A New Ascending Sensory Tract to the Calyces of the Honeybee Mushroom Body, the Subesophageal-Calycal Tract

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ABSTRACT
The mushroom bodies of the honeybee are important neuropils for learning and memory. Therefore, knowledge about their input and output connections is essential to understanding how these neuropils function. A newly described input tract to the mushroom body is presented here, which is called the subesophageal-calycal tract (SCT) and connects the subesophageal ganglion with the calyces of the mushroom bodies. The neuronal somata of the SCT neurons lie in one cluster between the lobula of the optic lobe and a neuropil area that is formed from the fusion of the tritocerebrum and the subesophageal ganglion. Within the subesophageal ganglion, the dendritic fibers of SCT neurons overlap with terminals of sensory neurons from the proboscis. Therefore, we conclude that the SCT neurons might process gustatory and mechanosensory information from the proboscis. Individual SCT neurons receive unilateral input within the subesophageal ganglion and may connect to either the ipsilateral or the contralateral mushroom body. On their way to the mushroom bodies, the SCT neuron axons meet the roots of the antennocerebralis tracts (ACTs) and from this point follow the same path as the median ACT neurons for a short distance. Within the calyces, the SCT neurons innervate two separate areas, a small area within the dorsal collar just below the lip and a part of the basal ring. Double-labeling experiments show that the projections of the SCT neurons do not overlap with the projections of the olfactory projection neurons and visual projection neurons from the dorsal medulla. The possible function of the SCT neurons and the relation of the SCT to known input tracts of the mushroom bodies in other insects are discussed. J. Comp. Neurol. 465:168–178, 2003.

Indexing terms: Apis mellifera; mushroom body; subesophageal ganglion; rhodamine dextran; confocal microscopy

The mushroom bodies in honeybees play an important role during appetitive learning of odors (Erber et al., 1980; Hammer and Menzel, 1998; Menzel, 2001; Menzel et al., 1974; Menzel and Müller, 1996). To understand how the mushroom bodies solve this task, knowledge of their anatomy, their intrinsic and extrinsic neurons, and their connectivity is essential. In bees, each mushroom body consists of two cup-like calyces, a pedunculus, and an α- and β-lobe (Mobbs, 1982). The latter are also called vertical lobe and median lobe, respectively (Strausfeld, 2002). Recent work suggests that the lower one-third of the α-lobe could be interpreted as a separate lobe (Strausfeld, 2002). The mushroom body is shaped by its intrinsic cells, referred to as the Kenyon cells, which are named after their discoverer (Kenyon, 1896). The dendrites of these neurons form the cup-like calyces, and their axons form the pedunculus and the lobes. The calyces are regarded as the main input region of the honeybee mushroom body, whereas the lobes and the pedunculus are regarded as the main output regions (Mobbs, 1982; Rybak and Menzel, 1993), but recent results show that the lower division of the α-lobe, the γ-lobe, may also receive inputs from afferent neurons.

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Received 10 January 2003; Revised 14 April 2003; Accepted 30 April 2003

DOI 10.1002/cne.10843
Published online the week of August 25, 2003 in Wiley InterScience (www.interscience.wiley.com).

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(Strausfeld, 2002). The calyces get olfactory and visual input from the antennal lobe via the antennal-cerebral tracts (ACTs; Mobbs, 1982) and from the optic lobes via three different optic tracts: the anterior-superior optic tract (ASOT), the anterior-inferior optic tract (AIOT), and the lobula tract (LOT; Ehmer and Gronenberg, 2002; Gronenberg, 1986, 2001; Mobbs, 1984). The olfactory and the visual projection neurons terminate within different calycal subcompartments: the olfactory projection neurons innervate the outermost part, the lip, whereas the visual projection neurons innervate the adjacent collar (Gronenberg, 1986, 2001; Mobbs, 1982). Both neuron types also innervate separate areas within the third calycal subcompartment, the basal ring (Gronenberg, 2001). However, the output neurons of the mushroom body respond not only to olfactory and visual stimuli, but also to gustatory stimuli (Grünewald, 1999; Maelchshagen, 1993). Therefore, one would postulate projection neurons that provide the mushroom body with gustatory information. The main gustatory center is located within the subesophageal ganglion (SEG; Mitchell et al., 1999), so such gustatory projection neurons should have their origin within the SEG. Neurons that connect the SEG with the basal ring of the calyces were mentioned by Mobbs (1985), but no evidence was shown. So far, only one neuron from the SEG is known to respond to gustatory stimuli, the VUMmx1 (ventral unpaired median neuron 1 of the maxillary neuromere; Hammer, 1993). The VUMmx1 innervates the basal ring and the lip of the calyces. All other neurons that have been described as connecting the SEG with the calyces of the mushroom bodies respond to odors and innervate the lip only (Abel et al., 2001). Vowles (1955) suggested that the calyces of bees (and ants) are connected with the SEG. However, this connection was described only on the basis of gross anatomical details, such as the structure of various tracts, so that a detailed description of the participating neurons is still needed. In this paper, we present the first description of a tract in the honeybee brain that connects the SEG with the calyces of the mushroom body. This tract has been named the subesophageal-calycal tract (SCT). To give a complete description of the SCT neurons, we show intracellular (SCT). To give a complete description of the SCT neurons, we show intracellular staining of individual SCT neurons within the SEG, showing processes of mushroom body intrinsic and extrinsic neurons, including axons and axon terminals of the SCT neurons (four preparations).

To visualize SCT neuron processes within the calyces of the mushroom body, the head capsule was opened above the SEG, the trachea were removed, and rhodamine dextran was applied in the SEG using the same tract-tracing technique as described above. This procedure labeled processes of the SCT neurons within the supraesophageal ganglion (10 preparations). In some preparations, neurobiotin was visualized with Cy3-conjugated streptavidin. The resulting staining of the SEG SCT neurons was the same as for rhodamine dextran (three preparations).

The dye was allowed to diffuse for 4 hours. Subsequently, the heads were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.2, for 2 hours at 20°C or overnight at 4°C. The brains were then dissected free, dehydrated in a graded series of ethanols, and cleared in methylsalicylate (Merck, Darmstadt, Germany). After they were mounted in Permount (Merck), the brains were examined under a fluorescence microscope (Leitz) with epifluorescence illumination.

**Intracellular staining of individual SCT neurons with rhodamine dextran**

The bees were anesthetized by cooling on ice and mounted in metal holders for the recording of subesophageal neurons. The tips of glass microelectrodes were filled with rhodamine dextran (5% dissolved in distilled water); the shafts contained 0.2 M K-acetate. With a micromanipulator (Eppendorf), the microelectrodes were inserted into the median SEG. Rhodamine dextran was injected into the cells by using 300-msec pulses of 5-nA depolarizing current at 2 Hz for 10–30 minutes. Electrode resistances ranged between 100 and 400 MΩ. After dye injection, the dye was allowed to diffuse for 1 hour. Subsequently, the heads were removed from the thorax and placed in 4% paraformaldehyde in PBS, pH 7.2, for 2 hours at 20°C or overnight at 4°C. The brains were then dissected free, dehydrated in a graded series of ethanols, and cleared in methyl salicylate (Merck). In this study, we filled seven individual SCT neurons. The arborizations of five of these neurons were stained completely in the SEG and the calyces of the mushroom body; the other two neurons were stained incompletely. The arborizations of one of these two neurons were not stained in the SEG, and the arborizations of the other neuron were not stained in the calyces of the mushroom body. Within the SEG, four SCT neurons innervated the ipsilateral side, and two SCT neurons innervated the contralateral side.

**Double labeling of SCT neurons and sensory neurons of the proboscis in the SEG**

To label SCT neurons and sensory neurons of the proboscis in the SEG simultaneously, rhodamine dextran was applied in the calyces of the mushroom body by using the same tract-tracing technique as described above, and fluorescein dextran (Molecular Probes; http://www.probes.com; D-3308) was applied to the maxillary or labial nerves, which both carry sensory neurons of the proboscis. Applying dye to the maxillary or labial nerves caused...
staining of sensory neurons and motoneurons of the respective nerves (two preparations for each double labeling). It was possible to distinguish between the projections of the sensory neurons and the motoneurons, because the projections of the latter have already been described (Rehder, 1989).

**Double labeling of SCT neurons and olfactory projection neurons in the mushroom body**

To label SCT neurons and olfactory projection neurons in the mushroom body simultaneously, rhodamine dextran was applied in the SEG, and fluorescein dextran was applied in the antennal lobe. Applying dye in the antennal lobe caused staining of olfactory projection neuron endings in the mushroom body via the m-ACT and the l-ACT (two preparations).

**Double labeling of SCT neurons and visual projection neurons in the mushroom body**

To label SCT neurons and visual projection neurons in the mushroom body simultaneously, rhodamine dextran was applied in the lobula and medulla of the optic lobe. Applying dye in the optic lobe caused staining of visual projection neuron endings in the mushroom body via the ASOT (three preparations).

**Confocal laser scanning microscopy and reconstruction**

The whole-mount preparations were viewed with two different confocal laser scanning microscopes, a Leica TCS-4D and Leica SP2, equipped with a Leitz microscope (DM RBE) and a krypton/argon laser light source (Leica TCS-4D) and a argon/helium laser light source (Leica SP2). Various objective lenses were used: 10× air, numeric aperture 0.45; 20× air, numeric aperture 0.6; 20× air, numeric aperture 0.7. For preparations containing Cy3- or rhodamine dextran-labeled fibers, the rhodamine filter (excitation wavelength 568 or 543 nm) was used. For preparations containing dual staining of SCT neurons and sensory neurons of the proboscis/olfactory projection neurons/visual projection neurons, the rhodamine filter (excitation wavelength 568 nm or 543 nm) and the fluorescein filter (excitation wavelength 488 nm) were used. Serial optical sections were imaged at intervals of 1–4 μm. Two-dimensional projections of series or parts of series were generated with Amira 2.3 software (Indeed, Houston, TX). In preparations with dual staining, projections were created for each channel by using different pseudocolours for each channel (red for rhodamine dextran and green for fluorescein dextran), and the images were subsequently merged. The digitized images were processed and—when required—modified to enhance contrast with Corel Photopaint and Corel Draw (Corel Corporation).

Labeled tracts, neuropils, and neuronal arborizations were reconstructed three-dimensionally with Amira 2.3 software (Indeed). Neuropils were labeled on subsequent pictures of an image stack from the confocal microscope, and, from this, three-dimensional reconstructions were generated. Individual neurons were traced semiautomatically on two-dimensional projections showing the frontal, sagittal, and horizontal orientations of an image stack to create a three-dimensional wire model of this neuron. In the next step, the volume was added to the wire model according to the thickness of the stained arborizations. Both the neuropil and neuron reconstructions could then be viewed simultaneously to reveal their spatial relationships.

**RESULTS**

**Neuronal somata location**

The neuronal somata of the SCT neurons lie in one cluster between the lobula of the optic lobe and a neuropil area that is formed from the fusion of the tritocerebrum and SEG (Fig. 1A). From a preparation in which almost all SCT neurons were stained, it was possible to estimate the number of the stained neuronal somata. Per side of the brain, approximately 100 SCT neurons were counted. The primary neurites run from this cell cluster in a bundle through the dorsal lobe. At the edge of the dorsal lobe, the axons ascend into the protocerebrum (Fig. 1A), and the dendrites project into the SEG (Fig. 1A,B). The course taken by the dendrites in the SEG together with the axonal projections form the tract described in this paper, the SCT. From the division point of the axons and the dendrites, the axonal projections of the neurons in the SCT follow the same path as cells in the median antennocerebralis tract (median ACT) for a short distance (see also under Brain projections).

**SEG projections**

The bundle within the SEG divides at the level of the median ventral tract (MVT) into two parts: One stays ipsilateral, and the other projects to the contralateral side by crossing the midline between the mandibular and the maxillary midline tract (MdMT and MxMT, respectively; Fig. 1A; see also Fig. 4). The ipsilateral and contralateral bundles innervate the same regions (mirror images) within the SEG. Both the ipsilateral and the contralateral bundles innervate the ventral part of the maxillary and labial neuromers of the SEG. Intracellular fills of single SCT neurons show that there are cells from the ipsilateral and contralateral neuronal soma clusters that innervate the same half of the SEG (Fig. 1B). SCT neurons arborizing in both halves of the SEG were not stained, indicating that individual SCT neurons stay to one side of the brain. The fibers of the SCT neurons have a smooth appearance within the ventral SEG. Most importantly, the ventral part of the maxillary and labial neuromers of the SEG are the areas that receive input from the sensory neurons of the proboscis (Rehder, 1989).

**Brain projections**

The axons of the SCT neurons run from the dorsal edge of the dorsal lobe to the roots of the mainly olfactory ACTs (Abel et al., 2001). At this point, the SCT joins the median ACT and ascends together with this tract into the protocerebrum (Fig. 1A,C). At the level of the α-lobe, the SCT divides from the median ACT and runs in a lateral turn, close to the posterior border of the mushroom body pedunculus, toward the calyces (Figs. 1C,D, 3B, arrow). The SCT divides into two bundles at the point where the visual projection neurons of the ASOT project within the “outer ring tract” into the calyces (Fig. 2A). These bundles rejoin between the bases of the median and lateral calyces, forming a shape similar to the eye of a needle through which
neurons of the ASOT travel. Intracellular fills of single SCT neurons show that the neurons run in only one of the two bundles; an SCT neuron, with an axon that divides in this area, was never stained. The axons of the SCT neurons enter the calyx neuropil exactly between the median and the lateral calyces.

**Arborizations within the calyces**

Within the calyces, the fibers of the SCT neurons run within the “inner ring tract.” From there, thin fibers project to the ventral part of the basal ring (Fig. 2A,B, small arrowheads) and to an area that seems to lie just...
below the lip region (Fig. 2A,B, large arrowheads). The innervated area within the basal ring is a little larger than that below the lip region (Fig. 2A). The fibers innervating the projection area below the lip run along the inner border of the calyx neuropil (Fig. 2A). All intracellularly filled SCT neurons (six) innervate both regions within the calyces, the ventral part of the basal ring as well as the area below the lip (Fig. 2C). The arborizations of one individually stained SCT neuron within the calyces were three-dimensionally reconstructed (Fig. 3). The fiber endings of the SCT neurons show bleb-like varicosities (Fig. 3), which resemble the varicosities of the olfactory and visual projection neurons within the calyces (Abel et al., 2001; Ehmer and Gronenberg, 2002; Gronenberg, 1986; Mobbs, 1982, 1984). Such varicosities are regarded as presynaptic specializations (Blenau et al., 1999; Ganeshina and Menzel, 2001; Yusuyama et al., 2002), which suggests that the fibers of the SCT neurons within the calyces represent the output of these neurons.

**Input of the SCT neurons**

The smooth appearance of the SCT neuron fibers within the SEG suggests that the SCT neurons receive their inputs within the SEG. Insofar as the SCT neurons arborize within the ventral part of the maxillary and labial neuromers of the SEG, which is innervated by the receptor neurons from the proboscis (Rehder, 1989), one could assume that the SCT neurons process sensory information from the proboscis. To test whether these neurons innervate overlapping areas within the ventral SEG, we made double stainings of the SCT neurons and the receptor neurons of the proboscis with two different fluorescent dyes. The proboscis of the honeybee is innervated by neurons of the labial and the maxillary nerves. The analysis of these double stainings shows that, within the ventral SEG, the arborizations of the SCT neurons are intermingled with the neurites of the sensory neurons of the labial nerve (Fig. 4A,C,D arrows) and of the maxillary nerve (not shown here). This observation suggests that the SCT neurons receive their input from the receptor neurons of the proboscis. To reinforce this conclusion, a comparison of the intracellular staining of two SCT neurons (Fig. 1B) and the mass staining of several SCT neurons (Fig. 1A,4) shows that the fine arborizations of the SCT neurons within the ventral SEG are not completely stained with the mass-staining method. The reason for this is most probably that, with the mass-staining method, the neurons do not take up enough dye to fill all of the fine arborizations. Therefore, it is likely that the area of overlap between the SCT neurons and the sensory neurons of the proboscis is actually larger than is evident in Figure 4.

**Fig. 2.** Confocal micrographs of the SCT neuron terminals within the calyces of the mushroom body. A,B: Two different confocal micrographs from one mass-staining preparation showing the areas in the calyces of the mushroom body that are innervated by the SCT neurons (dye application within the SEG). The arrow in A points toward the part of the SCT where it forms a hole through which the ASOT is running. C: A single SCT neuron innervates both areas within the calyces of the mushroom body, the one just below the lip (large arrowheads) and the other within the basal ring (small arrowheads). Note the bleb-like varicosities of the SCT neuron terminals in both projection areas. IC, lateral calyx; mC, median calyx; SCT, subesophageal-calycaler tract. Scale bars = 50 μm.
Output of the SCT neurons

The SCT neurons innervate two neuropil areas within the calyces. First, they innervate the ventral part of the basal ring, and, second, they innervate an area below the lip region. This would mean that the second area lies within the dorsal part of the collar, but a precise assignment is not possible, because the boundary between the lip and the collar is an estimate based on the outer constrictions of the calycal neuropil. Therefore, double stainings were made to describe the position of the innervated area by the SCT neurons in relation to the areas innervated by the visual and olfactory projection neurons.

The analysis of double staining of the SCT neurons and visual projection neurons shows that these cells innervate separate areas within the neuropil of the calyces of the mushroom body. There is no overlap between the areas innervated by the SCT neurons and the visual projection neurons. Within the basal ring, the SCT neurons innervate the middle part of the ventral area, whereas the ACT neurons innervate the adjacent inner part (Fig. 6). The second area that is innervated by the SCT neurons lies just below the area innervated by the ACT neurons, the lip. This means that the SCT neuron terminals do indeed innervate the dorsal collar.

DISCUSSION

Comparisons with other species

Although inputs to the mushroom bodies of the honeybee have been investigated previously (Abel et al., 2001; Gronenberg, 2001; Kenyon, 1896; Mobbs, 1982; Vowles, 1955), the SCT has not been described. In earlier studies, several tracts were mentioned that connect the SEG with the calyces of the mushroom bodies (Kenyon, 1896; Vowles, 1955), but the description was quite coarse or incomplete. In later studies, only the tracts of the antennal lobe (median ACT and lateral ACT) and the optic lobes (ASOT, AIOT, and LOT) were analyzed further (Ehmer and Gronenberg, 2002; Gronenberg, 2001; Mobbs, 1982). Mobbs (1984, 1985) claimed that a mushroom body input from the SEG exists and that such fibers terminate within the basal ring, but without showing any evidence. That Vowles (1955) described a tract similar to the SCT for ants but not for bees leads to the assumption that this tract was overlooked in bees because it consists of only 100 neurons. Furthermore, no tract similar to the SCT has been described for other holometabolous insects, such as moths, flies, and beetles. For flies, interneurons have been mentioned that run from the SEG within the median ACT into the mushroom body calyces, but no anatomical evidence has been provided (Strausfeld, 1976). However, for honeybees, evidence has been presented to show such neurons that run from the SEG within the median ACT.
into the mushroom body calyces (Abel et al., 2001). However, other than the SCT neurons described here, the neurons described by Abel et al. (2001) have their cell bodies within the SEG, and they seem to receive input not within the SEG but in the antennal lobes.

In some hemimetabolous insects within the group of the orthopteroidea, such as locusts, crickets, and grasshoppers, the tritocerebral tract (TT) shares several similarities with the SCT of the honeybee (Weiss, 1981). The SCT in the bee and the TT in orthopterans take similar routes through the brain. Both tracts run for a certain distance together with the median ACT into the protocerebrum and project behind the posterior border of the mushroom body pedunculus into the calyces. The neurons of the TT innervate their own region within the calyces in the same way that the SCT neurons do. The neurons of the TT innervate...
only the accessory calyx, whereas the olfactory projection neurons terminate exclusively within the primary calyx. Finally, the SCT neurons and the neurons of the TT receive similar inputs: both appear to receive inputs from the mouth parts (Ernst et al., 1977). There is, however, one difference between the neurons of the TT and the SCT neurons regarding the position of their dendritic projections. The neurons of the TT receive their inputs within the tritocerebrum, whereas the SCT neurons receive their inputs within the ventral SEG. However, the soma cluster of the SCT neurons is located near the tritocerebrum between the lobula and the neuropil area that is formed from the fusion of the tritocerebrum and the SEG. Possibly, the position of the dendrites could have been displaced in bees because of the fusion of the tritocerebrum and the SEG. The similarities of the TT of the orthopterans and the SCT of the honeybee suggest that these tracts could be homologous.

**Connectivity**

**Input.** Within the ventral SEG, the projections of the SCT neurons overlap with the projections of sensory neurons from the proboscis. Together with the smooth appearance of the SCT neuron fibers, this suggests that the SCT

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**Fig. 5.** Confocal micrographs and three-dimensional reconstruction of a double labeling of the SCT neurons and the visual projection neurons within the calyces of the mushroom bodies. The SCT neurons (red) were labeled by applying rhodamine dextran into the SEG; the visual projection neurons (green) were labeled by applying fluorescein dextran into the optic lobes. **A,B:** Confocal micrographs showing that the terminals of both neuron types do not overlap within the calyces of the mushroom body. The red arrowheads indicate the SCT; the green arrowheads indicate the ASOT. **C,D:** Three-dimensional reconstruction of the double labeling. **C:** Frontal view. **D:** Lateral view. ∂, anterior; Al, antennal lobe; ASOT, anterior-superior optic tract; β, β-lobe; a, anterior; Al, antennal lobe; ASOT, anterior-superior optic tract; d, dorsal; l, lateral; IC, lateral calyx; mC, median calyx; SCT, subesophageal-calycal tract; SEG, subesophageal ganglion. Scale bars = 100 μm.
The proboscis consists of five elements: the glossa and the paired labial and maxillary palps. Mechanosensory and gustatory inputs were found on all these elements (Galic, 1971; Whitehead and Larsen, 1976b). The contact-chemosensory sensilla contain five sensory neurons, as in other insects (Galic, 1971; Whitehead and Larsen, 1976b). The five neurons are a mechanosensory receptor neuron, which terminates at the base of the sensillum, and four receptor neurons, which project into the tip of the sensillum. One of the four receptor neurons responds to various carbohydrates (e.g., sucrose, glucose, fructose), and two others respond to various salts (e.g., NaCl, KCl, LiCl; Whitehead, 1978; Whitehead and Larsen, 1976a). The response spectrum of the fourth neuron was not determined, but it was suggested that it could respond to proteins or amino acids (Whitehead and Larsen, 1976a). Therefore, we conclude that the SCT neurons might receive mechanosensory and gustatory input from the receptor neurons of the proboscis. Because there are more gustatory than mechanosensory receptor neurons, it is possible that the SCT neurons receive mainly gustatory input from the proboscis.

**Output.** In that the SCT neurons connect the ventral SEG with the mushroom body calyces, one could assume that they provide the calyces with sensory information from the proboscis. This interpretation is supported by the fact that the terminals of the SCT neurons within the calyces show bleb-like varicosities. These are similar to the varicosities of other neurons that are known to synapse with Kenyon cells in the calyces of the mushroom bodies, such as olfactory projection neurons and the γ-aminobutyric acidergic (GABAergic) feedback neurons (Ganeshina and Menzel, 2001; Rybak, 1994; Schürmann and Elekes, 1987). It is interesting that Strausfeld (2002) described various Kenyon cells that innervate the dorsal region of the collar ("outer collar" of Strausfeld, 2002) and could, therefore, be the synaptic partners of the SCT neurons.

Several studies have shown that olfactory and visual neurons terminate in distinct and separate zones within the calyces of the mushroom bodies (Gronenberg, 2001; Mobbs, 1982). The present work shows that this is also the case for SCT neurons. Within the calycal neuropil, the SCT neurons terminate within two regions. The (putative gustatory) SCT neurons that possibly receive sensory information from the contact-chemosensory sensilla of the proboscis innervate a small area within the dorsal collar, just below the lip. Because the lip is innervated by the olfactory projection neurons, this means that the two chemosensory inputs are separated but in close proximity. On the other hand, there is a gap between the areas innervated by the SCT neurons and the visual projection neurons within the collar. The visual projection neurons innervate two layers of the collar, a broad layer in the middle of the collar and a thin layer in the outer area. This innervation pattern was described for visual projection neurons from the dorsal medulla (Ehmer and Gronenberg, 1982).
Gustatory and mechanosensory receptor neurons from the proboscis project via the labial and maxillary nerves into the ventral SEG, where they overlap with motor neurons from the proboscis. It is well established that processing of gustatory and mechanosensory information from the proboscis and the control of movements of the proboscis take place within the SEG (Mitchell et al., 1999).

However, gustatory stimuli have more functions than eliciting innate responses. Sucrose solutions also function as an appetitive reinforcer during olfactory learning and sensitize the responses to other stimuli, such as odors. On the other hand, aversive salty solutions function as negative reinforcers in olfactory conditioning experiments (Kim and Smith, 2000). It might, therefore, be necessary for the bee to evaluate the temporal relationship of olfactory and visual stimuli with respect to the contact-chemosensory input from the proboscis. The mushroom bodies are the most suitable place for such an evaluation, because they receive input from many sensory modalities in an ordered fashion. Therefore, one would postulate projection neurons that receive input from the proboscis within the SEG and synapse with afferent neurons within the mushroom body. One such neuron was found within the SEG: The putative octopaminergic VUMmx1 ascends from the SEG toward the brain, where it innervates the neuropils of the olfactory pathway, the antennal lobe, the mushroom body, and the lateral protocerebrum, and it was shown to mediate the appetitive reinforcer during olfactory conditioning (Hammer, 1993).

No neurons have been found so far that mediate the negative reinforcer during olfactory conditioning or that play a role during learning of visual cues. One might think that the SCT neurons solve these tasks. However, this hypothesis is not supported by the findings of this study.

First, neurons that mediate the negative reinforcer during olfactory conditioning should converge with the olfactory pathway, and neurons that play a role during learning of visual cues should converge with the visual pathway. However, this is not the case; the double-labeling experiments presented here clearly show that the SCT neurons do not converge with the ACT neurons within the calyces of the mushroom body. We also showed that the SCT neurons and the visual projection neurons from the dorsal medulla innervate separate areas within the calyces of the mushroom body. However, there are two other classes of visual projection neurons that innervate the calyces; visual projection neurons from the ventral medulla and from the lobula. The comparison of the innervation pattern of SCT neurons and visual projection neurons from the lobula shows that it is probable that these two neuron classes overlap within the calyces, and, thus, the SCT neurons might fulfill the convergence criterion for the visual pathway. Another possibility would be that Kenyon cells that are postsynaptic to the SCT neurons could have arborizations in more than one sensory domain of the calyx and thus combine two or more modalities.

Second, the SCT neurons do not synthesize a modulatory transmitter, such as octopamine, serotonin, or dopamine, but they might synthesize acetylcholine, a transmitter that is also used by sensory receptor neurons and olfactory projection neurons. Kreissl and Bicker (1989) show in their Figure 6d a number of tracts labeled with acetylcholinesterase. One of these tracts was identified as the median ACT, but another was not classified. A com-
comparison with the present data shows that the second labeled tract should be the SCT. Therefore, SCT neurons, as with most neurons of the median ACT, may synthesize acetylcholine. The SCT neurons thus appear to provide the mushroom body calyces with sensory rather than modulatory information.

Third, modulatory neurons have widespread arborizations that cover many neuropils, whereas the SCT neurons arborize within subareas of the SEG and the calyces of the mushroom body. We conclude that the SCT neurons are not modulatory neurons but rather sensory neurons that provide the mushroom body with sensory information from the proboscis. The role of this sensory input from the proboscis within the mushroom body remains to be determined.

ACKNOWLEDGMENTS

We are grateful to Dr. Michael Ibbotson for constructive comments on the article. We also thank Malte Westerhoff for cooperation regarding the development of important Amira tools for the three-dimensional reconstruction of single neurons.

LITERATURE CITED


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