

COMPARATIVE PHYSIOLOGY: LEARNING IN INVERTEBRATES¹

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INTRODUCTION

Of the more than one million described species of animals, fewer than 5 per cent possess a backbone and are known as vertebrates; the other 95 per cent comprise the invertebrates (1). Of the thousands of studies on animal learning published in the past decade, only some 5 per cent utilized invertebrates as subjects. There are hundreds of thousands of species that have never been introduced to a maze or a Skinner box nor watched at length by an ethologist—indeed, whole phyla have been totally neglected. Two of the many possible causes for this scientific bias are easy to identify: (a) man is anthropocentric, and (b) man is lazy.

Man is generally more interested in man than in any other animal, and the species he has studied most extensively have been those close enough to him in size and shape to excite his empathy and those of greatest economic and medical importance to him. Most of the invertebrates do not qualify strongly for his attentions. It is likewise true that the lower animals, for all of their beautiful structural and functional diversity, are not as easy to obtain, to house, to design experiments for, or to handle, as are many of the vertebrates. Scientists, like their experimental subjects, tend to obey Zipf's "Law of Least Effort". We know where to order white rats, what to do with them and what to expect of them; but which of us has ever seen a tunicate or a tartigrade?

Why then would anyone bother to study invertebrate behavior at all? For several reasons. First, the invertebrates are obviously less complex in structure than, say, the mammals; one might expect them therefore to be less complex in behavior too. The innate response patterns might well be stronger and hence more visible in invertebrates than in vertebrates, in which case the genetic substrata of behavior would be more available to experimental ma-

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nipulation. Second, the invertebrate nervous system is in many instances much more available to scalpel, electrode, and cannula than is the vertebrate central nervous system, an ideal situation for someone interested in correlating nerve activity with behavioral changes. Third, many of the animals have rather special talents: for instance, the facts that one can graft parts of one animal to another, that even very small portions of whole organisms can be kept alive and functioning fairly normally for long periods of time, that invertebrates can often regenerate lost sections of their bodies, and that they often reproduce asexually as well as sexually, make them valuable subjects for all sorts of "odd" (i.e. nonanthropocentric) experiments. Fourth, the invertebrates offer fertile testing grounds for any psychological theory that claims broad phylogenetic generality. And, at last, there are those scientists who pick the invertebrates because so little is known about them and, like Mount Everest, "they're there".

Scope.—The papers discussed in this review cover only six major animal groups: the ciliate protozoans, the coelenterates, the planarian flatworms, the annelids, the cephalopods (mollusks), and a few of the arthropods; there is little behavioral work published on other phyla.

This review has been profoundly influenced by two major publications, both of which deserve special mention. The first is the long-awaited *Structure and Function in the Nervous Systems of Invertebrates* by Bullock & Horridge, published in the spring of 1965 (2). A comprehensive handbook covering both the neuroanatomy and neurophysiology of the invertebrates has long been needed, and it is difficult to imagine two authors better suited to answering this need. That these authors whenever possible attempt to correlate behavioral changes with neurophysiological functioning suggests that the scientific study of behavior is now as important to biologists as it is to psychologists. Although, unfortunately, there are few literature citations in this two-volume set more recent than 1960, the work is obviously a classic and will serve as the standard reference in the field for many years to come.

The second item of major interest was the publication, in mid-1965, of a special supplement to the British journal *Animal Behaviour* containing the proceedings of the symposium on "Learning and Associated Phenomena in Invertebrates" held in Cambridge, England, in July of 1964. Ethologists and comparative psychologists from both sides of the Atlantic converged on Cambridge for what was probably the most stimulating and enlightening session on invertebrate behavior held anywhere in recent times. No better survey of the "state of the art" exists than can be found in this Supplement, and the present review is in many ways little more than a pale copy of the original.

DEFINITIONS

Can invertebrates learn? Few other questions in comparative psychology have engendered such heated and passionate discussions as has this one. Quite understandably, the question cannot be answered adequately until

some definition of the term "learning" is universally agreed upon, a most unlikely event at best. But the issues within the field cannot be placed in perspective unless the reader is given some explanation of the various types of behavioral changes that psychologists tend to measure when they study "learning" in animals. A great many biological scientists have spent a great amount of time in debating the taxonomy of the invertebrates and in placing these organisms on the twigs and branches of various hypothetical phyletic trees. It is unfortunate that psychologists have not spent comparable amounts of time on the systematics of behavior. Although some advances have been achieved at the human level (3), comparative psychology is still awaiting its Linnaeus. The definitions that follow are no more than an incomplete set of partial descriptions of behavioral events roughly arranged in an ascending order of complexity.

Innate responses.—The organism always comes with a highly complex pattern of ready-made responses; learning is what the environment imposes on this ready-made system. Before the psychologist can hope to demonstrate "learning" in his organisms in a given environment, he must make an extensive study of the animals' unlearned reactions in similar situations. No taxonomy of "learned responses" can be worth much unless it is based on a clear-cut and complete taxonomy of "unlearned responses". Neither taxonomy is available today.

Sensitization.—In its simplest form, sensitization is a mere lowering of all response thresholds due to prior stimulation and is not generally considered "true" learning at all. For instance, if we put a dog in a restraining harness and shock it, we will usually find that its response threshold to the onset of a light changes significantly simply because it has been shocked. If the punishment was strong enough, however, we can sometimes bring the dog back to the experimental room 2 or more years later and find that the mere act of putting it back in the harness causes a change in the animal's response to light, an example of "conditioned sensitization" (a primitive but very real form of learning).

Reactive inhibition.—When an organism is forced to make one response (such as turning right in a T maze), then is allowed a free choice of responses, it typically makes the opposite response (in this case, turning left), a phenomenon called "spontaneous alternation". If it is exposed to stimulus A but not to B, then allowed to approach either A or B, it usually picks B as if the prior exposure to A had created within it some sort of "stimulus satiation". According to Hull (4), whenever an animal makes a response, a momentary potential is built up that inhibits the repetition of that reaction. This "reactive inhibition", as Hull called it, is thought to be a very transient phenomenon, but some form of "memory" obviously seems involved, hence we may consider it a primitive form of learning.

Habituation.—If a stimulus that typically elicits an innate response is presented repeatedly, the response may weaken or even disappear. In those cases where the response decrement can be shown not to result from fatigue

or tissue damage, and particularly if the decrement is stimulus-specific and relatively enduring, habituation may be considered a simple form of learning.

Conditioned inhibition.—If an organism is repeatedly punished for making an innate or a learned response to a particular stimulus, it often stops making any response at all in the presence of that stimulus. In maze learning, for example, if the animal is severely punished for making the incorrect choice, it may soon refuse to run the maze at all. Since the effect is often quite long-lasting and stimulus-specific, conditioned inhibition is usually considered to be a form of learning.

Classical conditioning.—If an animal typically makes an innate or unconditioned response to a given stimulus, and if one repeatedly pairs a so-called “neutral” (conditioned) stimulus with the unconditioned stimulus, eventually the animal comes to respond to the conditioned stimulus much as it does to the unconditioned. The conditioned response given to the conditioned stimulus, however, is not always identical in form to the unconditioned response. If the conditioned stimulus is never paired with the unconditioned, but the two are merely presented independently but randomly, and if the animal nonetheless comes to give the conditioned response at the onset of the conditioned stimulus, “pseudoconditioning” rather than “classical conditioning” is said to have taken place, although Kimble (5) suggests that the only thing “pseudo” about this type of behavioral change is the name given to it. “Extinction” of the conditioned response usually occurs when the conditioned stimulus is presented repeatedly without the unconditioned. Considered by many behaviorists to be the “simplest” form of “true learning”, classical conditioning obviously includes elements of the more primitive behavioral changes discussed above. For example, during training the animal often shows first sensitization and then habituation both to the conditioned and to the unconditioned stimulus.

Avoidance conditioning.—In classical conditioning, the unconditioned stimulus is always presented whether or not the organism gives the conditioned response when the conditioned stimulus is presented. In avoidance conditioning, the animal can avoid the onset of a noxious unconditioned stimulus by giving the conditioned response as soon as the conditioned stimulus appears.

Complex associative learning.—There are a number of quite complex behavioral changes (such as maze learning or “operant conditioning”) that are recognized as being “true” learning by most theorists. The essential thing about all these behavioral changes is that the organism comes to discriminate between two or more stimuli and to associate different responses or response-consequences with the different stimuli. Jensen (6), for instance, considers associative learning to be the only “true” form and defines the term “learning” in rather an idiosyncratic manner that precludes even most types of classical and avoidance conditioning, a position that is considerably weakened by the fact that even such a simple type of behavioral change as sensitization often involves differential responding to two different stimuli.

Discussion.—It should be clear by now that the question “can invertebrates learn?” will not be answered in the same way by two theorists who hold different definitions of what learning actually is. What is perhaps not so clear is that two theorists who hold almost identical views on the matter may arrive at different answers if they use different measures of learning in a given situation. In most cases, learning is measured during the “acquisition” phase of training, usually as a significant increase in the probability that the appropriate response will occur. In a few instances, however, the strength of the conditioning is measured indirectly (and rather unreliably) in terms of the resistance the response shows to extinction. These two measures, when applied to the same situation, often show a zero or even a negative intercorrelation. We will need to keep this fact in mind as we plow through the statements, pro and con, concerning learning in the invertebrates.

PROTOZOANS

Far more complex in structure and function than once was imagined, the protozoans offer a subtle scientific challenge that, unfortunately, far too few psychologists have answered. There is little doubt that these single-celled animals are capable of quite complicated and often highly coordinated behavioral sequences; whether or not any of these sequences involve “true” learning is a matter still under debate. Although there were some early studies of perhaps doubtful validity on “learning” in *Amoeba*, most of the recent work has made use of the ciliates, particularly *Paramecium*.

Reactive inhibition.—In 1952, Lepley & Rice (7) reported evidence for the existence of reactive inhibition in paramecia. Their subjects were run in a series of narrow-channel T mazes and were forced to make 0, 1, or 2 forced turns prior to entering the choice-point. These authors obtained significant results indicating that spontaneous alternation did in fact occur, but reported that increasing the number of forced turns did not cause a corresponding increase in the tendency to alternate. In 1962, Lachman & Havlena (8) attempted to replicate the Lepley & Rice study, but failed to achieve the same results. Running their subjects in a series of wide-channel Y mazes with from 0 to 9 forced turns prior to the choice-point, they found no evidence at all for reactive inhibition. In 1965, however, Rabin & Hertzler (9) clarified this apparent contradiction by repeating both studies exactly. It is apparent from the results of this latter study that reactive inhibition can best be detected when the channels in which the animals run are quite narrow and when the choice-point is clear cut.

Complex associative learning.—In his talk at the Cambridge meetings in 1964, Jensen (6) summarized the early learning studies on paramecia and concluded that in all instances the “learning” obtained was due in fact to changes in the organisms’ environments rather than to changes inside the protozoans themselves. Jensen then aimed his analytical guns at the best-controlled set of studies purporting to show learning in paramecia, those performed by Gelber and her associates [described in (10)]. Briefly stated,

Gelber has shown that if a clean platinum wire is lowered repeatedly into the center of a culture of *Paramecium aurelia*, the protozoans typically avoid the wire. If the platinum is lightly baited with bacteria before it is dipped into the culture (a training trial), the paramecia now tend to approach the wire and cling to it. If after 15 such training trials, a clean wire is lowered into the culture, the paramecia now approach and cling to the bare wire if tested as long as 10 to 12 hours afterwards. In general, the more training trials the cultures are given, the greater is the number of paramecia that can be found clinging to the bare wire. Gelber has also reported a rather odd "reminiscence" effect in her animals. If a culture is tested with a clean wire at the end of 15 training trials, it shows less "learning" than if it is tested 2 or 3 hours later. The "retention curve" drops sharply at 4 hours, then climbs again to another peak at 9 hours after training, then drops to zero if the test trial is delayed until 19 hours after the training ceased. The first portion of this "retention curve" is somewhat similar to curves reported for higher organisms; the drop at 4 hours and the second peak at 9 hours are difficult to reconcile with the results obtained using higher organisms.

Jensen's criticisms center on two aspects of *Paramecium* behavior that he claims Gelber ignored in her research: First, the bacteria on the wire create a central zone of food-rich fluid into which the paramecia are innately drawn; and second, in the presence of food, paramecia tend to adhere to any surface that they may strike through random movements. When Jensen (11) added bacteria to the medium containing an untrained culture, he found significant wire-clinging behavior in animals that could not have made a prior "food-wire" association. It should be noted, however, that the amount of bacteria Jensen added to his untrained culture was apparently much greater (Gelber has estimated it was 1000 times greater!) than that Gelber used to reinforce her trained cultures, a methodological consideration Jensen has apparently overlooked. Katz & Deterline (12) replicated Gelber's early experiments and obtained similar "wire-clinging" behavior in their trained cultures. Immediately after the final training trial, they vigorously stirred the culture for an unreported period of time and then immediately dipped a clean wire into the culture for 3 minutes. They report the training had vanished presumably because the stirring removed the "food-rich" zone in the center of the culture. Since these authors failed to control for the effects of "vigorous stirring" on the behavior of paramecia, and since even an overtrained rat that was "well shaken" right after a final training trial might choose not to run the maze if returned to it immediately, the issue cannot be resolved on the basis of these studies alone.

At the Cambridge conference, Gelber (10) presented additional data that seem to invalidate Jensen's criticisms. In her latest work, Gelber gave the usual "bacteria-wire" training to two cultures containing 250 paramecia each and to one culture containing 500 animals. Three similar cultures received no training at all and served as controls. Although paramecia will undergo reproductive fission if given sufficient food, the amount of bacteria added dur-

ing training was insufficient to allow reproductive activity. Therefore, when training was complete, Gelber added sufficient bacteria to one of the smaller trained cultures and to a matched control culture to induce fission in all 250 animals in both groups. It is important to note that in both cases, the bacteria were added at the side rather than in the center of the culture. Ten hours later when all of the animals in both these groups had fissioned, all six groups were given one test with a clean platinum wire. Twelve minutes after the insertion of the wire, all of the trained groups had significantly greater numbers of paramecia clinging to the wire than did any of the control groups; furthermore, there were no differences between the trained culture containing the 500 freshly fissioned paramecia (all of which were daughter cells of the originally trained 250 animals) and the group containing the 500 trained animals none of which had fissioned.

Discussion.—In recent years, electron microscopy has revealed an order of internal complexity in the “primitive” unicellular organisms that could hardly have been dreamed of just a few short decades ago. Studies on the electrical properties of the cell membrane of the ciliates, in particular, have revealed that they have many similarities to those of nerve cells, including potential changes associated with activity and a threshold current for just perceptible changes in ciliary beat (2). Various authors have reported finding elaborate fibril systems throughout the animals’ bodies that often converge on a “motorium” that may or may not be involved in conduction of impulses and coordination of behavior (2). Whether one believes memory formation and storage to be neurophysiological or biochemical, or both, in nature, it seems likely that at least some of the more complex protozoans will be shown to possess the mechanisms necessary for learning.

In the final analysis, of course, the question “can protozoans learn?” will be settled on the basis of behavioral studies, not on the basis of what today’s anatomists and physiologists can find within the animals’ bodies. If Gelber’s work, and that of Rabin & Hertzler, holds up under replication, it will be up to the protozoologists to discover the internal mechanisms (including the necessary receptor systems) by which these behavioral alterations are mediated.

COELENTERATES

As Ross has pointed out (13), to anyone interested in the physiology of behavior, the sessile coelenterates are interesting objects of study because their nervous systems (so-called “nerve nets”) lack the centers and unipolar conduction pathways thought to be needed for the coordinated activities of higher animals, yet coelenterates display some remarkably well-integrated behavior patterns. To date, there is no unequivocal proof that classical conditioning and other forms of associative learning can be established in these animals, but some of Ross’s findings are quite suggestive. There can be no doubt about the evidence concerning habituation, however.

Habituation.—Rushforth and his associates have studied the habituation

of *Hydra*'s innate responses to mechanical stimulation (14, 15, 16) in some detail. *Hydra pirardi* exposed to 2.5 seconds of mechanical shaking every 16 seconds will show almost complete habituation in less than 3 hours of stimulation. The habituation is not due to muscular fatigue since animals fully habituated to mechanical stimulation still uniformly contract to light. Some effects of the habituation are evident 3 or more hours after training has ceased. Addition of live *Artemia salina* (brine shrimp) or of 10^{-5} M glutathione to the medium activates the feeding response and blocks the innate reflex both to mechanical and to photic stimulation. Rushforth (16) has also found that unlike *H. pirardi*, the species *H. pseudoligactis* is relatively insensitive to mechanical stimulation; animals formed from grafts between *Hydra* of the two species have behavior patterns intermediate between those of the parent species.

At the Cambridge meetings, Ross reported several quite interesting but previously unpublished attempts at establishing classical conditioning in sea anemones (13). *Metridium senile* shows an impressive mouth-opening reflex in response to food but not in response to low-frequency electrical impulses. Although pairing the electrical stimuli (conditioned stimulus) with the food (unconditioned stimulus) gave mostly negative or uncertain results, in a few cases apparent conditioning was observed in which the mouth opened to the electrical stimuli alone. In another set of experiments, Ross made use of two innate reaction patterns found in the sessile anemone *Stomphia coccinea*: the rather striking swimming reaction it makes on contact with the starfish *Dermasterias imbricata* and its closure response to strong mechanical pressure applied to its base. These two responses were paired in the following way: two seconds after starfish material had been applied to the anemone's tentacles, the closure response was elicited by pressing on the animal's base. The closure response inhibited the swimming response. After the two types of stimulation had been paired varying numbers of times with eight animals, each was tested for its reaction to the starfish material. None of the anemones showed a swimming response on the first test trial; to the contrary, each responded as if it had received a sharp mechanical stimulus applied to its base. However, one by one the animals began to swim again as the test trials were continued. In general, the more conditioning trials an animal received, the more test trials it took before the swimming reaction reappeared. Although Ross did not run sufficient pseudoconditioning controls, his results suggest that conditioned inhibition (and perhaps classical conditioning) can be demonstrated in coelenterates.

Discussion.—One of the difficulties in dealing with reactions such as the closure response in anemones is that the reaction lasts so long (up to several hours) that the inter-trial interval must perforce also be quite long. In light of the findings by Ratner on decapitated earthworms and Clark in decerebrate nereids (discussed below), that conditioning occurs when the interval between trials is very short but does not occur at longer intervals, one might

suspect that more clear-cut results with the "brainless" coelenterates might be obtained if an unconditioned response that had a very short recovery time were chosen.

PLATYHELMINTHES

In 1920, the Dutch biologist P. van Oye reported that he had been able to teach planarians to take an unusual route to food reward, a route that the animals apparently would not follow unless given the proper training (17). Had van Oye's study received the attention it deserved, it is likely that the question "can planarians learn?" would have been answered in the affirmative decades ago and much of the current controversy over this matter could have been avoided (18). But van Oye wrote in Dutch and his work appeared in a not-too-well-known European journal, thus his paper eluded even that great searcher of the invertebrate literature, Libbie Hyman, and it was not until Roman Kenk called the study to our attention a few years ago that van Oye's report became a visible part of the planarian literature. As it was, up until the late 1950's most of the scientists who attempted to train planarians used classical conditioning techniques and achieved at best a mixed bag of results. These early papers are described in Jacobson's two excellent reviews (19, 20).

Innate responses.—Several studies published in recent years have shown rather strong interactions between the planarian's innate reactions to various types of stimulation and its learned responses. Perhaps the most intriguing set of experiments has to do with the influence of short-term (circadian) and long-range (lunar) activity cycles on speed of learning. Best (21) noted that planarians cannibalized more at night than during the day, even when the animals had been kept in a light-proof, temperature-controlled environment for 6 months or more. Best's results probably explain Roe's findings that planarians learn a maze more rapidly during the afternoon than in the morning (22). Best also reported that worms trained in various situations showed a behavioral periodicity that approximated a semilunar cycle—the planarians did significantly more poorly when tested 7 or 21 days after the full moon than at any other time during the month, an effect that was constant across several hundred animals for periods of 6 months or more. Best notes that his laboratory at Colorado State University is a mile high, that background radiation there from cosmic rays and "solar wind" bombardment is several-fold more than at sea level, and that these physical phenomena exhibit more or less regular periods of fluctuation correlated with the astronomical periods. Best's explanation gains considerable credence from F. A. Brown's several papers showing that planarians are capable of detecting and reacting to very low levels of gamma irradiation and to weak electrostatic fields (23, 24, 25).

VanDeventer & Ratner (26) present data indicating that planarians become considerably more responsive than normal to photic stimulation at high temperatures and warn against the use of light as a conditioned stimulus in

conditioning studies. Their animals, however, responded fairly normally at room temperatures and only a group run at 90° F was clearly aberrant. Several authors have reported finding marked behavior differences among the various species of planarians (26, 27), including the fact that some species learn rapidly while others learn slowly, if at all. VanDeventer & Ratner (26) and H. M. Brown (28) both report that small animals are more responsive to light than large, and that small head regenerates are more reactive than small tails, a finding contradicted by several earlier and perhaps better controlled studies (see 19). In particular it should be noted that Brown does not report the species with which he was working and that he failed to obtain head-tail differences when using the intensity of light employed in most other conditioning studies.

Barnes & Katzung (29) showed that the orientation of the planarian when it receives dc shock as the unconditioned stimulus is quite important. Planarians given 150 light/shock conditioning trials while oriented with their heads toward the cathode reached maximal response rates rapidly, while those animals trained while oriented towards the anode showed no evidence of learning.

Planarians secrete a mucus trail as they move. Roe (22) reported that planarians trained in a Z maze chose to enter alleys containing slime trails significantly more often than arms containing no trails. In my own laboratory, we have recently shown that planarians have a strong preference for slimed areas both in a hexagonal maze and in an open field situation (27). Cannibalistic species show a stronger preference for areas in an open field that are covered with the slime trails of other species than for areas covered with their own slime. Most important of all, when planarians are run in apparatus that is carefully cleaned after each training trial, they show quite excited, abnormal behavior involving frequent headwaving and change of direction of locomotion; as soon as such an animal reaches a well-slimed area, it reverts to its normal type of smooth gliding movement. In the absence of slime, planarians become so negatively phototactic that attempts to train them in clean mazes in which the white alley is "correct" seldom meet with much success. Perhaps the greatest significance of the "slime" data lies in the fact that many of the experimenters who have had difficulties in training planarians have used well-cleaned apparatus (28, 30) while most of the experimenters who have obtained positive results have used "slimed" apparatus. In general, it is likely that much of the variability shown from one study to another, and from one laboratory to another, is due to the experimenters' failure to establish rigid control over all of the critical environmental factors that influence the worms' behavior.

Reactive inhibition.—Two recent studies (31, 32) indicate that, in marked contrast to the findings of earlier investigators (see 19), planarians do show spontaneous alternation when the conditions are right. Shinkman & Hertzler (31) gave each of 600 planarians from 0 to 5 forced right-hand turns in a

maze and then allowed the animals to turn either left or right at a T-shaped choice-point. Animals given 0 or 1 forced right-hand turns showed essentially random behavior at the choice-point; animals given 2 or more forced turns showed a significant tendency to turn in the opposite direction. The more forced turns the worms had to make prior to choice, the stronger was the tendency to turn in the opposite direction. P. Lee (32), using a somewhat different technique, obtained significant results after giving her animals but 1 forced turn prior to a free choice. As Shinkman & Hertzler conclude, any explanation of these results must be in terms of stored or persisting effects of the treatment. Their contention that the alternation effect increases as a function of the number of turns the animal must make is not yet proved, however, since the animals forced to make 5 turns prior to the free choice also traveled some 5 times as far through the maze as did animals that made only 1 forced turn, so that the distance the animal had to crawl prior to the final forced turn rather than the actual number of forced turns might have caused the observed alternations. As we will see later, there is evidence that this is indeed the case with insects.

Habituation.—Westerman reports that there are at least two types of habituation to light in planarians—a relatively temporary effect that shows up as a decrement in responding within a given day's trials, and a relatively lasting effect that appears as a response decrement across several days of testing (33, 34). The long-term habituation survives cutting and regeneration in both head and tail regenerates, and can be transferred to unhabituated animals via cannibalism. Westerman cautions that habituation can be achieved only when such factors as the animal's orientation in the training trough, the training regimen, the background illumination, and the intensity of any noxious stimulus also present (such as heat from the light source) are rigidly controlled. Bennett & Calvin (30) apparently found habituation to light under some circumstances, but since they do not report their findings in any statistical form, it is difficult to evaluate their results.

Conditioned inhibition.—In their study on maze learning in planarians, Best & Rubinstein (35) report that planarians could be trained to enter either the dark or the lighted arm in a Y maze but that shortly after the animals reached a peak rate of responding, they almost always showed a significant reversal and began picking the incorrect side with a greater-than-chance probability; following this, they often refused to run the maze at all. Best has called this phenomenon "conditioned lethargy" and points out that it is a common finding in higher organisms when punishment is employed in training (36). Both the lethargy and the reversal could be forestalled by decreasing the number of trials given the animal per day, by increasing the inter-trial interval, and by allowing the animal to escape from the confines of the maze between trials. Humphries & McConnell (37) confirmed these findings using a hexagonally shaped maze.

Classical conditioning.—When, in the early 1950's, Robert Thompson and

I decided to study learning in planarians, we chose classical conditioning as our paradigm since the planarian was "obviously" an extremely simple animal and conditioning was just as "obviously" the simplest form of learning! (We have, as the reader might suspect, learned a great deal about the complexities of both since those green and salad days.) I will describe our procedure briefly, since most subsequent conditioning studies have followed our procedure. For our experimental animals, the light was presented for 3 seconds, for the final second of which the shock was also present. Only those responses (a bodily contraction or noticeable turning of the head) made by the animal during the first 2 seconds of the conditioned stimulus, prior to the onset of the unconditioned stimulus, were recorded. The experimental animals showed a significant increase in responsivity over the 150 massed training trials. Animals given 150 trials of "light only" (no shock) showed a significant decrease in responsivity. A third group was given 150 trials of "shock only", with a test trial of light after each 5 shocks. We found a decline in responsivity to light on the test trials in these shock control animals, and by the end of training they were noticeably inferior to the experimental animals (38). Thompson and I believed we had demonstrated classical conditioning in planarians, but Jensen (6) has complained that our shock group was not a "true" control for pseudoconditioning since these animals received 150 shocks but only 30 test trials of light. Three recent studies appear to answer Jensen's objections. Baxter & Kimmel (39) found significant differences between a group of experimental animals given 250 paired light/shock trials and a control group given 250 unpaired presentations each of light and shock. When the two groups were subsequently given 25 extinction trials (light only), however, the experimental group dropped to the response level of the control animals within 10 trials. Baxter & Kimmel gave their animals 50 trials per day and counted as responses only body contractions, not cephalic turns. Crawford et al. (40) replicated this study giving their planarians but 8 trials per day and found significant differences between the conditioned and the pseudoconditioned animals both during training and during extinction. Vattano & Hullett (41) trained groups of planarians in four different ways: the experimental animals received the regular light/shock conditioning, a second group was given paired shock/light trials in which the shock always preceded the light ("backwards" conditioning), for a third group the light and shock were presented simultaneously, while a pseudoconditioning group received unpaired trials of light and shock. All but the final group received 250 presentations of light and shock (50 per day); the pseudoconditioning group received half that many. Following training (during which no data were taken), all the groups received 25 extinction trials of light only. In terms of the body contractions made during the extinction trials, the experimental animals were significantly superior to all other groups.

Extinction of a conditioned response often proceeds quite rapidly in rats (42) and in earthworms (see below), and animals often go into spontaneous extinction if given overtraining—two facts that explain why extinction data

are the least reliable measure of learning (43). Halas and his associates have performed a number of studies using resistance to extinction as the sole criterion of learning (44, 45). Halas concludes that classical conditioning was not demonstrated in his animals but, as I have pointed out in detail elsewhere (46), there are a great many drastic methodological differences between these studies and all others involving conditioning in planarians.

Bennett & Calvin attempted to achieve classical conditioning in planarians but concluded that their efforts were "unsuccessful" (30). Oddly enough, Bennett & Calvin draw their conclusion without reporting the statistics necessary to back it up, and a careful analysis of their data tells rather a different story. In their first experiment, the increase in response rate over 325 trials was from 18.4 per cent to 60 per cent, a change that is significant at the .001 level of confidence. In their second experiment, the increase was from 9.5 per cent to 64 per cent, a change that is significant at the .00006 level. It would appear that "success" means something quite different to Bennett & Calvin from what it means to most other scientists in this field (47).

If the claims, counterclaims, criticisms, and controversies outlined above have done nothing else, they should have alerted the reader to the fact that classical conditioning can be a most complex phenomenon to deal with. As John has said, "What is needed is a technique to establish a differentiated response, where a planarian makes one kind of response to one stimulus and another kind of response to another stimulus which is basically similar to the first one. Each animal then provides his own control with respect to the question of sensitization and pseudo-conditioning" (48, p. 181). As it happens, such a technique was recently reported.

When a planarian is exposed to the onset of a polarized field, as Pearl noted in 1903, that portion of the animal's body nearest the anode contracts, while the anterior of an animal that is not oriented directly towards either pole bends towards the cathode (49). Griffard & Peirce (50) took advantage of this response to dc in an experiment involving what seems to be "conditioned discrimination" in planarians. Light was employed as one conditioned stimulus, weak vibration as a second. For half the animals, light was always paired with a shock that forced the worm to turn right, while vibration was always paired with a shock that forced the worms to turn left. The other half of the subjects were trained with the opposite conditioned-unconditioned stimulus pairings. Each animal received 100 light/shock and 100 vibration/shock massed training trials. A test trial of either light alone or vibration alone was given at the end of each block of 5 training trials; a response was scored as "correct" if the animal's head turned 22.5° or more in the appropriate direction, as "incorrect" if the animal's head turned more than 22.5° in the opposite direction, and as "zero" if the head movement was less than 22.5° in either direction. Over the 200 training trials, correct responses rose from 17 per cent to 50 per cent, incorrect responses fell from 22 per cent to 19 per cent, and the "zeros", of course, declined markedly. The differences were significant beyond the .01 level of confidence. It is important to note that a

replication of the study was run in which the test trial responses were scored by an independent observer, who watched only the test trials and who was unaware of what constituted a correct or incorrect response for any subject. The results of the replication were virtually identical to those of the first study.

Complex associative learning.—Some 40 years after van Oye's classic study, Ernhart & Sherrick (51) reported that planarians could be taught a 2-unit T maze in which the animals had to learn to turn in one direction at the first choice-point, in the opposite direction at the second choice-point, in order to reach a darkened goal box. Best & Rubinstein (35) have criticized this study on the grounds that the final alley leading to the black goal well was probably darker than its alternative, a criticism that might apply to the second choice-point in the maze but probably not to the first. Best & Rubinstein avoided this difficulty by using a dry plastic Y maze that was flooded with water as a reward and by training some of their worms to approach the lighted alley at the choice-point, others to approach the darkened alley. Both groups showed significant learning followed by the "conditioned lethargy" mentioned earlier in this review. Roe (22) designed a hexagonally shaped maze to avoid handling the animals between trials, an apparatus that is essentially a continuous maze with six different choice-points. The maze was well slimed at all times. Of the 30 animals trained, 21 reached the rather stringent criterion Roe imposed. Humphries & McConnell (37) tested large numbers of planarians under several different training regimens in a well-slimed maze similar to Roe's. Worms given but 25–30 trials per day, two days a week, showed better learning than worms on any other schedule. Animals given random shock, or no shock at all, showed random black/white choice behavior even after 540 trials (although they showed extremely rapid learning when subsequently shifted to a true training schedule). Jacobson (52) tested planarians for their position preferences in a simple T maze, then trained the animals to go to their nonpreferred side. While the animals reached the criterion of 9 correct responses out of 10 continuous trials, retention data suggested that the habit was an unstable one. Corning (53) has reported similar findings. Unfortunately, both Jacobson and Corning cleaned their apparatus carefully after each trial, a procedure that we now know leads to erratic behavior in mazes as well as in conditioning troughs (27).

In light of the rather large amount of evidence suggesting that maze learning can be achieved fairly readily in planarians, it is surprising that Jensen devotes less than a paragraph to the matter in his Cambridge paper and mentions but one experiment, that of Best & Rubinstein. Jensen dismisses this study in two sentences by noting that it "had light as a cue", and that, "according to Pearl, light could produce turning towards or away from the light, depending on other factors which influence whether the positive or negative reaction is given . . ." (6). Since Best & Rubinstein trained some animals to approach the lighted alley, others to approach the dark alley,

could we not assume that the "other factors" in this case were, indeed, those factors that most people call "learning"?

The first report of operant conditioning in planarians was by Lee (54), who trained his experimental animals to interrupt a weak beam of light (and hence trigger a photocell) in order to turn off a noxious stimulus (a bright overhead light). Paired with each experimental animal was a yoked control animal that was exposed to the same light on/off conditions but whose actions had no control over the noxious stimulus. The behavior of the two groups was quite dissimilar. Best (21) has confirmed and extended Lee's findings, showing that the planarian's rate of responding is dependent on the exact location within the experimental chamber of the photocell beam. Best reports also that animals that are removed from the experimental chamber after their first 7 hours of training and allowed to sit quietly for 40 hours show the same high level of performance when returned to the chamber as do animals that have undergone continuous training for the 47-hour period. This finding, as Best points out, is similar to the "reminiscence" phenomenon reported by Gelber in her paramecia (10). Seydoux stated informally at the Cambridge conference that the behavioral differences between the experimental and control animals that Best and Lee reported finding only after training, were observed by her as soon as she put untrained animals into her apparatus. Since Seydoux's work was not reported in detail, however, no real comparisons can be drawn.

Cannibals and chemicals.—One of the reasons that the research on planarian learning has excited such controversy is surely the set of studies showing retention of learning in all the regenerated pieces of transected, trained planarians, a "transfer of training" effect when a naive planarian has cannibalized a trained one, and a similar "transfer" when RNA (ribonucleic acid) extracted from trained animals was injected into untrained subjects. These studies suggest that the engram (the "physical trace of a memory") may be primarily biochemical in nature, a suggestion that runs counter to most neurologically oriented learning theories.

The first demonstration that a headless invertebrate could remember was provided by Yerkes in 1912, who found that an annelid of the species *Allo-lobophora foetida* trained in a T maze continued to turn to the correct side when its first five segments (containing the pharyngeal ganglia) were removed (55). Heck, in 1920, using a similar apparatus and several other varieties of earthworms, showed that a decapitated animal could both retain a previously learned habit and learn the maze anew in as few trials as an intact animal (56). The first study showing that both head and tail sections of regenerated planarians retained prior training was by McConnell et al. (57). As each animal reached criterion in a conditioning situation, it was cut in half and allowed to regenerate for 1 month; then both segments were retrained to the original criterion. Head and tail regenerates showed equal and significant retention, a finding replicated for maze learning by Ernhart & Sherrick (51),

for classical conditioning by Corning & John (58), for habituation by Westerman (33, 34), in a variety of situations by several other authors (see 19, 20), and by at least 2000 high school students in Science Fair projects. Roe (22) found retention of a maze habit in head regenerates, but not in tails, as did Angyan (see 19), but more than half of Roe's animals were discarded prior to the final test and Angyan's animals were apparently trained to a very weak criterion prior to cutting. VanDeventer & Ratner (26) found what appeared to be greater retention of a conditioned response in regenerated heads than in tails, but also report that untrained regenerates showed much the same level of performance as did the regenerates of priorly conditioned animals. In this study, however, all of the regenerating worms were kept in darkness for long periods of time; it is possible that retention was confounded with dark adaptation. Brown (28) found what he considers little evidence either for learning or for retention following regeneration in a classical conditioning paradigm, but used clean troughs and such a weak criterion of learning that little retention could have been expected. In all cases in which significant retention following regeneration has been demonstrated, the planarians have been trained to a high level of performance before being cut in half.

The regeneration studies suggested to me that the chemical theories of memory storage just gaining recognition then (59, 60) might hold for planarians as well as for higher organisms, a suggestion my students and I put to the test in 1961 (61) by taking advantage of the fact that hungry planarians are cannibalistic. One group of "victim" planarians was given the standard light/shock conditioning; as soon as each animal reached criterion, it was cut in pieces and fed to untrained cannibals. A group of untrained "victims" was likewise cut up and was then fed to a second group of untrained cannibals. All the cannibals were assigned code numbers and were given conditioning trials by an experimenter who did not know which cannibal had eaten which victim. The planarians that had eaten trained victims were significantly superior to the control cannibals from the first 25 trials onward. This "cannibalistic transfer of training" was rapidly confirmed for classical conditioning by several other investigators (62, 63, 64), and by Westerman (33, 34) for habituation to light. Wells (65), who had obtained a significant transfer effect with classical conditioning, failed to achieve a transfer when using maze learning; however, he apparently did not feed his "victims" to his cannibals as soon as the victims reached criterion, and his training technique was somewhat different from that used by most other investigators.

There is now a substantial body of data suggesting that RNA may act not only as a part of the biochemical storage mechanism in most organisms (60), but as the "transfer agent" as well (27, 66). The first test of Hydén's "RNA hypothesis" in planarians came from Corning & John [(58); described in greater detail in (48)], who allowed some of their classically conditioned animals to regenerate in pond water while others regenerated in a weak solution of ribonuclease (RNase), an enzyme that hydrolyzes RNA. All the pond water regenerates, as well as the heads that regenerated in the RNase solu-

tion, demonstrated the expected retention; the RNase tail regenerates, however, showed complete "forgetting" of the prior learning, although they could be thereafter conditioned in approximately the same number of trials as could totally naive animals. Fried & Horowitz (67) have more recently shown that RNase injected directly into conditioned planarians causes an "erasure" of prior training that saline injections do not bring about, thus confirming a prior observation by John (58) in cats. Ribonuclease is such a destructive enzyme, however, that the "erasure" phenomenon might well have been due to fairly extensive internal damage to the animals. Therefore, in 1962, my associates and I decided to test the RNA hypothesis in a more direct way—by extracting RNA from conditioned planarians and injecting it directly into the bodies of untrained animals (68, 69). We found that worms injected with "trained" RNA were significantly superior to worms injected with: (a) RNA taken from pseudoconditioned animals; (b) RNA taken from planarians that received no training whatsoever; and (c) distilled water. There were a number of methodological difficulties with our experiment; however, Fried & Horowitz were able to replicate the study using an improved method of extracting the RNA (67). They also showed that RNA that had been treated with RNase prior to injection did not effect a transfer.

One very thorny problem concerning the cannibalism and injection studies has yet to be solved, at least with planarians: Is the "transfer effect" specific or general? That is, are specific "memories" being passed from one organism to another, or is it merely a generalized "activity level" that gets transferred? I have suggested elsewhere that both types of effect might be transferable, but Hartry et al. (70) have argued that their data indicate the effect is general and not specific. In their experiment, one group of cannibals ate conditioned worms, a second group cannibalized victims exposed just to light, a third group ate victims that had merely been "handled" in the conditioning trough without receiving either light or shock, a fourth group devoured worms exposed just to shock, while a fifth group cannibalized untrained victims. In terms of the average number of conditioned responses given by the animals in the first 25 trials after cannibalization, the first 3 groups were significantly superior to the last 2 groups. My own view is that these data show both a specific and a general transfer effect (47).

Several studies currently in progress are designed to settle the matter once and for all, at least as far as planarians are concerned. Parenthetically, it is interesting to note that two groups of investigators, working at opposite sides of the globe and stimulated to action by the planarian work, have seemingly resolved the issue as far as rats are concerned. In a recent set of studies both Jacobson and his colleagues at UCLA (71, 72) and Fjerdingsstad, Nissen & Røigaard-Petersen at the University of Copenhagen (73, 74) report they have achieved what appears to be a highly specific transfer of training by extracting RNA from the brains of trained rats and injecting it into untrained rats, then testing the injectees in a variety of situations.

Discussion.—Of the 60 or so experiments on planarian learning published

since van Oye's classic paper, all but 5 or 6 have provided evidence that "worms can learn". Anyone coming to the problem without strong theoretical or emotional biases (and with a more or less standard definition of "learning" in mind) might well be expected to decide the issue in the affirmative. The transfer data are more difficult to evaluate, in part because the chemical mechanisms involved are so little understood, in part because the psychological complexity of the learning process itself is so little appreciated. The fact that something that influences behavior gets transferred via cannibalism and RNA injection in planarians seems beyond doubt; if we accept the fact that specific memories can be transferred from one rat to another via RNA injection, it would seem rather illogical to deny the existence of a similar phenomenon in planarians.

ANNELIDS

A survey of the literature on learning in the annelids prior to 1963 can be found in Jacobson's recent review (19).

Innate behavior.—Arbit (75) found that innate diurnal activity cycles have an important influence on maze learning in *Lumbricus terrestris* and *Eisenia foetida*—both species of earthworm are physically more active and learn mazes more quickly at night than during the day. The clamworm *Nereis virens* had no similar innate cycles; however, if fed daily during a specific 4-hour period, it showed greater activity and more rapid learning during this 4-hour period than at any other time in the 24-hour cycle. Injecting or spraying the animals or their living quarters with such substances as curariform drugs, norepinephrine, tetraethylammonium, and hexamethonium (which block autonomic nervous system activity in mammals), or compounds structurally similar to acetylcholine or cholinesterase, had no effect either on the diurnal activity cycle or on spontaneous alternation in a T maze; however, spraying the worms' living quarters with lysergic acid diethylamide (LSD) caused a noticeable disorganization in the animals' behavior.

Reactive inhibition.—Arbit (75) was able to achieve spontaneous alternation in a T maze with *L. terrestris* and *E. foetida*, but failed to find evidence for reactive inhibition when the choice-point had 4 alleys instead of 2 (a combined Y and T maze). Apparently it is necessary for the animal to "bump its nose" at the choice-point for the reactive inhibition to show itself fully, a situation that does not obtain when a Y maze is used.

Habituation.—Clark (76) has demonstrated habituation of the withdrawal reflex in the tube-dwelling polychaete *Nereis pelagica* to such stimuli as mechanical shock, moving shadows, and a decrease or increase in light intensity. *Nereis diversicolor* and *Platynereis dumerilii* likewise show habituation of the withdrawal reflex to photic stimulation, but not to tactile stimulation presented either to the anterior or the posterior end of the animal [Evans, see (76)]. *N. pelagica*, a carnivore, shows an attack response to anterior tactile stimulation but a withdrawal reflex to posterior stimulation; the latter response habituates, but fairly slowly. Habituation to moving

shadows in *N. pelagica* is maintained for only a few minutes in the absence of any stimulation. However, if this animal is first prehabituated to moving shadows, and then one attempts to habituate it to sudden decreases in light intensity, a complex interaction occurs between the two habituation processes: the habituation to light is noticeably delayed but the habituation to moving shadows is maintained over long periods of time. This complex interaction does not occur in *N. diversicolor*. Decerebrate worms habituate as rapidly as intact animals even to visual stimuli (see 76), an odd finding since decerebration inevitably results in removal of the prostomial eyes, and no dermal light receptors have as yet been described in *Nereis*. These findings, as well as earlier studies by Clark (76), suggest that habituation is mediated at the level of the segmental ganglia in annelids.

Conditioned inhibition.—When *Nereis* is first placed at the entrance to a tube, it immediately crawls to the opposite end and searches around the exit. Evans (77) has demonstrated that this innate reflex can be inhibited by shocking the animal when it reaches the far end of the tube. *N. virens* acquires this conditioned inhibition in less than half the trials required for *N. diversicolor* and *Perinereis cultrifera*. If the supra-oesophageal ganglion is removed from trained *N. virens*, the decerebrate animals retain the conditioned inhibition as long as do intact animals. Worms decerebrated prior to training acquire the inhibition as rapidly as intact controls provided the trials are massed, although retention is very poor compared to controls.

Classical conditioning.—Both Ratner and his associates (78, 79, 80) and Wyers and his colleagues (81) have recently demonstrated classical conditioning in *L. terrestris* using a vibratory stimulus as the conditioned stimulus and a bright light (which causes a withdrawal response) as the unconditioned stimulus. Animals trained on a partial reinforcement schedule (where the unconditioned stimulus is presented only 50 per cent of the time) learn as fast as worms trained with 100 per cent reinforcement, but the partial-reinforcement animals show a greater resistance to extinction (81), a finding more or less in agreement with a similar study on planarians (40). Decerebrate earthworms can be conditioned only if the inter-trial interval is 10 seconds or less (80).

Complex associative learning.—As Jacobson has pointed out, there seems little doubt that annelids are capable of learning mazes (19). As far as interspecies differences are concerned, Evans (82) has shown that *N. virens* learns a T maze faster than *N. diversicolor* and *P. cultrifera*, a confirmation of earlier findings on conditioned inhibition in these three species. Evans further reports that the training schedule is of critical importance in maze learning. If *N. virens* is given an electric shock and returned to the start of the maze whenever it makes an incorrect response, but allowed at least 5 minutes in a darkened chamber when it makes a correct response, it reaches criterion rapidly and maintains a high level of correct responding for an indefinite period of time. If the animal is not given a rest period following a correct choice but is immediately returned to the start of the maze, it may

reach criterion but it typically shows a reversal immediately thereafter and often refuses to enter the maze at all. Clark (76) considers both the return to the darkened chamber and the long inter-trial interval to be rewarding, and both factors to be necessary for a consistent and sustained improvement in performance to take place. Since similar types of "conditioned lethargy" in planarians can be controlled by spreading the trials out in time (35, 37), it may be that whatever "reward"-value the 5-minute-long "rest period" may have is of less importance than the fact that the inhibition built up from running the maze can effectively be dissipated during this interval. That the inter-trial interval cannot be too long was shown by Datta (83), who obtained evidence for maze learning in earthworms with inter-trial intervals of 1 and 5 minutes but less evidence when the intervals were 25 or 125 minutes. Datta found some savings of the learning after 5 days of rest but none after 15 days.

Although several early studies indicated that oligochaetes (earthworms) retained a maze habit after decerebration, and that decerebrate naive animals could be trained in mazes, Evans (82) obtained contrary results with polychaetes. As Clark has pointed out (76), however, while decerebration of an animal such as the polychaete *Nereis* may or may not disrupt the retention of the learned response, it surely abolishes input from the important sense organs at the anterior of the animal and hence interferes with the sensory discrimination the animal must make in order to pick the correct alley. Clark suggests that the latter effect, not the former, may explain Evans' data. In confirmation of Clark's beliefs, Flint (84) has recently found that following an operation that "disconnects" the supra-oesophageal ganglion from the rest of the nervous system but that still allows sensory input to the sub-oesophageal ganglion and the ventral nerve cord, at least half of the trained animals showed retention of a previously learned maze habit. Naive animals subjected to this operation prior to training were unable to learn the maze, however. Clark concludes: "Storage of acquired information ('memory') does not depend upon the preservation of the structural integrity of the central nervous system, and, in particular, the supra-oesophageal ganglion, despite its structural complexity and relatively large number of nerve cells, does not serve as a unique memory storage centre" (76).

Discussion.—The similarities between learning in annelids and in flatworms are particularly striking. The major difference comes in the studies on learning and retention in decerebrate animals. To date, no one has published data showing evidence for learning in decapitated planarians, probably because light is the typical conditioned stimulus in conditioning experiments, and removal of the cerebral ganglia almost always entails removal of the eye spots too, and because the brainless planarian (unlike the decerebrate annelid) shows relatively little spontaneous movement until the brain has regenerated, hence it would be quite difficult to train planarians in either

situation. There is a possible further difference—while decerebrated earthworms show retention of a maze habit if tested immediately after the operation, Yerkes (55) reported that as the new brain grew back in his one decapitated subject, the animal progressively “forgot” the maze (a study that, alas, no one has since attempted to replicate). As Jacobson (19) has pointed out, presuming that Yerkes’ finding can be verified, much could be learned about the role of the oesophageal ganglia in learning by cutting off the *new* head of a trained, regenerated earthworm and then testing the headless worm a second time to determine whether the entire nervous system had forgotten the habit or whether the regenerated ganglionic tissue had merely “usurped control” of the animal’s behavior from lower centers.

MOLLUSKS

The only mollusks that have figured prominently in research on learning have been the squids and the octopods. The bulk of these experiments have involved the training of these cephalopods in rather complex learning situations since, as Wells has said, “In the case of *Octopus* there is now no doubt that the species can learn, whatever definition of learning one cares to employ” (85). Studies on reactive inhibition, habituation, classical conditioning, and most other “simple” forms of learning therefore are absent from the recent literature. Reviews of earlier experiments can be found in works by Wells (86) and Young (87).

Conditioned inhibition.—Newly hatched *Sepia* (a European squid) apparently recognize such prey as mysids innately, attacking this small crustacean to the exclusion of all other moving objects during their early life. Wells (88) indicates that for the first month after hatching, it seems to be impossible to teach *Sepia* not to attack mysids. By the middle of the second month of life, however, *Sepia* can be taught to leave mysids alone. Wells points out that the failure to learn by very young *Sepia* is correlated with the comparatively late development of the vertical lobe, known to be involved in learning and memory retention in the adult cuttlefish.

Work by several authors (reviewed in 85) shows that octopuses can be taught to make a variety of tactile, chemotactile, and visual discriminations. Most of these studies have required the animal to learn to approach one stimulus that is always paired with some reward (such as a piece of fish or a crab) while avoiding a second stimulus paired with electric shock. Although the training procedures employed are open to some criticism, these studies indicate that both a conditioned approach and a conditioned avoidance response can be achieved in *Octopus*. Further studies by Wells (85) suggest that octopuses fail to make conditioned discriminations when they must integrate information about the movement of parts of their own bodies when they learn. For example, blinded animals cannot distinguish between objects differing in shape (if texture is controlled) nor between objects differing in weight; removal of the statocysts in visually intact animals upsets

recognition of figure orientation since the animal then has no means of discriminating its own bodily orientation in space.

Complex associative learning.—That an organism's past history affects which cues it will pay attention to in a complex learning situation was recently demonstrated by Sutherland et al. (89). One group of octopuses was trained to make a shape discrimination, another group to make a size discrimination. Then both groups were presented with a second problem that could be solved either in terms of size or shape. After they had learned this second problem, the octopuses were all given transfer tests to determine which cues they had paid attention to. The animals that could solve both prior problems using size as a cue learned something about shape in the second problem, but less about it than the first group of animals. Sutherland et al. conclude that octopuses pay attention to various cues in their environment, even to those cues not necessary for solution of the problem before them, but at any given moment the animals attend to some cues more than to others. These findings are in agreement with an earlier study by the same authors (90) in which they showed that octopuses trained on an easy discrimination problem and then presented with progressively more difficult discriminations learned faster than octopuses given the same amount of training only on difficult problems. Apparently in the easier problems the animals learn which cues are important to the solution of the problems.

If rats and other higher organisms are deliberately overtrained on a brightness discrimination, they learn the reversal of this discrimination faster than do animals not given this overtraining (91, 92). In 1962 Mackintosh (93), using a very simple visual problem, failed to demonstrate the same effect in octopuses. That it was the experimental situation that was too simple, not the subjects, was shown by Mackintosh & Mackintosh a year later (94). Apparently in *Octopus* overtraining facilitates reversal learning only when there are irrelevant cues present in the learning situation. It would appear that during overtraining the animals learn to ignore all but the cues that are relevant to the solution of the problem; the animals not given this overtraining still pay some attention to the irrelevant cues, hence their learning is retarded during a reversal of the original problem.

One of the classic experiments in comparative psychology is the *Umweg* or detour problem in which the animal is shown a goal (such as food) but must turn away from the desired object and go around a barrier of some kind in order to reach the goal. The higher mammals have little difficulty in solving such problems, but chickens, for example, find it exceptionally difficult to turn their backs on the goal in order to get to it. A study by Wells (95) indicates that *Octopus* is more like a mammal than a chicken, at least as far as its detour behavior is concerned. Octopuses were shown a crab through a glass window in chambers either to the left or to the right of their home compartment. To get to the crab, the animal had to move down an opaque passage (out of sight of the food) and make either a left or a right turn at the end. The animals learned this problem readily. After unilateral

section of the optic nerves, an octopus trained before the operation continues to detour reliably to one side but usually fails to do so to the other. Wells concludes that octopuses solve this problem visually and that they do not remember the movements that they have made in detouring (85).

Bitterman (96) has recently proposed a theory of the evolution of intelligence that holds that the brain structures evolved by higher animals do not serve merely to replicate old functions and modes of intellectual adjustment but to mediate new ones. Bitterman has based his theory to a great extent on his own studies of reversal learning in various animals, experiments that suggest that organisms at the level of the fish and below fail to show progressive improvement over a series of reversal problems. A study by Mackintosh & Mackintosh (97) indicates that the octopus may well present certain difficulties to Bitterman's theory, for these invertebrates show precisely the type of progressive improvement in reversal problems that the rat and the monkey show. Indeed, the octopus is likely to prove to be rather a leathery morsel for many psychological theories to digest and assimilate. It is unfortunate that housing and maintenance difficulties prevent the more widespread use of the cephalopods in psychological laboratories.

Discussion.—Wells' Cambridge presentation could profitably be read by anyone interested in comparative neurophysiology (85). Pointing out that the brains of soft-bodied animals would have to be abnormally large if the brain were to exercise detailed control over the position of the whole of the animal's body, he concludes that motor control is hierarchic in these animals and that detailed proprioceptive information never penetrates to the highest centers in the brain. Wells believes that this necessary decentralization of control of motor responses is inevitable in animals where movement is unrestricted by joints, and that absence of any central monitoring of the position of parts of the body would mean that these animals could not be taught to make skilled movements, nor could their movements be brought under what Skinner has called "operant control". Thus, according to Wells, "the things that it is reasonable to expect flexible animals to learn to do are necessarily different from the things arthropods and vertebrates can learn" (85), a conclusion that surely will provide grist for the experimental mill in the future.

ARTHROPODS

Considering their enormous diversity and the size of the phylum, relatively few studies of learning in arthropods have been reported over the years and, as Bullock & Horridge (2) point out, those species studied most intensively by the behaviorists (the social insects) have for the most part been neglected by the neurophysiologists (and vice versa). The fascinating experimental possibilities offered by animals in this phylum can be illustrated by reference to two recent papers—Horridge's use of headless insects in learning situations (see below) and Corning's (98) technique of permanent

electrode implantation in and cannulation of the horseshoe crab *Limulus*. In the latter preparation, it is possible not only to record electrophysiological events in single ganglia during training but also, by pumping coolants through cannulae, to freeze the ganglia for biochemical analysis at any instant during learning. Drugs and radioactive isotopes can of course also be added directly to the ganglia through the cannulae whenever desired. It is difficult to imagine undertaking similar studies with higher organisms.

Reactive inhibition.—In a series of recent papers, Dingle (99–102) has demonstrated spontaneous alternation in the adult and larval forms of a variety of insects including the mealworm *Tenebrio* (a beetle larva), thus confirming prior reports of this latter-named insect (see 102). Dingle's experiments also confirm prior observations that the magnitude of the alternation effect decreases as the distance from the forced turn to the choice-point is increased, but also that the frequency of alternation increases as one lengthens the distance of a straight run the animal must make prior to encountering the forced turn. Previous studies (see 31) have suggested that increasing the number of forced turns prior to the choice-point increased the magnitude of the effect but in all these studies an animal making just one forced turn prior to the free choice situation always traveled a considerably shorter distance in the apparatus than an animal making several forced turns.

Dingle (102) has concluded, on the basis of his various experiments, that animals have an innate tendency towards nonrandom movement but that various stimulus inputs (such as the kinesthetic cues from a forced turn and visual patterns in the environment) cause the animal to deviate from its tendency to move straight ahead at all times. Unfortunately, Dingle's studies are open to rather severe methodological criticisms that tend to vitiate his conclusions. To begin with, as was pointed out earlier in this review, the shape of the testing apparatus is absolutely critical—spontaneous alternation usually does not occur if the alleys are too wide or if the "free choice" test point is not clear cut. Dingle apparently failed to find spontaneous alternation in two species of ants because the pathway used was too wide (99) and in several of his studies it is not at all clear that the animal was always aware it had reached a choice-point of any kind.

Complex associative learning.—Much of the early literature on learning in crustacea is summarized in Schoene's Cambridge paper (103). There seems little doubt that a variety of rather complex forms of learning occur in crabs, lobsters, and crayfish, including some rather interesting modifications of innate behavior patterns. Since a great deal more is known about the nervous systems and receptor organs of these animals than is true of many other species, and particularly since implantation techniques such as Corning's (98) should be applicable to crustacea, it is a pity that more behavioral research hasn't been done with these animals.

Experimental psychologists too often ignore the sorts of learning that animals show in natural settings, while the ethologists who observe animal

behavior "in the wild" often fail to "test the limits" of an organism's capabilities to adjust to controlled changes in its environment. In their Cambridge presentation, van Iersel & van den Assem (104) report a series of experiments on the diggerwasp *Bembix rostrata* that nicely takes into account some of the best features of both the experimental and the ethological approaches. After the female diggerwasp digs a nest in sandy soil, she leaves to catch a fly on which her egg will be laid. On several subsequent days, after the egg has hatched, the wasp brings as many as 15 flies back to the nest. Upon her return trips, *Bembix* locates the entrance to her nest by visual cues, apparently making use of visual stimuli both in the immediate vicinity of the nest and on the distant horizon. If some change has occurred in the cues around the nest, the wasp will make a reorientation flight over the nest site when next she leaves to catch another fly. By disrupting the nest site in systematic ways, van Iersel & van den Assem were able to show that the greater the disruption, the longer the subsequent reorientation flight will be. It would appear that the length of the flight is correlated with the gross amount of new information the wasp must absorb if she is to find her way back to the nest site successfully.

If a rat is trained to turn right in a T maze and then, once it has mastered this habit, is "reversed" and trained to go left, it typically makes a great many more errors on the reversal problem than it did during the original learning. Rats show progressive improvement over a series of reversal problems, but fish do not (96). Longo (105) trained cockroaches in a Y maze, giving them 10 trials per day. Some animals were "reversed" daily, some were "reversed" only at the end of 4 days; the latter animals made fewer errors daily than did the former, but neither group apparently showed the drastic increase in errors after the first reversal typical of both the rat and the fish. Since the roaches were not trained to criterion prior to reversal, it is difficult to know whether these findings represent yet another exception to Bitterman's theory (96) or not.

Classical conditioning.—Horridge's Cambridge paper (106) is, in my opinion, a most brilliant attempt at bridge-building between the disciplines of behavioral psychology and comparative neurophysiology. In an earlier paper, Horridge (107) reported that when a suspended headless cockroach or locust is arranged so that one leg receives regularly repeated electric shock for all the time that the foot falls below a particular position, the animal learns to hold its leg above that position. Similar results are obtained when the animals are trained on one leg and tested on another leg. In the cockroach, this transfer of training occurs more readily backwards than forwards along the ventral cord. Clearly, in the absence of a brain, the ventral ganglia are able to associate a position of the leg with punishment from the shock. At Cambridge Horridge reported several additional points concerning this preparation: leaving the head on the animal or removing it before or after training trials makes little difference, although headless animals are easier to work with; almost any noxious stimulus to the leg is

effective as an unconditioned stimulus in training; the learning observed during training rarely proceeds smoothly, but rather there are intermittent lapses and sudden improvements in performance; retention of the conditioning lasts for several hours after training ceases; the learning can be completely "erased" by applying electrical stimulation to the ventral connectives after training. Eisenstein & Cohen (108) extended these findings by showing that the same type of learning could be achieved in the prothoracic ganglion after it had been removed from the rest of the ventral cord (a finding Horridge subsequently confirmed); furthermore, there are a variety of different ways in which the leg can be raised in the isolated ganglion preparation—if the animal is prevented from using one method, it will adopt an alternative. Horridge (106) also cites interesting observations by G. Hoyle (109) indicating that postural learning in headless locusts can be used for electrophysiological analysis in rather an intriguing way. Recordings made of motor impulses to a leg-lifting muscle show spontaneous changes in frequency. If punishment is given to the leg (or to the leg nerve) whenever the frequency of these spontaneous impulses falls, then the average frequency rises and stays high for relatively long periods of time. If punishment is given whenever the frequency rises, then the frequency will fall. If the punishment is dissociated from changes in frequency, then the punishment has no effect on frequency. Horridge closes his paper with a lucid and insightful discussion of the problem of finding meaningful long-term electrophysiological changes that are correlated with behavior changes. This article should be "required reading" for anyone interested in the problem.

CONCLUSIONS

Can invertebrates learn? The bulk of the evidence reviewed above suggests that complex associative learning can be found as far down the phyletic scale as the planarians, and that simpler forms of learning (such as reactive inhibition and habituation) can be found even in the protozoans. Why then the dissenting opinions? Frankly, I believe it is because many of us have adopted (usually without realizing it) what I will call "the anthropocentric assumption", namely, the belief that man's behavior is somehow qualitatively superior to the behavior of all other forms of life. A century ago we could have contended that man was divinely ordained to be better than the other animals, but theological arguments no longer carry much weight in science and the Theory of Evolution strongly suggested that man differed from the rest of the animal kingdom in degree but not in kind. Rather than our giving up the implicit assumption that man is something rather special when we embraced the Theory of Evolution, I believe we simply hunted for measurable, quantitative differences that could be made to support our *a priori* belief in man's qualitative superiority. As it happens, humans have a noticeably more complex central nervous system than do other animals, and it would seem that behavioral complexity should somehow be correlated with neurophysiological complexity. How easy it apparently was (and how

comforting!) for us to adopt the viewpoint that the brain was all-important and that animals with but the simplest of neural structures (the planarians) or with no brain at all (the protozoans) should therefore be incapable of complex behavioral modifications no matter what the experimental data might seem to indicate. Of course, the stronger our emotional commitment to the "anthropocentric assumption", and the more "brain-bound" we are in hunting for a justification of our emotionality, the more likely it is that we will reject any evidence purporting to show the occurrence of learning in the lower phyla. For instance, a few years ago one of the leading antagonists in this controversy was asked why he thought that planarians couldn't possibly be trained. This comparative psychologist, whose theory of behavior predicts that no animal lower on the scale than the fish can learn, replied that planarians simply don't have enough neurons. When asked how many neurons he thought they had, he stated flatly that he didn't know, but however many it was, it wasn't enough. The "anthropocentric assumption" is probably much more widely held in the biological sciences than is generally recognized and must be guarded against vigorously if the behavior of the invertebrates is to be evaluated objectively rather than emotionally.

LITERATURE CITED

1. Barnes, R. D., *Invertebrate Zoology* (Saunders, Philadelphia, 632 pp., 1964)
2. Bullock, T. H., and Horridge, G. A., *Structure and Function in the Nervous Systems of Invertebrates* (W. H. Freeman, San Francisco, 2 vol., 1719 pp., 1965)
3. Melton, A. W., Ed., *Categories of Human Learning* (Academic Press, New York, 356 pp., 1964)
4. Hull, C. L., *Essentials of Behavior* (Yale Univ. Press, New Haven, Conn., 145 pp., 1951)
5. Kimble, G. A., *Hilgard and Marquis' Conditioning and Learning Revised* (Appleton—Century—Crofts, New York, 590 pp., 1961)
6. Jensen, D. D., *Animal Behavior*, 13, 9-20 (1965)
7. Lepley, W. M., and Rice, G. E., *J. Comp. Physiol. Psychol.*, 45, 283-86 (1952)
8. Lachman, S. J., and Havlena, J. M., *J. Comp. Physiol. Psychol.*, 55, 972-73 (1962)
9. Rabin, B. M., and Hertzler, D. R., *Worm Runner's Digest*, 7, No. 2, 46-50 (1965)
10. Gelber, B., *Animal Behavior*, 13, 21-29 (1965)
11. Jensen, D. D., *Science*, 125, 191-92 (1957)
12. Katz, M. S., and Deterline, W. A., *J. Comp. Physiol. Psychol.*, 51, 243-47 (1958)
13. Ross, D. M., *Animal Behavior*, 13, 43-53 (1965)
14. Rushforth, N. B., Burnett, A. L., and Maynard, R., *Science*, 139, 760-61 (1963)
15. Rushforth, N. B., Krohn, I. T., and Brown, L. K., *Science*, 145, 602-4 (1964)
16. Rushforth, N. B., *Animal Behavior*, 13, 30-42 (1965)
17. Van Oye, P., *Natuurw. Tijdschr. (Ghent)*, 2, 1-9 (Dec. 1920)
18. Austad, E., *Worm Runner's Digest*, 7, No. 2, 41-45 (1965)
19. Jacobson, A. L., *Psychol. Bull.*, 60, No. 1, 74-94 (1963)
20. Jacobson, A. L., *Animal Behavior*, 13, 76-82 (1965)
21. Best, J. B., *Animal Behavior*, 13, 69-75 (1965)
22. Roe, K., *Worm Runner's Digest*, 5, No. 2, 16-24 (1963)
23. Brown, F. A., *Biol. Bull.*, 123, 282-94 (1962)
24. Brown, F. A., *Biol. Bull.*, 125, 206-25 (1963)
25. Brown, F. A., and Park, Y. L. H., *Nature*, 202, 469-71 (1964)
26. VanDeventer, J. M., and Ratner, S. C., *J. Comp. Physiol. Psychol.*, 57, 407-11 (1964)
27. McConnell, J. V., Ed., *A Manual of Psychological Experimentation on Planarians (Worm Runner's Digest)*, Ann Arbor, Mich., 110 pp., 1965)
28. Brown, H. M., *Experimental Procedures and State of Nucleic Acids as Factors Contributing to "Learning" Phenomena in Planaria* (Unpublished doctoral thesis, Univ. Utah, Salt Lake City, Utah, 1964)
29. Barnes, C. D., and Katzung, B. G., *Science*, 141, 728-30 (1963)
30. Bennett, E. L., and Calvin, M., *Neurosci. Res. Program Bull.*, 2, No. 4, 3-24 (1964)
31. Shinkman, P. G., and Hertzler, D. R., *Psychon. Sci.*, 1, 407-8 (1964)
32. Lee, P., *Worm Runner's Digest*, 6, No. 1, 42 (1964)
33. Westerman, R., *Worm Runner's Digest*, 5, No. 2, 6-11 (1963)
34. Westerman, R., *Science*, 140, 676-77 (1963)
35. Best, J. B., and Rubinstein, I., *J. Comp. Physiol. Psychol.*, 55, 560-66 (1962)
36. Best, J. B., *Sci. Am.*, 208, 55-64 (1963)
37. Humphries, B., and McConnell, J. V., *Worm Runner's Digest*, 6, No. 1, 52-59 (1964)
38. Thompson, R., and McConnell, J. V., *J. Comp. Physiol. Psychol.*, 48, 65-68 (1955)
39. Baxter, R., and Kimmel, H. D., *Am. J. Psychol.*, 76, 655-69 (1963)
40. Crawford, F. T., King, F. J., and Siebert, L. E., *Psychon. Sci.*, 2, 49-50 (1965)
41. Vattano, F. J., and Hullett, J. W., *Psychon. Sci.*, 1, 331-32 (1964)
42. Walker, E. L., in *Nebraska Symposium on Motivation* (Levine, D., Ed., Univ. Nebraska Press, Lincoln, Neb., 284 pp., 1964)
43. McConnell, J. V., *Animal Behavior*, 13, 61-68 (1965)
44. Halas, E. S., James, R. L., and Knutson, C., *J. Comp. Physiol. Psychol.*, 55, 969-71 (1962)
45. James, R. L., and Halas, E. S., *Psychol. Record*, 14, 1-11 (1964)

46. McConnell, J. V., *Psychol. Record*, **14**, 13-20 (1964)
47. McConnell, J. V., *Worm Runner's Digest*, **7**, No. 1, 1-8 (1965)
48. John, E. R., in *Brain Function: RNA and Brain Function, Memory and Learning* (Brazier, M. A. B., Ed., Univ. California Press, Berkeley, 360 pp., 1965)
49. Pearl, R., *J. Microscop. Sci.*, **46**, 509-714 (1903)
50. Griffard, C. D., and Peirce, J. T., *Science*, **144**, 1472-73 (1964)
51. Ernhart, E. N., and Sherrick, C., *Retention of a Maze Habit Following Regeneration in Planaria* (D. maculata) (Paper read at Midwestern Psychol. Assoc., St. Louis, Missouri, May 1959)
52. Jacobson, A. L., *An Attempt to Demonstrate Transfer of a Maze Habit by Ingestion in Planaria* (Unpublished doctoral thesis, Univ. Michigan, Ann Arbor, Mich., 1962)
53. Corning, W. C., *J. Psychol.*, **58**, 131-39 (1964)
54. Lee, R. M., *Science*, **139**, 1048-49 (1963)
55. Yerkes, R. M., *J. Animal Behavior*, **2**, 332-52 (1912)
56. Heck, L., *Lotos*, **68**, 168-89 (1920)
57. McConnell, J. V., Jacobson, A. L., and Kimble, D. P., *J. Comp. Physiol. Psychol.*, **52**, 1-5 (1959)
58. Corning, W. C., and John, E. R., *Science*, **134**, 1363-64 (1961)
59. Hydén, H., *Proc. Natl. Acad. Sci. U.S.A.*, **49**, 618-24 (1963)
60. Hydén, H., in *Brain Function: RNA and Brain Function, Memory and Learning* (Brazier, M. A. B., Ed., Univ. California Press, Berkeley, 360 pp., 1965)
61. McConnell, J. V., *J. Neuropsychiat.*, **3**, Suppl. 1, S42-S48 (1962)
62. Smith, S. J., *Worm Runner's Digest*, **5**, No. 1, 49-54 (1963)
63. Wells, P. H., *Worm Runner's Digest*, **5**, No. 1, 58-59 (1963)
64. Shannon, L., and Rieke, J., *Worm Runner's Digest*, **6**, No. 2, 7-9 (1964)
65. Pickett, J. B. E., Jennings, L. B., and Wells, P. H., *Am. Zoologist*, **4**, No. 4, 158, (1964)
66. McConnell, J. V., *Harvard Rev.*, **3**, No. 2, 8-17 (1965)
67. Fried, C., and Horowitz, S., *Worm Runner's Digest*, **6**, No. 2, 3-6 (1964)
68. Zelman, A., Kabat, L., Jacobson, R., and McConnell, J. V., *Worm Runner's Digest*, **5**, No. 1, 14-21 (1963)
69. McConnell, J. V., *New Scientist*, **21**, 465-68 (1964)
70. Hartry, A. L., Keith-Lee, P., and Morton, W. D., *Science*, **146**, 274-75 (1964)
71. Jacobson, A. L., Babich, F. R., Bubash, S., and Jacobson, Ann., *Science*, **149**, 656 (1965)
72. Babich, F. R., Jacobson, A. L., Bubash, S., and Jacobson, Ann., *Science* (In press)
73. Fjerdingstad, E. J., Nissen, Th., and Røigaard-Petersen, H. H., *Scand. J. Psychol.*, **6**, 1-6 (1965)
74. Nissen, Th., Røigaard-Petersen, H. H., and Fjerdingstad, E. J., *Scand. J. Psychol.* (In press)
75. Arbit, J., *Animal Behavior*, **13**, 83-88 (1965)
76. Clark, R. B., *Animal Behavior*, **13**, 89-100 (1965)
77. Evans, S. M., *Animal Behavior*, **11**, 172-79 (1963)
78. Ratner, S. C., *J. Comp. Physiol. Psychol.*, **55**, 174-77 (1962)
79. Ratner, S. C., and Stein, D. C., *J. Comp. Physiol. Psychol.* (In press)
80. Ratner, S. C., *Animal Behavior*, **13**, 101-8 (1965)
81. Wyers, E. J., Peeke, H. V. S., and Herz, M. J., *J. Comp. Physiol. Psychol.*, **57**, 113-16 (1964)
82. Evans, S. M., *Animal Behavior*, **11**, 379-92 (1963)
83. Datta, L.-E. G., *Am. J. Psychol.*, **75**, 531-53 (1962)
84. Flint, P., *Animal Behavior*, **13**, 187-93 (1965)
85. Wells, M. J., *Animal Behavior*, **13**, 115-28 (1965)
86. Wells, M. J., *J. Exptl. Biol.*, **41**, 433-45 (1964)
87. Young, J. Z., *Biol. Rev.*, **36**, 32-96 (1961)
88. Wells, M. J., *Symp. Zool. Soc. London*, **8**, 149-69 (1962)
89. Sutherland, N. S., Mackintosh, N. J., and Mackintosh, J., *J. Genet. Psychol.* (In press)
90. Sutherland, N. S., Mackintosh, N. J., and Mackintosh, J., *J. Comp. Physiol. Psychol.*, **56**, 150-56 (1963)
91. Mackintosh, N. J., *J. Comp. Physiol. Psychol.* (In press)
92. Mackintosh, N. J., *Animal Behavior*, **13**, 129-34 (1965)
93. Mackintosh, J., *Quart. J. Exptl. Psychol.*, **14**, 15-22 (1962)
94. Mackintosh, N. J., and Mackintosh,

- J., *Quart. J. Exptl. Psychol.*, **15**, 236-42 (1963)
95. Wells, M. J., *J. Exptl. Biol.*, **41**, 621-42 (1964)
96. Bitterman, M. E., *Am. Psychologist*, **20**, 396-410 (1965)
97. Mackintosh, N. J., and Mackintosh, J., *Animal Behavior*, **12**, 321-24 (1964)
98. Corning, W. C., Feinstein, D. A., and Haight, J. R., *Science*, **148**, 394-95 (1965)
99. Dingle, H., *Ecology*, **43**, 727-28 (1962)
100. Dingle, H., *Animal Behavior*, **12**, 116-24 (1964)
101. Dingle, H., *Animal Behavior*, **12**, 137-39 (1964)
102. Dingle, H., *Animal Behavior*, **13**, 171-77 (1965)
103. Schoene, H., *Animal Behavior*, **13**, 135-44 (1965)
104. van Iersel, J. J. A., and van den Assem, J., *Animal Behavior*, **13**, 145-62 (1965)
105. Longo, N., *Am. J. Psychol.*, **77**, 29-41 (1964)
106. Horridge, G. A., *Animal Behavior*, **13**, 163-82 (1965)
107. Horridge, G. A., *Proc. Roy. Soc. (London)*, **157**, 33-52 (1962)
108. Eisenstein, E. M., and Cohen, M. J., *Physiologist*, **7**, 123 (1964)
109. Hoyle, G., in *The Physiology of the Insect Central Nervous System* (Treherne, J. E., and Beament, J. W. L., Eds., Academic Press, London and New York, 203 pp., 1965)