



M-2828

Free amino acids and biogenic amines in the brain of the carpenter ant, *Campanotus floridanus* (Buckley) (Hymenoptera, Formicidae)

F. Punzo* and D. F. Williams†

*Department of Biology, University of Tampa, Tampa, FL 33606, U.S.A.; and †Medical and Veterinary Entomology Research Laboratory, ARS, USDA, Gainesville, FL 32604, U.S.A.

Urea and ammonia together comprised 34.3–37.8% of the total ninhydrin-positive compounds in the brain of various developmental stages of *Campanotus floridanus*. The most abundant free amino acids were alanine (15.3–16.1%), proline (8.8–10.4%), aspartate (7.1–7.5%), glutamate (6.2–7.2%) and serine (4.3–5.9%). GABA levels increased between the pupal (1–4.1%) and adult worker stages. Taurine levels increased from 3.9% in larval brains to 6.5% in major workers. Brain concentrations of biogenic amines (dopamine, octopamine, noradrenaline and serotonin) increased between larval and adult stages. The possible relationships between neurochemical parameters and age polytheism are discussed.

Key words: Biogenic amines; *Campanotus floridanus*; Free amino acids.

Comp. Biochem. Physiol. 107C, 387–392, 1994.

Introduction

The functional significance of brain chemistry and whether or not specific compounds such as free (physiological) amino acids (FAA) and putative and classical neurotransmitters (NT) are involved in the control of specific behaviors have yet to be established (Ungar, 1973; Davidson, 1976; Punzo, 1985; Black *et al.*, 1987; Byrne, 1987). Some previous studies have shown that neurochemical changes do accompany certain behaviors. In vertebrates, for example, Flexner *et al.* (1967) demonstrated an impairment of memory in mice treated with antibiotics. Hambley and Rogers (1979) showed that the intracerebral injection of amino acids resulted in retarded learning in neonatal chicks. In arthropods, increases in RNA and protein synthesis within the CNS have been found to accompany learning in cockroaches (Kerkut *et al.*, 1972), mantids (Maldonado, 1980), grasshoppers (Punzo, 1980), decapod crustaceans (Punzo, 1983a), theraphosid spiders

(Punzo, 1988) and pompilid wasps (Punzo, 1991). In the spider wasp, *Pepsis formosa* (Say), brain RNA and protein synthesis were found to increase as a function of increasing encounter experience between the wasp and its spider host (Punzo, 1991). Cycloheximide-induced inhibition of neural protein synthesis has been shown to impair behavioral plasticity in mantids (Jaffe, 1980), grasshoppers (Punzo, 1980) and spiders (Punzo, 1988), as well as to disrupt innate phototactic behavior in some beetles (Punzo and Jellies, 1980).

These observations have led investigators to study neurochemical correlates of behavior in social insects. Many social insects exhibit ontogenetic caste-specific shifts in behavior (age polytheism), especially among workers, and these changes in behavior are frequently accompanied by neurochemical changes as well. For example, higher concentrations of dopamine (DA) were found in the brains of active nectar and pollen forager honeybees as compared to concentrations found in younger workers who function primarily as nurses or food storers (Free, 1965; Taylor *et al.*, 1992).

Correspondence to: F. Punzo, Department of Biology, University of Tampa, Tampa, FL 33606, U.S.A.

Received 24 September 1993; accepted 1 November 1993.

Juvenile hormone is also known to play a role in the regulation of age polytheism in honeybees (Robinson, 1987). Age polytheism is also well known in many species of ants where workers exhibit ontogenetic shifts in behavior functioning initially as tenders of the queen and brood and then shifting to reserves and eventually to active foragers (Wilson, 1971).

A thorough understanding of the cellular and molecular basis of behavior is essential for a more comprehensive analysis of how the CNS regulates the onset of age-dependent behavioral acts. In view of this, the present study was undertaken in order to identify and quantify the FAA and biogenic amines associated with the cerebral ganglion (brain) of the carpenter ant, *Camponotus floridanus* (Buckley), as an initial step in analyzing the relationship between neurochemistry and behavior in this economically important species. Such an analysis may have important implications for the development of new control procedures. Carpenter ants may invade buildings and other man-made structures causing extensive damage by excavating their tunnels in wood (Smith, 1965). Adult carpenter ants are characterized by three castes (workers, males and the queen) which have distinctive functions within the colony. The worker caste consists of minor and major workers with each performing specific tasks (Fowler, 1983). The minor workers are primarily involved in nest excavation and caring for the brood and queen, whereas the larger major workers guard the nest, engage in fighting any potential intruders and actively forage outside the nest. For a detailed discussion of the life history of carpenter ants, the reader is referred to Fowler (1983, 1986).

In this study, we determined the following neurochemical parameters associated with the brains of various developmental stages (larvae, pupae, minor and major workers) of *C. floridanus*: (1) the FAA composition; and (2) the concentration of biogenic amines: dopamine (DA), octopamine (OA), noradrenaline (NA) and serotonin (5-hydroxytryptamine, 5-HT).

Materials and Methods

The ants used in this study were from a laboratory colony originally obtained from the USDA fire ant project (Medical & Veterinary Entomology Research Laboratory, ARS, Gainesville, FL). The colony was maintained in a Williams Cell (Bishop *et al.*, 1980) at $23 \pm 1^\circ\text{C}/70 \pm 2\%$ RH as previously described by Punzo and Glancey (1985). Ants were fed on a diet of honey, sarcophaga pupae, mealworm larvae and egg yolk. This rearing method allows easy access to larvae, pupae and adults.

Analysis of free amino acids

The concentrations of FAA in the cerebral ganglion (brain) were determined according to procedures previously described by Punzo (1983b, 1987, 1990). Twenty replicates of five brains ($N = 100$) from 7-day old larvae, 3-day old pupae, minor and major workers were used for analyses. Five brains were pooled for each run. Brains were removed in physiological saline, transferred to a 1% picric acid solution and frozen for not more than 48 hr prior to FAA determinations. After thawing, samples were homogenized, transferred to centrifuge tubes and centrifuged at 2000 rpm for 45 min in a Sorvall Model RC-5B high-speed centrifuge. The supernatant was evaporated to a dry residue and analyzed as total ninhydrin-positive material at 570 nm against a glutamic acid standard. Two-dimensional HPTLC was performed using Whatman LHP-KF 20 \times 10 cm plates (Whatman Chemical Separation, New Jersey) using chloroform-ethanol-acetic acid (90:10:2 v/v) in one direction and chloroform-ethyl acetate (90:10 v/v) in the other. Individual amino acid spots were visualized with ninhydrin as described by Kileti and Lederer (1974). FAA concentrations were expressed in nmol/mg tissue based on tissue dry weights. To determine dry weights, freshly dissected brains were dried in a dessicator and weighed at 24 hr intervals. When the same weight was recorded for two consecutive trials, this weight was used as the dry tissue weight (Holman and Cook, 1982). Statistical procedures used in data analysis follow those described by Sokal and Rohlf (1981).

Analysis of biogenic amines

Each sample consisted of five brains pooled from larvae, pupae and workers as described above. Following removal, the optic lobes were removed and the remaining brain tissues were placed in a Beckman vial and frozen for subsequent analyses. Brain tissues were analyzed for the presence of DA, OA, NA and 5-HT using reverse-phase high performance liquid chromatography with electrochemical detection (HPLC-ED). Tissue samples were thawed, 50 μl of a 200 mM perchloric acid solution was added to each vial and samples were homogenized in a tissue homogenizer (American Scientific Co., Chicago). Following homogenization, an additional 40 μl of perchloric acid was added to each vial. Samples were then centrifuged at 9000 g for 4 min at room temperature. The supernatant from each sample was injected directly into a Beckman Model 020L column for HPLC-ED analysis as described by Taylor *et al.* (1992). The mobile phase used to elute

the amines contained 100 mM sodium dihydrogen orthophosphate, 2.5 mM octanesulfonic acid sodium salt, 12% acetonitrile and 0.3 mM EDTA disodium salt adjusted to pH 4.0 and filtered through a 0.45 μ m filter. Amine concentrations were expressed in pmol per brain for all developmental stages.

Results

The FAA composition and related ninhydrin-positive compounds found in the brain of various developmental stages of *C. floridanus* are shown in Table 1. Gamma amino butyric acid (GABA), well known as an inhibitory NT in many animal nervous systems (Davidson, 1976; Hokfelt *et al.*, 1984), decreases significantly from a mean of 9.4 nmol/mg in the larval brain to 4.4 nmol in pupae ($F = 9.85$, $P < 0.01$, Model I ANOVA). GABA levels then increase from 4.4 to 11.3 nmol/mg in minor workers ($P < 0.01$) and are found in the highest levels in the brains of major workers (20.1 nmol, $P < 0.01$). GABA was found to comprise 2.1–4.1% of the total ninhydrin-positive compounds in the brain of *C. floridanus* depending on the life cycle stage. Punzo and Glancey (1985) reported a value of 3% for minor workers of the red imported fire ant, *Solenopsis invicta*. A similar level of GABA was found in the brain of adults of the spider wasp,

P. formosa (Punzo, 1990). Somewhat higher values (9–22%) have been reported for spiders (Meyer *et al.*, 1980; Punzo, 1983b). Aspartate, glycine and glutamic acid, which have also been shown to function as classical and/or putative neurotransmitters (Davidson, 1976; Hokfelt *et al.*, 1984), together comprised 15.8–16.9% of the total ninhydrin-positive compounds in the brain of *C. floridanus*. No significant change in the concentration of these compounds occurred as a function of development in this ant. It should be pointed out that aspartate and glutamate are among the most abundant amino acids associated with mammalian neural tissues, and glutamate has been identified as an excitatory NT in invertebrates (Davidson, 1976).

The nitrogenous waste products (ammonia and urea) together comprised 37.8% of the total ninhydrin-positive compounds in larval brains, 37% in pupae, 34.4% in minor workers and 34.3% in major workers. This is in general agreement with results reported for other insects (Holman and Cook, 1982; Punzo 1983b, 1987, 1990). However, these compounds are generally present in lower concentrations in decapod crustaceans (Evans, 1973; Punzo, 1983a) and higher concentrations in arachnids (Meyer *et al.*, 1980; Punzo, 1983b).

Taurine levels also increased significantly from larval to adult stages (3.9–6.5%, $F = 5.1$, $P < 0.05$). It has been suggested that taurine is necessary for the normal development of the

Table 1. Free amino acids and other ninhydrin-positive compounds in the cerebral ganglion of various developmental stages of *Camponotus floridanus*

Compound	Mean concentration (nmol/mg)							
	Larvae	%	Pupae	%	Minor workers*	%	Major workers	%
Urea	131.2 (20.5)	29.5	125.1 (14.7)	28.1	119.7 (11.4)	25.1	126.7 (16.4)	25.7
GABA	9.4 (1.1)	2.1	4.4 (0.6)		11.3 (2.4)	2.3	20.1 (3.6)	4.1
Taurine	17.6 (3.6)	3.9	24.9 (5.1)	6.2	29.7 (4.7)	6.2	32.3 (5.2)	6.5
Ornithine	0.34 (0.01)		0.38 (0.02)		0.41 (0.03)		0.39 (0.02)	
Ammonia	37.3 (5.1)	8.3	39.7 (3.9)	8.9	44.8 (6.2)	9.3	42.6 (4.3)	8.6
Arginine	14.3 (3.1)	3.2	13.8 (2.9)	3.1	15.9 (1.7)	3.3	14.2 (2.6)	2.8
Aspartate	33.4 (2.9)	7.5	31.7 (3.8)	7.1	37.2 (5.1)	7.8	35.6 (7.2)	7.2
Glutamate	30.7 (4.4)	6.9	32.1 (3.7)	7.2	33.4 (6.8)	7.0	30.8 (5.9)	6.2
Glycine	10.5 (2.5)	2.3	9.7 (1.1)	2.1	10.1 (2.3)	2.1	12.3 (3.4)	2.4
Alanine	71.4 (12.1)	16.1	69.6 (11.3)	15.6	76.4 (14.1)	16.0	75.5 (9.3)	15.3
Histidine	7.1 (0.4)	1.5	7.2 (1.1)	1.6	6.9 (0.5)	1.4	7.4 (0.3)	1.5
Isoleucine	2.2 (0.2)		2.1 (0.3)		1.9 (0.3)		2.4 (0.5)	
Leucine	3.1 (0.4)		3.2 (0.1)		2.9 (0.2)		3.4 (0.2)	
Lysine	1.7 (0.2)		2.3 (0.3)		1.9 (0.1)		2.2 (0.2)	
Methionine	1.6 (0.3)		1.4 (0.1)		1.6 (0.1)		1.7 (0.2)	
Phenylalanine	0.3 (0.01)		ND†		0.4 (0.04)		0.7 (0.03)	
Proline	40.6 (8.7)	9.1	39.3 (9.1)	8.8	47.4 (11.3)	9.9	51.3 (7.9)	10.4
Serine	24.7 (5.8)	5.5	26.3 (4.9)	5.9	24.1 (7.1)	5.1	21.6 (5.3)	4.3
Threonine	1.9 (0.2)		2.5 (0.4)		2.3 (0.3)		2.5 (0.2)	
Tyrosine	2.4 (0.3)		7.8 (0.6)	1.7	6.8 (0.5)	1.4	6.9 (0.4)	1.4
Valine	2.5 (0.4)		2.2 (0.1)		1.7 (0.3)		2.1 (0.2)	
Total:	444.2		445.7		476.8 (0.4)		492.7 (0.4)	

Values represent means; numbers in parentheses represent (\pm SD). %: percent of total ninhydrin-positive compounds (only values > 1% are shown).

*Based on head capsule width: Minor workers (1.6–1.8 mm); Major workers (2.1–2.2 mm); †ND = not detected.

Table 2. Biogenic amines in the cerebral ganglion of various developmental stages of *Campanotus floridanus*.

Compound	Mean concentration (pmol/brain)			
	Larvae (n = 210)	Pupae (n = 140)	Minor workers (n = 150)	Major workers (n = 160)
Dopamine	9.8 (1.2)	17.3 (2.4)	20.4 (3.7)	22.7 (4.1)
Octopamine	4.6 (0.4)	6.6 (0.3)	6.1 (0.2)	5.6 (0.4)
Noradrenaline	1.9 (0.2)	2.3 (0.4)	3.6 (0.5)	3.9 (0.4)
5-Hydroxy-tryptamine	2.5 (0.4)	2.9 (0.3)	4.7 (0.4)	4.2 (0.2)

Values represent means (\pm SD). All samples conducted on whole brains minus the optic lobes.

arthropod CNS in general as well as for the regulation of developmental changes associated with metamorphosis in holometabolous insects (Bodnaryk, 1981). The levels of taurine reported in this study are comparable to those reported for other insects (Holman and Cook, 1982; Punzo, 1983b, 1987, 1990) and spiders (Meyer *et al.*, 1980), but considerably lower than values (24–30%) reported for decapod crustaceans (Evans, 1973; Punzo, 1983b).

The levels of biogenic amines found in the brain of *C. floridanus* are shown in Table 2. Dopamine concentrations increased significantly ($F = 19.6$, $P < 0.001$) from the larval to adult stages. Noradrenaline and 5-HT ($P < 0.05$) levels also increased as a function of developmental stage. Octopamine levels increased significantly between the larval and pupal stages ($P < 0.05$). Dopamine, NA, OA and 5-HT (serotonin) have all been identified as classical or putative neurotransmitters in the insect nervous system (Evans, 1980; Pandey and Habibulla, 1980; Clarke and Donnellan, 1982; Vieira and Aldegunde, 1993). The concentrations of biogenic amines previously reported for insect neural tissues have been quite variable (Evans, 1980). In brain tissue, OA levels range from 11.6 pmol/brain in locusts to 14.7 pmol in cockroaches. In the cockroach cerebral ganglion, the concentration of DA was found to be 26.8 pmol, and NA 5.5 pmol. In the honey bee, 5-HT levels increased from < 1.0 pmol in 2 to 4-day old pupae to > 5.7 pmol in adult foragers (Taylor *et al.*, 1992). DA levels were highest in 4-day old pupae, and decreased markedly in foragers. In the present study, DA levels continued to increase from the pupal to the adult worker stage (Table 2).

Discussion

The results of this study represent the first demonstration of definitive changes in brain chemistry as a function of development in carpenter ants. With respect to NTs, CADA

levels are lowest in larval, pupal and minor worker brains and then increase in major workers. Dopamine levels increase dramatically over the course of development between larval and adult stages, as well as noradrenaline and serotonin to a lesser degree. This suggests that changes in brain chemistry may be responsible for the manifestation of different behavioral repertoires in the various developmental stages of this ant. Larvae are basically immobile, have a relatively simple behavioral repertoire, and rely on workers to feed and care for them. During the pupal stage, larval tissues are reorganized into adult structures preparing the insect for its adult life. Emergent minor workers exhibit a more complex behavioral repertoire engaging in nest excavation and tending the queen and future brood. The next group of workers (major workers) is larger in size and exhibits an even more complex behavioral program characterized by active foraging outside the nest as well as defense of the colony. It is tempting to relate such functional changes in behavior to the changes in brain chemistry mentioned previously. Neurotransmitters are essential for the transmission of neural information involved in CNS integration and upon which all behavior is ultimately based. Future studies should focus on the use of pharmacological agents that can block or impair the synthesis of specific NTs and then assess to what extent these treatments result in behavioral deficits. This approach would yield valuable supporting evidence for a direct link between brain chemistry and behavior with important implications for control strategies against this economically important species.

Most of the previous work on neurochemical correlates of behavior in social insects has focused on honey bees. Worker bees undergo profound changes in morphology and behavior (Breed *et al.*, 1991). During the first 4–7 weeks of life, adult workers exhibit age polytheism progressing through a sequence of behavioral stages characterized by the performance of specific age-dependent tasks (Robinson, 1987; Winston, 1987). The youngest workers basically

engage in behavioral activities within the nest, whereas older workers leave the nest to forage for food. Subtle transitions between various behavioral stages prior to the onset of foraging are characterized primarily by changes in the relative frequencies in which certain behavioral acts are performed culminating in the final shift to behaviors specifically associated with foraging. Although these various behavioral stages exhibited by workers have been extensively studied, the physiological and neurochemical processes underlying them are poorly understood. Juvenile hormone is known to play a role in age polytheism in honey bees (Robinson, 1987) in that low concentrations are associated with the behavior of workers confined to the nest including grooming and brood care, whereas higher concentrations, which occur after 3 weeks of age, are associated with the onset of foraging behavior.

Biogenic amines have also been implicated in the regulation of insect behavior (Evans, 1980). In honey bees, low levels of DA are found in the head and thoracic segments of larvae (2–4 pmol/brain), increasing markedly in the pupal stage (35–140 pmol), decreasing again in young workers (20–25 pmol) and finally increasing once again in foragers (40 pmol) (Brandes *et al.*, 1990; Taylor *et al.*, 1992). In *C. floridanus*, major workers are associated with the highest levels of brain DA (22.7 pmol) as compared to pupae (17.3) and larvae (9.8) (Table 2). In honey bees, serotonin first appears in detectable concentrations at the onset of metamorphosis and increases significantly upon adult emergence. The highest levels of serotonin are associated with the brains of pollen foragers (6 pmol) as compared to those found for nurses (3.4 pmol), food storers (2.5), undertakers (3.2), guards and nectar foragers (4.2), and nonspecific foragers (4.7) (Taylor *et al.*, 1992). In this study, no significant differences were found in the concentration of serotonin in minor and major workers of *C. floridanus*, although worker levels were higher than those found in larvae and pupae.

In honey bees, changes that occur in the biogenic amine profiles as a function of development have been related to histological studies on the ontogenetic development and anatomical distribution of dopaminergic and serotonergic pathways in the brain (Schurmann and Klemm, 1984; Schafer and Rehder, 1989; Brandes *et al.*, 1990). Similar studies should be conducted on the ant brain as well. In this way, changes in neurochemical profiles can be directly linked to specific brain regions. This is important because previous studies have shown quite clearly that specific regions of the arthropod brain are associated with the regulation of specific behaviors (see review by Punzo, 1985).

For example, higher associative functions such as learning and memory are primarily associated with the corpora pedunculata (mushroom bodies, MB) of the protocerebrum (Punzo, 1980, 1988; Punzo and Malatesta, 1988). The alpha lobes of the MB have also been implicated in short-term memory processes in the honey bee (Menzel *et al.*, 1988). It has also been shown that the number of fibers within the MB of *Drosophila melanogaster* is significantly reduced in flies that have been reared in social isolation (Technau, 1984) indicating that social interactions can have profound influences on the development of the insect brain. Since social isolation can affect the morphology of the MB in flies, future studies are needed to assess the magnitude of similar isolation procedures on social insects. In addition, studies should be conducted to determine the relationship between development and age-specific changes in brain morphology and neuroanatomy in ants focusing on changes in overall brain volume, relative volumes of specific brain regions (protocerebrum, deutocerebrum and tritocerebrum), neuronal architecture within specific brain regions, volume and fiber number associated with the MB and optic lobes, and the elucidation of specific NT pathways utilizing immunoreactive histochemical procedures. Only in this way will we be able to fully understand the relationship between brain morphology, neurochemistry and behavior.

Acknowledgements—The authors wish to thank Jim Nation (University of Florida, Dept of Entomology & Nematology) for critical comments on an earlier draft of the manuscript, and Brian Garman (University of Tampa, Dept. of Mathematics) for consultation on statistical analyses.

References

- Bishop P. M., Banks W. W., Williams D. F., Stringer J. A. and Lofgren C. F. (1980) Simple nests for culturing imported fire ants. *J. Georgia Ent. Soc.* **15**, 300–304.
- Black I. B., Adler J. E., Dreyfus C., Friedman W. F., Lagamma E. F. and Roach A. H. (1987) Biochemistry of information storage in the nervous system. *Science* **236**, 1263–1268.
- Bodnaryk R. P. (1981) Developmental changes in brain taurine levels during metamorphosis of the moth, *Mamestra configurata*. *Insect Biochem.* **11**, 9–16.
- Brandes C., Sugawa M. and Menzel R. (1990) High performance liquid chromatography (HPLC) measurement of catecholamines in single honeybee brains reveals caste-specific differences between worker bees and queens in *Apis mellifera*. *Comp. Biochem. Physiol.* **97C**, 53–57.
- Breed M. D., Robinson G. E. and Page R. E. (1991) Division of labor during honey bee colony defense. *Behav. Ecol. Sociobiol.* **27**, 395–401.
- Byrne J. H. (1987) Cellular analysis of associative learning. *Physiol. Rev.* **67**, 329–439.
- Clarke B. S. and Donnellan J. F. (1982) Concentrations of some putative neurotransmitters in the CNS of quick-frozen insects. *Insect Biochem.* **12**, 623–638.

- Davidson N. (1976) *Neurotransmitter Amino Acids*. Academic Press, London.
- Evans P. D. (1973) Amino acid distribution in the nervous system of the crab, *Carcinus maenas*. *J. Neurosci.* **21**, 11-17.
- Evans P. D. (1980) Biogenic amines in the insect nervous system. In *Advances in Insect Physiology* (Edited by Berridge M. J., Treherne J. E. and Wigglesworth V. B.), pp. 317-467, Academic Press, New York.
- Flexner L. B., Flexner J. and Roberts R. (1967) Memory in mice analyzed with antibiotics. *Science* **155**, 1377-1383.
- Fowler H. G. (1983) The biology, economics and control of carpenter ants. In *Biology, Economic Importance and Control of Social Insects* (Edited by Vinson S. B. and Mauldin J. K.). Academic Press, New York.
- Fowler H. G. (1986) Biology, economics and control of carpenter ants. In *Economic Impact and Control of Social Insects* (Edited by Vinson S. B.). Praeger Press, New York.
- Free J. B. (1965) The allocation of duties among worker honey bees. *Symp. Zool. Soc. London* **14**, 39-59.
- Hambley J. W. and Rogers L. (1979) Retarded learning induced by intracerebral injection of amino acids in the neonatal chick. *Neuroscience* **4**, 677-684.
- Hokfelt T., Johansson O. and Goldstein M. (1984) Chemical anatomy of the brain. *Science* **225**, 1326-1333.
- Holman G. M. and Cook B. J. (1982) Physiological amino acids of the nervous system of the stable fly, *Stomoxys calcitrans*. *Comp. Biochem. Physiol.* **71A**, 23-27.
- Jaffe K. (1980) Effects of cycloheximide on protein synthesis and memory in praying mantids. *Physiol. Behav.* **25**, 367-371.
- Kerkut G. A., Emsen P. C. and Beesley P. W. (1972) Effect of leg-raising learning on protein synthesis and cholinesterase activity in the cockroach. *Comp. Biochem. Physiol.* **41B**, 635-645.
- Kileti G. and Lederer W. (1974) *Micromethods for the Biological Sciences*, pp. 212-227. Van Nostrand, New York.
- Maldonado H. (1980) Changes in brain peptides and memory consolidation in praying mantids. *J. Insect Physiol.* **26**, 1101-1110.
- Menzel R., Michelson B., Ruffer P and Sugawa M. (1988) Neuropharmacology of learning and memory in honey bees. In *Synaptic Transmission and Plasticity in Nervous Systems* (Edited by Hertig G. and Spatz C. H.), pp. 335-350. Springer, Berlin.
- Meyer W., Poehling H. M. and Neuoff V. (1980) Comparative aspects of free amino acids in the central nervous system of spiders. *Comp. Biochem. Physiol.* **67C**, 83-86.
- Pandey A. and Habibulla M. (1980) Serotonin in the central nervous system of the cockroach, *Periplaneta americana*. *J. Insect Physiol.* **26**, 1-6.
- Punzo F. (1980) Neurochemical changes associated with learning in *Schistocerca americana* (Orthoptera: Acrididae). *J. Kansas Entomol. Soc.* **53**, 787-796.
- Punzo F. (1983a) Localization of brain function and neurochemical correlates of learning in the mud crab, *Eurypanopeus depressus* (Decapoda). *Comp. Biochem. Physiol.* **75A**, 299-305.
- Punzo F. (1983b) Physiological amino acids in the brain of *Schistocerca americana* (Insecta), *Lycosa avida* (Arachnida) and *Eurypanopeus depressus* (Decapoda). *Comp. Biochem. Physiol.* **75C**, 399-402.
- Punzo F. (1985) Recent advances in behavioral plasticity in insects and decapod crustaceans. *Fla. Entomol.* **68**, 89-104.
- Punzo F. (1987) Physiological amino acids in the brain tissue of the lepidoptera, *Lepisma saccharina* and *Thermobia domestica* (Insecta, Thysanura). *Comp. Biochem. Physiol.* **88C**, 255-258.
- Punzo F. (1988) Learning and localization of brain function in the tarantula spider, *Aphonopelma chalcodes* (Orthognatha, Theraphosidae). *Comp. Biochem. Physiol.* **89A**, 465-470.
- Punzo F. (1990) The hemolymph composition and neurochemistry of the spider wasp, *Pepsis formosa* (Say) (Hymenoptera, Pompilidae). *Comp. Biochem. Physiol.* **96A**, 344-345.
- Punzo F. (1991) Neurochemical events associated with learning and hunting behavior in the spider wasp, *Pepsis formosa* (Say) (Hymenoptera, Pompilidae). *Fla. Scientist* **54**, 51-61.
- Punzo F. and Glancey B. M. (1985) Arginine concentration and free amino acids in the brain of various worker subcastes of the red imported fire ant, *Solenopsis invicta* (Hymenoptera, Formicidae). *Comp. Biochem. Physiol.* **81B**, 761-765.
- Punzo F. and Jellies J. (1980) Effects of cycloheximide-induced protein synthesis inhibition on the phototactic behavior of *Tenebrio molitor* (Coleoptera: Tenebrionidae) and *Popilius disjunctus* (Coleoptera: Passalidae). *J. Kansas Entomol. Soc.* **53**, 597-606.
- Punzo F. and Malatesta R. (1988) Brain RNA synthesis and the retention of learning through metamorphosis in *Tenebrio obscurus* (Insecta, Coleoptera). *Comp. Biochem. Physiol.* **91A**, 675-679.
- Robinson G. E. (1987) Regulation of age polytheism by juvenile hormone. *Behav. Ecol. Sociobiol.* **12**, 329-338.
- Schafer S. and Rheder V. (1989) Dopamine-like immunoreactivity in the brain and subesophageal ganglion of the honey bee. *J. comp. Neurol.* **280**, 43-58.
- Schurmann F. W. and Klemm N. (1984) Serotonin-immunoreactive neurons in the brain of the honey bee. *J. comp. Neurol.* **225**, 570-580.
- Smith M. R. (1965) House-infesting ants of the eastern United States: their recognition, biology and economic importance. *USDA-ARS Tech. Bull.* **1326**, 1-105.
- Sokal R. R. and Rohlf J. J. (1981) *Biometry*, 2nd ed. W. H. Freeman and Co., San Francisco.
- Taylor D. J., Robinson G. E., Logan R. J., Laverty R. and Mercer A. R. (1992) Changes in brain amine levels associated with the morphological and behavioral development of the worker honeybee. *J. comp. Physiol.* **170A**, 715-721.
- Technau G. M. (1984) Fiber number in the mushroom bodies of adult *Drosophila melanogaster* depends on age, sex and experience. *J. Neurogenetics* **1**, 113-126.
- Ungar G. (1973) Molecular mechanisms in central nervous system coding. In *Macromolecules and Behavior* (Edited by Ansell G. B. and Bradley P.), pp. 24-52. University Park Press, Maryland.
- Vieira R. and Aldegunde M. (1993) Serotonin and its N-acetylated and acidic derivatives in insect brain as determined by high-performance liquid chromatography with electrochemical detection. *J. Entomol. Sci.* **28**, 16-24.
- Wilson E. O. (1971) *The Insect Societies*. Belknap Press, Harvard University, Cambridge, Massachusetts.
- Winston M. I. (1987) *The Biology of the Honey Bee*. Harvard University Press, Cambridge, Massachusetts.