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Antennal Lobe Partitioning of Behaviorally Active Odors in Female Cabbage Looper Moths

J.L. Todd, T.C. Baker

Department of Entomology, Iowa State University, Ames, Iowa, 50011-3222, USA

A highly specific cobalt marking method for moth peripheral receptors [1] has led to great progress in characterizing the functional organization of the glomeruli within the male-specific macroglomerular complex (MGC) by focusing on first-order processing of female-emitted sex pheromones [1–4]. The first evidence for anatomically and functionally distinct glomeruli within the MGC was shown with *Manduca sexta* L. in a study of projection patterns of pheromone-sensitive interneurons [5]. The pathways of sensory axons into antennal lobe glomeruli of female moths have thus far been examined in only one noctuid species, *Spodoptera littoralis* (Boisd.), and these neurons were tuned to the conspecific major sex pheromone component [3]. The glomerular destinations of axons from sensory neurons specific for other behaviorally active odor blend components besides female-emitted sex pheromones have not heretofore been investigated for any moth species.

The main objective of the present study was to examine first-order processing of floral odors and male- and female-produced pheromone components in female cabbage looper moths, *Trichoplusia ni* (Hübner). Both the male courtship pheromone (one component of which is linalool) and an odor bouquet from flowers of *Abelia grandiflora* (phenylacetaldehyde, 2-phenylethanol, benzyl alcohol, benzaldehyde) have been shown to evoke upwind flight in *T. ni* females [6, 7], and single-neuronal recordings have revealed neurons that are tuned to individual components of these behaviorally active odor blends [8]. Female *T. ni* also have antennal neurons that exhibit a low threshold similar to that of the male's neurons [9] for the major component of the female-emitted sex pheromone, (*Z*)-7-dodecenyl acetate (*Z*7–12:Ac) [9], although the behavioral significance for this detection is not known. Because upwind flight in female *T. ni* can be caused by floral odors [6] or by the male courtship pheromone [7], we became interested in determining whether neurons tuned to components of these two odor blends project into overlapping or separate areas of the antennal lobe, thus stimulating upwind flight by activating the same olfactory pathways and perhaps the same sensation of odor, or by using different pathways that result in a unique type of odor quality integration, respectively. Rearing and maintenance of *T. ni* females is according to [8]. Descriptions of the cut-sensillum extracellular recording technique [10], cobalt marking method, and neuroanatomical reconstruction of the antennal lobes and axonal pathways follow in full the methodology described for male *T. ni* [4]. The odor stimuli used and their preparation and presentation follow that of [8]. The sustained pulsing of a sex pheromone component preferentially stains the neuron within the cut sensillum that is tuned to the pulsed component [1–4]; the specificity of the staining for other odorant-tuned neurons has not been investigated prior to this study.

The morphology of female *T. ni* antennal lobes was determined from 10-μm frontal sections, and measurements of the anterior-to-posterior (longitudinal) dimensions of 38 antennal lobes (Table 1) indicated that glomerular tissue spans 249.1±27.8 μm. Female moth brains have thus far reflected varied glomerular architecture [3, 12–14]. There was considerable variation in size among the individual glomeruli making up each lobe of female *T. ni*. The largest glomeruli were not consistently located at the entrance of the antennal nerve into the antennal lobe, as in male moths [1–4], in which two complexes of glomeruli are clearly present [1–5, 11]. Only a few females had one or more enlarged glomeruli, or a complex of ordinary-sized glomeruli, located at the dorsalmost aspect of the lobe, that were clearly separated from the more ventrally situated glomeruli. There was no identifiable, consistent arrangement of glomeruli having the appearance of the *T. ni* MGC [4] in conspecific females, nor did the ordinary glomeruli appear in a consistent location or with reproducible shapes from individual to individual based on light-microscopic examination of serial sections. Willis et al. [15] also found no stereotyped arrays of identifiable glomeruli in the antennal lobes of female *M. sexta*. In contrast, Rospars' [16] quantitative morphometric analysis of the glomerular organization of the antennal lobes of *Mamestra brassicae* indicated that the shape, size, and relative positions of glomeruli in both sexes were markedly constant, such that each glomerulus could be assigned a number and be recognized either within or between individual moths.

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We connected successfully with 341 trichoid sensilla on female *T. ni* antennae, as indicated by spontaneous background activity; however, the receptor neurons within only 71 of these sensilla were excited by one or more of the six odorants that we tested. Spontaneous firing of neurons within most of the sensilla that we sampled indicated at least three different spike amplitudes. Todd and Baker [8] have previously illustrated simultaneously recorded action potentials and DC potentials of female *T. ni* receptor neurons in response to the same set of stimuli used in the present study.

We successfully stained neurons with cobalt in 38 of the 71 sensilla wherein spike activity was recorded such that axonal projections could be traced into the antennal lobe (Table 1). In 31 of the 38 sensilla, a single receptor neuron was stained (single stain), and in seven sensilla, two neurons were stained (double stain; Table 1). In all of the single stains, the axon terminated within a single glomerulus. Regardless of whether neurons were tuned to one of the four floral compounds or to the male- or female-emitted pheromone component, they projected their axons into glomeruli located at the dorsalmost aspect of the lobe, closest to the antennal nerve entrance. No lateral or medial location ("addresses") were consistently targeted by neurons tuned to a particular component or to a type of odor (floral, courtship pheromone, sex pheromone). Thus, in female *T. ni*, it appears that the dorsalmost glomeruli in the antennal lobe process a relatively diverse array of odor signals, many of which are known to evoke upwind flight. The corresponding area in male moth antennal lobes is thus far exclusively devoted to input concerning the female-emitted sex pheromone [1–4] or to the breakdown product of the major sex pheromone component of the blend [1, 4], all of which are known to modulate upwind flight. The processing role of glomeruli in the central and ventral regions of the antennal lobe of female *T. ni* was not illuminated by using our six odorant stimuli.

Serial sections revealed that there does appear to be a longitudinal partitioning of axonal terminations from neurons tuned to the components that make up different odor blends (Table 1). Axons from neurons tuned to floral odorants tended to terminate in glomeruli located at the posterior of the antennal lobe, whereas projections from neurons transmitting information about the female-emitted sex pheromone component were located in glomeruli positioned closest to the anterior aspect of the lobe, as indicated by the center-point of each of the arborizations in the serial sections (Table 1). This pattern of partitioning was also evident when the size of the individual female's antennal lobe was taken into account (Table 1, right column). Glomeruli involved in processing information about the courtship pheromone were located more medially within the glomerular sac (Table 1).

There is a growing body of evidence that morphologically distinct glomeruli of the MGC [1–4], or even subunits of a single glomerulus [4], represent functionally distinct processing centers for incoming information about individual sex pheromone components; however, no such spatially consistent functional separation into single glomeruli seems to exist in female *T. ni* for individual floral compounds or for sex-pheromone or courtship-pheromone components. This does not mean that there might not be several groups of functionally specific glomeruli that collectively receive input from the same exclusive type of neuron as suggested by the longitudinal partitioning data above. Thus far, we have been unable to identify consistent and unique spatial locations of individual functionally distinct glomeruli in female *T. ni*.

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Table 1. Antennal lobe partitioning of axonal projections of odorant-specific sensory neurons in female *T. ni* trichoid sensilla

Stimulus	Attempted stains	Successful stains		Mean projection depth[a] in antennal lobe [μm ± SD]	Front-to-back length of projection [μm]	Relative projection depth per antennal lobe depth
		Single	Double			
Benzaldehyde	7	4	0	142.5 ± 24.7	15.0 ± 7.1	0.67 ± 0.1
Benzyl alcohol	14	9	1	187.8 ± 26.6	40.0 ± 15.8	0.78 ± 0.9
2-Phenylethanol	15	5	2	177.1 ± 45.4	42.9 ± 17.9	0.74 ± 0.2
Phenylacetaldehyde	14	5	0	166.3 ± 50.6	27.5 ± 9.6	0.61 ± 0.2
Linalool	10	2	2	125.0 ± 42.4	20.0 ± 14.1	0.53 ± 0.2
*Z*7-12:Ac	11	6	2	86.3 ± 13.1	22.5 ± 15.0	0.37 ± 0.1
Total attempts	71					
Total stains		31	7			

[a] Numbers increasing in size represent depths of glomerular tissue located toward the posterior of the antennal lobe; measurements based on single stains only

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Salinity and Vasotocin Immunoreactivity in the Brain of *Rivulus marmoratus* (Teleostei)

F. Nürnberger, H.-W. Korf

Zentrum der Morphologie, Klinikum, Goethe-Universität, D-60590 Frankfurt, Germany

M. Ather Ali

Département de Biologie, Université de Montréal, Montréal, Qué. H3C 3J7 Canada

D.L.G. Noakes

Institute of Ichthyology, University of Guelph, Guelph, Ont. N1G 2W1, Canada

As in higher vertebrates, vasotocin is also involved in osmoregulation in teleosts [1]. However, the specific vasotocinergic influences and actions in the extremely complex osmoregulatory system of teleosts are only partially deciphered [2]. In the present investigation we examined the effects of long-term acclimation to different salinities on the vasotocin system of *Rivulus marmoratus*, a teleost species inhabiting brackish waters of tropical mangrove forests [3]. Caused by tidal influences, this species is naturally exposed to fast changes in salinity ranging from freshwater to seawater conditions without showing behavioral alterations.

R. marmoratus was obtained from a breeding population continuously maintained (25 °C, LD 12:12, 1.6% salinity) by DLGN. Due to the fact that this species changes sex [4], only males and hermaphrodites could be investigated.

Three males and two hermaphrodites were transferred to freshwater (0% salinity), two males and three hermaphrodites to seawater (3.2%) for 27 days. Two males and three hermaphrodites left in brackish water (1.6%) served as controls. On day 28 all fish were fixed in Bouin's solution (early light phase). Their brains were embedded in paraffin, and 6-µm-thick serial sections were immunostained with antibodies against vasotocin or vasopressin, respectively (indirect peroxidase–antiperoxidase method [5]). Immunoreactivity was found only with the antibody against vasotocin and not with that against vasopressin. Appropriate tests for antibody concentration and specificity were carried out as described previously [6]. For evaluation of the cellular activity, the content of RNA in magnocellular hypothalamic neurons was determined semiquantitatively in one parallel series by the use of gallocyanin [7]. All preparations were examined and photographed with an Axioscope microscope (Zeiss, Jena). The gallocyanin-stained sections were analyzed densitometrically with the IBAS image analysis system (Kontron, Munich).

Vasotocin immunoreactivity was found in perikarya of the preoptic and lateral tuberal nuclei as well as in fibers coursing from these perikarya to the neural lobe of the pituitary forming intrahypophysial Herring bodies. All these structures showed a varying pattern of immunoreactivity with respect to the topographic distribution and the degree of habitat salinity. Pronounced differences in the immunoreactivity were detected in the pituitary: vasotocin-immunoreactive fibers and Herring bodies were abundant in seawater acclimated fish whereas their number was decreased in specimens acclimated to freshwater. The number of immunostained hypophysial fibers and Herring bodies was intermediate in fish kept in brackish water. No difference was observed between males and hermaphrodites (Fig. 1).

The vasotocin-immunoreactive perikarya in the preoptic and lateral tuberal nucleus of fish maintained in fresh- and brackish water were densely packed with intensely labeled granules, whereas these cells displayed a weak, homogeneous staining in specimens kept in seawater (Fig. 2). The hypophysial projections were stained most intensely in specimens kept in 3.2% salinity. This and the relatively large cell nuclei of the vasotocin-immunoreactive perikarya speak in favor of high synthetic activity of the vasotocin system in fish exposed to seawater. Moreover, the vasotocinergic perikarya of fish maintained in 3.2% salinity contained significantly higher amounts of RNA (Fig. 3). All findings suggest that the preoptic and lateral tuberal vasotocin system is actively involved in regulation of the salt-water balance.

Similar observations were made in specimens of the euryhaline medaka, *Oryzias latipes*, which were subjected to acute osmotic stress. When individuals of this species were acclimated to either