Comp. Biochem. Physiol. Vol. 92C, No. 2, pp. 337–342, 1989
Printed in Great Britain

0306-4492/89 $3.00 + 0.00

NEUROACTIVE COMPOUNDS IN THE BRAIN OF THE HONEYBEE DURING IMAGINAL LIFE

E. FUCHS,* J. H. DUSTMANN,† H. STADLER‡ and F. W. SCHÜRMANN§‖

*Division of Reproductive Biology, German Primate Center, Göttingen, FRG; †Institute for Bee Research of Lower Saxony, Celle, FRG; ‡Max-Planck-Institute of Biophysical Chemistry, Göttingen, FRG; and §Division of Cell Biology, I. Institute of Zoology, University of Göttingen, 3400 Göttingen, FRG. Telephone (0551) 39-54-06

(*Received* 4 *May* 1988)

Abstract—1. In the brains of worker honeybees (*Apis mellifera carnica*) corresponding to different stages in the life span, we measured the content of GABA, glutamate, acetylcholine, choline, norepinephrine, dopamine and serotonin.

2. The highest concentrations were found for GABA, glutamate and acetylcholine.

3. Biogenic amines occur in considerably lower concentrations in comparison to the above mentioned transmitters.

4. Age-correlated changes were found for different neuroactive substances.

5. GABA and glutamate show a well marked rise and fall of their concentrations with a maximum at day 10.

6. The results are discussed in comparison to other species and with respect to age polyethism of worker honeybees.

INTRODUCTION

Structural and physiological changes correlating with development and affecting various parts of the body are well-known in both vertebrates and invertebrates. Embryonic and postembryonic changes due to cell differentiation as well as growth and decline of cell populations implicate the dynamic nature of neuronal equipment and alterations of putative transmitters; neuromodulators (for definition see Hoyle, 1985). Thus, GABAergic neuron populations undergo a substantial programmed decline after a period of embryonic augmentation and growth in the vertebrate brain (Wolff, 1981). Biogenic amines in the vertebrate brain show fetal as well as considerable neonatal alterations (Coyle and Henry, 1973; Vaccari, 1980; Flügge *et al.*, 1986; for review see Coyle, 1973). Changes in concentrations of putative transmitters accompanying metamorphosis have been documented for a number of insects (Sanes and Hildebrand, 1976; Prescott *et al.*, 1977).

The knowledge concerning putative transmitter/modulator distribution and actions in the peripheral and central nervous system of invertebrates is steadily accumulating especially with regard to arthropodes (for review see Murdock, 1971; Klemm, 1976; Evans, 1980; Walker, 1982; David and Coulon, 1985; O'Shea and Schaffer, 1985). However, no particular attention has been paid to quantitative changes of neuroactive compounds in insect brains during the course of the life cycle. In a number of investigations, considerable neuromorphological and partially behavioural changes in adult holometabolous insects could be shown (Bieber and Fuldner, 1979; Technau, 1984; for review see Schürmann, 1987).

Because of their short life span and the dramatic behavioural changes, honeybees represent good models for investigating postimaginal alterations. For our study, we chose free-living non-laboratory animals and followed the concentrations of seven putative neurotransmitters/neuromodulators in whole brains of worker honeybees at different ages representative of the worker bee's imaginal life (Lindauer, 1952).

MATERIALS AND METHODS

All experiments were performed with *Apis mellifera carnica*, queen right worker bees of one stock, kept in the Institute for Bee Research of Lower Saxony, Celle, FRG. Six different charges of freshly emerged bees were marked by a colour dot on the dorsal thorax between 27 June 1986–8 August 1986. Marked bees were not separated from the stock but kept in the same hive. Samples of up to 10 bees for each age group (1, 6, 10, 19, 30, and 40 days old) were obtained between 10–12 a.m. Though not more than 10 animals were normally used for a given age group for biochemical measurements, a multitude of several hundred bees had to be marked to compensate for loss of bees caused by various reasons. Only five bees were left for the 40 day group. No six-week-old bees could be found in the hive on 8 August 1986 or later.

Animals were cooled on dry ice, the brains were rapidly removed and cleaned from surrounding tissue. Fresh wt was determined in 150 μl ice-cold 0.1 N $HClO_4$. After weighing they were frozen and stored at $-70°C$. For determination of transmitter and protein concentrations, the individual brains were sonicated. In duplicates of 5 μl aliquots of the homogenate protein concentrations were determined according to Lowry *et al.* (1951). Then the homogenates were centrifuged at 10,000 *g* for 10 min at 4°C. In the supernatants γ-aminobutyric acid (GABA) and glutamate were determined by microfluorimetric assays (Mansky *et al.*, 1982; Mansky and Wuttke, 1983). The detection limit in the GABA specific microfluorimetric assay was approximately 15 pmol/μg protein, and in the glutamate specific assay 20 pmol/μg protein. Norepinephrine and dopamine were

‖Author to whom correspondence should be addressed.

measured with internal standards by a radioenzymatic assay (Saller and Zigmond, 1978). The detection limit for both catecholamines was 2.5–3.0 fmol/μg protein. Epinephrine was not detected (detection limit 2.5 fmol/μg protein). Serotonin was determined in 20 μl aliquots by a high performance liquid chromatography (HPLC) method with electrochemical detection. The HPLC system consisted of a solvent delivery pump (LKB) and a Rheodyne injector. Serotonin was separated on a Lichrospher RP-18 column (125 × 4 mm) from Merck, Darmstadt, FRG. The mobile phase was 0.02 M citric acid, 0.02 M hydrogen phosphate, pH 3.5 with 6% methanol as organic modifier (Tusell *et al.*, 1982). The flow rate was 1 ml/min. Column effluents were monitored with coulometric detector (ESA 5100 A). The optimal working potential was +0.35 V, the potential of the guard cell was set to +0.4 V. Quantiation was made by reference to a calibration curve run at the beginning and at the end of each series of assays. The standard working solution was prepared freshly in weekly intervals from serotonin–creatinine sulfate (Sigma, Munich, FRG) stored at −20°C and thawed immediately before use. The detection limit was 0.15 pmol per injection.

For determination of choline and acetylcholine (ACh), aliquots of the supernatants were brought to pH 4.0 with 1 M KOH and after standing on ice for 10 min they were centrifuged at 10,000 *g* for 10 min at 4°C. Samples of 20 μl of these supernatants were analysed directly by a HPLC method described recently (Stadler and Nesselhut, 1986). In short, acetylcholine and choline were first separated by HPLC, then reacted in a biosensor (Biometra, Göttingen, FRG) with acetylcholinesterase and choline oxidase immobilized on sepharose. The resulting H_2O_2 produced by choline oxidase is subsequently detected electrochemically (Biometra, Göttingen, FRG). With this method, amounts as low as 0.1 nmol of choline or acetylcholine can be detected.

The results for all neurotransmitters are expressed as pmol/μg protein. For statistical evaluation, all data were subjected to the two-dimensional analysis of variance for repeated measures followed by a multiple *t*-test.

RESULTS AND DISCUSSION

We detected different quantities of neuroactive compounds (neurotransmitters/neuromodulators) in brains of adult worker bees (Table 1, Figs 2–4).

Most studies do not cover a wide range of neuroactive compounds. In spite of the wide range of differences in the magnitude of compounds between species and even between brain and thoracic ganglia (Breer, 1981; Breer and Heilgenberg, 1985), GABA and ACh appear to be the predominant neuroactive compounds in the bee brain as well as in the nervous systems of all insects and spiders (Meyer *et al.*, 1984). The concentration of ACh is relatively low in bee brains (2.56 pmol/μg protein) in comparison to housefly brains (7.6–43 pmol/μg protein), and locust brains (9.5–25 pmol/μg protein) (Clarke and Donellan, 1982), but still a magnitude higher than in vertebrates (Breer, 1981). The concentrations of GABA far exceed those of ACh in the brain of bees (ratio 1:18, this paper) and of locusts (ratio 1:28; Breer and Heilgenberg, 1985). On the other hand, very similar ranges for ACh and GABA have been reported for brains of *Musca*, *Sarcophaga*, and for the cotton leaf worm *Spodoptera* (Clarke and Donellan, 1982). As the transmitter layout varies from species to species and even within nervous system and brain parts (Prescott *et al.*, 1977; Mercer *et al.*, 1983), a general statement as to the preponderance of GABA or ACh cannot be made. The

Table 1. Concentrations of neuroactive compounds in nervous systems of different insect species and of spiders, expressed in pmol/μg protein

Species/Tissue	GABA	Glu	Ach	NE	DA	5-HT	OA	
Apis/ 6 days old, brain, suboesophageal ganglion	47.25	84.78	2.56	0.093	0.53	0.285		This study
Manduca pterothorax adult brain	10–110*		10.0†					*Kingan and Hildebrand (1985) †Prescott *et al.* (1977) data converted to pmol/μg protein
Sarcophaga/brain	18		17.7		0.056		0.03	Clarke and Donellan (1982)
Musca/brain	3.8		7.6	0.028	0.15	0.11	0.016	Clarke and Donellan (1982)
Spodoptera/nerve cord	29		12.4	0.01	0.22	0.064	0.066	Clarke and Donellan (1982)
Schistocerca/brain	15	476	25	0.012	0.19	0.37	0.4	Clarke and Donellan (1982)
Locusta/brain	268.75*		9.5†					*Breer (1981) †Breer and Heilgenberg (1985) data converted to pmol/μg protein
Periplaneta/nerve cord	19*	52*		0.04†	0.19†		0.11†	*Osborne and Neuhoff (1974) †Dymond and Evans (1979)
Araneae (spiders)/ Cephalothorax	98.95	35.19	1.188	0.0796	0.0824			Meyer *et al.* (1984)

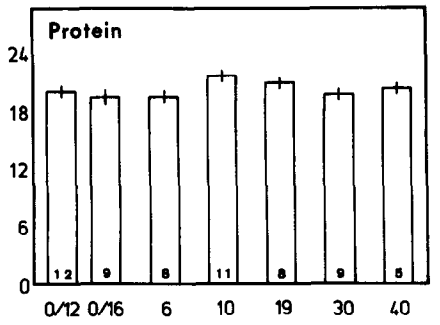

Fig. 1. Amount of protein in μg/brain (mean $\pm$ SEM) of different age stages (0–40 days), 0/12 and 0/16 animals obtained at 12 a.m. and 4 p.m. respectively. The number of brains per sample is given at the bottom of each column.

choline/ACh ratio varies considerably in different species (Clark and Donellan, 1982).

Glutamate is known to be an excitatory transmitter in the nerve–muscle junction of insects and likely to be a transmitter in the insect central nervous system (Walker, 1982). In the bee brain the amount of GABA and glutamate were found to be very similar. Glutamate is a precursor of GABA and occurs in similar concentration in the vertebrate nervous system (Breer and Heilgenberg, 1985).

A relatively low concentration of indolamines and catecholamines has been consistently reported in comparison to the above-mentioned transmitters. Our results are in accord with those of Clarke and Donellan (1982) on the brain of *Schistocerca*. Norepinephrine contents are considerably lower than those of 5-HT and dopamine. The ratios of these amines appear very similar for different species (for review see Nässel, 1987; David and Coulon, 1985). The small amount of measured 5-HT is reflected by the low percentage of immunoreactive cells (100 somata or 0.03% of brain cells) (Schürmann and Klemm, 1984; Klemm, 1985), whereas some thousand GABAergic cells occur in the bee brain (Schäfer and Bicker, 1986). Epinephrine could not be detected in the bee brain.

It is difficult to compare the concentrations of different transmitters/neuromodulators in various insect species as the data collected have been gathered using different methods or are expressed in different manners (see discussion in Evans, 1980; Clarke and Donellan, 1982; Nässel and Laxmyr, 1983).

One aim of this study was to investigate age-dependent transmitter changes during imaginal life of an insect. Worker bees progress through four stages during their 4–7 week life: after hatching they start with cell cleaning, followed by food and queen care, food storage and, finally, foraging (Lindauer, 1952; Robinson, 1987). Each new stage requires dramatic changes in behaviour. Following the sequence of hive life in the first three weeks, the subsequent foraging imposes a change in behaviour in worker bees which is usually not reversable (Robinson, 1987), in contrast to previous tasks in the hive which are not strictly age-correlated and subject to transitions. Behavioural changes are accompanied and supported by morphological, hormonal, and other biochemical changes (Fluri *et al.*, 1982; Robinson, 1987). Thus, juvenile hormone titer changes may be correlated with functional brain and behavioural changes (Robinson, 1987).

This study demonstrates for the first time age-dependent transmitter changes during the imaginal life span in an insect. We analysed seven neuroactive compounds in the bee brain from four different stages, as determined by their social functions in the hive period and foraging activities.

There is substantial evidence that GABA and acetylcholine are the major transmitters in the insect's central nervous system, exerting inhibitory and excitatory influences respectively on postsynaptic cells (Breer and Heilgenberg, 1985). The cellular function of amines in the central nervous system of insects remains, for the most part, obscure. Only a few studies have reported on the effects of biogenic amines in the honey bee brain (Mercer, 1987).

During whole imaginal life, protein amounts in the brain of honey bees were found to be constant (Fig. 1). We set the neuroactive compounds in relation to brain protein, as we noticed a considerable variation in wet wt values for samples of all selected ages, despite careful preparation and cleaning from adhesive tissues. The wet wt of nervous tissues is subject to considerable variations dependent upon age and/or physiological state (Evans, 1980).

GABA and glutamate concentrations (Fig. 2) followed an U-shaped curve with a maximum at day 10. At the beginning and at the end of the imaginal life, concentrations were comparable. The GABA concentrations at day 10 were significantly different from those at day 0 ($P < 0.004$) or day 40 ($P < 0.005$). Glutamate levels differ between day 0 and day 6, day 6 from day 10 ($P < 0.02$, 0.01) whereas day 0 and day 40 levels appear to be similar. Newly emerged workers showed up to the sixth day of their life a more or less constant ACh concentration. There may be a correlation between acetylcholine amount and hypopharyngeal/mandibular gland activity producing larval food in bees 6–12 days old. With increasing age,

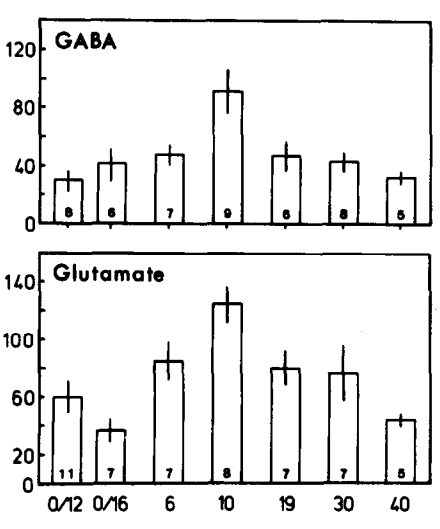

Fig. 2. GABA and glutamate concentrations (pmol/μg protein, mean $\pm$ SEM). Note concentration peak at day 10.

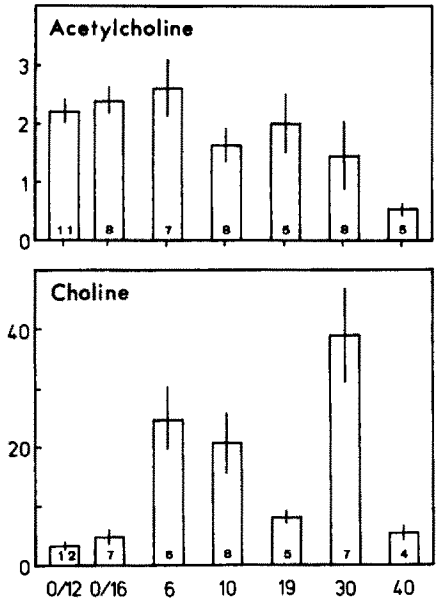

Fig. 3. Acetylcholine and choline concentrations. Compare with legends to Figs 1 and 2.

ACh concentration decreases (Fig. 3). In the oldest brains investigated (day 40) ACh concentrations were considerably lower than for day 0, day 6, day 10 ($P < 0.002$, 0.01, 0.05). Differences between day 0 and day 10 could not be verified statistically. Interestingly, choline levels (Fig. 3) have been found to be lower in comparison to ACh in newly emerged workers. Later they show an irregular pattern.

Among the biogenic amines (Fig. 4) investigated, norepinephrine concentrations demonstrated apparently the clearest alterations in correlation with the different age stages. The highest concentrations were measured in freshly emerged workers. Between day 6 and day 40, a rise and fall in concentrations can be observed, with a statistically significant difference between day 6 and day 19 ($P < 0.05$). For dopamine, a difference could only be verified between day 40 and the earlier stages, indicating a decline for the oldest workers whereas the concentrations of serotonin remained stable during imaginal life.

We consider our measurements to be a first step in gaining new knowledge on life span transmitter changes in the insect brain. Basically, we assumed that age-related transmitter changes occur due to turnover and/or availability in the course of altered physiological and behavioural needs. These might include recruitment of additional, large cell populations not required or only partially used during certain life periods, for instance during the dark hive period of worker bees. In fact, this assumption could be plausible in view of the large masses of optic lobe cells representing about 60% of the 1 million worker bee brain neurons (Witthöft, 1967). Extensive GABA- and GAD-immunoreactive neuropil strata have been recently described in the optic lobes stemming from local globule cells whereas GABAergic eye receptor cell endings do not occur (Schäfer and Bicker, 1986). The maxima of GABA and glutamate concentrations at day 10 do not coincide with outdoor life but rather mark the onset of nest building activities. Bees 10–12 days old perform their first orientation flights only in special situations of the colony. Normally, wax production and building of comb cells falls into that stage of worker bees 12–16 days old. At present, we cannot offer a sound explanation for the decline in acetylcholine levels. High amounts in young adults (hive bees) might reflect activities in cholinergic networks different from GABA-systems. There is evidence for a cholinergic nature of chemosensitive and mechanosensitive afferents in peripheral neuropils such as the antennal lobes (primary input center) and a widespread cholinergic interneuron system (Walker, 1982; Buchner *et al.*, 1986). These cholinergic cells may be involved intensively in the orientation within the hive.

The age-related decline of GABA, glutamate, and ACh levels may as well be due to neuronal degeneration and replacement of neurons by glial cells (Schürmann, 1980). The relatively low amine levels are a consequence of the small cell populations in the bee brain, e.g. 100 serotonergic neurons, which can be considered mainly as wide field neurons (Schürmann and Klemm, 1984). The lack of substantial amine level changes could signal that these systems react differently to behavioural changes and aging. If indeed the availability of neuronal amines is important for vital functions, then a loss of only a few non-redundant aminergic neurons could be crucial for survival and mortality, so that a gradual loss could not be expected or detected for the different stages under examination here.

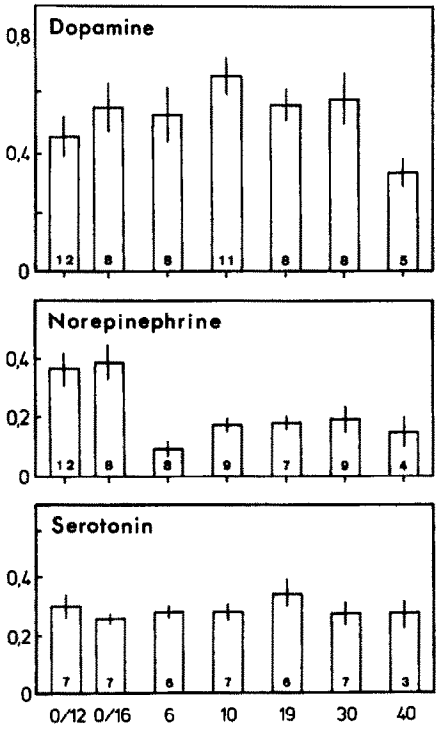

Fig. 4. Biogenic amines. Compare with legends to Figs 1 and 2.

Changes in transmitter concentrations accompanying aging have been reported for different mammalian species (see Giacobini *et al.*, 1982), mainly dealing with brain area specific alterations from the juvenile period up until maturity. A tendency to postembryonic augmentation of GABA and glutamate is obvious in various mammal brains (Agrawal *et al.*, 1968). These results cannot, however, be directly compared with ours as the data expression is not equivalent. Moreover, vertebrate brains show considerable growth of neurons as well as a decline in extracellular space, something not seen in the imaginal bee brain. Changes in the concentration of aminergic compounds in vertebrate brains comprise regional and age-dependent alterations (Hoffman and Sladek, 1980).

Our results clearly show that age-related changes of transmitter concentrations occur in mature worker bees. The meaning of these changes, especially in vital young animals, is obscure and cannot yet be satisfactorily ascribed to gross behavioural changes imposed by polyethism. Changes in transmitter layout may be important for the interpretation of morphological findings based on transmitter immunocytology such as neuron mapping. Further biochemical studies on transmitters should focus on a comparison of bee brain areas especially involved in sensory processing and in the organization of behavioral programs.

Acknowledgements—We wish to thank Mrs E. Schönberger (Celle) for her technical help in apiculture. We are grateful to Dr H. Breer (Stuttgart) for critical reading of our manuscript, and Mrs G. Schmidt for secretarial work.

REFERENCES

Agrawal H. C., Davis J. M. and Himwich W. A. (1968) Maturational changes in amino acids in CNS of different mammalian species. In *Recent Advances in Biological Psychiatry* (Edited by Wortis J.), Vol. X, pp. 258–265. Plenum Press, New York.

Bieber M. and Fuldner M. (1979) Brain growth during the adult stage of a holometabous insect. *Naturwissenschaften* **66,** 426.

Breer H. (1981) Comparative studies on cholinergic activities in the central nervous system of *Locusta migratoria*. *J. comp. Physiol.* **141,** 271–275.

Breer H. and Heilgenberg H. (1985) Neurochemistry of GABAergic activities in the central nervous system of *Locusta migratoria*. *J. comp. Physiol.* **157,** 343–354.

Buchner E., Buchner S., Crawfort G., Mason W. T., Salvaterra P. M. and Satelle D. B. (1986) Choline acetyltransferase-like immunoreactivity in the brain of *Drosophila melanogaster*. *Cell Tiss. Res.* **246,** 57–62.

Clarke B. S. and Donellan J. F. (1982) Concentrations of some putative transmitters in the CNS of quick-frozen insects. *Insect Biochem.* **12,** 623–638.

Coyle J. T. (1973) Biochemical aspects of the catecholaminergic neurons in the brain of the fetal and neonatal rat. In *Dynamics of Degeneration and Growth in Neurons* (Edited by Fuxe K., Olson L. and Zotterman Y.), pp. 425–435. Pergamon Press, New York.

Coyle J. T. and Henry D. (1973) Catecholamines in fetal and newborn rat brain. *J. Neurochem.* **21,** 61–67.

David J. C. and Coulon J. F. (1985) Octopamine in invertebrates and vertebrates. A review. *Prog. Neurobiol.* **24,** 141–158.

Dymond G. R. and Evans P. D. (1978) Biogenic amine in the nervous system of the cockroachy *Periplaneta americana*: association of octopamine with mushroom bodies and dorsal unpaired median (DUN) neurons. *Insect Biochem.* **9,** 535–545.

Evans P. D. (1980) Biogenic amines in the insect nervous system. *Adv. Insect Physiol.* **15,** 317–374.

Flügge G., Wuttke W. and Fuchs E. (1986) Postnatal development and sexual differentiation of central nervous catecholaminergic systems. *Biogenic Amines* **3,** 249–255.

Fluri P., Lüscher M., Wille H. and Gerig L. (1982) Changes in weight of the pharyngeal gland and haemolymph titres of juvenile hormone, protein and vitellogenin in worker honey bees. *J. Insect Physiol.* **28,** 61–68.

Giacobini E., Filogano G. and Vernadakis A. (1982) *The Aging Brain: Cellular and Molecular Mechanisms of Aging in the Nervous System*, pp. 1–284. Raven Press, New York.

Hoffman G. E. and Sladek J. R. (1980) Age-related changes in dopamine, LHRH and somatostatin in the rat hypothalamus. *Neurobiol. Aging* **1,** 27–37.

Hoyle G. (1985) Neurotransmitters, neuromodulators and hormones. In *Neurobiology* (Edited by Gilles R. and Balthazart J.), pp. 264–279. Springer, Berlin.

Kingan T. G. and Hildebrand J. G. (1985) Gamma-aminobutyric acid in the central nervous system of metamorphozing and nature *Manduca sexta*. *Insect Biochem.* **15,** 667–675.

Klemm N. (1976) Histochemistry of putative transmitter substance in the insect brain. *Prog. Neurobiol.* **7,** 99–169.

Lindauer M. (1952) Ein Beitrag zur Frage der Arbeitsteilung im Bienenstaat. *Z. vergleich. Physiol.* **34,** 299–345.

Lowry O. H., Rosenbrough N. J., Furr A. L. and Randall R. J. (1951) Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193,** 265–275.

Mansky T. and Wuttke W. (1983) Glutamate in hypothalamic and limbic structures of the diestrus, proestrus, overiectomised and ovariectomised estrogen primed rats. *Neurosci. Lett.* **28,** 51–56.

Mansky T., Mestres-Ventura P. and Wuttke W. (1982) Involvement of GABA in the feedback action of estradiol on gonadotropin and prolactin release: hypothalamic GABA and catecholamine turnover rates. *Brain Res.* **231,** 353–364.

Mercer A. R. (1987) Biogenic amines in the bee brain. In *Neurobiology and Behavior of Honeybees* (Edited by Menzel R. and Mercer A.), pp. 244–252. Springer, Berlin.

Mercer A. R., Mobbs P. G., Davenport A. P. and Evans P. D. (1983) Biogenic amines in the brain of the honeybee, *Apris mellifera*. *Cell Tiss. Res.* **234,** 655–677.

Meyer W., Schlesinger C., Poehling H. M. and Ruge W. (1984) Comparative quantitative aspects of putative neurotransmitters in the central nervous system of spiders (Arachnida: Araneidae). *Comp. Biochem. Physiol.* **78C,** 357–362.

Murdock L. L. (1971) Catecholamines in arthropodes: a review. *Comp. gen. Pharmac.* **2,** 234–274.

Nässel D. R. (1987) Serotonin and serotonin-immunoreactive neurons in the nervous system of insects. *Prog. Neurobiol.* **30,** 1–85.

Nässel D. R. and Laxmyr L. (1983) Quantitative determination of biogenic amines and Dopa in the CNS of adult and larval blowflies, *Calliphora erythrocepala*. *Comp. Biochem. Physiol.* **75C,** 259–265.

Osborne N. A. and Neuhoff V. (1974) Aminoacid and serotonin content in the nervous system, muscle, and blood of the cockroach *Periplaneta americana*. *Brain Res.* **80,** 251–264.

O'Shea M. and Schaffer M. (1985) Neuropeptide function: the invertebrate contribution. *A. Rev. Neurosci.* **8,** 171–198.

Prescott D. J., Hildebrand J. G., Sanes J. R. and Jewett S. (1977) Biochemical and developmental studies of acetylcholine metabolism in the central nervous system of the moth *Manduca sexta*. *Comp. Biochem. Physiol.* **56C,** 77–84.

Robinson G. E. (1987) Hormonal regulation of age polyethism in the honeybee, *Apis mellifera*. In *Neurobiology and Behavior of Honeybees* (Edited by Menzel R. and Mercer A.), pp. 266–279. Springer, Berlin.

Saller C. and Zigmond A. (1978) A radioenzymatic assay of catecholamines and dihydroxy-phenylacetic acid. *Life Sci.* **23,** 1117–1130.

Sanes J. R. and Hildebrand J. G. (1976) Acetylcholine and its metabolic enzymes in developing antennae of the moth, *Manduca sexta*. *Dev. Biol.* **52,** 105–120.

Schäfer S. and Bicker G. (1986) Distribution of GABA-like immunoreactivity in the brain of the honeybee. *J. comp. Neurol.* **246,** 287–300.

Schürmann F. W. (1980) Experimental anterograde degeneration of nerve fibres: a tool for combined light- and electron-microscopic studies of the insect nervous system. In *Neuroanatomical Techniques. Insect Nervous System* (Edited by Strausfeld N. J. and Miller T. A.), pp. 263–281. Springer, New York.

Schürmann F. W. (1987) The architecture of the mushroom bodies and related neuropils in the insect brain. In *Arthropod Brain* (Edited by Gupta A. P.), pp. 231–264. John Wiley, New York.

Schürmann F. W. and Klemm N. (1984) Serotonin-immunoreactive neurons in the brain of the honeybee. *J. comp. Neurol.* **225,** 570–580.

Stadler H. and Nesselhut T. (1986) Simple and rapid measurement of acetylcholine and choline by HPLC and enzymatic-electrochemical detection. *Neurochem. Int.* **9,** 127–129.

Technau G. M. (1984) Fiber number in the mushroom bodies of adult *Drosophila melanogaster* depends on age, sex and experience. *J. Neurogenet.* **1,** 113–126.

Tussel J. M., Sunol C., Artigas F., Martines E. and Gelpi E. (1982) Neurochemical studies on catecholamines and indolamines by high performance liquid chromatography with electrochemical detection. *Chromatographia* **16,** 112–116.

Vaccari A. (1980) Sexual differentiation of monoamine neurotransmitters. In *Biogenic Amines in Development* (Edited by Parvez H. and Parvez S.), pp. 352–357. Elsevier/North-Holland, Amsterdam.

Walker R. J. (1982) Current trends in invertebrate neuropharmacology. *Verh. Dtsch. Zool. Ges.*, pp. 31–59. Gustav Fischer, Stuttgart.

Witthöft W. (1967) Absolute Anzahl und Verteilung der Zellen im Hirn der Honigbiene. *Z. Morphol. Tiere* **61,** 160–184.

Wolff J. R. (1981) Evidence for a dual role as a synaptic transmitter and promoter of synaptogenesis. In *Amino Acid Neurotransmitters* (Edited by De Feudis F. V. and Mandel P.), pp. 459–465. Raven Press, New York.