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Review article

Chemical architecture of antennal pathways mediating proboscis extension learning in the honeybee

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Summary — The chief neuropiles mediating proboscis extension in response to antennal sugar water stimulation and also associative learning of an odour are the antennal lobes, the mushroom bodies, lateral parts of the protocerebrum, and the suboesophageal ganglion. This review focusses on the distribution of some classical neurotransmitters within olfactory pathways based on methods of chemical neuroanatomy. Chemosensory information processing and motor circuits for proboscis extension seem to include cholinergic projection and local interneurons, GABAergic interneurons, glutamatergic motoneurons, and aminergic neurons which link with extensive arborisations the neuromeres of the nervous system. Electrophysiological stimulation experiments and immunocytochemical investigations implicate octopaminergic ventral median neurons of the suboesophageal ganglion as a rewarding system in proboscis extension learning.

***Apis mellifera* / nervous system / neurotransmitter / receptor / learning**

INTRODUCTION

The proboscis extension reflex of the honeybee (*Apis mellifera* L) is an appetitive component of feeding behaviour that is elicited by touching one antenna with a droplet of sugar water. By pairing an initially neutral odour (conditioned stimulus) with the application of sugar water (unconditioned stimulus) to the proboscis, the reflex can be classically conditioned (Kuwabara, 1957; Menzel *et al,* 1974). The proboscis extension reflex is also subject to habituation (Braun und Bicker, 1992), a non-associative form of learning. The neurobiological analysis of learning requires the identification of brain areas and ultimately circuits responsible for neural plasticity. A subsequent cellular analysis of neural plasticity will depend on the identification of transmitter substances involved in synaptic transmission.

Based on the well-described functional neuroanatomy of chemosensory information processing pathways in the bee's nervous system (Pareto, 1972; Suzuki, 1975;

Arnold *et al,* 1985; Mobbs, 1985; Flanagan and Mercer, 1989; Rehder, 1989), this review will focus mainly on the chemical neuroanatomy of neuropiles, which participate in mediating or modulating the proboscis extension reflex. Chemical neuroanatomy describes the histochemical and immunocytochemical detection of neurotransmitter-related properties in the nervous system, bridging anatomy and neurochemistry.

The chief neuropiles mediating proboscis extension in response to antennal sugar water stimulation and also associative learning of an odour are the antennal lobes, the mushroom bodies, parts of the lateral protocerebrum, and the suboesophageal ganglion (Mobbs, 1985) (fig 1). The antennal lobes receive their sensory input predominantly from chemosensory receptors on the antennae, whereas antennal mechanosensory fibres are thought to project mainly into the dorsal lobes (Pareto, 1972; Suzuki, 1975). A quantitative ultrastructural study (Gascuel and Masson, 1991) has demonstrated that synaptic integration between sensory afferents, local interneurons and projection neurons is mainly confined to the spherical glomeruli which surround the central coarse neuropile. Groups, as well as certain individual glomeruli, can be identified (Arnold *et al,* 1985; Flanagan and Mercer, 1989) based on innervation by 4 different branches of the antennal sensory nerve (Suzuki, 1975). The principal output of the antennal lobes is carried *via* the median and lateral antennoglomerular tracts into the calycal lips of the mushroom bodies and into the lateral protocerebral neuropile (Mobbs, 1985). Cobalt staining has revealed additional but less prominent output tracts such as the mediolateral antennoglomerular tract and a small tract interconnecting the 2 antennal lobes (Arnold *et al,* 1985; Mobbs, 1985). Degeneration and dye backfilling studies of the antennal nerve

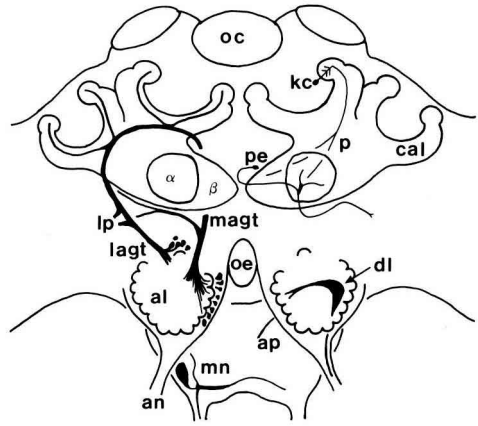

Fig.1. Schematic drawing of antennal and proboscis extension reflex pathways. Receptors of the antenna send sensory projections through the antennal nerve (an) into the antennal lobe (al) and dorsal lobe (dl). The suboesophageal ganglion below the oesophagus (oe) receives some direct antennal projections (ap) and its neuromeres contain the motoneurons (mn) mediating proboscis extension. Fibres of the median (magt) and lateral (lagt) antennoglomerular tracts project from the glomeruli of the antennal lobes (al) to the calyces (cal) of the mushroom bodies and into the lateral protocerebrum (lp). Neurites of the mushroom body intrinsic Kenyon cells (kc) project from their calycal input areas in a parallel arrangement through the pedunculus (p) and finally bifurcate into the β-lobe (β) and the α-lobe (α). A link between the mushroom bodies and the lateral protocerebrum is provided by neurons such as the pedunculus extrinsic neuron (pe) described by Mauelshagen (1993). Ocelli (oc). Scale: 100 m.

(Pareto, 1972; Suzuki, 1975; Arnold *et al,* 1985; Mobbs, 1885) have also revealed direct sensory projections into the posterior deuto- and tritocerebral parts of the brain, as well as into the suboesophageal ganglion (fig 1).

The suboesophageal ganglion is contiguous with the deuto- and tritocerebral parts

of the brain and arises from the embryonic fusion of the mandibular, maxillary, and labial neuromeres. It innervates the mouthparts and neck muscles, and serves as a relay station for the information flow between brain and cervical connective. The neuromeres receive sensory projections from the mouthparts and antennae and contain local and intersegmental interneurons and the processes of the motoneurons responsible for the extension of the proboscis (Rehder, 1989).

Relay neurons of the antennoglomerular tracts transmit chemosensory information into the mushroom bodies (Arnold *et al,* 1985; Mobbs, 1985). The shape and internal structure of this neuropile is largely reflected in the branching pattern of the intrinsic Kenyon cells. Fibres of the Kenyon cells project from their calycal input areas in a parallel arrangement through the pedunculus and finally branch into the α- and the β-lobe (fig 1). Besides the arborisations of extrinsic fibres that link the mushroom bodies to other brain areas, a worker bee's mushroom body contains ≈ 170 000 Kenyon cells (Witthöft, 1967; Mobbs, 1985). The Kenyon cells of both mushroom bodies constitute ≈ 1/3 of all neurons in the brain of the bee.

The detailed pathways from the mushroom bodies to the motor circuits of proboscis extension in the suboesophageal ganglion are largely unknown. Recently, a neural correlate of olfactory learning has been described by intracellular recordings from a single identified extrinsic neuron of the mushroom body neuropile (Mauelshagen, 1993). This PE1 neuron links the pedunculus with extensive arborisations in the lateral and median protocerebrum (fig 1). The PE1 neuron can serve as an example of how neural activity in the Kenyon cells might be transmitted to the lateral protocerebrum. A schematic circuit diagram can be found in the review of Mauelshagen and Greggers (1993). Descending pathways from the lateral protocerebrum to the suboesophageal ganglion remain to be investigated. The following section will provides an overview of classical neurotransmitter systems in the above-mentioned neuropiles at light-microscopical resolution.

ACETYLCHOLINE

The most reliable marker of cholinergic neurons is the presence of the ACh-synthesizing enzyme choline acetyltransferase (ChAT). Unfortunately, none of the commercially available antibodies against mammalian ChAT cross-reacted with the bee's enzyme (Kreissl and Bicker, unpublished observations). Therefore the current understanding of the organisation of cholinergic pathways has been derived from acetylcholinesterase (AChE) histochemistry combined with immunocytochemical localisation of nicotinic receptors (Kreissl and Bicker, 1989) as well as the autoradiographic mapping of α-bungarotoxin binding (Scheidler *et al,* 1990). A schematic drawing of AChE activity in the brain of the bee including some anatomical structures is provided in figure 2. Both the antennal and the dorsal lobe showed AChE activity. In the antennal lobe the activity was mainly confined to the glomeruli (fig 2), whereas the central neuropile was not stained. The sensory fibres of the antennal nerve, which project ventrally to the antennal lobe into the dorsal lobe, contributed largely to the AChE staining of the dorsal lobe neuropile (fig 2) which is the origin of those motorneurons responsible for the movement of the antenna (Pareto, 1972; Suzuki, 1975). Compared to the sensory fibres innervating the dorsal lobe, the sensory projections into the antennal lobe showed only a rather weak staining. Some glomeruli which received strongly AChE-stained fibres from the antennal nerve were, however, ob-

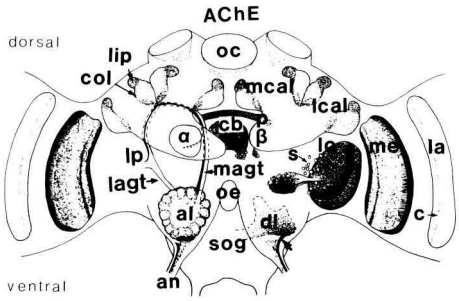

Fig 2. Schematic drawing of AChE activity in the brain. Staining in the optic lobes is included for the sake of completeness. The lamina (la), medulla (me), and lobula (lo) of the optic lobes display a layered AChE staining. The lobula is connected to the bulb-shaped optic tubercle. AChE active sensory fibres of the antennal nerve project into the dorsal lobe (dl) and the suboesophageal ganglion (sog) below the oesophagus (oe). The median (magt) and lateral (lagt) antennoglomerular tracts connect the glomeruli of the antennal lobe (al) with the lip area (lip) of median (mcal) and lateral calyx (lcal) of the mushroom bodies and with the lateral protocerebrum (lp). The collar (col) is a neuropilar compartment of the calyx receiving visual input. AChE active fibres leave the β-lobe (β) of the mushroom body. The central complex shows AChE activity in the pons (p) and central body (cb). Illustration modified after Kreissl and Bicker (1989). Scale: 100 m.

served at the entrance of the nerve into the lobe. Direct antennal sensory projections into the posterior deuto- and tritocerebral parts of the brain, as well as into the suboesophageal ganglion (Pareto, 1972; Suzuki, 1975; Arnold *et al,* 1985; Mobbs, 1985) (fig 1) showed AChE activity.

A high density of acetylcholine receptor immunoreactivity (AChR-IR) (Kreissl and Bicker, 1989) and α-bungarotoxin binding sites (Scheidler *et al,* 1990), was found in the antennal lobe. Once again, most of the binding of these 2 nicotinic AChR markers were confined to the glomeruli where the synaptic interactions are thought to occur. The combined data provided clear evidence for cholinergic information- processing circuits in the antennal lobe but the histochemical resolution does not yet allow the relative contributions of sensory receptors and local interneurons to be separated.

The calycal neuropile of the mushroom bodies (fig 2) has been subdivided into lip, collar, and basal ring regions (Mobbs, 1985). Antennoglomerular tract relay neurons enter the calyx *via* the inner ring tract and terminate mainly within the lip, while the collar receives input from the optic ganglia *via* the outer ring tract. The AChE staining of the mushroom bodies showed a clear compartmentalised pattern. While the somata and neurites of the mushroom body intrinsic Kenyon cells were not stained, the calycal neuropile showed AChE activity (fig 2). AChE activity was especially pronounced in the lip area, and could be attributed to fibres of the median antennoglomerular tracts (figs 1, 2) which have their main terminal arborisations within this area of the calycal neuropile. The AChE activity was not homogeneously distributed across the fibre population of the median antennoglomerular tract, but the fibres at the outer flanks of the tract exhibited strong AChE activity while those fibres in the core showed intermediate or no staining. Labelling in the basal ring, which receives projections from the tritocerebrum and suboesophageal ganglion is unaccounted for according to Mobbs (1985), but antennoglomerular tract fibres may also have contributed to the staining. Apart from the calyces, most parts of the pedunculus and the α- and β-lobes were devoid of staining. Some extrinsic fibres which leave the mushroom body neuropile through the β-exit did, however, express AChE activity (fig 2).

All parts of the mushroom body showed AChR-IR. The immunoreactivity in the caly-

ces reached its greatest density in the lip area. Since the somata within the calyces contained granular immunoreactivity and all neuropilar compartments of the mushroom body showed strong but fine immunoreactivity, the Kenyon cells seem to express nicotinic ACh receptors. The quantitative autoradiographic studies of Scheidler *et al* (1990) also described α- bungarotoxin binding sites in the calycal input areas of the mushroom bodies but compared to the expression of AChR-IR the lobes showed a more layered distribution of binding. The reasons for the slight discrepancy between AChR-IR and α-bungarotoxin binding sites in the lobes are not known. In conclusion, a particularly striking overlap of AChR immunoreactivity, α-bungarotoxin binding and AChE staining was found in the lip neuropile of the mushroom bodies, and this would suggest a cholinergic input into this neuropile *via* certain fibres of the median antennoglomerular tract.

GABA

Amino acid neurotransmitters can be visualised in nervous tissue directly with antibodies raised against the amino acid coupled to protein carriers (Storm-Mathisen and Ottersen, 1986), a technique which has been extensively used in the mammalian nervous system. Many studies have also been reported in insects including our immunocytochemical description of GABA immunoreactivity (GABA-IR) (Bicker *et al*, 1985; Schäfer and Bicker, 1986) in the brain of the honeybee. Studies with an antiserum directed against GABA have also been double checked with an antiserum against the GABA synthesising enzyme, glutamic acid decarboxylase (GAD) (Bicker *et al*, 1987). Staining patterns using antibodies against GABA and GAD were virtually identical, providing independent evidence for the specificity of transmitter immunocytochemistry.

GABA-IR was predominantly found in local interneurons and to a lesser extent in projection fibres. We estimated that ≈ 5% of the neurons in the brain and suboesophageal ganglion were GABA-IR. A high density of GABA-IR was found throughout the antennal lobe neuropile. The immunoreactivity seems to predominate in local interneurons whose somata cluster mainly in the lateral and dorsomedial soma rind (fig 3). GABA-IR projection fibres were found

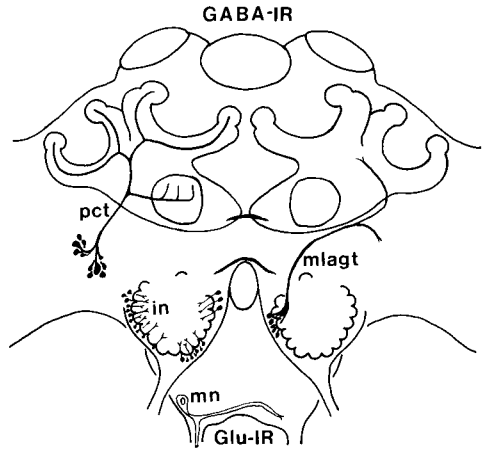

Fig 3. GABA-IR in antennal pathways of the central brain and glutamate-IR in the suboesophageal ganglion. The schematic drawing indicates immunoreactivity only in some conspicuous pathways. The antennal lobes contain GABA-IR local interneurons (in). GABA-IR projection neurons of the mediolateral antennoglomerular tract (mlagt) link antennal lobe with mushroom body and lateral protocerebrum. Antennal lobes and β-lobes of the mushroom bodies are connected by small immunoreactive commissures. Fibres of the protocerebrocalycal tract form a loop from the lateral exit point of the α-lobe to the calyces. Glu-IR motoneurons (mn) mediating proboscis extension are located in the neuromeres of the suboesophageal ganglion. Drawing compiled after data from Bicker *et al* (1985), Schäfer and Bicker (1986), and Bicker *et al* (1988). Scale: 100 m.

in a small commissure that interconnects the antennal lobes and in the mediolateral antennoglomerular tract, a small fibre tract linking antennal lobes with mushroom bodies and lateral protocerebral neuropile. A rather prominent group of GABA-IR mushroom body extrinsic fibres passes *via* the protocerebrocalycal tract (pct) from a lateral exit point of the α-lobe to the ipsilateral calycal input region (fig 3) (Bicker *et al,* 1985; Schäfer and Bicker, 1986). Intracellular recordings of honeybee mushroom body extrinsic neurons demonstrated that GABA acts, as in other animals, as a neuroinhibitory compound (Michelsen and Braun, 1987). Therefore it is possible that the pct fibres may function as an inhibitory feedback loop in the mushroom body system. Cross sections of the pct showed that it contains ≈ 110 GABA-IR fibres. The cell bodies of the pct fibres were found in 2 clusters in the soma rind dorsolaterally to the antennal lobe. Even though the pct neurons contribute largely to the GABA-IR in the mushroom bodies, other GABA-IR neurons invade the neuropile as well. At least 2 large extrinsic fibers that leave the α-lobe ventromedially expressed GABA-IR. A GABA-IR commissure linking the 2 β-lobes may conceivably mediate bilateral inhibitory interactions between the 2 mushroom bodies.

The neuropile of the suboesophageal ganglion contained a high density of GABA-IR. The majority of the ≈ 1000 labelled cell bodies belonged to interneurons because there were no labelled fibres in the nerves leading to the mouthparts.

GLUTAMATE

Physiological experiments have demonstrated that the excitatory transmitters at neuromuscular junctions of arthropods consist of the amino acid glutamate as classical transmitter. Immunocytochemical studies (Bicker *et al,* 1988) have confirmed that the majority of motoneurons in bees and locusts are glutamate-immunoreactive (Glu-IR), including the motoneurons in the suboesophageal ganglion (fig 3) and the antennal motoneurons of the dorsal lobe. In addition, a few Glu-IR cell bodies and fibres of interneurons descending to the suboesophageal ganglion have been found in the antennal lobe, suggesting that the insect nervous system may also employ glutamate as transmitter in central pathways. Faint Glu-IR was detected in a cluster of Kenyon cells, but it should be stressed that the staining intensity was much lower than that of the Glu-IR of motoneurons.

TAURINE

Taurine is one of the most abundant free amino acids found in insect nervous systems. The transmitter status of this rather enigmatic compound is not yet clear (Bicker, 1992), but physiological and neurochemical evidence argues against a role as classical transmitter and points towards a neuromodulatory role. I will review the cellular distribution of the neuroactive amino acid because discrete neuronal populations of the chemosensory information processing pathways have been found to be taurine-immunoreactive (Tau-IR). A report on the first immunocytochemical localisation of taurine in insects was published on the brain of the worker honeybee (Schäfer *et al,* 1988) in which taurine accounts for 16% of the total free amino acid pool, second only in concentration to glutamate (20%) (Frontali, 1964). Most of the prominent features of the distribution of Tau-IR were subsequently also found with another antiserum in the brains of fruit flies and locusts (Bicker, 1991).

Weak levels of Tau-IR appeared throughout the neuropile of the antennal lobe, whereas the cortical layers of the glomeruli in particular were more intensely stained (Schäfer *et al,* 1988). Some of the staining could clearly be attributed to the innervation of the glomeruli by sensory projections from the antennal nerve, but in many cases a clear separation of the contribution from sensory neurons and interneurons could not be achieved. The calyces of the mushroom bodies receive 2 Tau-IR sensory projections, one from the optic ganglia, and the other *via* relay neurons of the lateral antennoglomerular tract from the antennal lobe. Schäfer *et al* (1988) reported high levels of Tau-IR in all Kenyon cells of the worker honeybee showing, however, differences in the staining intensity among medial and peripheral Kenyon cell populations in the calyces. Immunoreactivity appeared more intense in the large diameter somata located peripherally than in the smaller medially located somata.

Finally, I would like to point out a general observation concerning the immunoreactivity to neuroactive amino-acid compounds: a detailed comparison of GABA-IR (Schäfer and Bicker, 1986), Glu-IR (Bicker *et al,* 1988), and Tau-IR (Schäfer *et al,* 1988) showed complementary distributions in the insect nervous system.

SEROTONIN

The distribution of serotonin (5-HT), a biogenic monoamine, has been investigated by histofluorescence (Mercer *et al,* 1983) and immunofluorescence (Schürmann and Klemm, 1984). The immunofluorescence study estimated that the bee brain contained ≈ 75 5-HT-IR cell bodies which were arranged in clusters. Immunoreactive fibres were present in most parts of the deuto- and protocerebrum, with the notable exception of the calyces of the mushroom bodies (fig 4). All 5-HT-IR fibres in the mushroom bodies were of extrinsic origin. Autoradiographic binding studies (Erber *et al,* 1991) showed a high density of binding sites for radiolabelled 5-HT in the mushroom bodies. A comparison of the immunocytochemical distribution of serotonin (Schürmann and Klemm, 1984) with binding studies revealed a mismatch in the calyces which bound the transmitter strongly but were not innervated by 5-HT-IR fibres.

Immunocytochemical staining of paraffin serial sections allowed the reconstruction of identified neurons in the antennal lobe and suboesophageal ganglion (Rehder *et al,* 1987). The glomeruli of the antennal

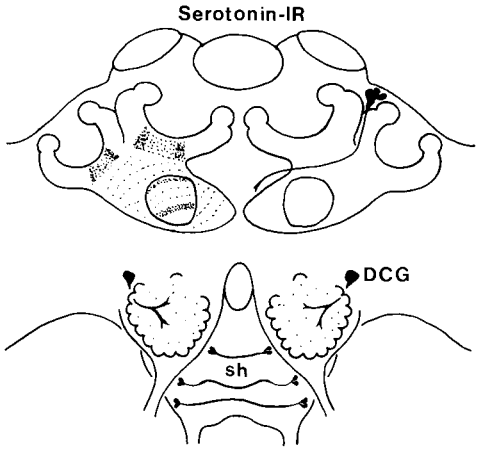

Fig 4. Serotonin-IR in antennal pathways of the central brain and suboesophageal ganglion. A single deutocerebral giant neuron (DCG) innervates the antennal lobes. The immunoreactivity in the mushroom bodies is completely of extrinsic origin and may originate from processes wound around the lobes, such as the clustered neurons shown on the right side. Each of the 3 suboesophageal neuromeres contains 2 pairs of immunoreactive neurons which form connections to the brain and thoracic ganglia. Drawing compiled after data from Schürmann and Klemm (1984) and Rehder *et al* (1987). Scale 100 m.

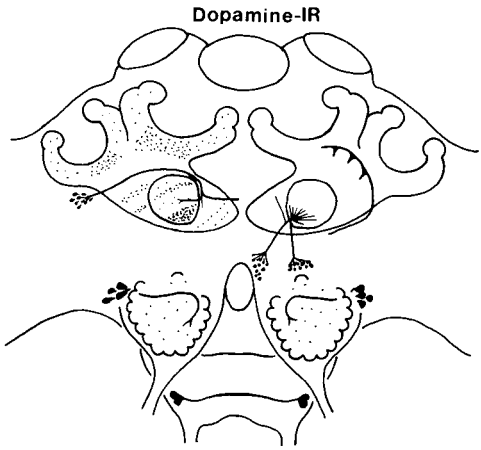

Fig 5. Dopamine-IR in antennal pathways of the central brain and suboesophageal ganglion. The antennal lobe is innervated by 4 immunoreactive neurons. Immunoreactive fibres in the mushroom bodies are of extrinsic origin and could be traced to 3 soma clusters. The suboesophageal ganglion contained groups of soma and an extensive network of fibres. Drawing compiled after data from Schäfer and Rehder (1989) and Schürmann *et al* (1989). Scale: 100 m.

lobe receive varicose immunoreactive fibres from a single identified neuron, the deutocerebral giant (DCG) (fig 4). This large interneuron interconnects the deutocerebral antennal and dorsal lobes with the suboesophageal ganglion and descends into the ventral nerve cord. Two pairs of bilateral immunoreactive neurons were identified in each of the 3 suboesophageal neuromeres (fig 4). The processes of these serial homologues generate an extensive network in the suboesophageal ganglion and projections into the brain and thoracic ganglia. The morphological studies were extended by double-labeling experiments with serotonin immunofluorescence after intracellular injections of a dye into the serial homologues. Intracellular recordings of 5-HT-IR serial homologues of the labial neuromere showed excitatory responses towards sugar water stimulation of the antenna (Hammer, personal communication), providing physiological evidence for the involvement of certain serotonergic pathways in the processing of antennal sugar water stimuli.

DOPAMINE

The distribution of the catecholamine dopamine has been mapped by histofluorescence techniques (Mercer *et al,* 1983) and by immunocytochemistry (Schäfer and Rehder, 1989; Schürmann *et al,* 1989). Dopamine immunoreactive (DA-IR) fibres were found in nearly all parts of the brain and suboesophageal ganglion except the optic lobe neuropile. Schäfer and Rehder (1989) estimated that there were ≈ 330 DA-IR somata in each brain hemisphere including the suboesophageal hemiganglion. The glomeruli and central neuropile of the antennal lobes were innervated by 4 large DA-IR processes (fig 5).

These fibres originated from 2 soma groups, each containing 2 somata, in the lateral deutocerebral soma rind posterior to the antennal lobe and close to the dorsal rim of the suboesophageal ganglion.

The lobes, pedunculi, and calyces of the mushroom bodies contained an uneven density of DA-IR fibres. All the immunoreactive fibres of the mushroom bodies were of extrinsic origin, most of which could be traced to 3 soma clusters (fig 5). The demonstration of DA-IR fibres in the cell body regions of the calyces may be a challenge to the view that insect neurons interact only in the neuropile.

The suboesophageal ganglion contained ≈ 30 stained cell bodies arranged in groups in the soma rind. An extensive network of immunoreactive fibres with widely overlap-

ping projection areas filled the neuropile of the suboesophageal ganglion.

OCTOPAMINE

Octopamine has been the most widely studied biogenic amine in physiological investigations of insect central nervous systems. A preliminary account of its immunocytochemical distribution recently appeared (Kreissl *et al,* 1991). With the exception of the pedunculi and large parts of the α- and the β-lobes of the mushroom bodies, varicose immunoreactive fibres invaded all parts of the brain and the suboesophageal ganglion. The calyces of the mushroom bodies received octopamine immunoreactive (OA-IR) innervation from the suboesophageal ganglion *via* the lateral antennoglomerular tracts (Kreissl *et al,* 1991) (fig 6). The pattern of octopamine immunoreactivity in the mushroom bodies contrasts rather sharply with autoradiographic studies which show a high density of octopamine binding sites in the pedunculi and β-lobes (Erber *et al,* 1991). Immunoreactivity in the glomeruli of the antennal lobe most likely derives from groups of somata clustered in the ventral median parts of the 3 neuromeres of the suboesophageal ganglion (fig 6). Most of the immunoreactive ventral median cells send their neurites dorsally through the midline tracts, whereas the neurites of a few cells follow the ventral cell body neurite tracts before entering the neuropile of the suboesophageal ganglion. The labial neuromere contained 3–5 somata in a dorsal median position, close to the exit of the cervical connective.

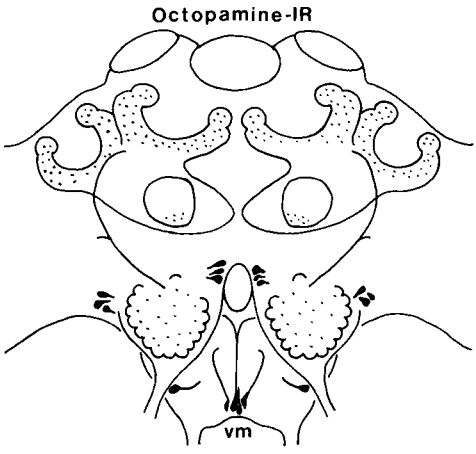

Fig 6. Octopamine-IR in antennal pathways of the central brain and suboesophageal ganglion. Antennal lobes are innervated from ventral median (vm) somata in the suboesophageal ganglion. The calyces of the mushroom bodies receive an immunoreactive innervation *via* the lateral antennoglomerular tracts. Drawing compiled after data from Kreissl *et al* (1991). Scale: 100 m.

DISCUSSION

The chemical neuroanatomy of transmitter systems in the brain of the honeybee has expanded rapidly since histofluorescence in conjunction with biochemical methods detected the distribution of biogenic amines (Mercer *et al,* 1983). Many of the classical transmitter substances of the mammalian brain have also been found in the bee. Unfortunately, rigorous physiological proof that any of the above-mentioned substances has a transmitter role in the nervous system of the bee is lacking. Since neurons of the pupal honeybee brain seem to express transmitter receptors and immunoreactivity when grown in dissociated cell culture (Kreissl and Bicker, 1992), physiological studies of neurotransmission are within reach. For example, bath application of the putative inhibitory neurotransmitter GABA to cultured neurons indeed caused an increased conductance membrane hyperpolarization (Bicker, unpublished observations). Similar observations have been reported for dissociated locust neurons (Giles and Usherwood, 1985).

Despite the growing body of information on the rather complex chemical architecture of the bee's brain, our knowledge is still incomplete. So far, the immunocytochemical studies have not revealed a classical neurotransmitter candidate in Kenyon cells. However, immunoreactivity to neuropeptides has been located in some Kenyon cell types (Schürmann and Erber, 1990; Eichmüller *et al,* 1991; Erber *et al,* 1991). It is possible that large parts of the insect nervous system employ only neuropeptides as transmitter substances.

Some areas of the honeybee brain show a matching of transmitter markers and receptor binding studies (Kreissl and Bicker, 1989; Erber *et al,* 1991), whereas in other areas there is an apparent mismatch. There are a variety of explanations for this phenomenon, which is also found in the mammalian nervous system. The cellular targeting of receptor subunits to synaptic areas may not function with complete efficiency, as indicated by the appearance of extrasynaptic receptors on insect neuron somata or muscle fibres. Since receptors are not only confined to synaptic areas, care should be taken in interpreting radioligand binding studies in terms of synaptic distributions. A rather simple solution to this dilemma would be to propose that neurotransmission occurs in areas where transmitter immunoreactivity and receptor binding overlap. Nevertheless, I would like to mention the caveat that at a light microscopical resolution transmitter immunoreactivity is not equivalent with release sites, and that receptor binding studies do not necessarily imply functional coupling of the binding site to ion channels or intracellular signal transduction.

Whereas vertebrate motoneurons use ACh as their transmitter, the insect motoneurons innervating skeletal muscles employ the amino acid glutamate as their excitatory transmitter. Some general principles are, however, common to the chemical architecture of the vertebrate and invertebrate brain. For example, most GABAergic neurons are intrinsic in such regions of the brain as the cortex, olfactory bulb, hippocampus, retina and cerebellum (Shepherd, 1983). Immunocytochemistry with antisera to GABA and GAD in the antennal lobes, mushroom bodies, suboesophageal ganglion and optic ganglia (Bicker *et al,* 1985, 1987; Schäfer and Bicker, 1986) revealed a similar organisation of GABAergic pathways in the insect brain, suggesting that the majority of GABAergic neurons function as parts of local inhibitory circuits and to lesser extent as projection neurons. Another common principle of brain organisation is the existence of aminergic neurons which are capable of influencing large areas of the brain (Bicker and Menzel, 1989). Several nuclei in the brainstem of the mammalian nervous system give rise to groups of thousands of widely projecting modulatory aminergic neurons (Moore and Bloom, 1978, 1979). However, even though the nervous system of the worker bee contains almost a million neurons (Witthöft, 1967) it is still possible to resolve single identified neurons such the 5-HT-IR deutocerebral giant (Rehder *et al,* 1987).

A knowledge of the chemical architecture of the bee's nervous system has proved especially useful in behavioural pharmacological studies of proboscis extension learning. A functional inactivation of monoaminergic systems by reserpine depletion which has been monitored by immunocytochemistry (Braun and Bicker, 1992) does interfere with processes required for associative learning (Braun and Bicker, unpublished observations). Systemic pharmacology influencing aminergic transmission has enabled memory storage to be dissociated from retrieval processes (Mercer and Menzel, 1982; Menzel *et al,* 1988, Bicker and Menzel, 1989) Octopa-

mine injections into the brain close to the calyces facilitated memory storage and retrieval during associative learning (Menzel *et al,* 1988).

The response decrement during a non-associative learning task, habituation of the proboscis extension reflex, depends on the satiation level of the experimental animal. Reserpine depletion experiments in combination with subsequent injections of octopamine or its metabolic precursor tyramine uncovered an octopaminergic mechanism in the state dependency of habituation (Braun and Bicker, 1992). Injections of neurochemicals into restricted areas of the brain combined with a detailed knowledge of its chemical architecture have provided clues on the structural basis of the reflex (Braun and Bicker, 1992). The reflex was abolished by injections of the cholinergic receptor blocker α-bungarotoxin into the antennal lobe, confirming the histochemical data which implicate nicotinic cholinergic transmission in the antennal lobe neuropile. Furthermore, feeding of the AChE inhibitor eserine retarded habituation of the proboscis extension reflex, demonstrating a facilitatory effect on cholinergic transmission within the neural circuits of the reflex. Bilateral injections of α-bungarotoxin into the calyces which receive cholinergic innervation *via* the median antennoglomerular tracts did not block the reflex, suggesting that the projection areas of the median antennoglomerular tract into the mushroom body neuropile are most likely not required for functioning of the reflex. Presumably the unconditioned reflex is mediated by direct pathways between the antennal lobe and the suboesophageal ganglion, bypassing the whole mushroom body neuropile. The relay stations in the antennal lobes, lateral protocerebrum, mushroom bodies and suboesophageal ganglion provide multiple loci for influencing excitability and plasticity of the reflex pathways.

It remains a challenging task to investigate the different forms of associative and non-associative neuronal plasticity by electrophysiological investigations at various stages of the reflex pathways. A neural correlate of learning was found in the PE-1 neuron of the mushroom body system in a reduced preparation (Mauelshagen, 1993). Depolarising intracellular stimulation of an identified ventral median neuron (VUMmx1) of the suboesophageal ganglion was sufficient to substitute for the unconditioned stimulus in the proboscis extension conditioning paradigm (Hammer, 1992). The soma position of VUMmx1 is among the octopamine-IR ventral median cell bodies in the maxillary neuromere, and its other anatomical characteristics are bilateral projections into the antennal lobes, the lateral protocerebra and *via* the lateral antennoglomerular tracts into the calyces (Hammer, 1991). A comparison with the immunocytochemical results (fig 6) justifies the suggestion that VUMmx1 is part of an octopaminergic system signalling the unconditioned sugar water stimulus in proboscis extension learning.

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Résumé — Architecture chimique des voies antennaires responsables de l'apprentissage de l'extension du proboscis chez l'abeille, *Apis mellifera* L. On déclenche le réflexe d'extension du proboscis chez l'abeille en appliquant une gouttelette de solution sucrée sur l'antenne. On peut appliquer au réflexe un conditionnement classique en associant une odeur initialement neutre (stimulus condi-

tionnel) à l'application d'une solution sucrée (stimulus inconditionnel). Les principaux neuropiles responsables de l'extension du proboscis en réponse à la stimulation antennaire par une solution sucrée et responsables également de l'apprentissage associatif d'une odeur sont les lobes antennaires, les corps pédonculés, les parties latérales du protocérébron et le ganglion sous-œsophagien. Cette revue traite plus particulièrement de la répartition de quelques neuromédiateurs classiques le long des voies antennaires, étudiée par les méthodes de le neuro-anatomie chimique. Plusieurs équipes de recherche ont employé une variété de techniques telles que l'histofluorescence, la localisation par immunocytochimie des médiateurs et de leurs enzymes de synthèse, la détection par histochimie de leur dégradation, la localisation des récepteurs par immunocytochimie et l'autoradiographie quantitative de la répartition des récepteurs pour cartographier les voies des médiateurs. Le traitement de l'information chimiosensorielle et les circuits moteurs de l'extension du proboscis semblent inclure les interneurones cholinergiques locaux et efférents, les inter-neurones gabaergiques, les motoneurones glutamaergiques et les neurones aminergiques qui relient, par leurs arborisations étendues, les neuromères du système nerveux. La discussion porte sur les études pharmacologiques du comportement, les expériences de stimulation électrophysiologique et les recherches en immunocytochimie qui impliquent les neurones médians ventraux octopaminergiques du ganglion sous-œsophagien comme éléments d'un système de récompense transmettant le stimulus inconditionnel de la solution sucrée lors de l'apprentissage de l'extension du proboscis.

système nerveux / neuromédiateur / récepteur / apprentissage

Zusammenfassung — Die chemische Architektur der antennalen Leitungswege, welche das Lernen der Rüsselstreckung bei der Honigbiene vermitteln. Der Kontakt einer Antenne mit einem Tropfen Zuckerwasser löst bei der Honigbiene (*Apis mellifera* L) den Rüsselreflex aus. Durch Paarung eines neutralen Duftreizes (bedingter Reiz) mit einer Zuckerwasser Applikation (unbedingter Reiz) an den Rüssel, kann dieser Reflex klassisch konditioniert werden. Die Neuropilbereiche, die den Rüsselreflex auf Zuckerwasserreizung und assoziativen Lernens eines Duftes vermitteln, sind die Antennalloben, die Pilzkörper, laterale Bereiche des Protocerebrums und das Unterschlundganglion. Dieser Übersichtsartikel untersucht die Verteilung einiger klassischer Neurotransmitter in den antennalen Bahnen mit Methoden der chemischen Neuroanatomie. Eine Reihe von Techniken wie Histofluoreszenz, immuncytochemische Lokalisation von Transmittern und transmittersynthetisierenden Enzymen, histochemische Detektion von Transmitterabbau, immunocytochemische Lokalisation der Rezeptoren und quantitative Autoradiographie der Rezeptorverteilungen wurden von mehreren Forschergruppen angewandt, um die Transmitterbahnen zu kartieren. Die chemosensorischen und motorischen Schaltkreise des Rüsselreflexes enthalten cholinerge Projektions- und lokale Interneuronen, GABAerge Interneuronen, glutamaterge Motoneuronen und aminerge Neuronen, welche mit ausgedehnten Verzweigungen die Neuromere des Nervensystems verbinden. Ergebnisse verhaltenspharmakologischer Studien, elektro-physiologischer Stimulationsexperimente und immuncytochemischer Untersuchungen implizieren octopaminerge ventral mediane Neuronen des Unterschlundganglions als Bestandteil eines Belohnungssystems, welches den unbedingten Zuckerwasserreiz beim Erlernen des Rüsselreflexes signalisiert.

Nervensystem / Neurotransmitter / Rezeptor / Lernen

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