between 44 and 67% of the projection neurons responding to Z7-12:OAc (a–d, f–h, k–q, s, t, w), Z5-12:OAc (f, h, j–l, n, s–u), or 12:OAc (e, l, n, s, t) have dendrite arborizations in the same MGC glomerulus as the axon terminals of receptor neurons specific for the same components (table 1). There was virtually no overlap between projection neuron dendrites and receptor neuron terminals within the MGC glomeruli for neurons responding to 11-12:OAc (c, e, h, l, n, s–u), Z7-14:OAc (e, f, h, j–l, n, o, r, t, u) or Z9-14:OAc

Figure 4. Reconstructions of all 23 anatomically characterized projection neurons in the antennal lobe of *Trichoplusia ni*. All reconstructions from $\pm$ frontal sections except (l), which was sectioned horizontally. Below each reconstruction, inserts showing the expected target glomeruli for olfactory receptor neuron terminals having the same response characteristics as the projection neuron are indicated by E and the glomeruli actually receiving projection neuron dendrites are indicated by O. Projection neurons responding to plant-related odours in addition to pheromones, are indicated by E2H and/or PAA.

(e, f, i, j–o, s–v). Thirty-three per cent overlap was found for projection neurons and receptor neurons responding to the behavioural antagonist Z7–12:OH (c, e, f, l, m, w) (table 1).

All projection neurons that responded to plant-related odours only, arborized in a single ordinary glomerulus. The three pheromone-responding projection neurons that arborized in ordinary glomeruli (u–w), responded in addition to plant-related odours. However, also 20% of the projection neurons arborizing exclusively in MGC glomeruli responded to plant-related odours (c, h, i, n).

In 13 neurons, the axonal pathway to the protocerebrum, as well as the protocerebral branching patterns, could be determined. Eight pheromone-responding projection neurons leaving the antennal lobe through the iACT all had medial cell bodies, whereas one plant volatile-responding projection neuron with an axonal projection through the iACT had a lateral cell body. The two pheromone-responding mACT projection neurons had lateral cell bodies, one oACT projection neuron had a lateral and two oACT projection neurons had medial cell bodies. Protocerebral arborization

Figure 5. Confocal images of 10 µm frontal sections through the male *Trichoplusia ni* antennal lobe. O, ordinary, sexually isomorphic glomeruli. Scale bar, 100 µm. (a) Macroglomerular innervation of neuron e. This neuron restricts its dendritic branches to glomerulus g of the MGC. At this level, only glomeruli a, b and g are visible. (b) MGC innervation of neuron c. The neuron's innervation is restricted to glomerulus e. All glomeruli except f are visible. (c) A section of the same neuron (c) slightly deeper in the antennal lobe. At this level glomerulus f appears beside glomerulus e, while glomerulus g has disappeared.

patterns could not be correlated with response characteristics of projection neurons.

4. DISCUSSION

The dendritic arborization patterns of output elements (projection neurons) from olfactory glomeruli have in the past been supposed to correspond to the specific terminal arbors of receptor neurons. In the present study, however, we show that in the moth *T. ni* response characteristics of pheromone-responding projection neurons only correlate to a very limited extent with the terminal arborization patterns of receptor neurons within the MGC.

In a number of noctuid moth species, including *T. ni*, physiologically distinct receptor neurons have been shown to project specifically to different MGC glomeruli (Hansson *et al.* 1992; Ochieng' *et al.* 1995; Todd *et al.* 1995; Hansson 1996; Berg *et al.* 1998). In several of these species, data have implied that projection neurons and receptor neurons displaying identical response characteristics do not as a rule extend their antennal lobe arborizations into the same MGC glomerulus or glomeruli (Anton & Hansson 1995; Hansson *et al.* 1994; Wu *et al.* 1996). In *T. ni*, we found only partially overlapping arborization areas of receptor neurons (Todd *et al.* 1995) and projection neurons (table 1). Neurons with the same response characteristics (for example, from figure 4a,b,d,g,p,q) did not have identical dendritic arborization patterns within the antennal lobe. The complexity of dendritic arborizations, as expressed by the number of MGC glomeruli targeted by a projection neuron, did not correlate with the number of pheromone components eliciting a response. Some generalist neurons had restricted arborizations (e.g. figure 4e,f,l), whereas some projection neurons specifically responding to a certain pheromone component had widespread arborizations in several glomeruli (e.g. figure 4p-r). All these observations show that there is a limited correspondence between the arrangements of projection neuron dendrites and olfactory receptor neuron terminals. These findings are partially at variance with the results obtained in a heliothine moth species. Vickers *et al.* (1998) described constant arborization patterns for projection neurons with the same response characteristics in *Heliothis virescens* and *Helicoverpa zea*. Out of 12 neurons stained in *H. virescens*, all innervated the glomerulus expected from the receptor neuron innervation pattern. Several of the neurons, however, also innervated non-predicted glomeruli (Vickers *et al.* 1998). The number of projection neurons sampled in *H. zea* was very small and the receptor neuron innervation patterns are not known.

Projection neurons responding to three of the *T. ni* pheromone components showed a considerably higher overlap with incoming receptor neurons of the same specificity than did projection neurons responding to the other three components. Could this pattern possibly hold the key to why we observe such a complicated neural arrangement? In another noctuid moth, *Spodoptera littoralis*, the female possesses receptor neurons to one of her own pheromone components (Ljungberg *et al.* 1993). These receptor neurons innervate a single, 'ordinary'-looking glomerulus situated right at the antennal nerve entrance, at a position equivalent to where the MGC is found in the male (Ochieng' *et al.* 1995). We have previously speculated that the single glomerulus observed in

Table 1. *Structure–response correlations for all stained projection neurons*

(Dendritic arborizations of projection neurons (PN) responding to pheromone components present in larger amounts in the female effluvium (**bold**) or to the behavioural antagonist (*Z7-12:OH*) display a greater degree of overlap with terminals of receptor neurons displaying identical specificity (33–67%) than projection neurons responding to minor pheromone components (0–12%).)

PN response to component	number of PNs stained	number of PNs arborizing in 'correct' glomerulus (%)	arborization in ordinary glomerulus
Z7-12:OAc	17	11 (67)	1
Z5-12:OAc	9	4 (44)	0
12:OAc	5	3 (60)	0
11-12:OAc	8	1 (12)	1
Z7-14:OAc	11	0 (0)	1
Z9-14:OAc	13	1 (8)	2
<i>Z7-12:OH</i>	6	2 (33)	1

the female might represent an early state in the evolution of the MGC in the male (Hansson 1995, 1996). Ancestral moth pheromone communication systems were probably less elaborate than we see today, and the male olfactory apparatus was correspondingly simpler, with fewer types and numbers of pheromone-detecting receptor neurons. As competition in the communication channel between species and between males may have forced the evolution of a much more complex detection apparatus, the receiving part of the brain correspondingly became more elaborate. The MGC increased in volume due to the increased numbers of receptor neurons invading it. New glomeruli may have split off to accommodate new receptor neuron types. In the male *T. ni* it is tempting to designate as 'evolutionary conserved' the closer correspondence between receptor neuron and projection neuron innervation that we see for the main pheromone component (Z7-12:OAc). As new glomeruli were added, the innervation patterns progressively departed from a strict match-up between input and output that might have been the basal condition. A reversed relationship can also be envisaged, where a complicated system through evolutionary time gets simplified. An hypothesis regarding speciation in Hawaiian *Drosophila* states that speciation is accompanied by the loss of male courtship elements and a decrease in discrimination by females (Kaneshiro & Boake 1987). If a similar model should be applied to moths, the female pheromone composition could get less complex over evolutionary time, and the male olfactory system could correspondingly get simpler.

Three of the pheromone-responding projection neurons in *T. ni* arborized in a single ordinary glomerulus. These neurons were not only excited by pheromones, but also by plant-related volatiles. In one-fifth of the projection neurons that send dendrites into the MGC, responses were elicited by plant-related odours in addition to pheromone components. These findings support the growing body of evidence that pheromone and non-pheromone pathways are not strictly morphologically separated in sex-specific and sexually isomorphic glomeruli, respectively.

The latter findings stress the important function of local neurons as mediators between receptor neurons and projection neurons in the antennal lobe (Christensen *et al.* 1993; Distler & Boeckh 1997). The widespread arborizations of local neurons in ordinary glomeruli might explain how pheromone-responding projection neurons can arborize in ordinary glomeruli whereas pheromone-

responding receptor neurons exclusively project into MGC glomeruli. The incoming neural message is distributed widely within the antennal lobe by local neurons.

The high percentage of stained projection neurons leaving the antennal lobe through the mACT and oACT is unusual. Most projection neurons in other noctuid moths and in *M. sexta* that have been characterized physiologically and anatomically were so-called P1a neurons, leaving the antennal lobe through the iACT (Christensen & Hildebrand 1987; Christensen *et al.* 1991; Kanzaki *et al.* 1989; Hansson *et al.* 1994; Anton & Hansson 1995). Because no obvious difference in the response patterns between neurons projecting through the mACT, oACT and the iACT was observed by us, this may suggest that the same physiological information is processed in parallel in different ascending pathways. Projection neurons innervating different areas in the protocerebrum might provide the same information to be used in different behavioural contexts or to be integrated with other sensory modalities.

The data from *T. ni* as well as from several other noctuid moths emphasize that the functional identity of glomeruli bestowed by receptor neurons is not strictly maintained at the level of projection neurons. Anatomically labelled lines are very rare in the central olfactory pathway, whereas channels carrying information about single components exist in parallel with channels that carry highly integrated information about blends. In comparison with results reported from *H. virescens* and *M. sexta*, the present study suggests subtle but important variation in the morphology of lepidopteran olfactory pathways. In some species, morphologically labelled lines are common, while in others they are scarce. Future investigations are needed to show if this variation is typical only of the pheromone detection system or whether it also occurs in other parts of the moth's olfactory pathways, such as the plant-odour information processing system.

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