

RECENT ADVANCES IN BEHAVIORAL PLASTICITY  
IN INSECTS AND DECAPOD CRUSTACEANS

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## SYNOPSIS

Although insects and other arthropods have traditionally been viewed as nonlearning creatures of instinct, many of their behaviors can be modified by experience. Recent interest in this subject has focused on the adaptive significance of behavioral plasticity. A valuable "spin-off" has been the use of learning in experiments to better understand the functioning of sense organs. Neurochemical correlates of learning in the grasshopper, *Schistocerca americana* and the mud crab, *Eurypanopeus depressus* include a significant increase in brain RNA and protein synthesis and a decrease in cholinesterase activity. Increase in RNA synthesis is associated with the corpora pedunculata and protocerebral bridge in insects, and the protocerebral bridge and central body in decapod crustaceans. This suggests that the function of the corpora pedunculata in the insect brain may be served by the central body in crustaceans.

"..... in our emotional estimate of the lower and the higher, we are also swayed by the potential ability of an organism to acquire new information ..... in the whole world, there is hardly a system more complicated than the central nervous organization underlying the behavior of an animal ..... One of the greatest achievements of phylogeny is to have constructed systems of this sort in such a way that they are still adaptively modifiable by an input occurring during the individual's life" (Konrad Lorenz 1969).

## INTRODUCTION

Neuroethological investigations in conjunction with advances in ecology over the last decade have demonstrated the importance of behavioral plasticity in many diverse animal groups (Davies and Krebs 1978, Ewert 1980). The ability of an organism to modify its behavioral response to varying environmental conditions is often essential to its survival (Alloway 1973, Punzo 1984, 1983a, 1980a). Learning is generally defined as adaptive changes in individual behavior occurring as the result of previous experience (Thorpe 1944, Alloway 1972).<sup>1</sup>

Insects and other arthropods have traditionally been viewed as non-learning creatures of instinct, characterized by relatively rigid and stereotyped behavior patterns (Chauvin 1947, Thorpe 1963). Nevertheless, although many insect behavioral repertoires are endowed with a rigidity of structure characteristic of Tinbergen's Fixed Action Patterns (1951)<sup>2</sup>, there have been many studies showing that the behavior of insects and other arthropods can be modifiable (see reviews by Schneirla 1951, Thorpe 1963, Corning and Lahue 1972, Alloway 1973, Krasne 1973). Upon closer inspection

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tion it should not surprise us that when changing environmental conditions alter the suitability of a behavioral response, some insects are able to adjust through fairly rapidly learned modifications of behavior.

Traditional categories of learning such as habituation (Alloway 1972, Eisensten and Peretz 1973), avoidance learning (Pritchatt 1968, Alloway 1972, Punzo 1974, 1980b, 1983a), trial-and-error learning (Thorpe 1944, Alloway 1973, Maldonado and Tablante 1975, Punzo 1980a), latent learning (Schneirla 1953, Lindauer 1967, Dyal and Corning 1973) and classical conditioning (Tekeda 1961, Nelson 1971, Corning and Lahue 1972, Alloway 1973) have been found in insects. In addition, the capacity for correcting behavior (Dingle 1962, Akre 1964), imprinting and olfactory conditioning (Thorpe 1963, Vowles 1964), reversal learning (Alloway 1973) and shock-avoidance learning in headless insect preparations (Horridge 1962) have been demonstrated as well.

In recent years, investigators interested in behavioral plasticity have been concerned generally with the adaptive significance of learning (Menzel and Wyers 1981, Pulliam 1981, Punzo 1984, Stanton 1984). The contributions of behavioral ecologists and neurobiologists have made it clear that any analysis concerning the adaptive significance of behavior must take into account: 1) the evolutionary history of the behavior; 2) processes related to the development and or modification (learning) of the behavior under present conditions; and 3) the environmental setting in which the behavior occurs. The ability of an organism to learn can also be used to obtain answers to questions concerning how the organism perceives its world, the role of CNS integration in the learning process (molecular mechanisms of learning), and what specific regions of the CNS are involved in the control of learning and memory (localization of brain function). It is in these areas that some of the most recent and interesting discoveries have been made.

#### LEARNING AND PERCEPTION IN A PRIMITIVE INSECT: ECOLOGICAL IMPLICATIONS

Despite the evidence for learning in insects, most research has been performed on relatively few species (Alloway 1973, Punzo 1980b) and has often dealt with what psychologists refer to as parameters of learning, such as the nature of reinforcement and the mechanisms of reward and motivation (Bitterman 1960, Corning and Lahue 1972, Ewert 1980). From a comparative viewpoint, however, the investigation of general trends and patterns of learning has been lacking. Thus, there is little or no information on the learning capacities of most insect and other arthropod species. Until my work (Punzo 1980a) on the silverfish, *Lepisma saccharina* (Thysanura: Lepismatidae) for example, no data were available on the Apterygota. I was interested not only in demonstrating the ability of this insect to learn, but also in determining the principle sensory modalities involved. One must know how a particular animal "perceives its world" in order to draw any significant conclusions about the possible adaptive significance of the behavior in question. Since this insect is a nocturnal forager (Smith 1970), I used a maze learning paradigm<sup>3</sup> with light as an aversive stimulus. Insects obtained randomly from laboratory stock cultures were placed in a darkened start chamber (Fig. 1, S), exposed to a cool fluorescent light source and then allowed to travel the maze until the darkened goal chamber (Fig. 1, G)

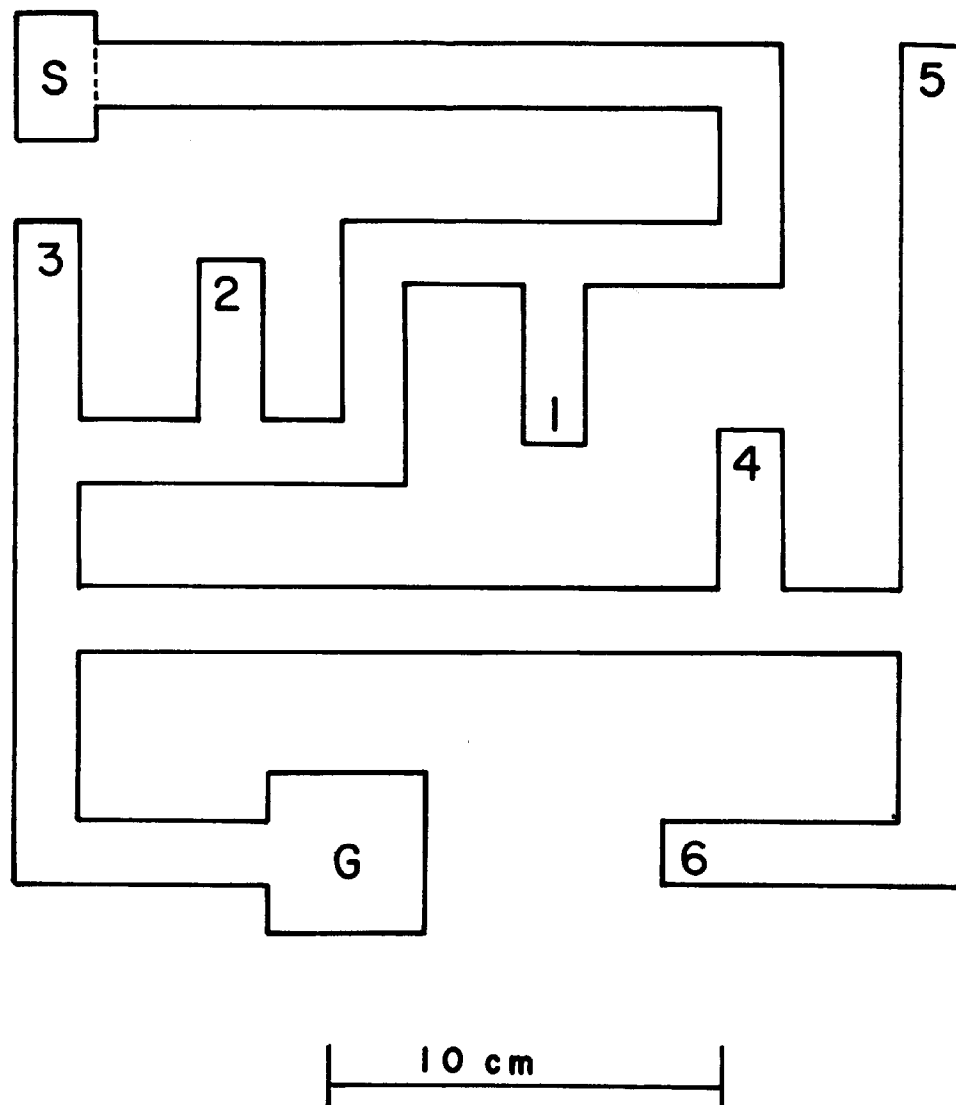


Fig. 1. Diagrammatic representation of the maze used to study learning in *Lepisma saccharina*. See text for explanation.

was reached. The number of blind alley errors for each trial was recorded. For a detailed description of the experimental procedure see Longo (1964) and Punzo (1980a). Five experimental groups were tested, each consisting of 10 insects. Group 1 insects (controls) were individually isolated in glass petri dishes and maintained under normal rearing conditions. Group 2 insects had both compound eyes covered with an opaque varnish (see Chauvin 1947).<sup>4</sup> The median caudal filament of Group 3 insects was removed between the first and second segments. Insects in Group 4 had only the cerci removed in a similar fashion. The antennae of Group 5 insects were removed at the joint between the pedicel and flagellum. Following these preparations, all insects were maintained individually in glass petri dishes and observed closely for one week prior to testing. Only those that remained healthy were used.<sup>5</sup>

The results for acquisition (learning curve, A) and extinction (return to variable behavior, E) procedures for Group 1 controls (all sense organs intact) are shown in Fig. 2. This represents the first demonstration of trial-and-error maze learning for the Thysanura. The criterion for learning (see Appendix) was attained after 13-14 days. The improvement in performance over 14 days was highly significant ( $F = 400.86$ ,  $df = 9$ ,  $p < .0001$ ). The results obtained for extinction (Fig. 2E) show a significant deterioration in performance after two days.

What are the ecological implications of learning in this species? Since *L. saccharina* is an active forager, "learning" search routes to food sources can optimize foraging by decreasing the expenditure of energy used to locate food and thereby increase its assimilation efficiency and survival capacity (Pulliam 1981). Silverfish are normally found in leaf litter, be-

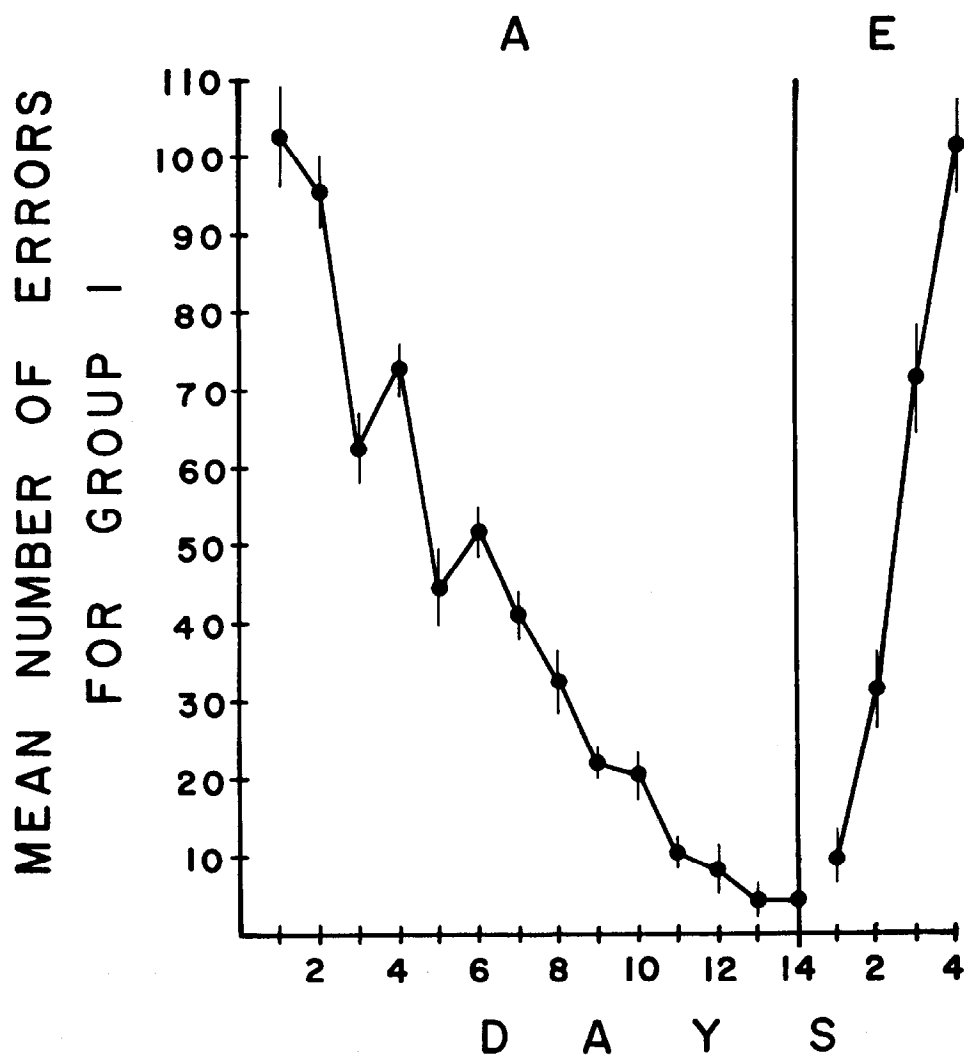


Fig. 2. Acquisition (A) and extinction (E) curves for *Lepisma saccharina*. Data points represent the mean number of blind-alley errors for 10 insects. Vertical lines represent  $\pm 1.0$  S.E.M. (data from Punzo 1980a).

neath rocks, within decaying logs and in buildings where they feed on decaying vegetation or plant products, paste and glue (Smith 1970).

The results obtained for *L. saccharina* after specific sense organs were removed (Fig. 3) show that those insects lacking cerci (Group 4) or antennae (Group 5) exhibit less proficiency in learning the correct maze route. On the other hand, there was no statistically significant difference among groups of insects lacking compound eyes (Group 2), a median caudal filament (Group 3) and intact controls (1). This evidence suggests that tactile and chemical cues play a more important role in foraging and avoidance behavior than do visual cues. Tactile and chemical sense organs are vital to an animal that frequents crevices found in soil, bark, rocks and buildings. Insects lacking antennae (Fig. 3, Group 5) or cerci (Group 4)

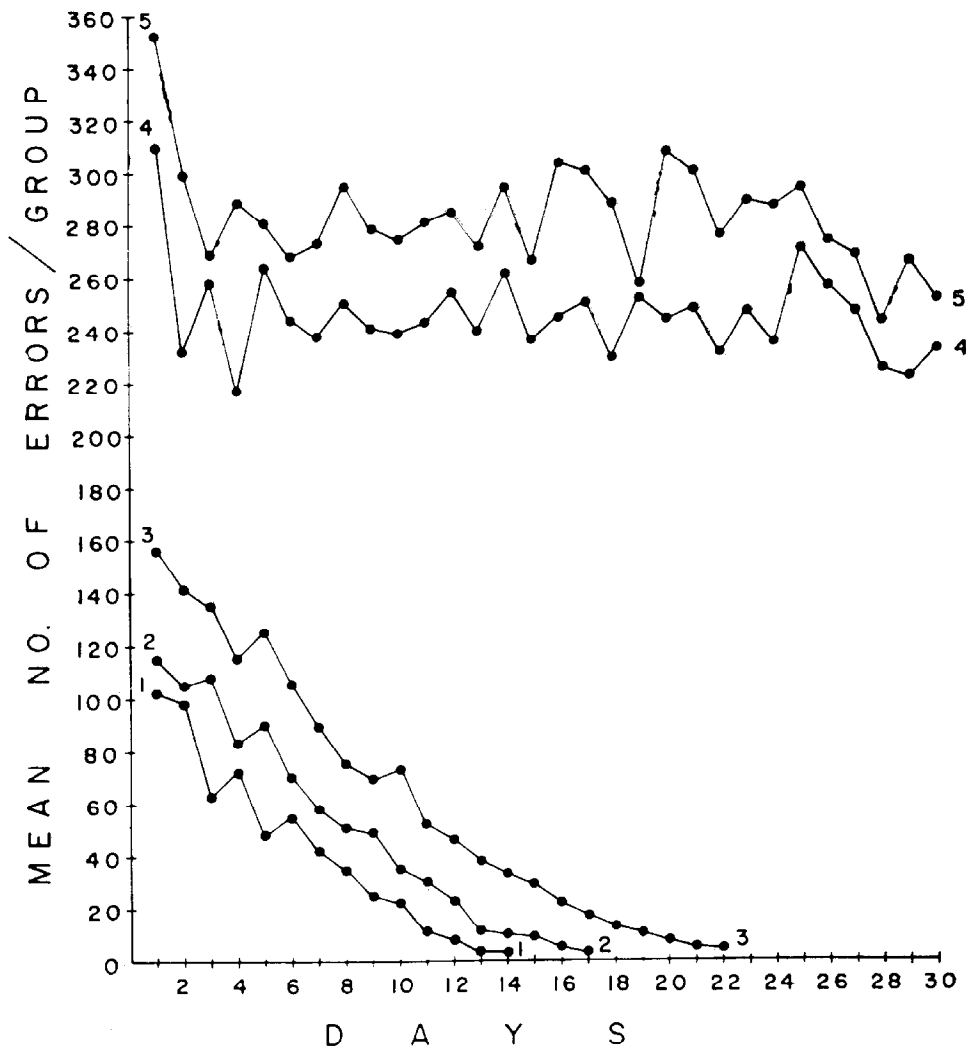


Fig. 3. Learning curves for *Lepisma saccharina* experimental groups. Data points represent the mean number of blind-alley errors for insects lacking functional compound eyes (Group 2), median caudal filament (Group 3), cerci (Group 4), antennae (Group 5), and control insects with all sense organs intact (Group 1) (data from Punzo 1980a).

showed no significant improvement in performance over a 30-day period, suggesting that olfactory and mechanoreceptors of the cerci are essential for maze learning and foraging behavior in this species.<sup>6</sup>

These results are consistent with the few previous reports present in the literature. The relatively rapid extinction shown by *L. saccharina* (Fig. 1) is also consistent with the rapid extinction of learned responses reported for roaches (Chauvin 1947, Longo 1964), honeybees (Menzel 1968) and grain beetles (Alloway 1972), and appears to be indicative of invertebrates in general (Corning and Lahue 1972, Dyal and Corning 1973, Krasne 1973). It should be noted that the maze utilized for *L. saccharina* is more complex than those generally employed for insect maze studies (see Thorpe 1963, Alloway 1972) with the exception of those used with some ants (see Stratton and Coleman 1973). The level of performance exhibited by *L. saccharina* suggests a rather well-developed capacity for learning even in insects of this primitive group.

#### NEUROCHEMICAL CORRELATES OF LEARNING AND LOCALIZATION OF BRAIN FUNCTION IN ARTHROPODS: ECOLOGICAL AND BEHAVIORAL IMPLICATIONS

The analysis of neurochemical changes associated with learning and their possible role in our understanding of molecular mechanisms of learning and memory and localization of brain function with respect to behavioral plasticity have received a great deal of attention over the last decade (see Davis 1976, Punzo 1983b, 1984). Biochemical alterations within the CNS such as increases in RNA and protein synthesis have been correlated with learning in vertebrates (see Hyden 1973, Glassman 1975, Punzo 1984) and invertebrates (see Punzo 1980b, 1983a). There is also substantial evidence that biochemical changes (macromolecular synthesis and amino acid metabolism) may somehow alter the structural (degree of dendritic branching, number of synaptic connections) and functional (postsynaptic thresholds) properties of neurons that participate in a learned behavior, thereby rendering such neurons more effective (lowering thresholds) in later trials (see Pusztaï and Adam 1974, Glassman 1975, Punzo 1984). It has been shown that information resulting from past experience that enters the brain (CNS) in the form of bioelectrical signals can initiate the synthesis of RNA and proteins that may function in the coding of such information. Although vertebrates have received the most attention, there is experimental evidence for the *in vivo*, *de novo* synthesis of informational macromolecules during learning in molluscs (Pusztaï and Adam 1974), decapod crustaceans (Punzo 1983a) and insects (Oliver et al. 1971, Kerkut et al. 1972, Maldonado 1980, Punzo 1980b). The disruption of learning and memory by inhibitors of protein synthesis such as cycloheximide also suggests a correlation between macromolecular synthesis and consolidation of memory (Borraco and Stettner 1976, Jaffe 1980, Punzo and Jellies 1980, Punzo 1983a). The following examples from my work will emphasize some of the ideas previously discussed.

#### NEUROCHEMICAL CORRELATES OF LEARNING

Adult males of the grasshopper, *Schistocerca americana* were obtained from a laboratory colony maintained at 28°C on a 12L : 12D photoperiod

regime. During training and testing periods, each insect was mounted in a restraining collar as described by Goldsmith et al. (1978). The experimental procedure used to train the insects is shown diagrammatically in Fig. 4.<sup>7</sup>

Ten hoppers were randomly assigned to experimental (P) or yoked-control groups (R) as previously designated by Goldsmith et al. (1978). P insects received a shock whenever they extended their metathoracic leg and contacted a salt solution. The R insect received a shock with the P insect regardless of its leg position. The total number of shocks was recorded over a 15-min period. Following this, the insects were left unstimulated for one hour and retested (retest P, retest R groups) for 15 min in order to assess their retention (see Fig. 5). The results are shown in Fig. 5. This species is clearly capable of avoidance learning<sup>8</sup> as reflected in the significant decrease in number of shocks.

Other experiments evaluated the magnitude of some of the important neurochemical changes associated with shock avoidance learning in the brains of *S. americana* (Punzo 1980b) as well as the mud crab, *Eurypanopeus depressus* (Crustacea: Decapoda) (Punzo 1983a). Of primary interest were changes in the concentrations of RNA and protein as well as changes in cholinesterase activity (ChE).<sup>9</sup>

A summary of these neurochemical correlates of learning is shown in Table 1. It is evident from these data that learning in both decapod crustaceans and insects is correlated with significant increases in the synthesis of brain RNA and protein and a decrease in ChE activity. The observed increases in RNA and protein further support the importance of macromolecular synthesis to the overall learning process (see Hyden 1973, Glassman 1975) and is consistent with what little information is available for insects (Kerkut et al. 1972, Jaffe 1980, Maldonado 1980). The correlation between brain ChE activity and learning is not clearly understood in vertebrates (Glassman 1975) and poorly documented at best in invertebrates (Oliver et al. 1971, Kerkut et al. 1972, Willner and Mellanby 1974). The decrease in ChE activity exhibited by *S. americana* and *E. depressus* (Table

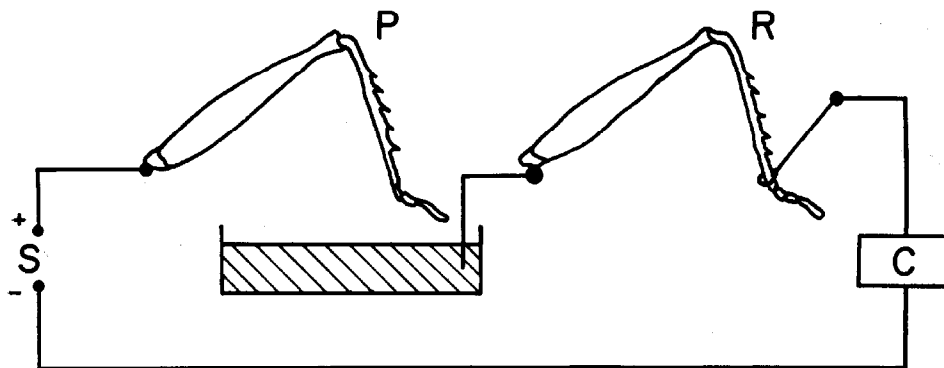


Fig. 4. Diagrammatic representation of the metathoracic legs of an experimental (P) and yoked-control (R) insect pair during training. The P leg receives a shock when it contacts the salt solution (stipled area) and completes a circuit so that the R leg also receives a shock. Power is supplied by a stimulator (S) and the number of shocks during training is recorded on an automatic counter (C). This procedure was adopted from Goldsmith et al. (1978).

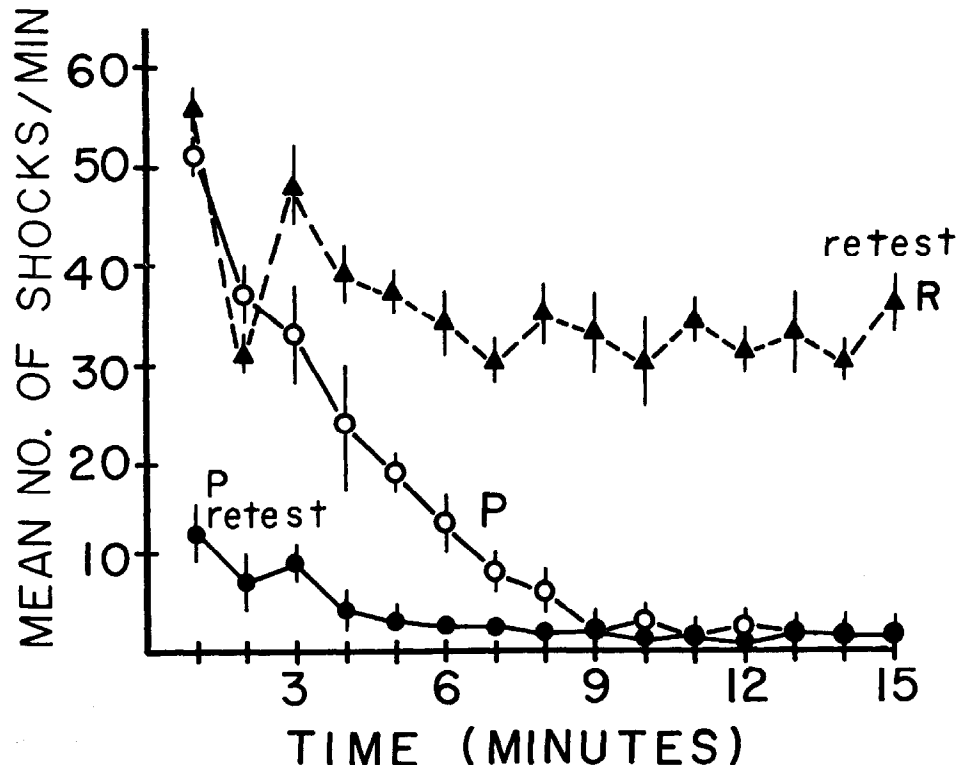


Fig. 5. Shock-avoidance learning for experimental (P), and experimental retest (retest P) and yoked-control retest (retest R) *Schistocerca americana* adults. Data points represent the mean number of shocks for 10 insects over a 15-min training period. Vertical lines represent  $\pm 1.0$  S.E.M. No significant difference was found between the performance of R insects during their initial training period with that exhibited during their retest period and only the retest R group is shown in the figure (Data from Punzo 1980b).

1) lends support to the notion that depressed ChE activity may increase synaptic efficiency in those neuronal pathways involved in the learning process (see Hyden 1973, Ewert 1980).

The importance of RNA and protein synthesis to the overall ability of an animal to modify its behavior based on past experience is also supported by studies showing that inhibition of protein synthesis within the CNS can significantly impair learning and retention capabilities. Cycloheximide—induced inhibition of protein synthesis resulted in a significant impairment of the learning process in praying mantids, grasshoppers and decapod crustaceans (see Jaffe 1980, Punzo 1980b, 1983a). In addition to the effect of protein synthesis inhibition on learned behaviors, recent studies have also demonstrated that macromolecular synthesis is important for the expression of innate responses as well. A fundamental question that has been asked metaphorically and never tested experimentally, concerns the functional relationship (if any) between instinct and the retrieval of learned information. Molecular mechanisms that could serve as models for the storage of information in nervous systems should also be involved in experiential learning and instinctive behavior and thus have access to the genome.<sup>9</sup> Punzo and Jellies (1980) showed that cycloheximide induced protein syn-



TABLE 1. NEUROCHEMICAL CORRELATES OF LEARNING IN THE BRAINS OF *Schistocerca americana* (INSECTA) AND *Eurypanopeus depressus* (DECAPODA). VALUES FOR INCORPORATION OF H<sup>3</sup>-LABELED URIDINE (RNA) AND LEUCINE (PROTEIN) ARE EXPRESSED AS COUNTS/MIN/ $\mu$ g. CHOLINESTERASE (ChE) ACTIVITY EXPRESSED AS  $\mu$ mol ACETATE RELEASED/HR/100  $\mu$ g PROTEIN (DATA REVISED FROM PUNZO 1980b, 1983a).

Species	RNA <sup>a</sup>	Protein	ChE activity	p <sup>b</sup>
<i>S. americana</i>				
Nontrained controls	370 $\pm$ 7	79 $\pm$ 3	4.30 $\pm$ 0.12	NS 0.01
Yoked-controls (R)	391 $\pm$ 10	83 $\pm$ 6	4.10 $\pm$ 0.06	
Experimental (P)	441 $\pm$ 9	99 $\pm$ 4	3.21 $\pm$ 0.14	
<i>E. depressus</i>				
Nontrained controls	429 $\pm$ 4	91 $\pm$ 7	7.63 $\pm$ 0.03	NS 0.01
Yoked-controls (R)	437 $\pm$ 2	97 $\pm$ 3	7.51 $\pm$ 0.14	
Experimental (P)	519 $\pm$ 8	123 $\pm$ 4	4.87 $\pm$ 0.07	

<sup>a</sup>All values represent means for 10 animals plus or minus standard error.

<sup>b</sup>Level of significance computed using a Student t test. NS = not significant.

thesis inhibition resulted in a significant impairment of the innate phototactic responses of the beetles *Tenebrio molitor* and *Popilius disjunctus*, though other behaviors (motility and feeding) and metabolism appeared normal. Davis (1976) suggested that the neural circuitry of nervous systems is determined by the genotype and that an individual organism has certain behavioral limits as well as predispositions. However, there is evidence that synaptic connections for many neural pathways are not completely functional or "soldered" at birth (Hyden 1973). That is, though not all neural (synaptic) connections are specified, those responsible for mediating innate behaviors may be. The neuronal circuitry must be persistent enough to account for the encoding of phylogenetic memory (instinct) and the resultant behaviors elicited toward specific stimuli without previous experience. The mechanism of protein synthesis provides a tenable hypothesis for the soldering of such synaptic connections. If informational macromolecules can determine the dynamics of pre- and post-synaptic membrane relationships, then it is possible that the continued manifestation of certain behavioral responses (including innate behaviors) is dependent upon the periodic synthesis of such molecules.

#### LOCALIZATION OF BRAIN FUNCTION

The analysis of macromolecular synthesis associated with learning provides an excellent method to study localization of brain function. There is little known about the localization of brain function in arthropods, though there have been studies on behavioral deficits resulting from brain lesions or the elicitation of specific behaviors via electrical brain stimulation (Vowles 1964, Krasne 1973, Punzo 1983a).

Following the shock-avoidance technique discussed above, levels of incorporation of H<sup>3</sup>-labeled uridine into RNA in specific brain regions of

*S. americana* and *E. depressus* were determined.<sup>10</sup> The results obtained for *S. americana* (Fig. 6), show that significant increases in RNA synthesis associated with learning are localized in the protocerebrum<sup>11</sup>, especially in the corpora pedunculata and protocerebral bridge. No significant increases in RNA were found in the central body of the protocerebrum or in the deutocerebral or tritocerebral brain regions. In similar studies on the decapod brain (Punzo 1983a), which is characterized by the absence of corpora pedunculata, significant increases in RNA synthesis were found in the protocerebral bridge and central body. This suggests that the function of the central body in the decapod brain may be served by the corpora pedunculata in the more advanced Insecta. In any event, these studies represent the first analysis of localized brain regions associated specifically with learning in arthropods, and demonstrate the importance of the protocerebrum in the learning process.

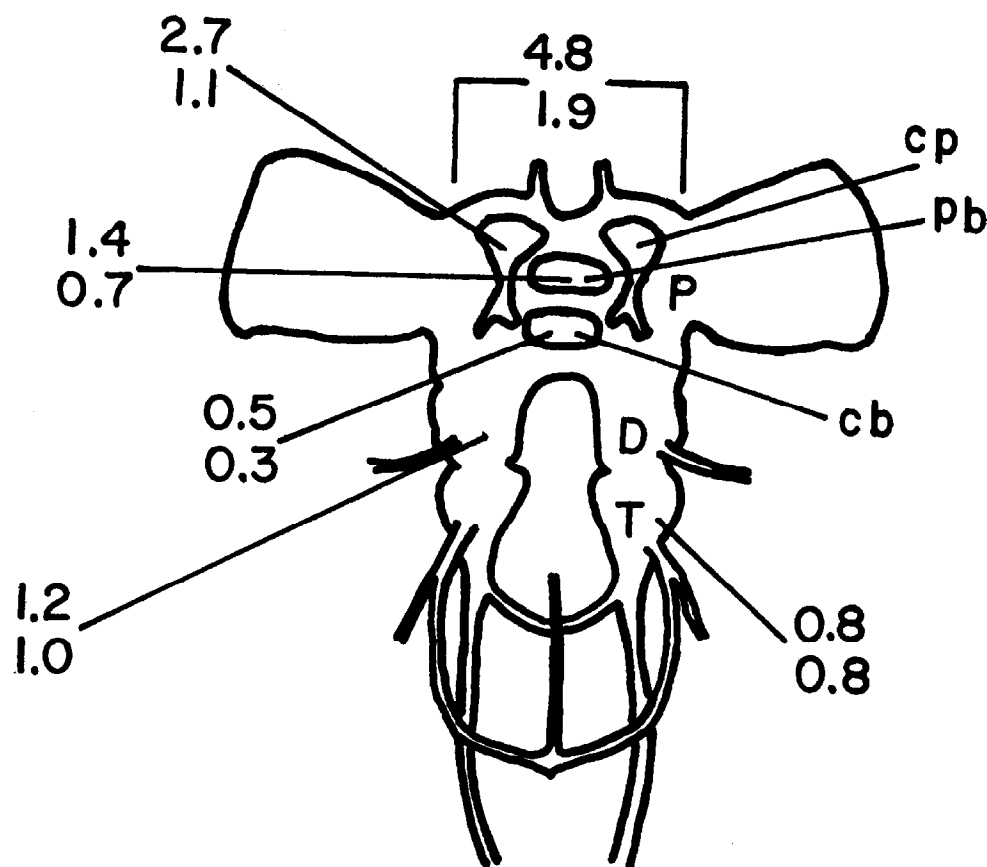


Fig. 6. Effects of shock-avoidance learning on the incorporation of  $H^3$ -labeled uridine into RNA in several brain regions of *Schistocerca americana*. Each pair of values represents the mean for uridine incorporation for 10 insects expressed as counts/min x 10,000. The lower value of each pair refers to RNA activity in nontrained controls, and the upper value refers to RNA activity for that brain region in insects exposed to the learning procedure. P (protocerebrum); D (deutocerebrum); T (tritocerebrum); cp (corpus pedunculatum); Pb (protocerebral bridge); cb (central body).

## CONCLUSIONS

The demonstration of avoidance learning in *L. saccharina*, *S. americana* and *E. depressus*, and the important neurochemical parameters involved, although valuable in and of themselves, also provide a basis for further studies on learning and memory in arthropods. To understand the learning capacities of any species, it is necessary to understand the ways in which it uses learning during its life. Closed, rigid and innate Fixed Action Patterns characteristic of arthropods in general may be adaptive especially for organisms inhabiting relatively stable and homogeneous environments (Davies and Krebs 1978). Factors favoring such closed behavioral systems have been previously listed by Alcock (1979) as follows:

1. short life spans
2. strong selective pressures and circumstances that favor correct initial responses to particular stimuli (such as responses to predators, courtship sequences, etc.)
3. situations in which the costs of an initial "behavioral mistake" are too high (resulting in decreased survivorship)
4. constancy in the structural and/or biotic features of the environment

However, learning can be adaptive if: 1) the organism is able to survive the consequences of initial "mistakes"; 2) there is a sufficient life span to allow for behavioral plasticity; 3) the organism inhabits an unpredictable and heterogeneous environment; and 4) the benefits of such an open behavioral system outweigh the increased metabolic costs of a more complex neural template. The dichotomy between the "advantages" of closed versus open systems has been expressed by Mayr (1974) as follows:

"Under what circumstance is a closed genetic program favored and under what others an open one? . . . . . to state it more generally, selection should favor the evolution of a closed program when there is a reliable relationship between a stimulus and only one correct response . . . . . On the other hand, noncommunicative behavior leading to an exploitation of natural resources should be flexible, permitting an opportunistic adjustment to rapid changes in the environment."

A key word in the above quotation is "opportunistic". It is evident that behavioral evolutionary pathways have led to an "opening of the genetic program" (Mayr 1974) which have allowed an increased role for the use of information acquired by experience. The advantages to any organism capable of learning more efficient foraging and/or escape routes, structural features of its environment that can be utilized to ensure optimal choice of habitat, as well as possible shifts in food preferences based on changes in the availability of certain food items and conspecific recognition, are obvious, and have been documented elsewhere (see Eibl-Eibesfeldt 1970, Dyal and Corning 1973, Mayr 1974, Alcock 1979, Lloyd 1981). I hope that future investigations on the adaptive significance of learning and memory in arthropods will bridge the individual contributions of neurobiologists, behavioral ecologists and psychologists and begin to construct a unified theme with respect to the adaptive significance of closed versus open behavioral systems.

## APPENDIX

<sup>1</sup>Over the years, there have been numerous philosophical arguments between comparative psychologists, ethologists and neurobiologists concerning acceptable formal definitions of learning. These arguments are intensified by the fact that it is frequently difficult (if at all possible) to reconcile formal learning theories developed primarily by experimental psychologists such as E. L. Thorndike (1932), B. F. Skinner (1938), C. L. Hull (1943), and H. F. Harlow (1949) (for a thorough discussion of these and other learning theorists, see Bitterman 1960, Hilgard and Bower 1966), who based their ideas primarily on laboratory experiments utilizing white rats, and stochastic models for learning, with the rather complex and adaptive behavioral interactions exhibited by invertebrates toward their environments under natural conditions. Frequently, debates have centered around what Dyal and Corning (1973) refer to as the search for "grade A certified" learning. While formal definitions of learning can vary (see Hilgard and Bower 1966, Lorenz 1969), one of those most frequently cited by biologists is the definition given by Thorpe (1963):

"We can define learning as that process which manifests itself by adaptive changes in individual behavior as a result of experience" (p. 27).

This definition incorporates the adaptive significance and nature of the learning process and is the one that will be implied throughout this paper.

<sup>2</sup>Fixed action patterns (FAP) represent genetically based and species-specific behaviors or motor patterns that are elicited by specific sign stimuli (SS) or releasers in the environment. They develop without the benefit of learning and are generally attributed to fixed neuronal circuits within the CNS referred to as innate releasing mechanisms (IRM). The reader is referred to Eibl-Eibesfeldt (1970) and Alcock (1979) for further discussion of the concept of FAPs, IRMs and their relationship to theories of instinctive behavior.

<sup>3</sup>The maze was modified according to an earlier model originally designed by Turner (1913) for studies on roaches and which has subsequently been used by numerous investigators. It was constructed of aluminum and consisted of 6 blind alleys. The top of the maze was covered by a transparent glass plate. A light source consisting of a 15-watt cool fluorescent lamp was placed directly over the center of the maze at a height of 12 cm (Punzo 1980a).

<sup>4</sup>In addition to the compound eyes (principle photoreceptors), *Lepisma saccharina* possesses prominent cephalic antennae (olfactory and mechanoreceptors), posterior abdominal cerci and a well-developed median caudal filament (mechanoreceptors).

<sup>5</sup>In order to ascertain the maze-learning capacity for this species (acquisition), each insect was placed in the maze start chamber for a period of one minute. A restraining panel between the start chamber and the main body of the maze was then removed, enabling the insect to enter the maze. The insect was allowed to traverse the maze in order to reach the darkened goal chamber and thereby avoid the aversive light stimulus. Records were kept of the total number of blind-alley errors made by each insect for all experimental groups. An error was defined as such if more than one-half of the body entered a blind alley (Schneirla 1953). The insect remained in the goal chamber for one min, retrieved and returned to the start chamber for the next trial. Each insect received 10 trials per day until the criterion for learning (two days of 5 consecutive trials of 10 errors or less) was achieved.

The procedure for extinction trials was identical to that described above for acquisition except that the cover on the goal chamber was removed so that the insect could no longer avoid the light. Extinction procedures were

conducted in order to assess the retention capacity of this insect (memory) and were continued until the behavior of Group 1 insects approached the random behavior exhibited by them on the first day of acquisition trials.

The floor and walls of the maze were treated with a 1% acetone solution following each trial for all insects in order to eliminate any possible utilization of odor cues.

<sup>6</sup>Analysis of variance showed a highly significant difference between Groups 4 and 5, and among Groups 1, 2 and 3 ( $F = 265.27$ ,  $df = 2/27$ ,  $p < .0001$ ).

<sup>7</sup>A wire lead placed anterior to one metathoracic leg allowed free flexion for training. The other metathoracic leg was tethered to a support rod and prevented from moving. A piece of fine copper wire was placed around the metathoracic tibia of the free leg and extended below the tibia so that the end of the wire made contact with a 0.9% NaCl solution positioned below the insect when the leg was not flexed. Contact with the salt solution resulted in a shock of square wave pulses of 20V amplitude and 30 msec duration at a frequency of 6 Hz. The power source was a Thornton AGC stimulator (Fig. 4, S). The measurement for shock-avoidance learning was the change in the number of shocks received over a 15-min training period (see Goldsmith et al. 1978, Punzo, 1983a). The number of shocks received was automatically recorded on a Simpson Model 87R counter (Fig. 4, C).

<sup>8</sup>Analysis of variance indicated that the improved performance exhibited by the P group was highly significant ( $F = 34.22$ ,  $df = 1/14$ ,  $p < .01$ ). In addition, retention of the learned response was clearly demonstrated by the retest P insects following a one hr intertrial period. Retest P insects averaged only 13.8 shocks during the first minute of retesting as opposed to the 51.2 shocks received during the first minute of the initial training period. This improvement in performance was shown to be significant by a direct-difference Student *t* test ( $t = 3.24$ ,  $df = 9$ ,  $p < .01$ ). Analysis of variance also indicated a significant difference between the performance of the P group and retest R group ( $F = 29.73$ ,  $df = 1/14$ ,  $p < .01$ ).

<sup>9</sup>It is well documented that neurons are characterized by significantly higher rates of RNA synthesis than are other somatic cell types (Maldonado 1980). RNA is essential to the expression of gene function. External factors such as hormones are known to activate genes resulting in protein synthesis. It has been suggested that environmental and behavioral factors could activate genes as well (Hyden 1973, Davis 1976). Behavioral events, either learned or instinctive, could trigger conformational changes in neuronal proteins directly or through differential gene activation.

<sup>10</sup>Neurochemical changes were assayed using radioisotope  $H^3$ .  $H^3$ -labeled uridine (for RNA) and leucine (for protein) were diluted with insect saline and injected into experimental animals through the cervical region before training procedures as described previously by Kerkut et al. (1972) and Punzo (1980b). For a discussion of the radioassay procedures, see Oliver et al. (1971) and Punzo (1980b). Protein determinations were conducted according to the method of Lowry et al. (1951). Brain ChE activity was determined by the method of Willner and Mellanby (1974) and expressed as micromoles of acetate released per hr per 100  $\mu g$  of protein.

<sup>11</sup>The arthropod brain (cerebral ganglion) is generally divided into three major anatomical regions: the protocerebrum (concerned primarily with the innervation of the compound eyes and ocelli, and the control of higher associative functions); deutocerebrum (innervation of the antennae); and tritocerebrum (innervation of the mouthparts). In insects the protocerebrum contains: paired corpora pedunculata, a central body, protocerebral bridge and optic lobes. Corpora pedunculata are absent in the decapod brain. For a

comparative treatment of brain anatomy in the major arthropod groups the reader is referred to Bullock and Horridge (1965).

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#### LITERATURE CITED

- AKRE, R. D. 1964. Correcting behavior by insects in vertical horizontal mazes. *J. Kansas Ent. Soc.* 37: 169-86.
- ALCOCK, J. 1979. *Animal behavior: an evolutionary approach*. 2nd ed., Sinauer Assoc., Inc., Massachusetts.
- ALLOWAY, T. M. 1972. Learning and memory in insects. *Annu. Rev. Ent.* 17: 43-56.
- . 1973. Learning in insects except Apoidea. Pages 131-67 *In* W. C. Corning, J. Dyal and A. O. D. Willows, Eds. *Invertebrate learning*, Vol. 2, Plenum Press, New York.
- BARRACO, R., AND L. STETTNER. 1976. Antibiotics and memory. *Psychol. Bull.* 83: 242-302.
- BITTERMAN, M. E. 1960. Toward a comparative psychology of learning. *Amer. Psychol.* 20: 396-410.
- BULLOCK, T. H., AND G. A. HORRIDGE. 1965. Structure and function in the nervous systems of invertebrates. Vol. II: The Arthropoda. Pages 1165-1272, W. H. Freeman Co., San Francisco.
- CHAUVIN, R. 1947. Etudes sur le comportement de *Blatella germanica* dans divers types des labyrinthes. *Bull. Biol. Fr. Belg.* 61: 92-128.
- CORNING, W. C., AND R. LAHUE. 1972. Invertebrate strategies in comparative learning studies. *American Zool.* 12: 455-69.
- DAVIES, M. B., AND J. R. KREBS. 1978. Introduction: ecology, natural selection and social behavior. Pages 1-20 *In* J. R. Krebs and N. B. Davies, Eds. *Behavioral ecology: an evolutionary approach*, Blackwell Sci. Publ., London.
- DAVIS, W. J. 1976. Plasticity in invertebrates. Pages 430-62 *In* M. R. Rosenzweig and E. Bennett, Eds. *Neural Mechanisms of learning and memory*, MIT Press, Massachusetts.
- DINGLE, H. 1962. The occurrence of correcting behavior in insects. *Ecology* 43(4): 727-28.
- DYAL, J. A., AND W. C. CORNING. 1973. Invertebrate learning and behavior taxonomies. Pages 1-48 *In* W. C. Corning, J. A. Dyal and A. O. D. Willows, Eds. *Invertebrate Learning*, Vol. 2, Plenum Press, New York.
- EIBL-EIBESFELDT, I. 1970. *Ethology, the biology of behavior*. Holt, Rinehart and Winston, New York, pp. 207-19.
- EISENSTEN, E. M., AND B. PERETZ. 1973. Comparative aspects of habituation in invertebrates. Pages 214-71 *In* H. Peeke and M. Herz, Eds. *Habituation*. Vol. 1: behavioral studies, Academic Press, New York.
- EWERT, J. P. 1980. *Neuroethology*. Springer-Verlag, New York.
- GLASSMAN, E. 1975. Macromolecules and behavior. Pages 667-78 *In* S. Snyder, Ed. *Biochemistry and behavior*, MIT Press, Massachusetts.
- GOLDSMITH, C. M., HEPBURN, H., AND D. MITCHELL. 1978. The retention of an associative task after metamorphosis in *Locusta migratoria*. *J.*

- Insect Physiol. 24: 737-41.
- HILGARDE, E. R., AND G. BOWER. 1966. Theories of learning. University Park Press, Maryland.
- HYDEN, H. 1973. Changes in brain protein during learning. Appleton, Centurt and Crofts, New York.
- JAFFE, K. 1980. Effects of cycloheximide on protein synthesis and memory in praying mantids. *Physiol. Behav.* 25: 367-71.
- KERKUT, G. A., EMSON, P., AND P. BEESLEY. 1972. Effect of leg-raising learning on protein synthesis and ChE activity in the cockroach CNS. *Comp. Biochem. Physiol.* 41B: 635-45.
- KRASNE, F. B. 1973. Learning in Crustacea. Pages 49-130 *In* W. C. Corning, J. A. Dyal and A. O. D. Willows, Eds. *Invertebrate learning*, Vol. 2: Arthropods and gastropod molluscs, Plenum Press, New York.
- LAGUELTE, L. 1969. Learning in spiders. *American Zool.* 9: 145-52.
- LINDAUER, M. 1967. Recent advances in bee communication and orientation. *Annu. Rev. Ent.* 12: 439-70.
- LLOYD, J. E. 1981. Sexual selection: individuality, identification and recognition in a bumblebee and other insects. *Florida Ent.* 64: 89-118.
- LONGO, N. 1964. Probability learning and habit reversal in the cockroach. *American J. Psychol.* 177: 29-41.
- LORENZ, K. 1969. Innate bases of learning. Pages 35-36 *In* H. Hyden, K. Lorenz, H. Magoun, W. Penfield and K. H. Pribram, Eds. *On the biology of learning*, Harcourt, Brace and World, New York.
- LOWRY, D. H., N. ROSEBROUGH, D. L. FARR, AND R. RANDALL. 1951. Protein measurements using the Folin—Phenol reagent. *J. Biol. Chem.* 193: 265-75.
- MALDONADO, H. 1980. Changes in brain peptides and memory consolidation in the praying mantis. *J. Insect Physiol.* 26: 339-44.
- , AND A. TABLANTE. 1975. Mnemonic factors in the learning process of praying mantids. *J. Insect Physiol.* 21: 1101-10.
- MAYR, E. 1974. Behavior programs and evolutionary strategies. *American Sci.* 62: 650-58.
- MENZEL, E. W., AND E. J. WYERS. 1981. Cognitive aspects of foraging behavior. Pages 355-78 *In* A. C. Kamil and T. D. Sargent, Eds. *Foraging behavior: ecological, ethological and psychological approaches*. Garland Press, New York.
- MENZEL, R. 1968. The honeybee's memory of spectral colors. *Z. Vergl. Physiol.* 60: 82-102.
- NELSON, M. C. 1971. Classical conditioning in the blowfly, *Phormia regina*. *J. Comp. Physiol. Psychol.* 77: 353-68.
- OLIVER, G., J. TABERNER, T. RICK, AND G. A. KERKUT. 1971. Changes in GABA level, GAD and ChE activity in the CNS of an insect. *Comp. Biochem. Physiol.* 38B: 529-35.
- PRITCHATT, D. 1968. Avoidance of electric shock by the cockroach *Periplaneta americana*. *Anim. Behav.* 16: 178-85.
- PULLIAM, H. R. 1981. Learning to forage optimally. Pages 379-88 *In* A. C. Kamil and T. D. Sargent, Eds. *Foraging Behavior: ecological, ethological and psychological approaches*. Garland Press, New York.
- PUNZO, F. 1974. Oxygen deprivation as a drive state in an aquatic insect: Family Dytiscidae. *Anim. Learning Behav.* 2(1): 31-33.
- . 1980a. Analysis of maze learning in the silverfish, *Lepisma saccharina* (Thysanura: Lepismatidae). *J. Kansas Ent. Soc.* 53(3): 653-61.
- . 1980b. Neurochemical changes associated with learning in *Schistocerca americana* (Orthoptera: Acrididae). *J. Kansas Ent. Soc.* 53(4): 787-96.

- . 1983a. Localization of brain function and neurochemical correlates of learning in the mud crab, *Eurypanopeus depressus* (Decapoda). *Comp. Biochem. Physiol.* 75A: 299-305.
- . 1983b. Physiological amino acids in the brain of *Schistocerca americana* (Insecta), *Lycosa avida* (Arachnida) and *Eurypanopeus depressus* (Decapoda). *Comp. Biochem. Physiol.* 75C: 399-402.
- . 1984. Neurochemical correlates of learning and role of the basal forebrain in the brown anole, *Anolis sagrei* (Lacertilia, Iguanidae). *Copeia* (in press).
- , AND J. JELLIES. 1980. Effects of cycloheximide induced protein synthesis inhibition on the phototactic behavior of *Tenebrio molitor* (Coleoptera: Tenebrionidae) and *Popilius disjunctus* (Coleoptera: Passalidae). *J. Kansas Ent. Soc.* 53(3): 597-606.
- PUSZTAI, J., AND G. ADAM. 1974. Learning phenomenon in the giant neurons of the snail *Helix pomantia*. *Comp. Biochem. Physiol.* 47A: 165-71.
- SCHNEIRLA, T. C. 1953. Modifiability of insect behavior. Pages 723-47 In K. D. Roeder, Ed. *Insect physiology*. John Wiley and Sons, New York.
- SMITH, E. L. 1970. Biology and structure of some California bristletails and silverfish. *Pan-Pac. Ent.* 46(3): 212-25.
- STANTON, M. L. 1984. Short term learning and the searching accuracy of egg-laying butterflies, *Anim. Behav.* 32(1): 33-40.
- STRATTON, L. O., AND P. COLEMAN. 1973. Maze learning and orientation in the fire ant, *Solenopsis saevissima*. *J. Comp. Physiol. Psychol.* 83: 7-12.
- TAKEDA, K. 1961. Classical conditioning in the honeybee, *J. Insect Physiol.* 6: 168-79.
- THORPE, W. H. 1944. Types of learning in insects and other arthropods. *British J. Psychol.* 34: 66-76.
- . 1963. *Learning and instinct in animals*. Clarendon Press, Oxford, pp. 81-96.
- TINBERGEN, N. 1951. *The study of instinct*. Clarendon Press, Oxford.
- TURNER, C. H. 1913. Behavior of the roach (*Periplaneta americana*) in an open maze. *Biol. Bull.* 25: 348-65.
- VOWLES, D. M. 1964. Olfactory learning and brain lesions in the wood ant, *Formica rufa*. *J. Comp. Physiol. Psychol.* 58: 105-11.
- WILLNER, P., AND J. MELLANBY. 1974. Cholinesterase activity in the cockroach CNS does not change with training. *Brain Res.* 66: 481-90.