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Neuromodulation by serotonin and octopamine in the honeybee: behaviour, neuroanatomy and electrophysiology

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Abstract. The biogenic amines serotonin (5HT) and octopamine (OA) exist in the bee and can modulate neuronal activity and behaviour. 5HT-like and OA-like immunoreactivities can be found in most neuropils of the brain. Binding sites for the two amines are also present in most brain neuropils. The highest density of binding sites for [^{3}H]serotonin and [^{3}H]octopamine was found in the mushroom bodies. In some brain areas, especially the mushroom bodies, mismatches exist between binding sites and immunoreactivities, suggesting that the two amines also bind to neuropils which are not directly innervated by 5HT-like or OA-like immunoreactive neurons. The action of the two amines on behaviour in the bee is antagonistic. In the antennal pathway, proboscis and antennal responses to olfactory and gustatory stimuli are enhanced by OA and reduced by 5HT. In olfactory conditioning experiments, storage and retrieval of the learned signal can be enhanced by OA and reduced by 5HT. The specificity of these effects depends on the site of amine application in the neuropil. In the visual system the direction specificity of the visual antennal response is enhanced by OA and reduced by 5HT after topical application or injection into the lobula, the third optic ganglion. Correlates for the behavioural modulation can be found in higher-order visual interneurons. While OA application can mimic the stimulation of the bee with sugar water, the behavioural conditions leading to the release of 5HT are not yet known.
Key words. Insect; honey bee; behaviour; neuromodulation; serotonin; octopamine.

Introduction

Animals have to cope with unpredictable and changing environmental conditions. They have to find food, territories, and partners; they have to escape enemies and environmental threats. The appropriate behaviours to solve these problems are initiated and controlled by the brain which has the vital task of determining, sometimes within fractions of a second, the best response to a given constellation of inputs. Neurobiological research on arthopods has shown that nervous systems can solve these adaptive tasks by modulating the properties of sensory cells, nerve cells and even muscles[1, 5, 28]. This modulation of single network elements can in turn change the properties of the whole network and consequently can modify behaviour[5]. We have chosen the honeybee to study these questions because the behaviour, neuroanatomy and physiology of this insect have been analysed in great detail by generations of biologists. Behavioural analyses using stereotyped and adaptive behaviours can be performed under controlled laboratory conditions and it is possible to attribute specific behavioural functions to parts of the bee's nervous system[21, 24, 55]. Research in the last few years has shown that biogenic amines, which are present in the bee nervous system, play an important role in behavioural modulation in other animal groups[5]. The analyses of the processes by which biogenic amines modify behaviour in the bee therefore have significance for other nervous systems as well.

The biogenic amines octopamine (OA) and serotonin (5HT) can function in invertebrates as neurotransmitters, neuromodulators and neurohormones. The modulatory effects of the two amines on sensory receptors, interneurons, muscles and behaviour have been reviewed extensively[1, 5, 17, 28, 31, 76]. Therefore, a brief summary of some common effects of OA and 5HT in the nervous system of different arthropod groups will suffice.

The amines can modulate the sensitivity of sensory receptors and receptor organs[73]. In chelicerates[11, 66, 94], crustaceans[19, 72] and insects[74, 104] it has been shown that different classes of sensory receptors can be modulated by the two amines. In many cases the sensitivities of the receptors are enhanced, but there is also experimental evidence for a depression of sensory responses by both amines[72]. A functional distinction of the effects of OA and 5HT at the sensory periphery so far has not been possible because in many cases the two amines differ only in the degree of modulation.

At the level of interneurons 5HT and OA can have opposite modulatory effects. A well documented example is the modulation of posture in the lobster where 5HT and OA modulate the activity of premotor- and motoneurons in opposite ways[39, 46]. OA has activating and enhancing effects in a number of interneurons which were tested in different preparations[75, 91, 92, 96, 99]. Although the effects of OA and 5HT were not tested systematically in many preparations, there is some indication that 5HT can suppress neuronal activity and OA

can have opposite effect[12, 13, 36, 39, 46]. As electrophysiological measurements of modulatory effects in single neurons are limited to larger cells, only a minority of the cells which are involved in behaviour have so far been analysed.

Behavioural analyses provide sensitive assays to quantify the effects of putative modulators. In a number of studies in invertebrates a similar pattern of 5HT and OA effects was found in different animal groups. The application of OA can elicit specific behaviours[13, 42, 71, 91, 93] or modulate, usually enhance, behavioural responses[2, 42, 49, 64]. The elicitation of flight activity in the locust after local OA application and the induction of a 'flexed' posture in lobsters are good examples of the release of specific behaviours by OA. In different invertebrates an enhancement of motor activity and of behavioural responses to sensory stimuli was found after OA application[42]. Although the enhancement of behavioural responses after OA application is the most frequently found effect, inhibition of ongoing behaviour by OA has also been described[64]. In many cases the behavioural functions of 5HT appear to be opposite to those induced by OA. Habituation can be mimicked by 5HT, sensitization by OA; flexion in the lobster is induced by 5HT, extension by OA; and the posture of an insect induced by 5HT is opposite to that produced by OA[5, 36, 49, 50]. Although opposite behavioural consequences of OA and 5HT are well documented in different invertebrate groups, both amines can also modulate behaviour synergistically in the same animal[26].

At the level of the muscles OA and 5HT often have similar modulatory effects. The characteristics of muscle contraction, such as contraction amplitude and rate of contraction and relaxation, can be enhanced by 5HT and OA. The two amines can modulate transmitter release presynaptically and act postsynaptically on the mechanisms of contraction[3, 32, 34, 43, 46, 67, 69, 101, 103].

Very little is known about the actual changes of 5HT or OA concentrations in nervous tissue, muscles and haemolymph under natural conditions. In the bee, for instance, there is detailed knowledge about the changes of amine concentrations during the lifetime of the animal, but the behavioural significance of these findings remains unclear[35, 38, 98]. A positive correlation between stress and OA levels was found in a number of insects, and in a study with bees 5HT levels were also enhanced after stress[14, 15, 38, 41, 102]. In locusts OA levels change differently in different neuropils after the start of flight[16]. Yet, the studies of amine concentrations have so far contributed little to an understanding of the function of the two amines.

The difficulties in understanding the functions of OA and 5HT are in part due to the variety of experimental approaches, the different animals used and the divergent types of tested behaviours. Therefore, it appears necessary to analyse in the same species the effects of the two amines at different levels, from behaviour to the single cell. In this respect studies in the bee are useful because they can include measurements of amine concentrations, immunocytochemical stainings, OA and 5HT binding studies in the nervous system, electrophysiology of single cells and cell populations, analyses of different behaviours, and measurements of modulation at the sensory and motor periphery.

Putative neuromodulators, immunoreactivity and receptor binding studies in the bee

Various experimental approaches have been used to identify putative neuromodulators in the brain of the bee. Immunocytological studies using antibodies against different putative neuroactive compounds have contributed significantly to this analysis. Neuroanatomical descriptions for the bee brain exist for serotonin-[77, 82, 90], dopamine-[84, 88], octopamine-[48], GABA-[6, 82], glutamate-[7], taurine-[8, 83] FMRFamide-[18, 89] and CCK/gastrin-like[44, 65] immunoreactivities. Histochemical investigations of the distribution of biogenic amines and acetylcholinesterase complete these studies[47, 61]. Although the immunocytological analyses do not prove the existence of certain neurotransmitters in defined populations of neurons, they provide us with very useful hypotheses about the distribution of putative neuromodulators in the brain. In addition to these analyses biochemical methods have been used to demonstrate the presence of these substances in the bee brain and the distributions of binding sites for some transmitters have been identified[35, 38, 61, 86, 87, 98].

The concentrations of putative neuromodulatory compounds in the bee brain have been measured for noradrenaline, dopamine, octopamine and serotonin. The absolute concentrations of the four neurotransmitters in the whole bee brain vary considerably in the different studies. These variations are probably due to the different methods used and to heterogeneities of the bee groups[98] and differences between bee colonies[35, 38, 61, 98]. The highest concentrations were found for dopamine (12 to 40 pmol/brain) and serotonin (6 to 21 pmol/brain). The concentration of OA is smaller (7 to 8 pmol/brain), and that of noradrenaline was the lowest (1 to 4 pmol/brain). The concentrations of the putative neuromodulators vary considerably in the different neuropils of the brain. The highest concentrations were found in the alpha-lobes, the outputs of the mushroom body system[61], being up to 25 times (noradrenaline) higher than in the whole cerebral ganglion. For the other analysed amines the concentrations were between 4 (dopamine) and approximately 20 times (OA and 5HT) higher in the alpha-lobes than in the whole cerebral ganglion. Apparently, the alpha-lobes of the mushroom body system differ from the rest of the brain in their concentrations of putative neuromodulators. The function of these compounds in the alpha-lobes can be

tested in behavioural and electrophysiological experiments (see below). In addition to the variations in the neuropils, the concentrations of putative neuromodulators in the bee depend on the age of the animals, the season, stress factors and the colony the bees were taken from[38].

The target sites for putative neuromodulators in the bee brain can be characterized by analysing the neuroanatomical distribution of binding sites for the respective compound. The distributions of binding sites for [^{3}H]serotonin, [^{3}H]octopamine and other ligands in the bee brain have been analysed with autoradiographic methods[10,86,87]. The distributions of transmitter binding sites for the putative neuromodulators 5HT and OA differ in several neuropils from the immunocytochemical or histochemical data. A comparison between 5HT and OA binding sites and immunohistological findings is shown in table 1.

The specificity of binding for [^{3}H]serotonin in the bee brain is high (97%). The density of specific binding for [^{3}H]serotonin (20 nM) ranges from approximately 50 fmol/mg wet weight in the antennal lobe to over 600 fmol/mg wet weight in the calyx area of the mushroom bodies. The vertebrate 5HT antagonist methysergide inhibits [^{3}H]serotonin (10 nM) binding very effectively. The IC_{50} is 3 nM for methysergide which is even lower than the IC_{50} for 5HT (10 nM). Other vertebrate 5HT ligands like ketanserin, 8-OH-DPAT, and buspirone inhibit [^{3}H]serotonin binding only partially[86].

Binding specificity for [^{3}H] octopamine (9 nM) in the bee brain is also high (94%). Like 5HT, binding for OA is highest in the mushroom bodies. The lowest specific binding was found in the lamina, the highest binding density in the pedunculus of the mushroom bodies. Phentolamine, a vertebrate alpha-adrenergic blocker which acts in insects as OA antagonist, can displace [^{3}H]octopamine by 93% in all brain areas except for the mushroom bodies, where the displacement was only 70%. This finding indicates that the OA receptors in the mushroom bodies differ in their binding characteristics from those in the rest of the brain[86].

The high density of binding for [^{3}H]octopamine and [^{3}H]serotonin in the mushroom bodies is probably a consequence of the high neural density in this neuropil. The density of intrinsic neurons in the mushroom bodies of the fly was estimated to be 3 to 5 times higher than in the optic lobes[95]. Similar relations are found in the bee. Similarily, the binding densities for [^{3}H]octopamine and [^{3}H]serotonin in the mushroom bodies and optic lobes differ by a factor of approximately 3.

The comparison between 5HT-like immunoreactivity (IR), OA-like IR and the binding sites for the two amines shows a complete or partial overlap in the optic

Table 1. Comparison of immunoreactivities with binding sites

Brain region		Specific binding (fmol/mg wet weight ± SD) of 10 nM [^{3}H]serotonin	Comparison 5HT-like IR with 5HT binding sites	Specific binding (fmol/mg wet weight ± SD) of 9 nM [^{3}H]octopamine	Comparison OA-like IR with OA binding sites
Optic ganglia					
Lamina	min	68 ± 11	+/−	17 ± 3	+/−
	max	102 ± 9			
Medulla	min	119 ± 16	+/−	61 ± 6	+
	max	140 ± 46		97 ± 10	
Lobula	min	181 ± 27	+/−	78 ± 5	+
	max	254 ± 17		93 ± 10	
Deutocerebrum					
Dorsal lobe		196 ± 14	+	58 ± 14	+
Antennal lobe	min	48 ± 17	+/−	31 ± 6	+/−
	max	56 ± 12		59 ± 10	
Mushroom bodies					
Calyx	min	312 ± 61	−	89 ± 5	+
	max	634 ± 62		172 ± 32	
Pedunculus	min	333 ± 66	+	285 ± 21	−
	max	540 ± 61		454 ± 73	
α-lobe	min	236 ± 4	+	85 ± 27	+/−
	max	363 ± 4		368 ± 58	
β-lobe		487 ± 45	+	446 ± 55	−
Central complex	min	121 ± 21	+	116 ± 14	+
	max	243 ± 40		297 ± 43	

Comparison of the neuroanatomical distribution of 5HT- and OA-immunoreactivities with the neuroanatomical distribution of binding sites for [^{3}H]serotonin and [^{3}H]octopamine as determined by autoradiograms of the bee brain. Minimum and maximum specific binding for different neuropils and the respective standard deviations are shown. 12 bee brains were measured for each of the two experimental series. The columns 'comparison' indicate the degree of correspondence between immunoreactivity and binding sites: + good correspondence; +/− partial overlap; −mismatch between binding sites and immunoreactivity. For details refer to text.

lobes (see table 1)[48,86,90]. The projection areas of the IR-neurons in the optic lobes are smaller than the areas displaying binding for the transmitters. The relatively uniform labelling of the optic ganglia with [^{3}H]serotonin and [^{3}H]octopamine suggests that the two amines can bind in all optic ganglia, as well as in neuropils which are not directly innervated by neurons displaying 5HT-like IR or OA-like IR. A similar partial or complete overlap was found for the deutocerebrum and the central complex.

In the mushroom bodies of the bee clear mismatches between 5HT-like IR, OA-like IR and the respective binding sites are obvious. The 5HT and OA immunoreactive neurons in the mushroom bodies are extrinsic neurons which connect the intrinsic mushroom body cells with the protecerebrum and the other parts of the brain. No 5HT-like IR was found in the calyces of the mushroom bodies[90], although these neuropils still exhibit very high densities of specific binding for [^{3}H]serotonin. In contrast to the calyces, the correlation between binding sites and 5HT-like IR in the other neuropils of the mushroom bodies is very good. For OA, on the other hand, no IR was found for the pedunculus or beta-lobe of the mushroom bodies[48], while the highest density of [^{3}H]octopamine binding is present in these neuropils. Another mismatch is the high concentration of OA in the alpha lobe (142 pmoles/mm^3)[61] and the small degree of OA-like IR in this neuropil[48,86].

We conclude from all these experiments that receptors for 5HT and OA are present in all neuropils of the bee brain. High densities of binding sites are present in some mushroom body neuropil areas which are not innervated by neurons displaying the respective IR. These findings suggest that the two amines OA and 5HT can bind in all neuropils of the brain even in areas where the release sites are remote from the target sites. The high receptor densities in neuropils which are not directly innervated by the respective neuromodulatory neurons could enable neuromodulators to act over relatively long diffusion distances.

Behavioural assays to test putative neuromodulators

Behavioural analyses are very useful to test the effects of putative neuromodulators in the bee. The actions of the neuromodulators at the sensory periphery, at the level of interneurons, at the motor output, and at the level of muscles controlling behaviour, can all be observed in behavioural experiments. A putative neuromodulator can either be introduced into the haemolymph or injected locally into defined neuropil areas, which makes it possible to distinguish between global and local effects.

Free flying bees display a large variety of different behaviours which have the potential to be used for such experiments. Unfortunately, experiments in which free flying bees are treated with putative neuromodulatory substances have the disadvantage that the animals return from the hive to the experimental setup at varying time intervals. Sometimes it takes hours until an animal can be tested after the treatment. This makes it very difficult to compare the effects of pharmacological treatments in different animals. In addition, most electrophysiological experiments have to be done under behaviourally restricted laboratory conditions which are only partly comparable to conditions in the field. However, behavioural tests of putative neuromodulators in the bee can be performed in the laboratory while the animals are kept in small tubes compared to the environment of electrophysiological recordings. Under these conditions two different types of behaviour, proboscis and antennal reflexes, have proven to be very effective in the analysis of neuromodulator functions[21,23,56].

Proboscis extension occurs in response to gustatory or olfactory stimuli. Bees respond with proboscis extension when a drop of sugar water is applied to one antenna. Stimulation of the antenna and proboscis with sugar water leads to sensitization of the proboscis extension reflex which can then also be elicited by water vapour. Both reflex responses can be used to analyse signal processing in the olfactory pathway[23,57,58,60].

The proboscis reflex to sugar water can be conditioned very effectively, for example by using a floral odour as conditioning stimulus and sugar water as a reward. In this learning paradigm an odour is presented to the bee; during this stimulus proboscis extension is elicited by touching an antenna with sugar water. The reflex response of proboscis extension is then rewarded by a small amount of sugar water. Presentation of the odour some time after pairing leads to proboscis extension in response to the conditioned stimulus. Depending on the season, up to 80% of the bees can be conditioned in one trial. This paradigm is very effective in analysing the physiological mechanisms of memory storage and retrieval in the bee[21,24,54,57,58,62].

Antennal movements, a second type of behavioural response that can be studied under laboratory conditions, occur in response to a number of different stimuli. The animals respond to gustatory or olfactory stimuli by movements of both antennae toward the stimulus source. Rapid scanning movements of the antennae are superimposed on these stimulus-directed responses. These antennal responses can be used to analyse neuromodulation in the olfactory pathway, for instance in the antennal lobes and mushroom bodies[21,27,25,97].

Objects in the range of one antenna are scanned by frequent brief touches of the antenna. The most frequent touch durations are often shorter than 10 ms. The mechanosensory afferents of the antenna converge in the dorsal lobe of the deutocerebrum where they overlap with the motoneurons controlling antennal move-

ments. The mechanical scanning behaviour of the antenna, therefore, can be used to analyse neuromodulatory effects at the level of the sensomotor interface in the dorsal lobe[25,45].

The visual antennal reflex is very useful for analysis of neuromodulation in the visual system of the bee. A vertically moving stripe pattern induces directed movements of the antennae. A pattern moving upward leads to antennal movements which are directed downward, while a downward moving pattern induces upward antennal movements. This reflex in the laboratory is very similar to the antennal movements of the bee during landing under free flying conditions[21,22,25,27].

Modulation of visual behaviour by seorotonin and octopamine

The visual antennal reflex can be measured with an optoelectronic device which allows the quantification of the modulatory effects of 5HT and OA, or other tested compounds. The antennal response is specific for the two directions of a vertically moving stripe pattern[25]. The difference of the antennal angles for the two directions of the stimulus can be used as a measure of the direction specificity of the response[23]. This direction-specific antennal response (DAR) is modulated when 5HT or OA is applied to the brain surface[23]. Figure 1 shows the dose- and time-dependent response changes for this experimental paradigm.

The application of 5HT to the brain surface leads to a significant dose-dependent decrease of the DAR which lasts at least one hour. The application of saline leads to a continous reduction of the DAR which is very similar to the response reductions in untreated bees (fig. 1)[23]. The same experiments were done with OA, where the resulting dose- and time-dependencies differ from those for 5HT (fig. 1). After application of OA at a concentration of 10^{-6} M the DAR is significantly higher compared to lower and higher concentrations. The dose-response curve has the shape of a bell with a maximum at 10^{-6} M. Like 5HT, the modulatory effects of OA last for the duration of the experiment. Apparently, 5HT and OA modulate the direction-specific antennal response in an antagonistic way.

Physiological experiments in the bee have shown that many motion-sensitive neurons in the lobula display direction-specificity[40]. The effects of 5HT and OA, therefore, could be due to neuromodulatory effects in the optic neuropil of the lobula. This hypothesis was tested by injecting small volumes (approximately 500 pl) of 5HT or OA at different concentrations into the lobula. The direction-specific antennal response was tested as in the previous experiments[23]. Figure 2 shows a summary of this experimental series. Similar to the topical application of 5HT, the injection of 5HT into the lobula leads to a time- and dose-dependent

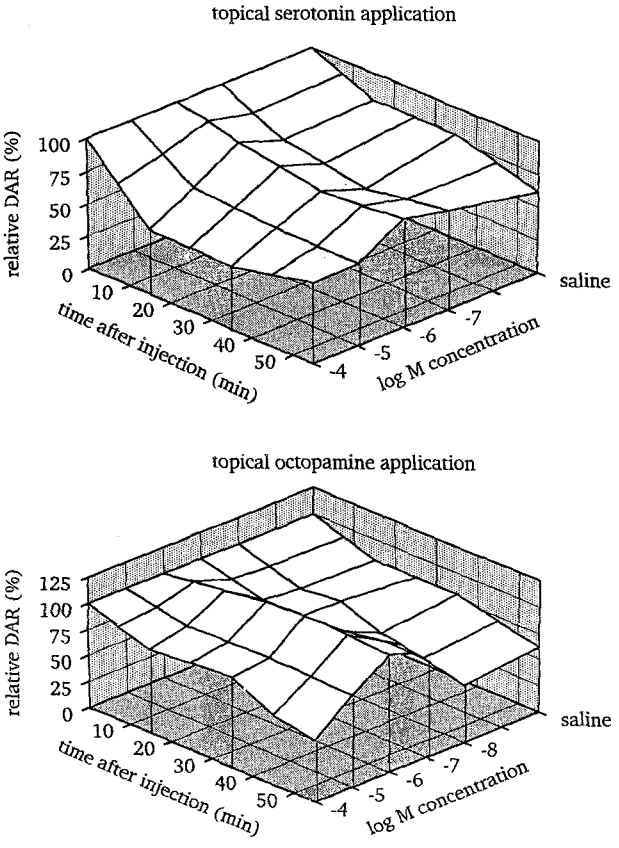

Figure 1. Combined dose- and time-dependencies of the visual antennal response after topical application of 500 nl 5HT or OA over the brain. As a measure of the visual antennal response the relative direction-specific antennal response (DAR) is shown. At the beginning of the experiment the DAR was measured; all other values refer to this initial measurement (=100%). To illustrate the temporal and concentration dynamics of the response changes, error bars are not indicated in the figures. For details of the method of measurement see reference 23. Large DAR values indicate that the antennae display a strong direction specificity when a vertically moving stripe pattern is presented in upward and downward motion to the compound eyes.
The axis 'time after injection' indicates at 0 the relative response shortly before application and then the responses up to 60 min after application. The axis 'log M concentration' indicates the concentration of 5HT or OA in the 500 nl drop applied on the brain surface. 'Saline' indicates the response changes of a control group which was treated with saline. In the 5HT experiments 7-10 animals were tested for each concentration; in the OA experiments 8 bees were tested in each group.

decrease of the DAR which lasts at least 30 min (fig. 2). The modulatory effects of OA show a complex dose and time dependence (fig. 2). At lower concentrations (10^{-8} M and 10^{-7} M) the DAR is enhanced immediately after injection, while for 10^{-6} M the enhancement is first apparent 15 min after injection.

From these experiments it is clear that local injections of 5HT and OA into the lobula also modulate the visual antennal response, similar to the experiments with topical application. Injection of the two amines into the lamina and medulla, the first and second optic ganglion in the bee brain, have shown no modulatory effects of

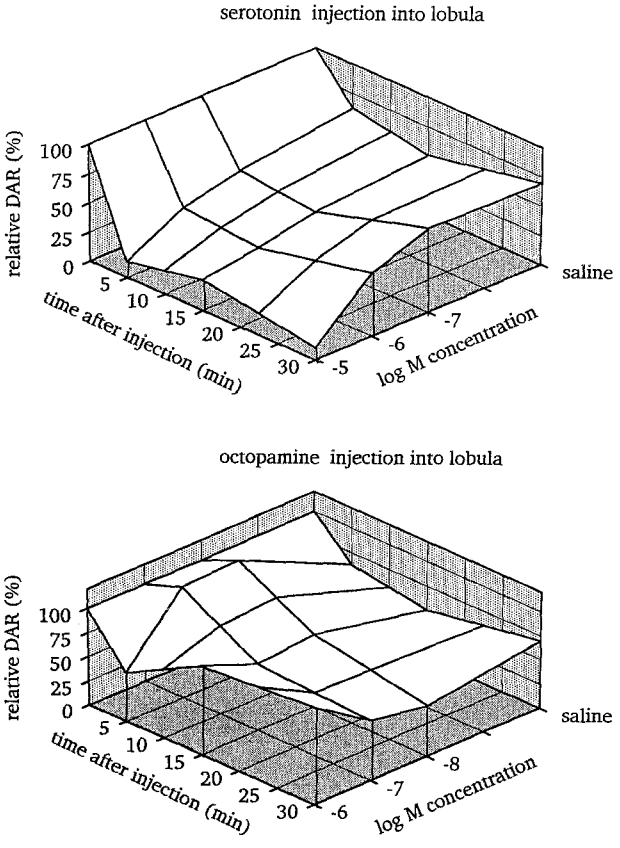

Figure 2. Combined dose- and time-dependencies of the visual antennal response after pressure injection of 500 pl 5HT or OA into the lobula, the third optic ganglion of the bee brain. The ipsilateral antennal response on the injected side is shown. All other details are as in figure 1.
The axis 'time after injection' indicates at 0 the relative response shortly before application and then the responses up to 30 min after application. The axis 'log M concentration' indicates the concentration of 5HT or OA in the 500 pl drop injected into the lobula. 'Saline' indicates the response changes of a control group which was injected with saline. In the 5HT experiments 7–9 bees were tested for each concentration; in the OA groups 9–10 bees were tested in each group.

OA in these neuropils, while the effects of 5HT were weaker but similar to those in the lobula.

Electrophysiological recordings in the visual system

The physiological effects of the two amines on visual neurons can be measured with different electrophysiological techniques: a) electroretinogram recordings (ERG) can reveal perpipheral modulation of the photoreceptors; (b) the recording of field potentials in the optic ganglia can be used to determine the modulation of populations of visual interneurons; and (c) intracellular recordings of single motion-sensitive cells in the lobula can analyse the modulation at the single cell level.
The response of retinula cells are modulated by 5HT and OA. In contrast to the findings in the behavioural experiments where 5HT and OA had antagonistic effects, the sustained amplitudes of ERG are significantly enhanced compared to saline after topical application of both 5HT and OA. After the application of OA, the ERG intensity-response curve for the sustained response is shifted by 0.7 log units towards lower intensities, indicating a sensitivity increase of the photoreceptors.
Field potentials evoked by moving stripe patterns were recorded in the lobula after local application of 5HT and OA. From the behavioural experiments we would expect that application of 5HT leads to a reduction of field potentials induced by a motion stimulus, while OA induces an enhancement of the evoked potentials. Both hypotheses were correct. 5HT reduces field potentials, while they are enhanced by OA[45].
Single cell recordings were made from motion-sensitive cells projecting from the lobula to the protocerebrum or to the contralateral hemisphere. These cells respond to vertical movement stimuli presented to the compound eyes. The neurons can respond to ipsilateral or contralateral movement stimuli, or to stimuli presented to both eyes. During the intracellular recordings first saline and then OA or 5HT were applied to the ipsilateral lobula to test the effects of these neuromodulators on single motion-sensitive cells[45]. 5HT injections (500 pl, 10^{-5} M) reduced the spontaneous activity of many recorded neurons. In addition, the direction specificity of 11 out of 12 cells was reduced or totally blocked[45]. The effects of OA were variable as one would expect from the behavioural experiments shown in figure 2.

Behavioural modulation in the antennal pathway

Antennal afferents project from the antenna to the antennal lobe and to the antennal motorneuropil in the dorsal lobe. While olfactory signals are processed in the antennal lobe, the dorsal lobe receives mechanosensory information from the antenna[51,53,70,97]. Interneurons from the antennal lobe project to the mushroom bodies[55,63] which play an important role in olfactory memory formation[5,24,54–58]. A large number of pharmacological experiments investigating the effects of 5HT and octopamine on learning exist for the mushroom bodies. In most of these studies one-trial odour conditioning of the proboscis reflex was used as a behavioural assay[5,55,57,60,62].
With this learning paradigm the effects of putative neuromodulators on storage and retrieval of learned information can be tested separately. The application of a neuroactive compound **before** one-trial conditioning will mainly affect the storage of information (storage test), while the application **after** conditioning, shortly before testing the animal, will interfere with the retrieval of information (retrieval test)[5,55,57,60,62]. In both types of tests OA and 5HT had opposing effects, similar to the experiments in the visual system. Local injection of OA into the calyx of the mushroom bodies enhances the storage and retrieval processes, while local injections of 5HT into the mushroom bodies reduce the storage and retrieval of information (see table 2). The effects of OA

during conditioning appear to depend on the state of the animals. Olfactory conditioning is not changed in motivated animals, but enhanced or restored in satiated animals. Recently it was shown that OA injections into the calyces of the mushroom bodies in the bee can substitute for the unconditioned stimulus during olfactory conditioning[37].

OA and OA-agonists synephrine and chlordimeforme also enhance the sensory responses of the proboscis reflex to water or water vapour when applied into the haemolymph or injected into the protocerebrum. A similar effect was found after local injection of OA into the dorsal lobe[57,58]. The motor component of the proboscis reflex, as measured by the amount of water and sugar water intake, is also enhanced by OA application into the hemolymph and into protocerebrum. Local injections of OA into the antennal lobe, dorsal lobe and alpha-lobe have similar effects on the motor component of the reflex[57].

Although a complete survey of different injection sites in the antennal pathway and systematic tests of different concentrations of OA and 5HT have not been done so far, it is clear that the two amines here also have functionally antagonistic effects (see table 2).

Table 2. Summary of 5HT and OA effects in the honeybee

Tested response	Drug application	5HT	OA	References
Proboscis extension to water vapour and water	Topical application and local injection into brain and mushroom bodies	↓	↑	5, 58, 60, 78
Odour conditioned proboscis reflex	Injection into mushroom bodies			5, 58
– storage test	– pedunculus	↓	↑	
	– α-lobe	↓	↑	
– retrieval test	– pedunculus	↓	↑	
	– α-lobe	↓	↔	
Visual antennal reflex				23
– direction specificity	Topical application on brain surface	↓	↑	
	local injection into			
	– lobula	↓	↑	
	– medulla	↓	↔	
	– lamina	↓	↔	
Electroretinogram (sustained component)	Topical application on brain			45
– amplitude		↑	↑	
– sensitivity		↔	↑	
Field potentials measured in lobula, evoked by moving stripe patterns	Topical application or local injection into lobula	↓	↑	45
Field potentials measured in α-lobe				59
– evoked by light flashes to compound eyes	Iontophoretic application in lobula	Not tested	↑	
– evoked by antennal stimulation	Iontophoretic application in mushroom bodies	Not tested	↓	
Direction specificity of single motion-sensitive cells at the output of the lobula and in the protocerebrum	Local injection into lobula	↓	↑↓	45
Frequency of antennal motoneuron action-potentials	Local injection into dorsal lobe	↓	↑	Pers. comm. Pribbenow
Contraction amplitude, contraction rate and relaxation rate of antennal scapus muscles	Superfusion	Not tested	↑	20

A summary of behavioural and physiological experiments in which the modulatory effects of 5HT and OA were tested in the bee. The tested responses ranged from behaviour through electrophysiology to the contraction properties of muscles. The arrows in the columns 5HT and OA indicate the observed response changes after 5HT or OA application: ↑ increase of the response; ↓ reduction of the response; ↑↓ (column OA, direction specificity of motion-sensitive cells) ambiguous responses; ↔ no change observed. The references refer to the text.

Conclusions

The biogenic amines OA and 5HT can modulate neuronal activity at the level of photoreceptors, interneurons and motoneurons in the honeybee. Behavioural responses are modulated as a consequence of these neuronal changes. A modulatory action of OA is also obvious in antennal muscles. A summary of experiments on OA and 5HT effects in the bee is shown in table 2. The common effect of 5HT on behaviour, inter- and motoneurons is a **reduction** of responsiveness and sensitivity. The most frequent effect of OA at all levels of the nervous system is an **increase** of activity, responsiveness or sensitivity. The effects of 5HT in behavioural and electrophysiological experiments are very constant and reproducible. This distinguishes 5HT effects from those of OA.

The modulatory action of OA apparently depends on the state of the tested animals. In animals which are in an aroused state OA has no or only minor effects. A state of arousal and increased responsiveness in bees can also be induced by stimulation with sugar water. On the other hand, some of the stimulatory effects of sugar water, which can be mimicked by OA application, can be blocked by phentolamine, an OA antagonist. The sensitizing effect of OA and sugar water was found with olfactory and visual responses, In addition, OA can facilitate retrieval and storage during olfactory conditioning. Application of OA in the calyces of the mushroom bodies can even substitute for the unconditioned stimulus in olfactory conditioning[37]. These experimental findings demonstrate that both amines have widespread, functionally antagonistic effects in the central nervous system of the honey bee. The working hypothesis that OA is released during sugar water stimulation is supported by many experiments[37, 55, 58]. The functional role of 5HT and the behavioural contexts which lead to 5HT release, on the other hand, remain unclear. The common 5HT effect, a reduction of responsiveness and sensitivity, could play a role during circadian activity. It is necessary to test this hypothesis in controlled experiments.

The mismatch between neuropil areas with appropriate binding sites and 5HT-like or OA-like immunoreactivities shows that both amines can bind and possibly act in brain neuropils which are free of immunoreactivity. This is especially obvious in the mushroom bodies where we find very high densities of binding sites but no corresponding immunoreactivity in some neuropils. This indicates a possible neuromodulatory and neurohormonal role of both amines. The pharmacological profiles of OA and 5HT receptors in the bee brain are not clear at the moment. Comparative studies on OA receptors in locusts demonstrate that peripheral OA receptors differ from those in the brain[29, 30, 79, 81]. Recent findings in locusts suggest that OA receptors in the brain represent a specific receptor class[81]. Whether the OA receptors in the bee brain are similar to those in locusts has to be tested. Pharmacological experiments indicate that bee and locust 5HT receptors differ from those found in vertebrates[9, 100]. The differences between vertebrate and insect 5HT receptors should be analysed in future experiments. These questions can be addressed by using single electrode voltage clamp, patch clamp and tissue culture techniques[4, 55, 85]. The honeybee is a good subject for such investigations because the large variety of observable behaviours and the possibility of measuring neuronal responses which correlate directly with behaviour are essential prerequisites for such studies.

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