Aminergic Control and Modulation of Honeybee Behaviour

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Abstract: Biogenic amines are important messenger substances in the central nervous system and in peripheral organs of vertebrates and of invertebrates. The honeybee, *Apis mellifera*, is excellently suited to uncover the functions of biogenic amines in behaviour, because it has an extensive behavioural repertoire, with a number of biogenic amine receptors characterised in this insect.

In the honeybee, the biogenic amines dopamine, octopamine, serotonin and tyramine modulate neuronal functions in various ways. Dopamine and serotonin are present in high concentrations in the bee brain, whereas octopamine and tyramine are less abundant. Octopamine is a key molecule for the control of honeybee behaviour. It generally has an arousing effect and leads to higher sensitivity for sensory inputs, better learning performance and increased foraging behaviour. Tyramine has been suggested to act antagonistically to octopamine, but only few experimental data are available for this amine. Dopamine and serotonin often have antagonistic or inhibitory effects as compared to octopamine.

Biogenic amines bind to membrane receptors that primarily belong to the large gene-family of GTP-binding (G) protein coupled receptors. Receptor activation leads to transient changes in concentrations of intracellular second messengers such as cAMP, IP_3 and/or Ca^{2+}. Although several biogenic amine receptors from the honeybee have been cloned and characterised more recently, many genes still remain to be identified. The availability of the completely sequenced genome of *Apis mellifera* will contribute substantially to closing this gap.

In this review, we will discuss the present knowledge on how biogenic amines and their receptor-mediated cellular responses modulate different behaviours of honeybees including learning processes and division of labour.

Key Words: Serotonin, dopamine, octopamine, tyramine, honeybee, behaviour, division of labour, amine receptors.

1. INTRODUCTION

Biogenic amines play a pivotal role in the control and modulation of complex behaviours both in vertebrates and invertebrates. The group of biogenic amines consists of seven main members. Whereas dopamine (DA), serotonin (5-hydroxytryptamine, 5-HT), and histamine (HA) are present both in the vertebrate and invertebrate nervous systems, two pairs of biogenic amines are rather specific to either vertebrates or invertebrates. The catecholamines norepinephrine and epinephrine are important regulators of vertebrate physiology. In invertebrates, however, these amines appear to be substituted by the phenolamines tyramine (TA) and octopamine (OA), which control and modulate similar physiological functions [10,11,40,44,45,106,140].

DA and 5-HT are present in high amounts in the vertebrate central nervous system (CNS). A failure of the dopaminergic system can lead to motor or movement disorders, addiction, paranoia, and schizophrenia [91,162] (for reviews see [166,169]). Serotonin modulates emotion, mood, sleep and appetite. In humans, 5-HT has been implicated in the aetiology of neurological diseases including depression, anxiety and schizophrenia. In addition, 5-HT has an impact on migraine, hypertension, pulmonary hypertension, eating disorders and vomiting (for reviews see [72,75]). HA is considered an important mediator of allergy and inflammation in vertebrates [76,160]. In the vertebrate CNS, HA is synthesised from a small population of neurons located in the posterior hypothalamus. These neurons project to most cerebral areas and have been implicated in cardiovascular control, hormonal secretion, thermoregulation and memory functions [160].

When it comes to studying the function of aminergic systems, invertebrates have a number of advantages over vertebrates. Obviously, invertebrate nervous systems are less complex than those of vertebrates. In addition, the biogenic amines seem to have similar functional properties in vertebrates and invertebrates. Among the invertebrate species, honeybees (*Apis mellifera*) in particular are ideal subjects to experimentally examine the functional role of biogenic amines. These insects possess a remarkably rich behavioural repertoire. Bees, for example, learn sensory cues very fast and reliably. In addition, they display a complex division of labour (for reviews see [57,62,99]).

In the honeybee and in other invertebrate species, biogenic amines act as neurotransmitters, neuromodulators or neurohormones (for reviews see [10,37,40,135,136]). In this review, we summarise the present knowledge on how DA, TA, OA and 5-HT contribute to neuronal functions and behaviour of bees. Insights from molecular cloning approaches of biogenic-amine receptors will be provided. The role of HA in honeybees will not be discussed in detail, because only limited information is available on this amine. Generally, HA has been established as the neurotransmitter that is

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released from insect photoreceptor cells in response to illumination (for reviews see: [118,119,172]). Once released, it activates chloride currents in postsynaptic monopolar cells [66,67,164]. These HA-gated chloride channels have been cloned from *Drosophila melanogaster* [55,188]. Apart from photoreceptors, HA modulates mechanosensory transduction in *Drosophila* [97]. Evidence for HA acting as an inhibitory transmitter in the honeybee antennal lobe was provided by a Ca^{2+} imaging study [142]. The spontaneous activity and the odour responses of antennal lobe projection neurons were strongly reduced during bath application of HA [142].

We will start this overview with a brief introduction to the bee-brain anatomy, before we discuss the amines, their receptors and their behavioural functions in the honeybee.

2. THE HONEYBEE BRAIN

The brain of the honeybee consists of three major regions: protocerebrum, deutocerebrum and tritocerebrum. The protocerebrum contains the optic lobes (lamina, medulla and lobula), a pair of mushroom bodies (MBs) and the central complex (Fig. (**1A**)). The optic lobes receive and process sensory information from the compound eyes. Each MB consists of a lateral and a median calyx, which are connected to the α- and β-lobes *via* the pedunculus (Fig. (**1B**)). The MBs comprise a large number of densely packed intrinsic neurons, called Kenyon cells (after Kenyon, [80]), whose dendritic extensions form the cup-shaped calyces of the MBs. Three subpopulations of Kenyon cells have been distinguished based on their highly distinctive morphological profiles: outer compact cells, which are later in development pushed outward by non-compact cells, and inner compact cells, which, in turn, push the non-compact cells outward and which reside at the centre of each calycal cup (Fig. (**1B**), [49]]). The calyces are the main input regions of the MBs for visual, olfactory and mechanosensory information, whereas the α- and β-lobes are the output regions [32,100,109,141, 151,157]. Insect MBs are generally believed to be higher-order sensory integration centres necessary for insect learning and memory [38,62,69,98,100,170]. The central complex comprises a group of neuropils in the centre of the insect brain. It consists of the protocerebral bridge and the central body. The latter is subdivided into an upper division, a lower division and a pair of noduli. The central complex connects both brain hemispheres, coordinates information from both brain sides, and is presumably involved in motor control [70,171].

The deutocerebrum harbours the antennal lobes (ALs) and the dorsal lobes [165]. The ALs contain approximately 160 glomeruli and receive sensory inputs from olfactory receptor cells on the antennae, whereas antennal mechanosensory fibres mainly project to the dorsal lobes [174].

The tritocerebrum is composed of two relatively small bilateral lobes at the base of the brain. These are adjacent to the subesophageal ganglion (SOG). The compartments of the tritocerebrum are known to have sensory and motor connections to the mouth and the digestive tract. The SOG is a relay station between the brain and the ventral nerve cord. It processes sensory inputs from the proboscis and from the brain and projects to the motor neurons of the proboscis and mandibles [110]. Important modulatory neurons, such as ventral unpaired median neurons (VUM neurons), have their somata in the SOG [86].

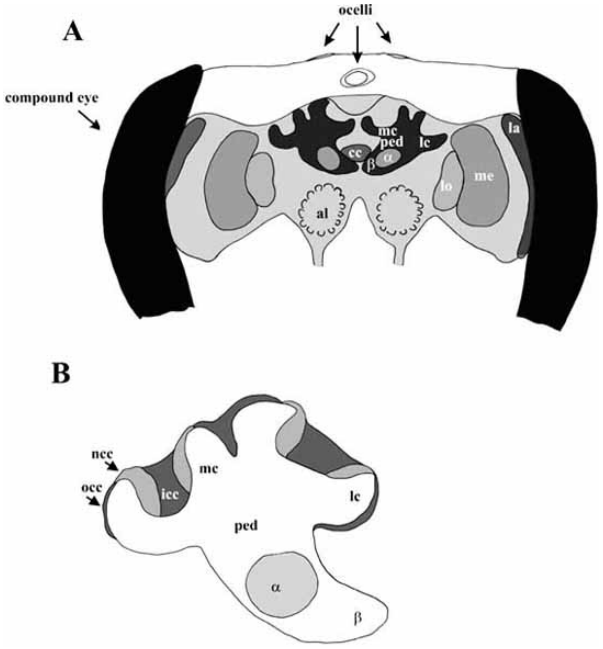

Fig. (1). A. Schematic drawing of major compartments of the honeybee brain. The protocerebrum contains the optic lobes, the lamina (la), medulla (me) and lobula (lo), a pair of mushroom bodies (mb) and the central complex (cc). Each mushroom body consists of a lateral calyx (lc) and a median calyx (mc), which are connected *via* the pedunculus (ped) to the α-lobe (α) and the β-lobe (β). The antennal lobe (al) is part of the deutocerebrum, which also contains the dorsal lobe (not shown). The tritocerebrum (not shown) is composed of two small bilateral lobes at the base of the brain.

B. Schematic drawing of a mushroom body with the three subpopulations of Kenyon cells. The outer compact cells (occ) are born first and later pushed outward by the non-compact cells (ncc). These cells are later pushed outward by the inner compact cells (icc), which reside at the centre of each calycal cup (after [47]).

3. DISTRIBUTION OF BIOGENIC AMINES IN THE BRAIN OF ADULT HONEYBEES

Immunocytochemical studies have shown that biogenic amines are only synthesised by a relatively small number of interneurons in the insect brain [70]. Antisera raised against DA, OA and 5-HT specifically stain these interneurons, which often possess widespread projections. When biogenic amines are released from these neurons, they modulate the activity of target neurons in various parts of the brain [8,60,70]. Biochemical and pharmacological studies [53,68, 106,143,152,75,181] have shown that DA and 5-HT are present in high concentrations in the bee brain (DA: 12-40 pmol/brain; 5-HT: 6-21 pmol/brain), whereas the amount of OA is lower (2-10 pmol/brain). The amount of TA was recently determined in bees [144]. It varied between 0.3 and 2 pmol/brain and is considerably lower than that of OA.

Only trace amounts of norepinephrine have been detected in the bee brain [53,106].

3.1. Dopamine

Approximately 330 DA-immunoreactive somata have been identified in each brain hemisphere and the SOG [147,159]. Most of these somata are clustered. One cluster is located below the lateral calyx of the MB. Two clusters are located in the anterior-ventral protocerebrum. Additional DA-immunoreactive somata are organised in small groups around the protocerebral bridge, below the optic tubercles, proximal to the ventral margin of the lobula, and in the lateral and ventral somatal rind of the SOG.

The MBs and the central body are surrounded by a dense network of DA-immunoreactive fibres. The processes project into the MB neuropil and into the somatal rind, where they synapse onto Kenyon cell bodies [17]. In the deutocerebrum, the ALs are innervated by fine projections of DA-immunoreactive interneurons [147,159]. With this innervation pattern and because single DA-immunoreactive neurons can have wide projection fields, it is assumed that DA participates in modulatory processes rather than in local neuronal interactions [104]].

3.2. Octopamine and Tyramine

The distribution of OA-immunoreactivity has been investigated in great detail [7,86]. Five cell clusters containing more than 100 OA-immunoreactive somata have been identified in the honeybee brain. These comprise a number of neurosecretory cells, a cell cluster located mediodorsal to the AL, a group of cells distributed on both sides of the protocerebral midline, another group between the lateral protocerebral lobes and the DLs, and single somata on either side of the central body [7,86]. The distribution pattern of OA in the bee brain is particularly interesting, because an identified octopaminergic neuron (VUM_{mx1}, [60]) is important for olfactory conditioning (see below). The soma of this neuron is located in the SOG. VUM_{mx1} has an amazingly extensive axonal projection with ramifications in the ALs, the calycal region of the MBs, the lateral protocerebrum and the SOG.

Similar to dopaminergic fibres, OA-immunoreactive fibres innervate most neuropils of the brain [86,163]. The strongest labelling was observed in the central complex [36]. In the MBs, a striking compartmentalisation was described: the calyces are innervated by extrinsic varicose OA-immunoreactive fibres, whereas large parts of the α- and β-lobes do not show OA-immunoreactivity at all [7,86].

In contrast to OA, the distribution of TA in the honeybee brain has not been studied at the cellular level. However, because TA is the precursor of OA during biosynthesis (see below), TA is expected to be present at least in all OA-containing cells. In addition, TA-containing neurons were identified in the CNS of *Drosophila* larvae, using specific antibodies raised against *p*-TA [117]. Interestingly, some of these neurons were distinct from OA-containing neurons [117]. Further evidence for the existence of cells that specifically contain TA but not OA comes from the analysis of the enzymes which are required to synthesise TA or OA. In the first step, the amino-acid tyrosine is decarboxylated to TA by tyrosine decarboxylase (TDC; [92]). TA can be hydroxylated on the β-carbon of the side chain by tyramine β-hydroxylase (TβH), thus generating OA [112]. Recently, two TDC genes were cloned from *Drosophila melanogaster*, *dTdc1* and *dTdc2* [29]. The gene *dTdc1* is expressed non-neuronally, whereas *dTdc2* is expressed in neurons. Interestingly, several clusters of cells in the central brain hitherto not known to contain TβH were detected in a genetic screen with *dTdc2-GAL4* lines. Recently, TA was shown to modulate transepithelial Cl^- conductance in *Drosophila* Malpighian (renal) tubules [19]. In this non-neuronal tissue, *dTdc1*expression and TA, but not OA, was detected [19,29]. Since the anti–TA antibody is commercially available [19,117], one may expect that the distribution of tyraminergic cells will soon be described in the honeybee as well.

3.3. Serotonin

The honeybee brain contains approximately 75 serotonin (5-HT)-immunoreactive somata [158] (for a review see [7]). They reside in the optic lobes, in the median and in the dorsal protocerebrum [158]. Immunoreactive fibres are present in all brain regions and in the SOG. A stratified staining was observed in the optic lobes, with the highest intensity in the lobula [158]. A net of 5-HT-immunoreactive fibres innervates the MBs, the central body and the ALs. Only the MB calyces, the protocerebral bridge and a small region of the central body are devoid of 5-HT [158].

3.4. Histamine

The brain of the honeybee contains about 150 histaminergic neurons [21]. The axons of these neurons innervate most parts of the protocerebrum except the mushroom bodies [21]. Photoreceptor fibers terminating either in the lamina or in the medulla as well as axons emanating from ocellar photoreceptors also contain HA [21].

4. GENERAL STRUCTURAL AND FUNCTIONAL PROPERTIES OF AMINE RECEPTORS

Apart from HA-gated ion channels [54-56,188] which will not be covered in this review, all biogenic amine receptors characterised in invertebrates so far belong to the superfamily of G-protein coupled receptors (GPCRs, for reviews see [10,11,126,180]). GPCRs possess a conserved topology. Each polypeptide has an extracellular N-terminus followed by seven transmembrane (TM) segments and an intracellular C-terminus. The N-terminus often contains consensus motives for N-linked glycosylation [128,168]. Cysteine residues in the cytoplasmic tail of the proteins may become post-translationally palmitoylated. The X-ray structure of bovine rhodopsin was recently solved, which strongly supports the general *bauplan* of GPCRs [124] (for a review see [52]). GPCR binding to biogenic amines occurs in a binding pocket formed by the TM bundle [74]. Specific residues in different TM segments interact with functional groups of the biogenic amines. In particular, an aspartic-acid residue (D) in TM3, serine residues (S) in TM5 and a phenylalanine residue (F) in TM6 contribute to ligand binding [168,178]. Ligand binding induces changes in the core structure of the receptor protein. Particularly TM3 is thought to participate in receptor activation by changing the relative orientation to TM6 [20]. In

addition, a sequence motif at the cytoplasmic interface of TM3 ([D,E]R[Y,W]) is involved in receptor activation. Receptor desensitisation occurs when serine or threonine residues of the protein are phosphorylated and β-arrestins bind to the phosphorylated receptor [28,89,96,123].

Once activated, the receptors interact with heterotrimeric G-proteins [22,113]. The high specificity of the receptors for particular subtypes of G-proteins is decisive for controlling downstream effectors. It has been attempted to identify characteristic sequence motives in GPCRs to predict which G-proteins may couple to a particular receptor [11,22,71,111, 182]. This, however, is a complex task, because a single GPCR may couple to different G-proteins and may show agonist-dependent changes in coupling to G-proteins [129,130].

As a result of GPCR activation, the concentrations of intracellular second messengers, especially $[cAMP]_i$ or $[Ca^{2+}]_i$, are altered. When the activated GPCR binds to a G_s-type protein, the $G_{\alpha s}$ subunit stimulates adenylyl cyclase activity (Fig. (**2A**)). This leads to the production of cAMP from ATP. The rise in $[cAMP]_i$ activates cAMP-dependent protein kinase (protein kinase A, PKA). PKA phosphorylates serine and threonine residues of target proteins and thereby modifies the properties of various cytosolic or membrane-bound proteins. In contrast to adenylyl cyclase stimulation, several neuroactive substances are known to inhibit the activity of the enzyme. This effect is mediated by interaction of GPCRs with inhibitory G-proteins (G_i).

Another pathway activated by GPCRs leads to an increase in $[Ca^{2+}]_i$. Such receptors stimulate $G_{q/o}$ proteins. Subsequently, phospholipase C (PLC) is activated (Fig. (**2B**)). PLC hydrolyses membrane-bound phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). The IP_3 freely diffuses and binds to IP_3 receptors on the membrane of intracellular Ca^{2+} stores. These receptors are ligand-gated Ca^{2+} channels, which release Ca^{2+} after binding of IP_3. In contrast to IP_3, DAG remains associated with the membrane, where it activates protein kinase C (PKC). Full enzymatic activity of PKC, however, requires the presence of DAG and Ca^{2+}. Similar to PKA, PKC phosphorylates a variety of proteins on serine and threonine residues, which alters the functional properties of these proteins.

In summary, GPCR activation by biogenic amines generates graded cellular responses depending on the second messenger pathways involved. The different second messenger pathways may also be activated in parallel within the same cell when the respective receptors and coupling partners are present. Such co-activation potentially leads to either amplification or diminishment of cellular responses to a given stimulus and provide a molecular basis for "coincidence detection" [90,187].

4.1. Dopamine Receptors in the Honeybee

In vertebrates, two classes of DA receptors have been defined based on sequence similarity, functional characteristics and pharmacological profiles: D1- and D2-(like) receptors (for reviews see [26,78,105,120]. D_1 and D_5 receptors constitute the D1 subfamily and activate adenylyl cyclase. Members of the D2 subfamily (i.e., D_2, D_3, and D_4 receptors) either inhibit adenylyl cyclase and/or modulate Ca^{2+} and K^+ channel activity.

In the honeybee, three DA receptors have been characterised so far: AmDOP1 (***Apis mellifera* dop**amine receptor **1**, [14,115]), AmDOP2 [73,114] and AmDOP3 [4]. Sequence comparison showed that AmDOP1 is closely related to vertebrate D1 receptors (Fig. (**3**), [14,115]). Activation of AmDOP1 expressed in HEK 293 or insect *Sf*21 cells by DA or 6,7-ADTN leads to the production of cAMP. The benzazepines SCH 23390 and SKF 38393, which bind to mammalian D1 receptors with nanomolar affinity, are much less potent at the AmDOP1 receptor (Table (**1**), [14]). The AmDOP2 receptor and its *Drosophila* orthologue, DAMB [50,65], are more similar to mammalian α_1-adrenergic- and insect OA-receptors than to DA receptors (Fig. (**3**), Table (**2**), [73,114, 115]). They thus form a distinct group of "invertebrate type" DA receptors (INDR) [73,115]. Functionally, however, AmDOP2 and DAMB resemble D1-type receptors, because they up regulate $[cAMP]_I$ [73,114].

The expression patterns of Am*dop*1 and Am*dop*2 receptor genes were analysed by *in situ* hybridisation. Am*dop*1 mRNA was detected in many neurons of the adult worker honeybee brain, including mushroom body intrinsic neurons, neurons of the central complex, the ALs and dorsal lobes, and neurons of the optic lobes [14,88]. During pupal development, Am*dop*1 is highly expressed in newly born Kenyon cells of the MBs [88]. In workers and drones, Am*dop*2 mRNA is expressed in the inner and outer compact cells of the MBs (Fig. (**1B**)), whereas expression levels in non-compact cells were variable and increased with age [73]. During pupal development, Am*dop*2 is expressed in the compact cells [88]. Interestingly, the differential expression pattern of Am*dop*2 correlates with age-dependent changes in worker-bee behaviour [73].

Recently, a cDNA encoding a D2-like receptor (AmDOP3) was cloned (Fig. (**3**), [4,84,115]). Activation of AmDOP3 receptors results in the downregulation of $[cAMP]_i$, a property characteristic of D2-like receptors [4]. *In situ* hybridisation revealed that Am*dop*3 mRNA is widely expressed in the brain [4]. The cellular expression patterns of the three honeybee DA receptors are overlapping but not completely identical. This suggests that D1 : D2 receptor interactions are possible in some neurons, including subpopulations of MB neurons [4].

Consistent with the widespread projections of dopaminergic neurons, *in situ* hybridisation experiments and radioligand-binding studies confirmed that DA receptors are widely distributed in the honeybee brain and that receptor densities vary with age [4,14,15,73,82-85,88]. More specifically, the density of tritiated benzazepine (e.g. [^{3}H]SCH 23390) binding sites increases dramatically during the first two days of adult life [83]. This is the time when multiple changes occur both in the brain and in the behaviour of honeybees [1,95,131,185]. When interpreting these data, however, one should keep in mind that the benzazepine R(+)-SCH 23390, although being a specific vertebrate D1-receptor antagonist, does not bind with high affinity to AmDOP1 (K_i = 250 nM, [12]). Furthermore, SCH 23390 is not particularly potent in inhibiting DA-induced cAMP elevation *via*

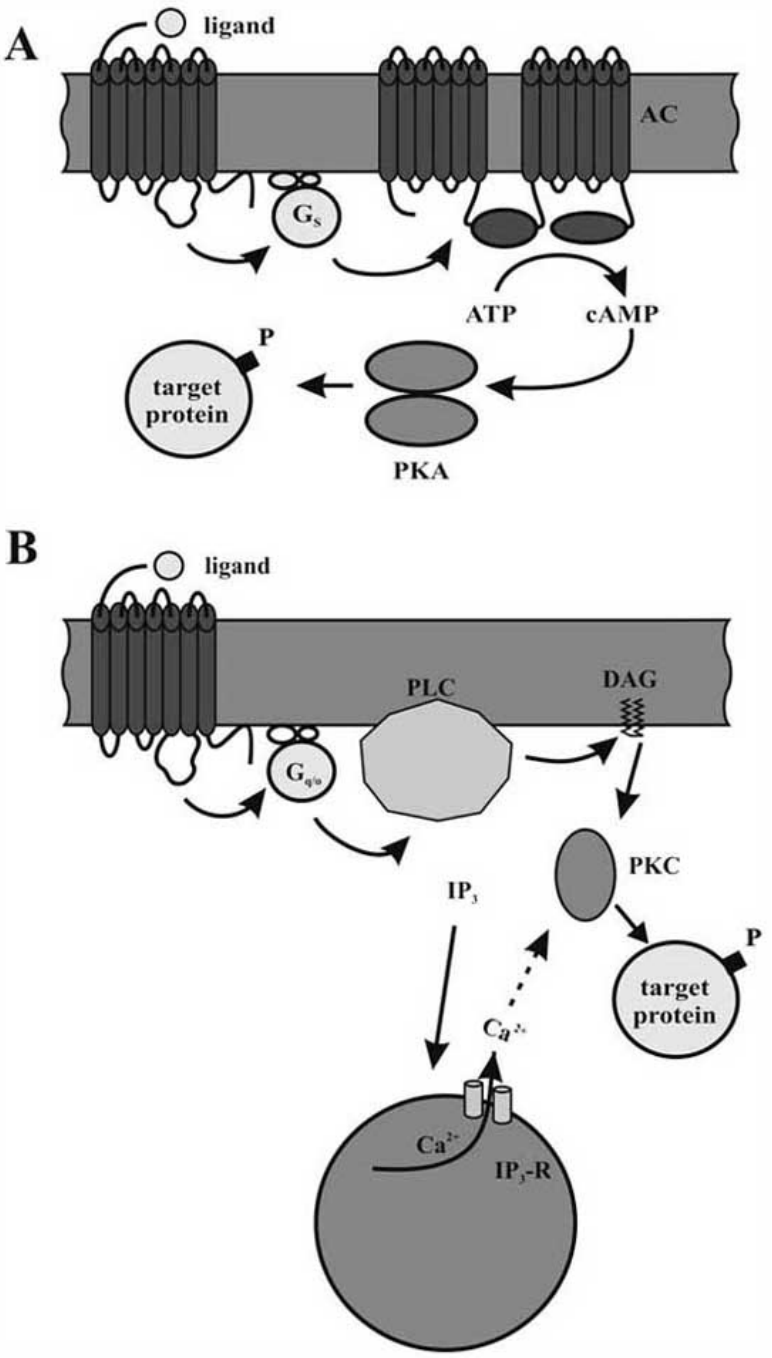

Fig. (2). Signalling cascades following activation of a G-protein coupled receptor (GPCR). Biogenic amine receptors are activated after binding of the respective ligand. The activated GPCR in turn can activate a stimulatory G-protein (G_s) and thus initiate a cAMP signalling pathway (**A)** or it can activate a G-protein of the $G_{q/o}$ family ($G_{q/o}$), which couples the amine receptor to IP_3/DAG signalling pathways (**B)**.

A. The activated G-protein stimulates an adenylyl cyclase (AC), which catalyses the production of cAMP from ATP. The increasing intracellular concentration of cAMP activates cAMP-dependent protein kinase (PKA). This kinase can phosphorylate a number of target proteins on serine and threonine residues.

B. Members of the $G_{q/o}$-protein-family activate phospholipase C (PLC). This enzyme hydrolyses phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to specific receptors (IP_3-R), which form ligand-gated ion channels in the membrane of the endoplasmatic reticulum. Opening of IP_3-Rs causes an efflux of Ca^{2+} from the endoplasmic reticulum into the cytoplasm. DAG and the increased intracellular Ca^{2+} levels lead to activation of protein kinase C (PKC) which, like PKA, can phosphorylate different target proteins.

Table 1A. Pharmacology of Dopamine Receptors in *Apis mellifera*: Binding to Brain Membrane Homogenate

	Specificity in vertebrates	Brain membrane homogenate: DA-sensitive [^{3}H]LSD binding site (K_i [nM])	Brain membrane homogenate: [^{3}H]SCH 23390 binding site (K_i [nM])	Brain membrane homogenate: [^{3}H]spiperone binding site (K_i [nM])
Biogenic amines				
DA		22	30,800	300
Norepinephrine		+/-	n.d.	n.d.
5-HT		7,000	548,000	18,300
TA		+/-	≥1,000,000	45
OA		+/-	892,000	18
Dopamine receptor agonists				
6,7-ADTN	DA receptor agonist	78	n.d	n.d.
R(+)-SKF 38393	D_1 agonist	-	3,200	n.d.
Dopamine receptor antagonists				
R(+)-Lisuride	D_2 agonist, D_1 antagonist	4.7	n.d.	n.d.
Chlorpromazine	D_2 antagonist	48	208	n.d.
cis(Z)-Flupentixol	DA receptor antagonist	150	218	n.d.
Spiperone	selective D_2 antagonist	+/-	25,400	0.17
R(+)-SCH 23390	selective D_1 antagonist	+/-	9.5	456
S(+)-Butaclamol	DA receptor antagonist	89	13,800	55.5
Haloperidol	D_2, D_3, and D_4 antagonist	+/-	n.d.	n.d.

Values are from [15] for the dopamine-sensitive [^{3}H]LSD binding site, from [75] for the [^{3}H]SCH 23390 and [^{3}H]spiperone binding sites. Abbreviations: +/- = intermediate; - = little or no effect; n.d. = not determined.

Table 1B. Pharmacology of Dopamine Receptors in *Apis mellifera:* Binding in Cell Cultures

	Specificity in vertebrates	AmDOP1 in HEK 293: [^{3}H]LSD binding (K_i [nM])	AmDOP1 in *Sf*9: cAMP production (EC_{50} or IC_{50} [nM])	AmDOP2 in *Sf*9: □amp production (EC_{50} or IC_{50} [nM])
Biogenic amines				
DA		56	360	2,200
Norepinephrine		3,100	n.d.	58,000
5-HT		3,600	n.d.	-
TA		9,900	n.d.	-
OA		110,000	n.d.	n.d.
Dopamine receptor agonists				
6,7-ADTN	DA receptor agonist	93	650	5,100
R(+)-SKF 38393	D_1 agonist	4,200	n.d.	-

(Table 1B. Contd....)

	Specificity in vertebrates	**AmDOP1 in HEK 293: [^{3}H]LSD binding (K_i [nM])**	**AmDOP1 in *Sf*9: cAMP production (EC_{50} or IC_{50} [nM])**	**AmDOP2 in *Sf*9: □amp production (EC_{50} or IC_{50} [nM])**
Dopamine receptor antagonists				
R(+)-Lisuride	D_2 agonist, D_1 antagonist	4.3	+/-	+/-
Chlorpromazine	D_2 antagonist	15	n.d.	+
cis(Z)-Flupentixol	DA receptor antagonist	17	200	3.8
Spiperone	selective D_2 antagonist	64	2,200	8,500
R(+)-SCH 23390	selective D_1 antagonist	250	8,100	17,000
S(+)-Butaclamol	DA receptor antagonist	77	540	81
Haloperidol	D_2, D_3, and D_4 antagonist	390	n.d.	+

Values are from [14] for AmDOP1 in HEK 293 and from [104] for AmDOP1 in *Sf*9 and AmDOP2 in *Sf*9. Abbreviations: + = effective; +/- = intermediate; - = little or no effect; n.d. = not determined.

either AmDOP1 (IC_{50} = 8.1 μM, [114]) or AmDOP2 (IC_{50} = 17 μM, [114]). This makes it difficult to decide which type of receptor was actually labelled in the binding studies performed on bee-brain tissue.

Taken together, the expression of DA receptors in the MBs, important centres of sensory integration, suggests a major role of this transmitter in honeybee behaviour. The presence of DA receptors in the central complex, in the ALs and in the optic lobes implies that the dopaminergic system affects sensory perception and is involved in the control of motor patterns.

4.2. Tyramine Receptors in the Honeybee

The function of TA in the honeybee is still unclear, but since a honeybee TA receptor was cloned (AmTYR1, ***Apis mellifera* tyr**amine receptor **1**, Table (**2**), [13]), this question has received special attention. AmTYR1 shares high homology with TA receptors from *Drosophila* (DmTYR, ***Dro**sophila **m**elanogaster* **tyr**amine receptor [146]), *Locusta* (Loc-TYR, ***Loc**usta* **tyr**amine receptor, [179]), and *Bombyx mori* [121]. Activation of heterologously expressed AmTYR1 and of TA receptors in membrane homogenates of honeybee brains inhibits forskolin-induced cAMP synthesis [13,116].

Am*tyr*1 mRNA is expressed in the somata of most neuropils in the honeybee brain. Particularly the MB intrinsic neurons, the somata surrounding the ALs and the DLs, and the first and second optic chiasmata express Am*tyr*1 mRNA [13]. Because the Am*tyr*1 gene is expressed in the ALs and MBs, it can be assumed that TA is involved in sensory integration and possibly in learning and memory processes. This hypothesis is supported by recent behavioural and pharmacological experiments (see below).

Expression of Am*tyr*1 mRNA changes during bee development. The mRNA level increases in the late pupal stages (P6 to P8) and then remains stable until the bees emerge. During adult life, Am*tyr*1 expression increases in foragers compared to newly emerged bees [116].

4.3. Octopamine Receptors in the Honeybee

The first OA receptor from the honeybee was cloned a few years ago (AmOA1, ***Apis mellifera* octop**amine receptor **1**, [58] below). This receptor shares a high degree of amino-acid sequence-similarity to OA receptors cloned from other insects and molluscs [58]. Originally, insect OA receptors were classified on the basis of second messenger changes induced in a variety of intact tissue preparations. OCT-1 (**oct**opamine 1) receptors cause an increase in $[Ca^{2+}]_i$, whereas OCT-2A, OCT-2B and OCT-3 receptors activate adenylyl cyclase [41-44,47,134,136,138]. Such a classification system, however, is problematic when more than one receptor subtype is present in the same tissue preparation. Therefore, Evans and co-workers proposed a new classification system for insect OA receptors into "α-adrenergic-like OA receptors (OctαRs)" and "β-adrenergic-like OA receptors (OctβRs)" based on their similarities in primary structure and in signalling properties with vertebrate adrenergic receptors [46,94].

The functional properties of the AmOA1 receptor were investigated after its expression in HEK 293 cells. Low concentrations of OA (≥10 nM) induced oscillation of $[Ca^{2+}]_i$. At high ligand concentrations (≥1 μM), single, slowly declining Ca^{2+} responses were observed [58]. In addition to Ca^{2+} signalling, high concentrations of OA caused a rather moderate production of cAMP in AmOA1-expressing cells. Thus, based on its amino acid sequence as well as on its cellular signalling capabilities, AmOA1 is a member of the OctαR (or former OCT-1) receptor subfamily [11,46] (Table **2**). It is, however, unlikely that AmOA1 is the only neuronal octopamine receptor expressed in the bee [137], since orthologues of the *Drosophila* OctβRs can be identified in the completely sequenced honeybee genome.

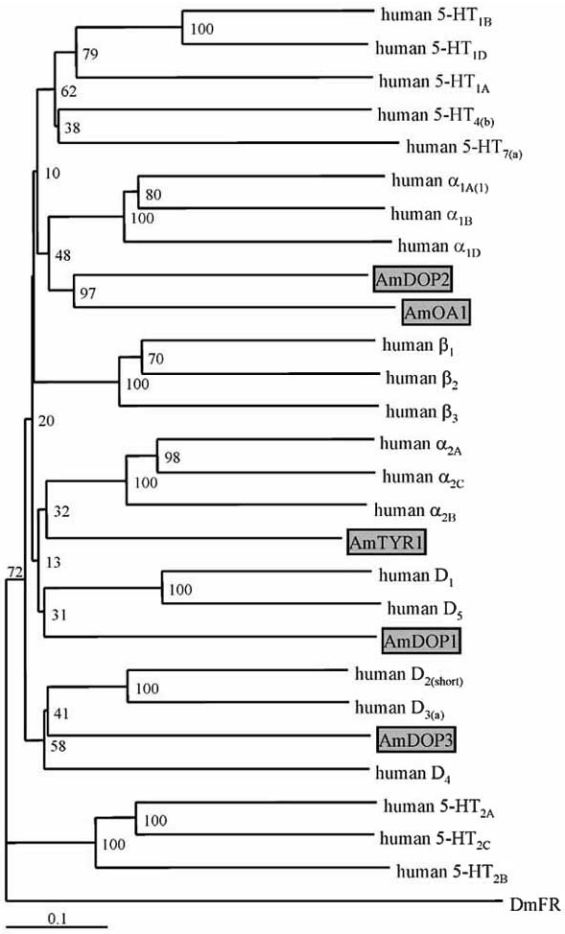

Fig. (3). Phylogenetic relationship between biogenic amine receptors of the honeybee and human aminergic receptors. Alignments were performed with the complete amino-acid sequence of each receptor. The receptor sequences, followed by their GenBank accession numbers (#), are listed below in the order illustrated: human 5-HT_{1B} (#NP_000854), human 5-HT_{1D} (#NP_000855), human 5-HT_{1A} (#NP_000515), human 5-HT_4 isoform b (#NP_000861), human 5-HT_7 isoform a (#NP_000863), human α_{1A}-adrenergic isoform 1 (#NP_000671), human α_{1B}-adrenergic (#NP_000670), human α_{1D}-adrenergic (#NP_000669), *Apis mellifera* dopamine 2 (AmDOP2; #NP_001011567), *A. mellifera* octopamine 1 (AmOA1; #NP_001011565), human β_1-adrenergic (#NP_000675), human β_2-adrenergic (#NP_000015), human β_3-adrenergic (#NP_000016), human α_{2A}-adrenergic (#NP_000672), human α_{2C}-adrenergic (#NP_000674), human α_{2B}-adrenergic (#NP_000673), *A. mellifera* tyramine 1 (AmTYR1; #NP_001011594), human D_1 (#NP_000785), human D_5 (#NP_000789), *A. mellifera* dopamine 1 (AmDOP1; #NP_001011595), human D_2 isoform short (#NP_057658), human D_3 isoform a (#NP_000787), *A. mellifera* D2-like dopamine (AmDOP3; #NP_001014983), human D_4 (#NP_000788), human 5-HT_{2A} (#NP_000612), human 5-HT_{2C} (#NP_000859), human 5-HT_{2B} (#NP_000858), and *Drosophila melanogaster* FMRFamide (DmFR; CG2114-PA; #AAF47700). The genetic distance between sequences was calculated with ClustalX (version 1.81). A neighbor-joining tree was constructed with ClustalX by using 1000-fold bootstrap re-sampling. The resulting tree was displayed graphically by TreeView using the divergent *D. melanogaster* FMRFamide receptor as an outgroup. The numbers at the nodes of the branches represent the percentage bootstrap support for each branch. The scale bar allows conversion of branch lengths in the dendrogram to genetic distance between clades (0.1 = 10% genetic distance).

In situ hybridisation to sections of the honeybee brain showed that the Am*oa*1-gene is expressed in intrinsic MB neurons, in somata of the ALs and optic lobes, and in somata of the SOG [58]. The distribution of binding sites for [^{3}H] OA in tissue sections of the honeybee brain has been analysed with autoradiographic methods (Fig. (**4**), [37]). Specific and high labelling densities were observed in the MBs, especially in the pedunculus and in the α- and β-lobes. Interestingly, these brain regions are not innervated by octopaminergic neurons (see above) [7,36,37,86]. There are several possible explanations for this observation. (1) OA may act over long distances in a neuromodulatory way [37]; (2) the immunocytochemical staining did not reveal all of the finer processes of octopaminergic neurons; and (3) not all of the [^{3}H]OA binding sites are functionally relevant. The OA receptor antagonist phenolamine displaced ~93% of [^{3}H]OA binding in all brain areas except the MBs (~70% displacement). This result suggests that OA receptors in the mushroom bodies are pharmacologically different from those in the rest of the brain [37] and thus provides further evidence for the existence of more than one OA receptor in the bee brain. Radioligand binding studies to membrane preparations of different brain regions have also been performed [31]. A high-affinity OA receptor agonist, [^{3}H]-NC-5Z, binds to membrane fractions from MBs and optic lobes. In samples prepared from other parts of the brain and the SOG, less binding was observed [31].

Recently, Farooqui and co-workers applied the RNAi technique to examine the function of AmOA1 in the ALs [48]. Injection of AmOA1 dsRNA into the tissue led to approximately 80% inhibition of the olfactory acquisition responses and to approximately 50% reduction in memory recall [48].

In conclusion, phylogenetic analyses performed by different authors (Fig. (**3**), [46,73,137]) suggest that the insect OctαRs (including AmOA1) are closely related to vertebrate α_1-adrenergic receptors, whereas insect OctβRs (no honeybee orthologue characterised so far) are most closely related to vertebrate β-adrenergic receptors. In contrast, the AmTYR1 receptor is not related to the classical OA receptors mentioned above. Instead, AmTYR1 and other insect TA receptors seem to form a sub-group which is related to the vertebrate α_2-adrenergic receptors (Fig. (**3**), [73,137]).

4.4. Serotonin Receptors in the Honeybee

In mammals, the effects of 5-HT are mediated by 13 distinct GPCRs and a ligand-gated ion channel (for reviews see [72,87]). The receptors are divided into seven groups, based on their sequence homologies and signalling properties. The 5-HT_1 receptors couple preferentially to $G_{i/o}$ and inhibit cAMP synthesis. 5-HT_2 receptors couple preferentially to $G_{q/11}$ and activate PLC. The rise in $[IP_3]_i$ subsequently elevates $[Ca^{2+}]_i$. 5-HT_3 receptors are ligand-gated ion

Table 2. Comparison of honeybee receptors with vertebrate receptors and characteristics of these honeybee receptors.

Honeybee receptor (endogenous agonist)	Most closely related vertebrate receptor class	Effect of activation	Occurrence in the brain	References
AmDOP1 (DA)	DA D_1/D_5 receptors	cAMP↑	ubiquitously	[12, 88, 114]
AmDOP2 (DA)	$_1$-adrenergic receptors	cAMP↑	restricted to the mushroom bodies	[73, 88, 114]
AmDOP3 (DA)	DA D_2 receptors	cAMP↓	ubiquitously	[4]
AmTYR1 (TA)	α_2-adrenergic receptors / 5-HT_1 receptors	cAMP↓	ubiquitously	[13, 116]
AmOA1 (OA)	α_1-adrenergic receptors	Ca^{2+}↑	ubiquitously	[48, 58]

channels. 5-HT_4, 5-HT_6, and 5-HT_7 receptors couple to G_s and stimulate adenylyl cyclase activity.

In insects, the serotonergic system seems to be similarly complex (for reviews see[145,177]). Phylogenetic analysis of the receptor sequences suggests that most of 5-HT-receptor subtypes evolved before the divergence of invertebrate and vertebrate branches. Unfortunately, no honeybee 5-HT receptor has been described at the molecular level so far.

However, 5-HT-sensitive binding sites have been characterised in membrane preparations of honeybee brains [16]. The pharmacological properties of these potential 5-HT receptors differ considerably from those of mammalian receptors [16]. Radioligand binding studies revealed a relatively uniform distribution of [^{3}H]5-HT binding-sites in each of the three optic ganglia (Fig. (**5**), [36]). As has been described for OA, there is an interesting mismatch between the distribution of 5-HT-immunoreactivity and [^{3}H]5-HT binding sites. The

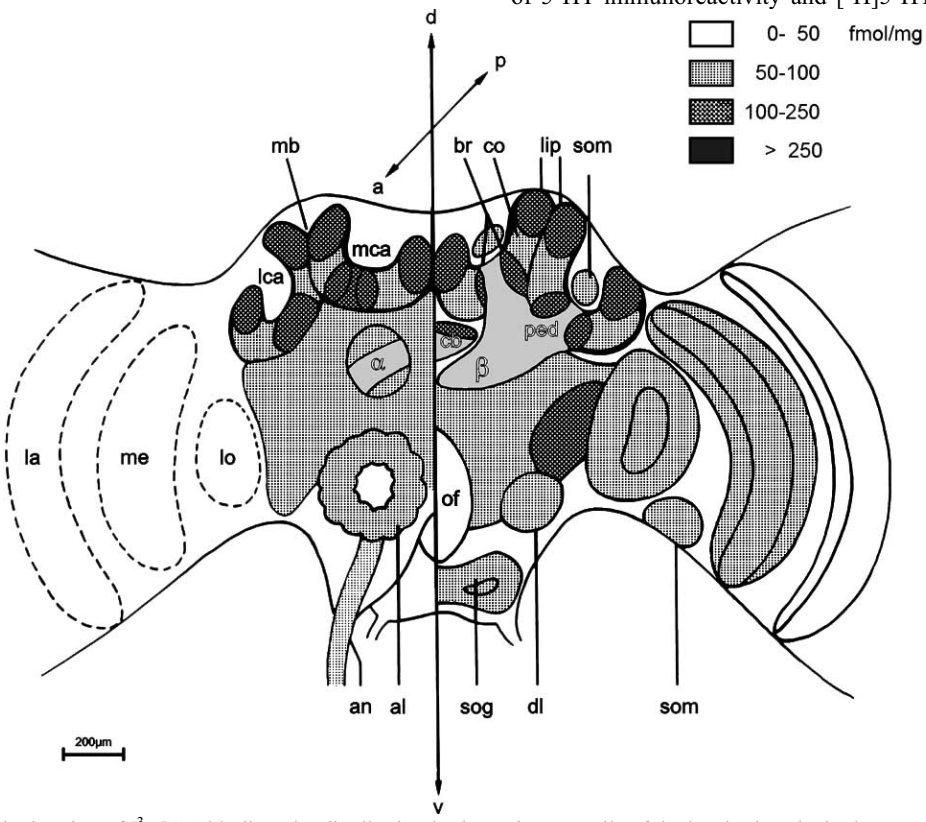

Fig. (4). Schematic drawing of [^{3}H]OA binding-site distribution in the main neuropils of the bee brain. The brains were incubated with 9 nM [^{3}H]OA and the densities of radioactive labelling are indicated by the degree of shading. The left part of the figure shows anterior parts of the brain whereas the right part shows the posterior parts of the bee brain. Abbreviations: α alpha lobe, β beta lobe of the mushroom bodies. *a* anterior axis, *al* antennal lobe, *an* antennal nerve, *br* basal ring of the calyx, *cb* central body, *co* collar of the calyx, *d* dorsal axis, *dl* dorsal lobe, *la* lamina, *lca* lateral calyx, *lip* lip of the calyx, *lo* lobula, *mb*, mushroom body, *mca* median calyx, *me* medulla, *of* oesophageal foramen, *p* posterior axis, *ped* pedunculus of the mushroom body, *sog* subesophageal ganglion, *som* layer of somata, *v* ventral axis. The figure was taken from [122] with friendly permission of the publisher, © Springer-Verlag 2002.

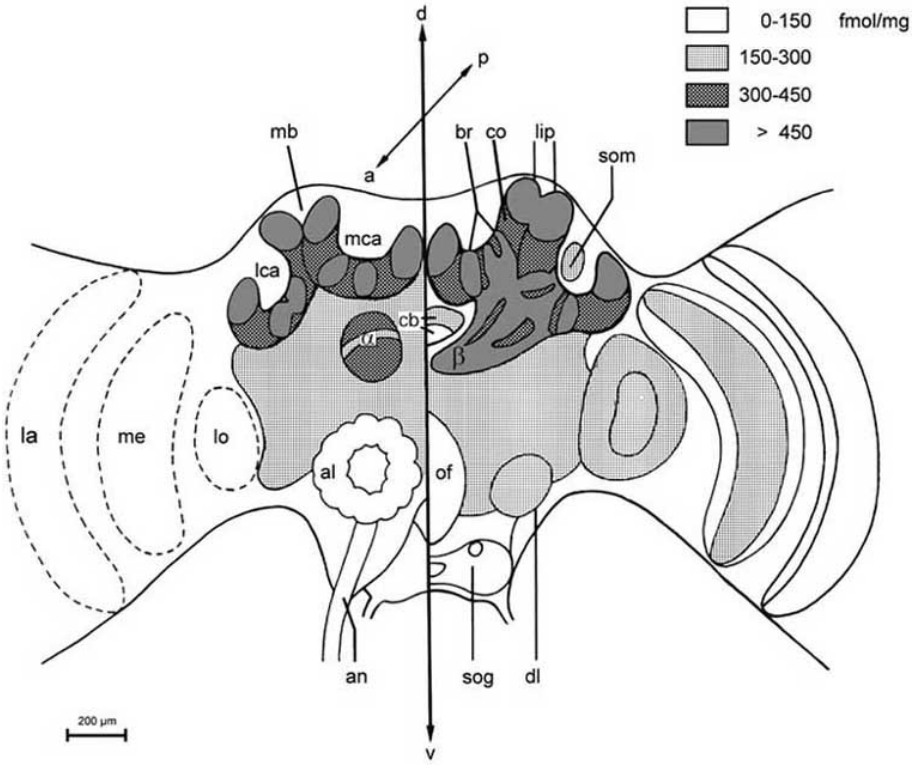

Fig. (5). Schematic drawing of [^{3}H]5-HT binding-site distribution in the bee brain. The brains were incubated with 10 nM [^{3}H]5-HT and the densities of radioactive labelling are indicated by the degree of shading. The left part of the figure shows anterior parts whereas the right part shows the posterior parts of the bee brain. Abbreviations as in Fig. (**4**). The figure was taken from [122] with friendly permission of the publisher, © Springer-Verlag 2002.

lips of the MB calyces are strongly labelled with [^{3}H]5-HT [36], but no 5-HT-immunoreactivity was observed (for a review see [7]). These findings suggest that 5-HT can bind to receptors expressed in brain regions which are remote from its release sites [36].

5. MODULATION OF HONEYBEE LEARNING AND MEMORY BY BIOGENIC AMINES

From the previous chapters, it is obvious that biogenic amines exert a multitude of effects in insects. They can act on different levels: at the sensory periphery, at the level of interneurons and brain compartments, and at the level of motor output and muscles. In the honeybee, biogenic amines are important modulators of learning and division of labour. Both forms of behaviour can be tested under laboratory conditions or in the field. The impact of biogenic amines has been studied in honeybees either by systemic application, i.e. feeding, injection into the haemolymph or onto the brain, and by local microinjections into defined brain compartments.

Each of these methods has advantages and disadvantages. The advantage of feeding experiments is that the animal does not have to be wounded for application of the substances. However, the fed compounds enter the digestive tract where they are eventually metabolically modified. In addition, it is not clear whether the substances reach the respective brain areas where they should induce modulatory effects. An advantage of microinjecting compounds is that the amines can be applied to a particular brain area where they are supposed to act.

Generally, some caution is appropriate when interpreting data obtained from injection or feeding experiments for the following reasons. 1) Most experiments have tested the effect of a drug which was injected in excess of intrinsic drug titres. Under such conditions, it is difficult to conclude that a particular substance serves to modulate or mediate a specific function, because an excessive amount of a substance may disturb a balanced system such that even opposing effects may be initiated [102]. 2) We often find complex dose-response relationships for biogenic amines, such as U-shaped dose-response functions. An excess of a substance may therefore antagonise its effect under physiological concentrations. 3) In most cases, the injected substances are not specific for a single receptor subtype. Therefore, it is difficult to assess which receptor subtypes are actually affected. Even a cross-talk between different aminergic systems (e.g., TA *vs*. OA) seems possible when the injected ligand concentrations are high enough. 4) Another problem is that there is little knowledge on the diffusion rates, half lives or the bioavailability of the injected substances in the bee. 5) Finally, the drug effects may depend on the condition of the bee and factors such as arousal, satiation level, age and gustatory responsiveness may influence the drug action.

Learning and memory are very general forms of plasticity in the nervous system. They have been conserved across

distant animal species. Thus, certain features of the cellular and molecular processes underlying learning and memory are similar in vertebrates and invertebrates. Because learning and memory are rather difficult to study in complex vertebrate brains, invertebrates, such as the honeybee, offer a promising alternative for studying brain functions at the cellular and molecular level.

Learning is an essential part of honeybee life. Foraging bees have to remember the location of their hive and different food sources. Once they arrive at a flower, they have to remember how they find their way to nectar or pollen. Honeybees learn conditioned stimuli of different modalities very fast and establish long-lasting memories under free-flying conditions and in the laboratory (for reviews see [9,57,99]). Different established paradigms for testing non-associative and associative learning in honeybees use the proboscis extension response (PER, for a review see [98]). When the antennae of a bee are stimulated with sucrose solutions of sufficient concentration, the animal reflexively extends its proboscis in expectation of food (Fig. (**6**)). While some bees are very sensitive to gustatory stimuli and even show the PER after stimulation with water or low sucrose concentrations, other bees are rather insensitive and only display the PER when their antennae are stimulated with 30% sucrose [149].

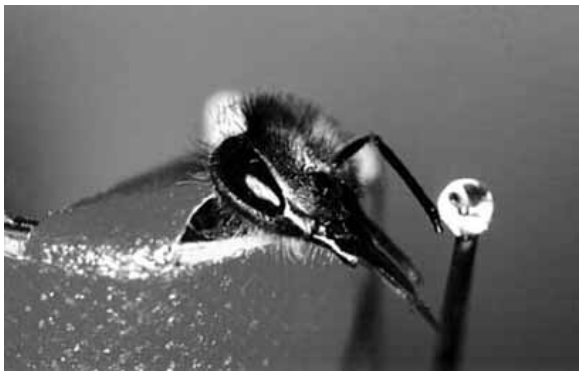

Fig. (6). Proboscis extension response (PER) in the honeybee. When the antennae of a fixed bee are stimulated with a droplet of sucrose solution above the individual response threshold, the bee reflexively extends its proboscis in expectation of food. This behaviour is employed for different non-associative and associative learning paradigms.

5.1. Non-Associative Learning

Repeated stimulation of the antennae with a low sucrose concentration leads to habituation of the PER [24,64,148]. Habituated bees can be dishabituated by stimulating the antennae with a high sucrose concentration, such as 30% sucrose. There is convincing evidence that some biogenic amines are involved in these processes. TA appears to increase the rate of habituation, when fed 12 h before testing the bees. Animals fed with TA needed fewer trials than controls to achieve complete habituation of the PER [24]. Interestingly, the OA receptor agonist chlordimeform had the same effect as TA [24], suggesting a similar role for both amines in habituation of the PER. This would contradict the general assumption that TA and OA are "antagonistic modulators of behaviour" [140]. Originally, it was assumed that the effects of TA were mediated by the activation of OA receptors either after biochemical conversion of TA to OA or by direct binding of TA to OA receptors [24]. Considering the existence of specific TA receptors, however, some experiments need to be repeated using ligands binding specifically to either TA or OA receptors.

Sensitisation can be tested as PER to antennal stimulation with odours [101,102]], water vapour [12] or gustatory stimuli [105]. Normally, bees do not show proboscis extension in response to antennal stimulation with an odour or water vapour. When bees are stimulated with a high sucrose concentration, such as 30% sucrose, shortly before the odour is presented to their antennae, they can be sensitised to the odour. Successful sensitisation leads to a PER after subsequent antennal stimulation with an odour [12,101]. Similarly, bees can be sensitised to antennal stimulation with water [105]. Application of OA, either into the haemolymph or into the dorsal lobe, increased responsiveness to water and thus mimicked the effect of sucrose stimulation [103]. In contrast, sensitisation to odours was neither affected by OA nor by DA in honeybees whose nervous system had been depleted of biogenic amines by reserpine [102]. In addition to their effects on non-associative learning, biogenic amines exert complex effects on associative learning, which has been studied more extensively than non-associative learning in the bee.

5.2. Associative Learning

To examine associative learning under laboratory conditions, olfactory and tactile conditioning of the PER have been employed (Fig. (**7**)). In both paradigms, the conditioned stimulus (CS, which could be an odour or a flat object that can be touched by the antennae) is paired with an unconditioned stimulus (US, which is sucrose in this case). First, spontaneous responsiveness to the CS is measured by applying the odour to the antennae of a bee or by moving the tactile stimulus into the scanning range of the bee antennae. The conditioning trial begins when the bee can smell the odour or when it scans the tactile object. While the bee experiences the CS, the PER is elicited by applying a small droplet of sucrose solution to its antennae (US). When the bee extends its proboscis, it is allowed to drink a small volume of sucrose solution as reward. Usually, a few pairings of CS and US suffice for the formation of a memory, which lasts for days (Fig. (**7**), [9,35], for a review see [99]).

Using such tests, it has been demonstrated that DA selectively inhibits the retrieval of learned information without affecting the storage process. Injection of DA into the brain or brain compartments *after* conditioning led to a reversible decrease in conditioned responses (CRs). In contrast, DA injections *prior* to conditioning had no effects on memory [8,93,100,102-105,107]. Serotonin can reduce both memory storage and retrieval when injected *prior* to conditioning [8,100,104,105]. When 5-HT is injected *after* conditioning, responses are only slightly reduced. The role of TA in associative learning has not been studied.

OA appears to play a decisive role in acquisition and memory formation in bees. Application of OA improves olfactory acquisition, memory formation and retrieval. Sati-

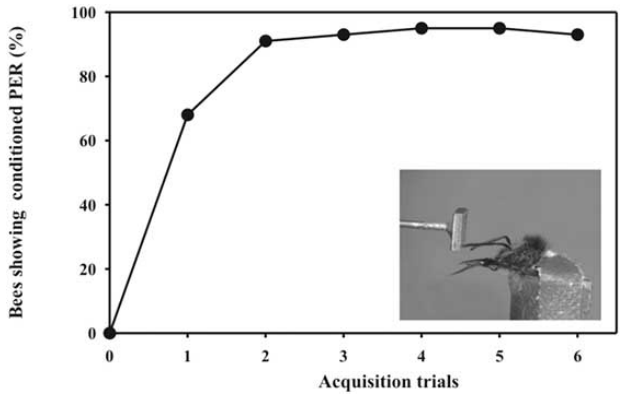

Fig. (7). Acquisition curve in tactile antennal learning. The x-axis shows the number of acquisition trials. The y-axis displays the percentage of bees showing the conditioned proboscis extension response (PER). After only two conditioning trials, the bees reach a stable plateau in their learning performance. The inlet displays a bee showing conditioned proboscis extension while it scans the conditioned stimulus, a small metal plate.

ated bees normally do not display any associative PER learning. OA injections into the haemolymph of satiated bees can restore the "motivation" to learn. In bees whose nervous system had been depleted of biogenic amines by reserpine, OA significantly improved the acquisition performance but not retention [102]. Local injections of OA into the calyx or the α-lobe of the MBs enhanced memory formation [104]. When the OA-receptor antagonist mianserin was injected into the AL, it strongly reduced acquisition and retrieval [48]. A similar but irreversible effect was observed when the expression of the AmOA1 receptor was down-regulated by injection of Am*oa*1 dsRNA [48].

Evidence for the role of OA in memory formation at the cellular level originates from the analysis of the VUM_{mx1} neuron (ventral unpaired median neuron 1 of the maxillary neuromere [60]). The VUM_{mx1} neuron depolarises in response to the presentation of sucrose rewards to antennae and proboscis. Current injection into the VUM_{mx1} neuron can substitute for the sucrose reward during olfactory conditioning [60,63]. VUM_{mx1} belongs to a group of OA-immunoreactive neurons, the cell bodies of which are located ventrally in the SOG [86,163]. It is assumed that VUM neurons release OA, which could then mediate the US in PER conditioning (for reviews see [25,61,63,140]). Whether or not TA is co-released with OA from activated VUM neurons can currently not be assessed.

Besides these striking effects on learning behaviour, the different biogenic amines also act on division of labour, which is a central feature of social insects.

6. BIOGENIC AMINES AND DIVISION OF LABOUR

Division of labour is a key feature of honeybee social life. Worker honeybees show a complex age-dependent division of labour, which is also referred to as "age polyethism" [161,184,186]. Young bees work in the centre of the nest and care for the brood and the queen. Bees in their second week of life engage in the processing of nectar and pollen and in comb building. In their third week of life, bees begin to forage after spending a couple of days as guarding bees at the hive entrance. A small number of bees are concerned with the removal of dead brood from the hive, before they start foraging; this is referred to as "undertaking behaviour". The performance of most of these tasks depends on the age of the worker bee [132,161,186]. Division of labour, however, also occurs among bees of similar age. Foragers are a good example of this phenomenon. While some bees collect pollen, other bees of the same age collect nectar and again other bees collect both pollen and nectar. A small number of bees collect water, which is necessary to cool the hive and to maintain the climatic conditions of the nest. A few bees collect propolis, a resinous substance which is used to repair the hive [161,186]. Thus, the division of labour in a honeybee colony is very plastic. Small changes in the amount of brood, in the foraging resources or in the weather conditions may greatly change the pattern of division of labour in a colony [186].

6.1. Age-Dependent Division of Labour

To gain insight into the contribution of biogenic amines in age-dependent division of labour, amine titres have been determined in the brains of bees performing different tasks inside and outside the hive and of bees which differ in age. DA, OA and 5-HT are present in the brains of larvae, pupae and adult honeybees [53,68,81,83,106,175]. During the transition from larval to pupal stage, the levels of all three biogenic amines generally increase [175]. In adults, biogenic-amine titres increase with age, with the highest concentrations being found in foragers [68,152,156,181]. Whether the differences in biogenic-amine titres between bees of different ages are related to age differences or whether they are related to the different tasks the bees perform is difficult to test. Single-cohort colonies, which only consist of same-aged bees, are very helpful for distinguishing between these alternatives. Schulz and Robinson [152] showed that differences in the titres of DA, OA and 5-HT in MBs of foragers and nurse bees were related to age, whereas in the AL, the differences were related to different tasks. Unfortunately, TA titres have not yet been measured in single-cohort colonies. Another way of studying the role of biogenic amines in division of

labour is to manipulate amine titres and to determine the behavioural effects. Thus it was shown that OA induced bees to forage precociously, whereas TA had the opposite effect [153].

6.2. Octopamine and Juvenile Hormone

Juvenile hormone (JH) and OA are decisive factors in regulating the onset of foraging behaviour [133]. Typically, JH titres increase with age in adult bees, so that foragers have higher levels of JH than younger bees. Treating younger bees with methoprene, a JH analogue, accelerates the onset of foraging [18]. Removal of the *corpora allata*, the organs synthesising JH, delays the initiation of foraging [173]. This effect can be reversed by methoprene [173]. These findings suggest that JH controls the pace at which bees become foragers.

It is generally assumed that OA and JH regulate each other and modulate the onset of foraging behaviour and the initiation of other tasks [77,154] (for a review see [155]). Foragers have high titres of both JH and OA, particularly in the ALs [152,155]. Treating 1-day-old bees with methoprene causes increased levels of OA in the ALs 12 days later compared to controls, and leads to precocious foraging [154]. When bees whose *corpora allata* had been removed were fed OA, they became normal foragers. These experiments suggest that OA probably acts downstream of JH. On the other hand, OA was shown to increase JH release from the *copora allata in vitro* in a dose-dependent manner [77]. The details of the complex interaction pattern of JH and OA remain to be resolved. Usually, a close interaction of both neuroactive substances is required for adequate initiation of foraging behaviour.

6.3. Age-Independent Division of Labour

Whereas DA, OA, and 5-HT were shown to be decisive regulators of age-dependent division of labour, the role of these amines in age-independent division of labour is less clear. Božič and Woodring [23] described that the levels of the three amines differed between foraging-aged bees when they performed different tasks. These bees are usually foragers, "dancers" or "followers". After returning from a foraging bout, "dancers" perform waggle dances to inform other foragers about the location of a food source. Some foraging-aged bees in the hive follow the dancers and beg for small food samples. These bees are "followers". Throughout the season, the titres of DA, OA and 5-HT were higher in dancers than in followers. This may be related to richer sources of sensory input they collect from the environment or to the locomotor activity they perform during foraging. To confirm these initial results on age-independent division of labour, additional measurements of biogenic-amine titres in same-aged bees are necessary.

7. HOW DO BIOGENIC AMINES AFFECT DIVISION OF LABOUR?

There are different hypotheses on how division of labour in honeybee colonies is organised. The most widely accepted hypothesis is that division of labour is achieved by differences in response thresholds of individual bees to stimuli that are associated with specific tasks [5,6,122,132,176]. Individuals with a low response threshold for a task-related stimulus are the first to start the respective job, once their response threshold is exceeded. Individuals with higher response thresholds perform the task only if the stimulus intensity increases even further. Recent experiments support this hypothesis and demonstrate how biogenic amines can modulate response thresholds for certain stimuli.

7.1. Response Thresholds for Gustatory and Olfactory Stimuli

Foragers differ not only in the material they collect but also in their response thresholds to gustatory stimuli presented to their antennae [125]. One week-old bees which have a high sensitivity for sucrose are most likely to later collect water or pollen, whereas bees with lower gustatory sensitivity collect nectar. Biogenic amines alter these gustatory response-thresholds. Both OA and TA significantly decrease the threshold [150]. This is another example of a similar action of OA and TA, which is in contrast to the assumption that OA and TA are "antagonistic modulators of behaviour" [140].

The opposite effect was observed for DA [150], whereas 5-HT had no effect (Scheiner, personal observation). Thus it is conceivable that response thresholds for foraging-related stimuli are adapted to the conditions in the hive and in the environment by changing levels of biogenic amines.

The spontaneous PER to antennal stimulation with an odour is often used as an indicator of olfactory sensitivity [100,101,105]. OA injection into the brain or brain compartments was shown to decrease olfactory response thresholds. DA and 5-HT injections had no effect on odour responsiveness [101,103,105] but increased response thresholds to water vapour [12,100,103]. The role of TA in honeybee olfaction is currently being analysed.

OA also affects response thresholds for the odour of brood (brood pheromone) [3]. When hive bees were fed with OA for several days, their response thresholds for brood pheromone decreased. The animals subsequently increased their foraging activity [3], but did not change the rate of corpse removal, another flight-related task [2]. Interestingly, OA treatment also did not increase attendance in the queen's retinue [2]. Since retinue behaviour is mediated by queen mandibular pheromone, elevated brain levels of OA apparently did not cause a general increase in responsiveness to odour stimuli. Although OA treatment enhanced the foraging response to brood pheromone, it decreased the cell capping response, a component of brood care [2]. This selective modulation of different responses to brood pheromone suggests that OA does not generally increase sensitivity to brood pheromone and demonstrates a rather specific octopaminergic modulation of pheromone-mediated behaviour. In honeybee strains differing in their hygienic behaviour [167], OA affects the sensitivity to the odour of diseased brood. Bees of the hygienic strain discover diseased brood quickly and remove it from the hive. In contrast, animals of non-hygienic strains need more time and higher stimulus intensities to perform the task. OA treatment of bees from the unhygienic strain resulted in increased electroantennogram (EAG) responses to odours originating from diseased brood. In bees

of the hygienic strain, however, OA did not change the sensitivity of the bees. They most likely had reached satiation in their sensitivity, because the OA-receptor antagonist epinastine [139] led to a reduction of EAG amplitude in these bees. In contrast, epinastine had no effect on unhygienic bees. This is a good example of how strongly the impact of a biogenic amine depends on the state of a bee.

7.2. Visual Sensitivity

Honeybees rely heavily on their visual senses, particularly at foraging age. They can recognise the flight paths to a foraging source, they look for potential food sources they have been informed about by their hive mates, and they have to discover the way to the pollen or nectar sources once they have arrived at a flower.

The direction-specific visual antennal reflex is a useful tool to study neuromodulation in the visual system of the bee. A stripe pattern which is moved upwards in front of a fixed bee induces downward antennal movements, whereas a downward moving pattern induces antennal movements in the opposite direction. The difference in the antennal angles for the two directions of the stimulus can be used as a measure of the direction specificity of the response [34,36,37]. This antennal reflex, when induced under controlled laboratory conditions, is very similar to the antennal movements of bees under free-flying conditions [33,39]. OA injections into the lobula can enhance the direction-specificity, whereas injections of 5-HT reduce this specificity [34,36, 37]. These experiments suggest that 5-HT and OA modulate motion-sensitive neurons in the lobula in an opposite way. The physiological changes of movement-sensitive neurons in the lobula during application of transmitters were measured by intracellular electrical recordings. Whereas 5-HT injections reduced spontaneous activity in many movement-sensitive neurons, OA application did not affect these responses [36]. DA appears not to be involved in the modulation of honeybee vision. The role of TA has yet to be tested.

7.3. Motor Activity and Locomotion

A decisive component of foraging behaviour consists of active scanning movements of the bee to analyse the surface of a flower and to use tactile information to identify a food source [79]. Antennal scanning behaviour can be characterised by the frequency of antennal contacts with an object, which can be measured electronically [127]. Comparable to the visual system, both 5-HT and OA acted antagonistically in the dorsal lobe, the sensory motor-centre of the antenna. OA stimulated antennal scanning activity, whereas 5-HT reduced it [127].

Another parameter of motor activity is the latency of PER after sucrose stimulation. Biogenic amines are most likely involved in PER, because the latency of PER was increased when bees were depleted of biogenic amines. Interestingly, DA restored the latency of the response to normal levels whereas injection of OA had no rescuing effect [102].

CONCLUSION

The four biogenic amines DA, TA, OA and 5-HT are present in different quantities in the bee brain and differ widely in their behavioural roles. Although OA is only present in small amounts in the honeybee brain, it modulates numerous behaviours. Together with JH, OA is involved in the initiation of foraging behaviour. OA also plays a decisive role in other forms of division of labour by modulating the sensitivity for specific stimulus modalities. In addition, OA can increase both acquisition and retrieval of information and thus controls learning and memory formation. DA and 5-HT, both of which are present in high amounts in the honeybee, often have inhibitory effects on behaviour. In associative olfactory learning, DA reduces the retrieval of learned information. 5-HT inhibits both storage and retrieval of information. The role of TA is less clear, probably because TA has mainly been considered as the biochemical precursor of OA rather than being a neurotransmitter itself.

In contrast to the general assumption that OA and TA act antagonistically [140], we presented data showing that both amines have similar functions. This finding can be explained either by the fact that the behavioural changes which were observed after TA application are a result of OA receptor activation or that TA may have both agonistic and antagonistic effects to those of OA depending on the behaviour investigated. More experiments, particularly with ligands highly specific for either OA or TA receptors will help to unequivocally unravel the functions of the two amines.

The signals mediated by biogenic amines are transformed into cellular responses by signalling cascades triggered by amine-specific GPCRs. Hitherto, a couple of these receptors have been molecularly identified and functionally characterised after heterologous expression. At present, it can be speculated that honeybees express a multitude of receptor genes with a complexity comparable to that of vertebrate systems. Forthcoming studies should deal with the comprehensive molecular identification, biochemical and pharmacological analyses, and the determination of the cellular expression patterns of these receptors. Such studies are urgently awaited and will help to further unravel the contribution of amine-induced signalling cascades to honeybee behaviour. We are convinced that results can be expected in reasonable time, because the availability of the honeybee genome sequence facilitates the molecular analysis of the system significantly. Biogenic-amine research in the honeybee will thus provide important new insights into various fields in the near future. Among these, the control of learning behaviour and division of labour are the most challenging ones. The DNA microarray technology [27,59,183] and molecular knock-down technologies, such as the antisense technique [51] and the RNA interference (RNAi) technique [48], are attractive developments with promising perspectives for honeybee molecular genetic studies. These techniques in combination with behavioural experiments will help to elucidate the physiological functions of the various biogenic-amine receptor subtypes in the bee. Thus, the study of aminergic signalling in the honeybee will shed further light on the role of amine modulators in complex behaviours of animals of all phyla.

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