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# A confined taste area in a lepidopteran brain

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**Abstract**

Knowledge about the neuronal pathways of the taste system is interesting both for studying taste coding and appetitive learning of odours. We here present the morphology of the sensilla styloconica on the proboscis of the moth *Heliothis virescens* and the projections of the associated receptor neurones in the central nervous system. The morphology of the sensilla was studied by light microscopy and by scanning- and transmission electron microscopy. Each sensillum contains three or four sensory neurones; one mechanosensory and two or three chemosensory. The receptor neurones were stained with neurobiotin tracer combined with avidin-fluorescein conjugate, and the projections were viewed in a confocal laser-scanning microscope. The stained axons entered the suboesophageal ganglion via the maxillary nerves and were divided into two categories based on their projection pattern. Category one projected exclusively ipsilaterally in the dorsal suboesophageal ganglion/tritocerebrum and category two projected bilaterally and more ventrally in the suboesophageal ganglion confined to the anterior surface of the neuropil. The bilateral projecting neurones had one additional branch terminating ipsilaterally in the dorsal suboesophageal ganglion/tritocerebrum. A possible segregation of the two categories of projections as taste and mechanosensory is discussed.


*Keywords:* *Heliothis virescens*; Proboscis; Sensilla styloconica; Taste neurone projections; Suboesophageal ganglion; Tritocerebrum

## 1. Introduction

The biological importance and early evolutionary origin of the sense of taste is apparent from its ubiquitousness throughout the animal kingdom. The major role is regulation of feeding behaviour like detection and discrimination of food sources and toxic items. In some animals, the sense of taste may also be involved in oviposition and pheromone communication, which is well demonstrated in insects (Städler and Roessingh, 1991; Ramaswamy et al., 1992; Bray and Amrein, 2003). Reflecting the function, the taste organs of insects (contact chemosensilla) are present on the mouthparts, antennae, tarsl, ovipositor and wings (Dethier, 1976; Pollack, 1977; De Jong and Städler, 2001). They generally appear as hair-formed structures comprising a thick cuticle wall surrounding an inner lumen with the dendrites of two to four gustatory neurones (Altner and Altner, 1986; Krenn, 1990; Singh, 1997; Pollack and Balakrishnan, 1997; Walters et al., 1998). A mechanosensory neurone is usually attached to the base of the hair. The apical part of the cuticle is perforated allowing non-volatile compounds to enter the lumen and stimulate the receptor neurones. One prominent type of contact chemosensillum in herbivorous lepidopteran species is the sensillum styloconicum located on the proboscis. As described in *Vanessa cardui* (Nymphalidae), *Choristoneura fumiferana* (Clem.) (Tortricidae) and *Rhodogastria bubo* Walker (Arctiidae) these sensilla contain three or four sensory neurones, of which two or three are gustatory and one is mechanosensory (Altner and Altner, 1986; Krenn, 1990; Walters et al., 1998). Electrophysiological recordings have shown that each gustatory receptor neurone in a contact chemosensillum seems to be specified for one taste category including sugars, salts, water, deterrents and in some cases amino acids (Hodgson, 1957; Wolbarsht and Dethier, 1958; Evans and Mellon, 1962; Shiraishi and Kuwabara, 1970; Dethier, 1976; Liscia and Solari, 2000; Chyb et al., 2003; Thorne et al., 2004).

Revealing the neuronal pathways of the taste system is important for studying how gustatory information is coded in the central nervous system, leading to the different responses of feeding behaviour. The taste pathways are also interesting with respect to their role in appetitive learning where sucrose is used as the unconditioned stimulus (Hammer and Menzel, 1995; Hartlieb, 1996; Skiri et al., 2005). To understand the neural mechanisms of appetitive learning it is important to trace the involved neural pathways (taste and olfaction) and reveal their neuronal connection. Whereas the olfactory pathways have

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been extensively studied in several insect species (Hildebrand and Montague, 1986; Menzel et al., 2005; Stocker, 2001; Mustaparta, 2002), the taste pathways are less explored. In the honey bee *Apis mellifera* as well as in the flies *Phormia regina* and *Drosophila* the axonal projections from bimodal contact chemosensilla on the external mouthparts have been traced to the suboesophageal ganglion (SOG) (Rehder, 1989; Murphey et al., 1989; Edgecomb and Murdock, 1992). In the flies the taste neurones seem to project in a different region than the associated mechanosensory neurones, and in *Drosophila* the phagostimulant (trehalose) neurones project in a different area than the deterrent (caffeine) neurones (Thorne et al., 2004; Wang et al., 2004). An additional projection area is shown for the sensory neurones associated with the pharyngeal contact chemosensilla of *Drosophila* (Stocker, 1994). These neurones project in the tritocerebrum which is located anterior-dorsally to the SOG and are connected to the labro-frontal nerve (Strausfeld, 1976). The anatomical border between these two areas (SOG and tritocerebrum) is indefinable in higher insects, but they are still ascribed to specific behavioural tasks (Chaudonneret, 1987; Altman and Kien, 1987; Rajashekhar and Singh, 1994b). One octopaminerge neurone connecting the taste- and the olfactory system has been identified in the honey bee as the ventral unpaired medial neurone (VUM-mx1) which plays a major role in appetitive learning in this species (Hammer, 1993). This interneurone, with dendritic-axonal arborisations in the olfactory neuropils, responds to sucrose, it has the soma in the ventral SOG and dendritic arborisations in the dorsal SOG and tritocerebrum.

In the noctuid moth *Heliothis virescens*, the interest is to reveal the physiological properties and the anatomical pathways involved in contact chemosensation as well as the neuronal connections between this pathway and the olfactory centres. The olfactory system is well studied as concerns the receptor neurone specificity and the central pathways (Almaas and Mustaparta, 1991; Berg et al., 1998; Stranden et al., 2003; Skiri et al., 2004; Røstelien et al., 2005; Mustaparta and Stranden, 2005). Olfactory learning of relevant plant odours is also demonstrated in this species by the use of the proboscis extension reflex (Skiri et al., 2005). Electrophysiological recordings from the contact chemosensilla on the proboscis have been performed showing responses to various phagostimulants and potential deterrents (Blaney and Simmonds, 1988). The morphology of these sensilla has not been studied, and the projections of taste neurones are not known in any adult lepidopteran species. The present study describes the morphology of the s. styloconica on the proboscis of *H. virescens* and the axonal projections of the associated sensory neurones in the SOG and tritocerebrum. The axons and the target areas are shown in three-dimensional reconstructions.

## 2. Materials and methods

### 2.1. The animals

The moths were imported as pupae from a laboratory culture at Novartis Crop Protection, Basel, Switzerland. Before emerging the pupae were separated according to sex and put in a glass container (hight: 18 cm, width: 12 cm, depth: 17 cm) covered by a perforated plexiglass. The container with pupae was kept in a Refritherm 6 E incubator (Struers) at a reversed photoperiod (14 h light and 10 h dark) and at a temperature of 22–23 °C. When emerged, the adults were placed into a plexiglass cylinder (height: 20 cm, diameter: 10 cm) covered by a perforated lid. The moths could feed ad. lib. on a 0.15 M sucrose solution. The moths were one to three days old when used in the experiments.

### 2.2. Electron microscopy

The outer morphology of the s. styloconica were examined with a scanning electron microscope (SEM) at 15–25 kV (JEOL JSM-25S). The proboscis of two individuals were fixed in a buffered 0.135% glutaraldehyd-3.6% formaldehyd solution (0.1 M phosphate buffer, pH 7.4) for 3 days at 4 °C dehydrated in ethanol and left to air dry for 1 day. The material was then mounted onto SEM viewing aluminium stubs using a carbon adhesive tape. The carbon tape was further connected to the aluminium stubs by carbon pasta. The material was coated with a 30 nm thick layer of gold–palladium (JOEL FINE COAT ion sputter JFC-1100) before examination in SEM.

The inner morphology of the s. styloconica on the proboscis was examined with a transmission electron microscope (TEM) at 60 kV (JEOL JEM-1200). The proboscides of two individuals were cut at the base and immediately fixed in buffered 2.5% glutaraldehyde (0.1 M phosphate buffer, pH 7.4) over night at 4 °C. The material was subsequently postfixed in buffered 1% osmium tetroxide ($OsO_4$) for 1.5 h at room temperature, dehydrated in ethanol and embedded in a medium mixture of Durcupan (Fluka) through progressive series of durcupan/propylene oxide (1/5, 1/3, 1/1 and 1/0). By the use of an ultratome (ULTRACUT, Reichert-Jung) equipped with a diamond knife (Diatome), serial ultrathin sections (70 nm) were made and collected on one-hole grids (Tebra 1GC 12H/10H) covered with a pioloform membrane (Pioloform, Agar Scientific LTD). Finally the sections were stained with uranyl acetate for 30 min and rinsed in distilled water before being stained with lead citrate for 5 min (standard procedure).

### 2.3. Staining of the receptor neurones

The insects were mounted in a plastic tube and wax was used for further immobilization of the head and the mouthparts. The proboscis was uncoiled, the two galeae separated and fastened to the wax with tungsten cramps. The distal part of the two galeae was covered with distilled water and the proximal part was covered with vaseline to avoid drinking. In 15 preparations one to five s. styloconica were cut. This was carried out under a drop of distilled water and left for 10 min for osmotic distension of the cut dendrites. A few crystals of neurobiotin tracer (SP-1120, Vector Labortories, Inc.) were subsequently dissolved in the water. The preparation was placed in a petri dish moistened with a wet piece of paper. The petri dish was placed in the refrigerator for 7 days at 4 °C to