Mini-Review

Biochemistry of Insect Learning: Lessons from Bees and Flies

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Recent advances in the study of learning in insects are examined with an emphasis on two of the most powerful model systems, the honeybee (Apis mellifera) and the fruit fly (Drosophila melanogaster). The honeybee exhibits easily manipulated feeding behavior coupled with extremely high mnemonic fidelity. The size of the honeybee brain has allowed for electrophysiological analysis of the neural correlates of behavior, sometimes with single cell resolution, as well as identification of critical brain regions. Drosophila has proved to be invaluable in the genetic dissection of learning. Through analysis of learning and memory mutants the biochemistry of critical steps has been elucidated and the temporal phases of memory in the fly have been described. Two regions of brain neuropil are essential for olfactory learning in these species: the antennal lobes and the mushroom bodies. In spite of similarities, temporal, and possibly biochemical aspects of learning differ markedly between these organisms. Copyright © 1996 Elsevier Science Ltd.

INTRODUCTION

The insects are rich in behaviors of stunning complexity. A bumblebee calculates the potential profits and her needs before visiting a certain type of flower (Heinrich, 1979; Real, 1991). An assassin bug uses the carcass of a termite which it has already consumed to fish near a nest entrance for new prey (McMahan, 1983). The distant locations of host plants scattered throughout the jungle are learned by the Heliconius butterflies that methodically visit them every day (Gilbert, 1975). How much of these behavioral patterns are learned and how much is inherited?

The concept of learning and what constitutes a learned response is intuitive to most people but in practice a precise definition is difficult. A modification of behavior, or behavioral plasticity, which is produced by previous experience is a simple definition of learning. Nonassociative learning occurs in response to one stimulus alone, and two of the most commonly studied forms are habituation (learning to ignore a cue) and sensitization (becoming more responsive to an otherwise innocuous cue). Associative learning, which includes classical conditioning, involves the temporal linkage of one stimulus to another, thus enabling one stimulus to elicit a response normally reserved for the other. By necessity, the degree of learning is assayed through memory, which is not a foolproof strategy as a perfect lesion in one will render the other undetectable. Even so, a time course of animal performance after training can sometimes separate a problem in acquisition (learning) from one in retrieval (memory).

This review will examine recent results obtained using two of the most popular organisms for studying insect learning: the honeybee, Apis mellifera, and the fruit fly, Drosophila melanogaster. The emphasis will be placed on the biochemical processes and neuroanatomical centers underlying insect learning and the mechanisms by which memories are stored. The parallels between learning in these two organisms are emphasized by recent research suggesting that shared mechanisms of neural plasticity link many different organisms (Kandel and Abel, 1995).

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LEARNING AND HONEYBEE BEHAVIOR

To address the problem of how learning occurs in any insect, the first task is to establish a training paradigm in which the degree of learning can be assessed. Honeybees remember the location, scent, color and even time of day which signal a food reward (von Frisch, 1967). Not only do the animals learn to associate a scent with a sugar reward after a single trial, but this can be unlearned, a necessary adaptation to foraging for a changing resource. The learned association of an odor with reward can also be demonstrated using the proboscis extension reflex (PER), as an index of the animals expectation of food (Bitterman et al., 1983; Erber et al., 1980). The food reward is called the unconditioned stimulus or US, and will evoke a response by itself. The stimulus used as a predictor for the US, in this case an odor, is called the conditioned stimulus (CS) because animal response to the CS is changed (conditioned) by association with the US. When sugar water (the US) is touched to the antennae, the bee reflexively extends its proboscis in anticipation of feeding, and is then fed a droplet of sugar water. If the CS is presented slightly before or with the US (forward pairing), the bee readily learns the predictive value of the scent and will extend its proboscis when the scent alone is presented. However, if the CS is presented after the US (backwards pairing), the bee does not recognize any predictive value in the CS and ignores it (Hammer and Menzel, 1995; Menzel, 1990). A conditioned PER can also be transferred from one CS to another one by presenting the new CS slightly before the already conditioned one, a phenomenon termed second order conditioning (Bitterman et al., 1983). Although a conditioned PER can be demonstrated after a single training trial, multiple trials are required for memory that lasts more than a few days. Conditioning can be extinguished by presentation of either stimulus in an unpaired fashion; in general, more recent memories, ones formed in response to smaller food rewards, and those resulting from fewer training trials are more easily extinguished (Hammer and Menzel, 1995). These observations parallel the deductions of von Frisch regarding the mental capabilities of bees based on their feeding behavior (von Frisch, 1967). Thus, the acquisition characteristics of the PER mirror some of the types of learning that occur in free-flying bees.

NEURAL BASIS OF LEARNING IN THE BEE

Two regions of neuropil, the antennal lobes and mushroom bodies (also known as the corpora pedunculata), are essential for olfactory conditioning and the initial memory of this conditioning (Erber et al., 1980). The antennal lobe is the primary site in the CNS in which olfactory information is received, as shown in Fig. 1. Olfactory sensory neurons terminate in the neuropil of its glomeruli, and interneurons in the antennal lobe form tracts that convey the information to the mushroom bodies and the lateral protocerebrum (Mobbs, 1985). The mushroom bodies are massive structures in the honeybee, formed from 170,000 Kenyon cells (intrinsinc neurons) whose dendrites form a dense neuropil called the calyx just beneath the dorsal surface of the brain (Mobbs, 1982). The mushroom body calyx is subdivided into regions which receive input from olfactory, visual and mechanosensory systems (Mobbs, 1982; Rybak and Menzel, 1993). Kenyon cell axonal tracts project anteriorly to form a bundle called the peduncle, and each axon forks, sending a branch medially to form the β lobe, and dorsally to form the α lobe. The polarity of the Kenyon cells and extrinsic neurons, i.e. non-Kenyon cell interneurons that establish connections within this structure, is deduced from the morphology of these neurons alone (see Mobbs, 1982; Rybak and Menzel, 1993). Although there is a consensus on the major direction of information flow within the mushroom body – the calyx being the main input region and the lobes conveying output – some interneurons appear to bring input into the lobes and Kenyon cell axons synapse with one another in the peduncle and lobes, thus providing a source of input to the axonal tracts of mushroom body cells (Mobbs, 1985).

Extrinsic neurons which have dendritic fields in the α or β lobes and project to other regions of the brain have been studied for clues to their function and the nature of
the information they carry (Bicker et al., 1985; Groenberg, 1987; Mauelshagen, 1993; Mobbs, 1982; Rybak and Menzel, 1993). Some extrinsic neurons have dendrites in the mushroom body lobes, axonal projections running back to the calyces, and are immunoreactive for the neurotransmitter GABA (Groenberg, 1987; Rybak and Menzel, 1993). Their unique positioning has led to speculation that they are involved in providing feedback to the calyces (Mobbs, 1982). If these interneurons project to different Kenyon cells than those which provide their input, they could form a system for comparing information from different sensory modalities. Alternatively, feedback could be a mechanism for maintaining an altered state, or for producing a time delay allowing comparison of input at different times (Mobbs, 1982; Schurmann and Elkes, 1987). Each of these theories is supported to some extent by electrophysiology. Convergence of sensory input certainly occurs in the mushroom bodies; studies of electrical activity indicate that the majority of extrinsic neurons respond to multiple sensory modalities (Groenberg, 1987; Mobbs, 1985). Sensory stimulation also results in long lasting changes in neuronal activity of mushroom body cells (Erber, 1978; Groenberg, 1987).

Recording from an identified extrinsic neuron, the PE1, which has dendrites in both the peduncle and α lobe and output in the lateral protocerebrum, detected changes in electrical activity dependent on classical conditioning protocols (Erber, 1978; Mauelshagen, 1993). This suggests that a conditioning-dependent change in the electrical correlates of sensation occurs either within the PE1 itself or within cells which feed into the PE1. The lateral protocerebrum, a region of the brain located laterally in the mushroom body peduncle and lobes, may also play a role in learning and memory. It receives information from sensory neuropils as well as from the mushroom body, and thus could evaluate or store information (Hammer and Menzel, 1995; Mauelshagen, 1993).

**ACQUISITION**

Using the PER, the early process of acquisition in the bee has been dissected in detail. The signal from the US appears to be relayed to a segmentally repeated octopaminergic interneuron in the suboesophageal ganglion, the ventral unpaired medial neuron, (VUMmx1) (Hammer, 1993; Hammer and Menzel, 1995). This neuron has an amazingly extensive axonal projection, with ramifications in the antennal lobes, the calycal region of the mushroom bodies (solid arrows in Panel A), as well as the suboesophageal ganglion and lateral protocerebrum. The VUMmx1 depolarizes in response to the application of sugar to antennae or tarsi. In addition, after PER conditioning it will depolarize in response to the CS alone (Hammer, 1993). Direct injections of octopamine into either the antennal lobe or the calyces of the mushroom bodies can substitute for sugar application in PER conditioning, as can depolarization of VUMmx1, but both of these treatments must obey the rules of forward pairing for CS and US; the odor must be presented slightly before or concurrent with the depolarization of the VUMmx1 or the injection of octopamine for conditioning to occur (Hammer, 1993). Thus, the VUMmx1 has the characteristics of an interneuron mediating the US, and furthermore, its firing in response to the CS following conditioning provides a mechanism by which second order conditioning could occur: by depolarization of VUMmx1 the animal creates a cellular model of the US every time it detects the CS, thus obviating the need for presentation of a real US. The regions to which the VUMmx1 has its most extensive projections, the antennal lobes and the calyces of the mushroom bodies, have been implicated in early stages of memory by cooling studies, and both are sites where integration of olfactory and gustatory information could occur (Mobbs, 1985). Protein kinase A (PKA) in the antennal lobe is transiently activated immediately after octopamine injection or sucrose application to the antennae, suggesting that octopamine may exert its effect through activation of adenyl cyclase (Hildebrandt and Muller, 1994).

**MEMORY PHASES IN THE BEE**

Acquisition is only the initial step in the learning process. Memory itself is dynamic, and temporal phases of memory have been documented in vertebrates and invertebrates. These usually consist of two or three short and intermediate-term phases, lasting from seconds to hours, and a long-term phase which may be first evident hours after training and can last for days or a lifetime. This latter phase has in most cases been shown to be dependent on protein synthesis (Davis and Squire, 1984; DeZazzo and Tully, 1995; Goelet et al., 1986). Bees also exhibit both short and longer term memories, and these can be dissociated to some degree by experimental protocol. Cooling of the animal shortly after learning will impair the formation of memory, but memory passes within minutes into a form which is insensitive to cooling (Erber et al., 1980; Menzel, 1990). Local cooling experiments revealed that the antennal lobes are the earliest brain neuropil necessary for the processing of associative learning (up to 3 min) followed by the calyces and α lobes of the mushroom bodies to about 7 min after training. Subsequent to this, memory is resistant to cooling. Longer lasting and cold resistant memories are divided into two categories: an intermediate term memory which lasts for a day or so and can be triggered by a single learning trial, and a concurrent long term memory (LTM), which can last for weeks, but only appears when multiple training trials are used (Hammer and Menzel, 1995). There is currently little information to allow the dissociation of intermediate term memory and LTM in the bee. In contrast to many other systems, a protein synthesis-dependent long term memory phase has not been found in the honeybee (Wittstock et al., 1993; Wittstock and Menzel, 1994). Bees may be unusual in not having
a protein synthesis-dependent long-term memory phase, or the failure to find this component of memory may be a function of the conditions under which the experiments have been done: bees were tested for retention after only 24 h, a time which may not be long enough to reveal the shift from short-term to long-term memory.

THE ROLE OF NEUROTRANSMITTERS

Although it appears that octopamine inputs to the mushroom body calyx are important there is no strong octopamine binding activity in the calycal neuropil, but there is very strong binding activity throughout the peduncle and both lobes (Erber and Homberg, 1987; Erber et al., 1993). This raises the question of where octopamine introduced into the calyx is acting. As its release by VUMnx I is at a distance from receptors in the peduncle, it was speculated that octopamine was neuromodulatory or neurohormonal in nature (Erber et al., 1993). A similar mismatch in serotonin binding activity and serotonin levels is observed in the mushroom body: the calyx has high binding activity, although serotonergic innervation has not been found in this region (Schurmann and Elkes, 1987). Local injection of serotonin reduces both learning and the ability to retrieve memories, suggesting that it can function in the mushroom body (Erber et al., 1993). Although aspects of mushroom body pharmacology, such as the importance of octopamine, seem quite certain, the precise role of neurotransmitters and the locations where they function are still unresolved.

DROSOPHILA: HOW TO LEARN WHAT A FLY KNOWS

The paradigms most often used to assay Drosophila learning (see Davis, 1996 for a detailed review) are of a much different nature than the PER used in bees, although the neurobiology of this reflex has been studied extensively in the fly Phormia regina (Dethier, 1976) and its conditioning demonstrated in Drosophila (Duerr and Quinn, 1982). First, Drosophila are not highly motivated foragers, nor do they have reason to use great mnemonic skills on a daily basis. In spite of this, habituation, sensitization and associative learning can all be demonstrated within the context of several Drosophila behaviors, including courtship (reviewed in Hall, 1994). A male fly learns to avoid all females after an unproductive encounter with a non-receptive (i.e. mated) female, however; flies with impaired learning show less courtship suppression than wild type flies (Hall, 1994). Both appetitive (Tempel et al., 1983) and aversive (Quinn et al., 1974) conditioning of Drosophila to odors have been accomplished, and the robust nature and ease of use of the later have made it widely used for evaluating learning and memory. Training consists of placing about 100 individuals in a tube lined with a copper grid. As an odor is drawn through the tube, an electric shock is applied to the grid. A different odor is then provided, without shock. Flies are subsequently placed in a T-maze and allowed to choose between the two odors; the percentage avoiding the odor paired with shock is usually above 80% (Tully and Quinn, 1985). This assay has allowed for direct questions about the biochemistry of learning and the function of the mushroom body to be asked using Drosophila subjected to pharmacological, genetic or developmental damage.

DISRUPTIONS OF THE MUSHROOM BODY

Much of Drosophila learning research has focused on trying to resolve the function of their mushroom bodies. These are much reduced compared with the bee, having only about 2500 cells per hemisphere (Balling et al., 1987). As shown in Fig. 1, they consist of a small dendritic calyx beneath the Kenyon cell bodies, and axonal tracts which form the peduncle and project to the anterior edge of the brain where they divide to form the dorsally oriented α lobes, and paired, medially directed β and γ lobes (Power, 1943).

Several structural mutants in the mushroom bodies have been isolated in an attempt to directly address the function of central brain regions (Heisenberg, 1980; Heisenberg et al., 1985). For two of the best studied of these, mushroom body deranged (mbd) and mushroom body miniature (mbm), decrements in olfactory learning correlate with the degree of mushroom body disruption. Surprisingly, in two different visual learning paradigms, association of color with noxious agitation and change of flight orientation in order to avoid punishment, the mbd mutant showed normal learning (Heisenberg et al., 1985). Another clever strategy for disrupting the mushroom body was based on its unique developmental timing. In the fly this structure is formed from neuroblasts which proliferate at an atypically late stage (Ito and Hotta, 1992). If hydroxyurea is fed during the first instar, these dividing cells are killed with amazingly little damage to other structures, and the adult fly is apparently normal but missing the Kenyon cells, peduncles and lobes (de Belle and Heisenberg, 1994). These animals can detect the shock as well as the odor, but are unable to form conditioned olfactory memories. Nonetheless, these flies function normally in visual learning of flight orientation (de Belle and Heisenberg, 1994).

LEARNING AND BIOCHEMISTRY

Behavioral mutants of Drosophila have been obtained by searching for lines which display abnormal responses in an aversive learning paradigm (Dudai et al., 1976), and by using enhancer trapping to target genes specifically expressed in the mushroom bodies (Davis, 1993). Both of these methods have produced flies with defects in the cyclic AMP signal transduction pathway. The first identified learning mutant, dnc (dnc1) (Dudai et al., 1976), was found to be lesioned in cAMP phosphodiesterase (Byers et al., 1981; Davis and Kiger, 1981). Dnc
flies show both reduced learning and a rapid loss of what they do learn (Dudai, 1983; Tully and Quinn, 1985), a phenotype which can be rescued in adult animals transformed with a heat shock inducible dnc cDNA by heating the adults (Dauwalder and Davis, 1995). The dnc gene product is widely expressed in neural tissue, but levels of expression are extraordinarily high in the lobes of the mushroom body and in the calycal neuropil (Nighorn et al., 1991). A second learning mutant, rute-baga (rut), was found to be deficient in Ca\(^{2+}/\text{CaM}\)-dependent adenylyl cyclase activity (Levin et al., 1992; Livingstone et al., 1984). This cyclase is homologous to type I cyclase of mammalian brain, and is responsive to both G-protein and Ca\(^{2+}/\text{CaM}\) stimulation (Levin et al., 1992). The rut flies have a phenotype very similar to dnc flies, and both fly types have essentially normal sensitivity to the odors and shock used in training, so their poor performance cannot be attributed to sensory deficits. Given the opposite biochemical effects of mutation in the rut cyclase versus the dnc phosphodies-terase, why are their effects on learning so similar, and why do dnc/rut double mutants not rescue learning even though they have normal cAMP levels (Livingstone et al., 1984; Tully and Quinn, 1985)? The answer may be that precise modulation of cAMP level is more important to neural plasticity than absolute amount. Alternatively, high cAMP levels in dnc flies may cause down regulation of a critical component in the signaling pathway, one which is insufficiently stimulated in rut flies because of lack of cAMP (see Davis and Dauwalder, 1991). As with the dnc gene product, the rut cyclase is strongly expressed in mushroom bodies, but its distribution is highest in the lobes and peduncle, with expression in the calyx no stronger than the low level seen in the rest of the brain (Han et al., 1992).

Not surprisingly, mutations in the catalytic subunit of the cAMP-dependent protein kinase A, a gene called DCO, also cause a learning and memory phenotype (Skoulakis et al., 1993), as does transformation of flies with a construct producing a peptide that specifically inhibits DCO activity (Drain et al., 1991). PKA effects its actions in two ways: it regulates the activity of cyto- plasmic proteins by phosphorylation, and the activated catalytic subunit is transported into the nucleus where it can regulate gene expression by phosphorylating transcrip- tion factors (see Goelet et al., 1986). Flies without a wild type copy of DCO exhibit very reduced learning but little deterioration of the memory which they do acquire. The DCO gene product is high in the mushroom body lobes, and it is also strong in the calyx (Skoulakis et al., 1993). This raises an interesting incongruity: dnc, rut and DCO gene products are all involved in cAMP signal transduction, function in learning and are highly expressed in the mushroom body, but their distributions within this structure are not the same. The rut cyclase has been proposed as a molecular coincidence detector by virtue of its sensitivity to both G-protein-coupled sig- nals and Ca\(^{2+}/\text{CaM}\) levels (Abrams and Kandel, 1988), but its level is low in the calyx of the mushroom body, the region which receives the most sensory input. One possible explanation is that the rut adenylyl cyclase does not process information in the calyx at all, but a distinct enzyme does. Another possibility is that integration of sensory inputs does involve the rut cyclase, but it takes place in the lobes and peduncles, at a distance from the most obvious site of sensory input. A third possibility is that the rut cyclase functions in multiple roles in the mushroom body, but these require different amounts of enzyme activity. Perhaps a high level of cyclase in the lobes and peduncles is necessary for one process, but a much lower level is required in the calyx for a different one. Mutations in rut provide some support for the idea that this protein functions at multiple levels. Immediate and 60 min retention were evaluated in a series of P- element derived rut lines which had varying reductions in the amount of gene product produced. As rut levels dropped, retention was affected before acquisition was (Han et al., 1992).

Lesions in a signaling pathway as widely used as cAMP cause problems other than an associative learning defect: dnc mutants have reduced fertility (Byers et al., 1981), and both dnc and rut mutants show abnormal use- dependent synaptic plasticity at the neuromuscular junction (Zhong and Wu, 1991), aberrant habituation and sensitization of PER (Duerr and Quinn, 1982) and rut flies showed altered habituation of a grooming reflex (Corfas and Dudai, 1989). It is unknown at this time if these neural defects of dnc and rut flies are mechanistically related to the associative learning defect. Analysis of yet another learning mutant may lead to the mechanism of adenylyl cyclase activation and the involvement of other signaling pathways. Amnesiac (amn) was isolated as a mutation with somewhat impaired acquisition but very rapid loss of memory (Quinn et al., 1979; Tully and Quinn, 1985). Amn flies have a frame shift mutation in a gene which codes for a neuropeptide similar to the vertebrate pituitary adenylyl cyclase-activating polypeptide (PACAP) (Feany and Quinn, 1995). Mammalian PACAP induces rapid depola- rization at the Drosophila neuromuscular junction, as well as an enhancement of a K\(^{+}\) current with a very long latency (Zhong and Pena, 1995), effects which are diminished in rut flies, and also deficient in flies with lesions in the Ras/Raf signaling pathway (Zhong, 1995). The coactivation of two pathways by a neurotransmitter which can induce synaptic plasticity suggests a potential role for multiple pathways in learning. Parallel pathways have been previously suggested (Davis, 1993), because flies null for rut function can still learn, although not at levels approaching normal.

**MEMORY PHASES OF DROSOPHILA**

The olfactory learning assay formed the basis for a search for memory phases in Drosophila, which have short and intermediate-term memories of minutes to
hours, and more permanent long-term memory. These proved to be resolvable by a combination of training regimens, pharmacological treatments and genetic manipulation. Administration of cold anesthesia shortly after training serves to erase memory, much as it does in bees (Quinn and Duda, 1976; Tully et al., 1994). This marked a shift between an early, cold-sensitive memory phase lasting under 1 h, followed by consolidation into an anesthesia resistant memory (ARM), which lasts about 1 day. Long-term memory begins several hours after training, and lasts for over 1 week (Tully et al., 1994). An uncloned memory mutant, radiish (rsh), has normal learning and long-term memory but is defective in ARM, exhibiting a very rapid decrement of memory within the first 24 h of training but normal memory decay thereafter (Folkers et al., 1993; Tully et al., 1994).

The training regimen used to induce memory proved to be critical for the formation of long-term memory in Drosophila, and required multiple training trials with an intervening rest period of 15 min (spaced training, Tully et al., 1994). Equivalent or even greater repetition of training without rest periods (massed training) would not induce long-term memories (Yin et al., 1995). Long-term memory could also be blocked by treatment of flies with the protein synthesis inhibitor cycloheximide. If a block of protein synthesis eliminates long-term memory, what are the training induced biochemical changes that govern this process? Research in Aplysia showed that training-induced protein synthesis involves the cAMP-response-element-binding factor (CREB) (Dash et al., 1990), a transcription factor which is a PKA target (Armstrong and Montminy, 1993). Drosophila CREB (dCREB) is believed to have multiple splice forms, two of which showed antagonistic influences on the formation of long-term memory. One form, dCREB2-b, blocked long-term memory when placed under a heat-shock promoter and induced before training (Yin et al., 1994), but a different splice form, dCREB2-a, appears to promote the formation of long-term memory, even allowing formation of these memories under unfavorable training regimens, i.e. massed training. This has led to the hypothesis that both forms of dCREB2 are produced by training but dCREB2-b has a shorter half life, and rest periods are necessary to allow the establishment of a ratio of these transcription factors which is favorable for memory formation (Yin et al., 1995).

These findings begin to address the mechanism of how short and long term memories are formed in Drosophila, but what constitutes the actual changes in cell biochemistry or structure, and the cellular sites of memory formation and storage are still unknown. Shorter term memories are presumably formed at least in part by cAMP driven PKA phosphorylation of as yet unknown targets; perhaps ion channels, whose modification alters the electrical properties of the cell, or cytoskeletal or cell adhesion proteins that can change the physical properties of a synapse.

Both ion channels and the kinases which modulate them have been implicated in the process of learning and memory. The abnormal synaptic plasticity of dnc mutants can be largely ameliorated by blocking K+ channels (Delgado et al., 1992). Interestingly, two K+ channels subject to cAMP modulation have been identified in dissociated Kenyon cells (Wright and Zhong, 1995). Flies with mutations in the K+ channel gene ether-a-go-go (eag) fail to learn in the courtship paradigm, as do flies in which the enzyme calmodulin kinase II (CaM K II) is blocked by expression of a peptide inhibitor (Griffith et al., 1994). CaM K II is very abundant in neural tissue, and it can phosphorylate eag in vitro. Calmodulin regulation of this enzyme is lost after autophosphorylation, a property which led to the proposal that CaM K II could store information by alterations in its activity state (Lisman and Goldring, 1988). The neuromuscular junction of Drosophila is the most accessible site for assaying the effects of mutations on synaptic function, and a block of CaM K II both reduces synaptic plasticity and increases excitability there (Wang et al., 1994).

Longer term memory is likely to involve more permanent changes in cell structure, such as alterations in axonal projections or remodeling of synapses. Alterations in both axonal projections and synaptic morphology can be observed at the neuromuscular junctions of animals with mutations in K+ channels (Jia et al., 1993) or with blocked CaM K II (Wang et al., 1994). It remains to be seen if the effects of these mutations on the electrical properties or on the structure of cells (or both) from the basis for changes in behavioural performance.

DISCUSSION

We have attempted to summarize the progress made in the honeybee and Drosophila systems, and now must ask the question, are the systems comparable and complementary? How conserved are the mechanisms of learning and memory between the two species, and can they be applied to insects in general? There appears to be a great deal of variation in the duration of different memory phases from one organism to another, making comparison of phases based on timing alone difficult (see DeZazzo and Tully, 1995). For example, sensitivity to disruption by cold anesthesia links the shorter-term phases, but bee memory is anesthesia-resistant after 10 min, and in Drosophila after 1 h. Does this indicate that there is a change in the timing of a similar biochemical event, or is a totally different process occurring in each organism? At present, this question can not be answered, but Drosophila mutations such as amn and rsh which are specific for short-term memory phases make this phase of learning accessible in at least one insect.

The parallels between long-term memory in a wide variety of organisms suggest that aspects of its formation are likely to be conserved. The commonality of a protein synthesis requirement for long-term memory, as well as the apparent involvement of CREB transcription factors in mediating this synthetic activity argue for a conserved
mechanism of memory consolidation. Indeed, gene knock out of mouse CREB causes a lesion specific to long-term memory (Bourtchuladze et al., 1994), and blocking of Aplysia CREB eliminates training-dependent changes in synaptic strength (Dash et al., 1990). The failure to find a protein synthesis-dependent memory phase in bees creates a difficulty in comparing information from these two systems. It is possible that bees do not have a protein synthesis-dependent long-term memory phase, but the training regime and temporal assay requirements for demonstrating this phase in Drosophila are so specific that further examination of the bee system is needed before this conclusion is final. It is worth noting that a protein synthesis requirement has been demonstrated in another insect, the praying mantis (Jaffe, 1980).

What is the actual function of the mushroom body, and is this function the same in all insects? The convoluted appearance of the bee mushroom body and its prominence in the brain led to the belief that it was analogous to the human cortex, and thus the seat of insect intelligence (references in Mobbs, 1982; Power, 1943). Early anatomists believed that the mushroom bodies were involved in social behavior, as they were larger in social insects such as bees, ants and termites (Hows, 1974). Interestingly, the transition from nurse bee to forager causes an increase in the volume of the mushroom body neuropil (Withers et al., 1993), and Drosophila reared in isolation lose greater numbers of mushroom body axons as they age compared to flies reared with company (Balling et al., 1987; Heisenberg et al., 1995).

We can be certain that the mushroom body has a central role in the process of olfactory learning based on cooling and ablation studies, and the fact that many proteins required for olfactory learning are highly expressed in it. The strength of antennal lobe connections to the mushroom body, and the observation that the size of the mushroom body is related to the degree of development of the antennal lobe, but not to the sophistication of the visual system, have led some researchers to consider it a secondary olfactory neuropil (Mobbs, 1985; Weiss, 1974). It is apparently unnecessary for some forms of visual learning: as mentioned before, flies with ablated or malformed mushroom bodies do well on visual tasks. Clearly, both the biochemistry and neural requirements of insect learning may differ based on the sensory modalities used for training. In fact, the diffuse nature of the neural substrates of learning can be demonstrated in a rather dramatic fashion: a headless insect will learn to change leg position to avoid a shock (Booker and Quinn, 1981; Horridge, 1962). What we learn from using the methods presented here may represent only a subset of the neural and biochemical correlates of learning. Nevertheless, the study of olfactory learning in bees and flies represent two very productive and powerful methods for gathering information on the process of learning, and the mechanism of memory.

REFERENCES


