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DANIEL WOJCIK

## ARGININE CONCENTRATION AND FREE AMINO ACIDS IN THE BRAIN OF VARIOUS WORKER SUBCASTES OF THE RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA* (HYMONOPTERA: FORMICIDAE)

FRED PUNZO\* and B. MICHAEL GLANCEY†

\*Department of Biology, Division of Science and Mathematics, University of Tampa, Tampa,  
FL 33606, USA (Tel: 813-253-3333); and

†Insects Affecting Man and Animals Laboratory, ARS, USDA, Gainesville, FL 32604, USA

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**Abstract**—1. The free (physiological) amino acid (FAA) composition of the brain of minor workers of the red imported fire ant (RIFA), *Solenopsis invicta*, was conducted as well as an analysis of brain arginine concentrations in various worker subcastes.

2. Nitrogenous waste products (urea and ammonia) together comprised 33% of the total ninhydrin-positive compounds.

3. GABA, taurine, aspartate, glutamate and glycine together constituted 24% of the total ninhydrin-positive compounds.

4. The most abundant FAA were alanine (16%), proline (12%), aspartate (7%) and glutamate (6%).

5. No significant differences in brain arginine concentrations were found within a given head size class, within a given worker subcaste or between various subcastes, suggesting that arginine does not represent a neurochemical correlate with respect to the developmental switch in the behavioral repertoire shown by *S. invicta* worker subcastes.

6. Brain arginine concentrations reported for *S. invicta* are compared with those reported for other arthropods.

### INTRODUCTION

The cellular and molecular bases of learning and memory, as well as other aspects of behavioral plasticity, have received a great deal of recent attention (see reviews by Bullock, 1984; Punzo, in press). Neurochemical correlates of learning such as increases in brain RNA and protein synthesis, first demonstrated in vertebrates (see reviews by Ungar, 1973; Glassman, 1975), have more recently been documented for molluscs (Pusztai and Adam, 1974), decapod crustaceans (Punzo, 1983a) and insects (Jaffe, 1980; Maldonado, 1980; Punzo and Jellies, 1980; Punzo, 1980, 1983a). There is also experimental evidence that such macromolecular syntheses somehow contribute to structural (degree of dendritic branching, number of synaptic connections) and functional properties of neurons that participate in learned responses, thereby rendering such neurons more effective (lowering thresholds) in later trials (Kandel and Schwartz, 1982). Therefore the importance of documenting specific neurochemical events within animal central nervous systems toward a more complete understanding of the behavioral acts which they accompany is obvious.

In addition to overall changes in brain RNA, protein and cholinesterase activity (ChE), which accompany the learning process, certain amino acids have also been shown to play an important role in CNS integration, functioning as putative neurotransmitters and/or modulators of pre- and post-synaptic events (see reviews by Murdock, 1971; Davidson, 1976; Klemm, 1976; Usherwood, 1978). Thus, although an analysis of the free amino acid

(FAA) composition of the brain and other regions of the CNS can provide a valuable initial step in the identification of amino acids that may mediate important neuronal events during behavioral acts, relatively little information is available on amino acid profiles specifically within arthropod nervous systems. FAA profiles have been reported for the peripheral nerve, cerebral ganglion and thoracic ganglion of the crab, *Carcinus maenas* (Evans, 1973), the brain of the mud crab, *Eurypanopeus depressus* (Punzo, 1983b), a few spiders (Meyer *et al.*, 1980; Punzo, 1983b), and insects such as praying mantids (Maldonado *et al.*, 1976), the stable fly, *Stomoxys calcitrans* (Holman and Cook, 1982), and the locust, *Schistocerca americana* (Punzo, 1983b). One of the most exciting recent developments has been the implication of the amino acid arginine in learning and memory consolidation in the praying mantis, *Stagmatoptera biocellata* (D'Alessio *et al.*, 1982). In this study a significant increase in brain arginine was found to accompany habituation learning. In addition, the injection of arginine prior to training completely abolished the normal amnesic effects of anoxia in this insect. This strongly suggests that arginine plays an important role in habituation learning/memory consolidation in this species. On the other hand, other amino acids such as lysine, alanine and histidine were unable to inhibit the amnesic effect of anoxia.

As a result of the work of D'Alessio *et al.* (1982), and the important implications thereof, we decided to investigate the FAA composition of the brain in the red imported fire ant (RIFA), *Solenopsis invicta*

Buren (Hymenoptera: Formicidae). This introduced species has become a serious pest throughout the southeastern United States and is noted for its aggressive behavior and painful sting [for a complete review of fire ant biology see Lofgren *et al.* (1975)]. This species has been the object of numerous and diverse control programs for over two decades due to its economic and medical importance. Indeed, in light of the recent realization by investigators in the fields of insect toxicology, integrated pest management and biological control concerning the potential significance that an understanding of insect brain chemistry holds toward the development of new and more efficient control strategies for insect pests in general (Worthy, 1984), coupled with the D'Alessio *et al.* (1982) study, the analysis of arginine as well as other FAA levels in the brain of *S. invicta* is an important initial step and logical starting point for the overall elucidation of brain chemistry in this species.

It must also be pointed out that *S. invicta* workers (worker subcastes) are characterized by an age polyethism (Mirenda and Vinson, 1981) that has been correlated with head capsule width (Wilson, 1978). These worker or behavioral subcastes consist of: (1) brood tenders (1–15 days after eclosion), which function primarily in feeding and tending the queen and brood; (2) foragers (+30 days), which actively forage for food outside of the nest; and (3) reserves (16–30 days), which transfer food from the returning foragers to the brood tenders. Within each behavioral subcaste can be found a range of head capsule widths as described by Mirenda and Vinson (1981): minor workers (0.55–0.75 mm), media workers (0.78–1.31 mm) and major workers (>1.31 mm).

In view of the recent advances concerning neurochemical correlates of behavioral plasticity in arthropods (see review by Punzo, in press) and the implication of arginine as an important requisite for memory consolidation in mantids, as well as the ontogenetic switch in behavioral preference shown by worker subcastes of *S. invicta*, the present study was conducted in order to:

- (1) analyze the FAA composition of the brain in minor workers;
- (2) establish baseline data for the concentration of arginine in the brains of each behavioral worker subcaste: brood tenders (BT), reserves (R) and foragers (F);
- (3) ascertain whether there are any significant differences in arginine concentrations between the behavioral subcastes that might account for differences in behaviour; and
- (4) compare brain arginine concentrations for *S. invicta* with those previously reported for other arthropods.

#### MATERIALS AND METHODS

*Solenopsis invicta* workers were obtained from laboratory colonies originally obtained from the USDA (Insects Affecting Man and Animals Research Laboratory, ARS) fire ant project, Gainesville, FL. Colonies were maintained in Williams cells (Bishop *et al.*, 1980) at  $25 \pm 2^\circ\text{C}/65 \pm 3\%$  RH, on a diet of honey-water, dipteran pupae and cockroaches. For a detailed description of rearing methods see

Table 1. Physiological amino acids in the brain of minor workers\* of the red imported fire ant, *Solenopsis invicta*

Compound	Mean brain concentration† (nmol/mg) $\pm$ SD	% total
Urea	130.9 $\pm$ 21.6	25
GABA	15.7 $\pm$ 3.8	3
Taurine	31.4 $\pm$ 6.7	6
Ornithine	0.37 $\pm$ 0.02	
Ammonia	45.1 $\pm$ 4.3	8
Arginine	15.5 $\pm$ 0.9	3
Aspartate	36.8 $\pm$ 7.1	7
Glutamate	32.1 $\pm$ 3.8	6
Glycine	12.1 $\pm$ 2.1	2
Alanine	81.7 $\pm$ 18.1	16
Histidine	7.4 $\pm$ 2.1	1
Isoleucine	2.8 $\pm$ 0.5	
Leucine	3.1 $\pm$ 0.3	
Lysine	2.0 $\pm$ 0.1	
Methionine	1.6 $\pm$ 0.7	
Phenylalanine	0.02 $\pm$ 0.01	
Proline	62.3 $\pm$ 8.4	12
Serine	24.1 $\pm$ 6.2	4
Threonine	2.5 $\pm$ 0.6	
Tyrosine	1.1 $\pm$ 0.1	
Valine	2.4 $\pm$ 0.3	
Total	510.9	

\*Based on head capsule width (0.55–0.75 mm) (Mirenda and Vinson, 1981).

†Values represent concentrations (nmol/mg tissue) for 100 brains based on dry weights:  $0.037 \pm 0.001$  mg/brain. Only values > 1% are shown.

Bishop *et al.* (1980) and Mirenda and Vinson (1981). This method allows for easy identification and access to BT, R and F behavioral subcastes.

#### Free amino acid analysis

FAA determinations were carried out as previously described by Punzo (1983b, c). One hundred brains were pooled for FAA analysis. Brains were removed in physiological saline according to the procedure described by Maldonado *et al.* (1976), transferred to a 1% picric acid solution and frozen for not more than 96 hr before analysis. After thawing, samples were homogenized in a glass tissue homogenizer, transferred to centrifuge tubes and centrifuged at 2000 rpm for 20 min in a Sorvall model RC-5B high-speed centrifuge. The supernatant was evaporated to a residue and analyzed as total ninhydrin-positive material at 570 nm against a glutamate standard using a Bausch & Lomb Spectronic 20 spectrophotometer. Two-dimensional HPTLC was performed using Whatman LHP-KF 20  $\times$  10 cm plates (Whatman Chemical Separation, New Jersey) using chloroform-ethyl acetate (90:10 v/v) in one direction and chloroform-ethanol-acetic acid (90:10:2 v/v) in the other. Individual amino acid spots were visualized with ninhydrin and identified by their  $R_f$  values as described by Sherma (1981). FAA concentrations were expressed as nmol/mg tissue based on dry tissue weights (Table 1). To determine dry weights, freshly dissected brains were dried in a desiccator and weighed at 24-hr intervals. When the same weight was recorded for two consecutive trials, this value was taken as the dry tissue weight (Holman and Cook, 1982).

#### Arginine determinations

Ants were killed in a  $\text{CO}_2$ -acetone mixture and the brains removed as described by Maldonado *et al.* (1976). Groups of five brains were pooled from each behavioral subcaste and homogenized in 1 ml of distilled water in a glass homogenizer (A.H.T. Co., Philadelphia, PA) provided with a motor-driven Teflon pestle. Ten replications were conducted for each pooled sample for all behavioral subcastes (see Table 2). Homogenate samples were centrifuged at 80,000 g for 1 hr as described by Punzo (1983b). Independent arginine determinations were made from the super-

Table 2. Concentrations of arginine (nmol/mg tissue) in the brain of various worker subcastes of *Solenopsis invicta*. Values based on dry tissue weights (brain weights:  $0.037 \pm 0.001$  mg/brain)

Samples*	Brood tenders			Reserves			Foragers		
	0.75†	1.0	1.5	0.75	1.0	1.5	0.75	1.0	1.5
1	18.6	16.4	12.7	17.5	20.2	15.7	14.3	13.8	19.3
2	20.4	19.1	16.6	15.7	17.2	18.6	17.2	19.8	16.3
3	14.5	16.7	21.4	14.6	18.5	13.7	15.8	14.7	16.2
4	17.3	20.5	12.7	15.9	13.2	17.9	16.9	13.4	17.1
5	12.7	16.2	18.2	19.6	13.1	14.7	19.1	17.4	13.5
6	16.8	15.2	13.8	13.9	15.8	17.5	16.6	14.7	18.1
7	19.2	12.5	14.4	15.8	11.5	14.3	16.2	10.8	17.1
8	14.1	16.9	10.2	18.1	17.3	20.2	12.4	14.1	16.3
9	18.7	11.3	13.5	11.5	16.4	9.8	10.7	15.4	10.3
10	12.2	15.8	14.5	18.6	8.2	15.5	17.3	9.7	13.8

\*Each sample (1-10) represents a pool of five ant brains.

†Head capsule widths in mm: 0.75, 1.0, 1.5 (Wilson, 1978).

nantant (test solution) according to the Sakaguchi procedure as reported by Tomlinson and Viswanatha (1974). This technique allows for reliable determination of arginine concentrations as low as  $0.1 \mu\text{M}/\text{sample}$ . One milliliter of 0.1% naphthol (in 50% ethanol) and 10% KOH were added to 1 ml of the test solution and mixed in a warm water ( $25^\circ\text{C}$ ) shaker bath. One milliliter of 5% urea was added with continuous shaking, followed by the addition of 2.0 ml of potassium hypobromite. A reagent blank was prepared simultaneously using distilled water. After allowing the test solution to stand for 20 min at room temperature, the percentage absorbance at 520 nm was measured on a Bausch & Lomb Spectronic 20 spectrophotometer. The arginine content of the test solution was determined by comparing its percentage absorbance against a standard reference curve for a  $1.0 \mu\text{M}/\text{l}$  stock solution of arginine. Brain arginine concentrations were expressed as nmol/mg tissue as described by Punzo (1982, 1983c).

The data were analyzed using analysis of variance (ANOVA) according to the general linear model procedure of SAS (1982). A Waller-Duncan  $K$ -ratio  $t$  test was used to test the main effects on the means.

#### RESULTS AND DISCUSSION

The physiological (FAA) amino acids found in the brains of RIFA minor workers as well as other ninhydrin-positive compounds are listed in Table 1. Ammonia and urea together accounted for 33% of the total ninhydrin-positive compounds. In comparison, these nitrogenous waste products accounted for 20% in the brains of the mud crab, *Eurypanopeus depressus*, 32% in the locust, *Schistocerca americana*, and 53% in the wolf spider, *Lycosa avida* (Punzo, 1983b). Holman and Cook (1982) reported values of 31% (brain) and 26% (thoracic ganglion) for the stable fly, *Stomoxys calcitrans*. These studies indicate that urea and ammonia are present in higher concentrations in terrestrial insects and arachnids than in marine decapods.

GABA, taurine, aspartate, glutamate and glycine, which have been shown to function as putative neurotransmitters in animal central nervous systems (Davidson, 1976), together constituted 24% of the ninhydrin-positive compounds found in the brain of *Solenopsis invicta*, Table 1). These putative neurotransmitters accounted for 25% in *S. americana* (Punzo, 1983b), 25% in *S. calcitrans* (Insecta) (Holman and Cook, 1982), 28% in *L. avida* (Arachnida) and 43% in the decapod, *E. depressus* (Punzo, 1983b). Aspartate and glutamate are among the most abundant FAA in the mammalian brain, and glutamate

has been implicated in playing an important role as an excitatory synaptic transmitter in the CNS of invertebrates (Kravitz *et al.*, 1970) and vertebrates (Davidson, 1976). GABA has been shown to function as an inhibitory synaptic transmitter in crustacean peripheral stretch receptor neurons (Davidson, 1976) as well as in the CNS of arthropods, molluscs and vertebrates (see reviews by Klemm, 1976; Usherwood, 1978). There is evidence that taurine and glycine function as potent inhibitors in mammalian spinal cord motoneurons (Sieghart and Karobath, 1974), although their role in invertebrates is largely unknown. Evans (1973) reported that taurine comprised 30% of the FAA in the CNS of the crab, *Carcinus maenas*. This is in agreement with the high levels of taurine found in the CNS of the spiders, *Marpissa muscosa*, *Araneus diadematus* and *Pardosa amentata* (Meyer *et al.*, 1980). High taurine concentrations have also been correlated with morphological changes in the brain occurring during pupal-adult metamorphosis in the armyworm, *Mamestra configurata* (Bodnaryk, 1981).

The arginine concentrations found in the brains of various worker subcastes are listed in Table 2. Analysis of variance indicated that there were no significant differences in arginine concentration within a given head size class ( $F = 0.86$ ,  $df = 2/29$ ), within a given subcaste ( $F = 0.26$ ) or between the various subcastes ( $F = 0.85$ ). This strongly suggests that although arginine is a correlate of habituation learning in mantids (D'Alessio *et al.*, 1982), it apparently is not correlated with the developmental switch in behavioral repertoires shown by *S. invicta* worker subcastes. Differences in brain arginine concentrations cannot account for the behavioral shift from brood/queen tender to reserve to forager. As previously discussed, amino acids such as glycine, glutamate and aspartate (Davidson, 1976), as well as other ninhydrin-positive compounds, including taurine and GABA (Usherwood, 1978), have been shown to function as neurotransmitters in vertebrate CNS integration and may well serve a similar function in invertebrates. Neurochemical changes such as this may be correlated with concomitant changes in behavior. The direct effects of certain amino acids on neural events at the cellular level have been well documented in vertebrates (see review by Davidson, 1976). Dicarboxylic amino acids (L-glutamic, aspartic and cysteic acids), for example, have pronounced excitatory effects on nerve cells whereas neutral

Table 3. Arginine concentration in the central nervous system of various arthropods

Species	Tissue	Arginine concentration (nmol/mg) $\pm$ SD	Reference
Insecta			
Hymenoptera			
<i>Solenopsis invicta</i> *	brain	15.5 $\pm$ 0.2	
Orthoptera			
<i>Schistocerca americana</i>	brain	21.4 $\pm$ 3.8	Punzo (1983b)
Diptera			
<i>Stomoxys calcitrans</i>	brain	20.6 $\pm$ 3.7	
	thoracic ganglion	51.1 $\pm$ 22.3	Holman and Cook (1982)
Arachnida (Aranea)			
<i>Lycosa avida</i>	brain	2.1 $\pm$ 0.2	Punzo (1983b)
Crustacea (Decapoda)			
<i>Eurypanopeus depressus</i>	brain	11.8 $\pm$ 1.0	Punzo (1983b)
<i>Carcinus maenas</i>	brain	10.7 $\pm$ 0.8	
	thoracic ganglion	11.2 $\pm$ 0.5	Evans (1973)

\*Values represent the mean  $\pm$  SD for all behavioral (worker) subcastes: brood tenders, reserves and foragers.

amino acids (glycine, taurine) show widespread depressant effects. It has been suggested that some amino acids in animal brains may serve to regulate or modulate the 'readiness' of nerve cells and their subsequent ability to respond to conventional neurotransmitters (Evans, 1973; Davidson, 1976; Maxwell *et al.*, 1978). It is not difficult to extrapolate these effects at the cellular level to effects at the organismal level. In addition, changes in metabolic pathways of amino acid synthesis within the CNS may have profound effects on individual neurons, clusters of nerve cells and overall behavior patterns of animals (Murdock, 1971; Ungar, 1973; Davidson, 1976). General changes in the concentrations of amino acids in the hemolymph have been shown to occur in insects as a function of development (Tomoda *et al.*, 1976; Bradfisch and Punzo, 1977), and as a result of insemination in queens of *S. invicta* (Toom *et al.*, 1976). However, it is not known to what extent these changes in hemolymph amino acid profiles reflect alterations in the FAA composition of the CNS. Future studies on the concentration of dicarboxylic and neutral amino acids in the brains of worker subcastes of *S. invicta* may provide additional valuable information and insight toward an understanding of possible neurochemical events correlated with behavioral changes in this species. The role of arginine in memory consolidation in mantids may represent a relatively restricted, species-specific function. So little is known concerning general levels of amino acids in arthropod nervous systems that species comparisons across various behavioral acts are difficult to assess at this time.

Table 3 shows a comparison of the brain arginine content of *S. invicta* workers with arginine concentrations reported for the CNS of other arthropods. Although comparisons across species are few, spiders appear to have rather low arginine concentrations. Meyer *et al.* (1980) reported arginine concentrations of less than 0.07 nmol/ml from the CNS of six species of spiders. Arginine concentrations are markedly higher in decapod crustaceans and insects. However, the functional significance of this, if any, is as yet unknown. Although arginine has not been implicated as a putative neurotransmitter *per se*, its role in memory consolidation in praying mantids (D'Alessio *et al.*, 1982) may be related to general protein metabolism and the increased proteinemia within the CNS

associated with locomotor activity previously reported for this species (Maldonado *et al.*, 1976).

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