Food for Thought: Honeybee Foraging, Memory, and Acetylcholine

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The belief that experience leaves a physical trace in the brain, while long-standing (1), has been difficult to substantiate. Among the many difficulties inherent in searching for such a trace is that, because a given experience could hardly encompass the totality of both potential sensory stimulation and its behavioral importance, any particular experience should not produce a wholesale anatomical change throughout the brain. An experience may produce many small changes in the brain that are widely distributed across a complex network, reflecting the multiple perceptual, emotional, motor, and other features that are involved in an event. Such "distributed-localized" changes may be difficult to detect, especially if structural changes are on a very small—for instance, subsynaptic—scale. It would seem that one would need to greatly reduce the size of the "haystack" to find such mnemonic "needles." On the other hand, broad and prolonged complex experience might leave a larger residue, thus increasing the size or number of "needles."

Memory's impact on the brain need not be only anatomical; it might additionally (or only) be chemical. Thus, in principle, gross assays of the levels of central nervous system (CNS) neurotransmitters, such as glutamate, or neuromodulators, such as acetylcholine, could reflect the effects of experience, and perhaps denote something about memory itself. Indeed, pioneering studies used prolonged "environmental enrichment" (EE) to detect both anatomical and chemical changes that occurred in response to experience (*2*–*4*). Groups of rats (for instance, $n = 12$) were housed in large cages containing "toys," mazes, and the like, which were changed daily; this constituted the EE condition. Comparison groups included "social housing" (three animals in a plain cage) as well as isolated rats in standard cages. After a period as short as about 30 days, the EE treatment, in both newly weaned and adult animals, produced both thicker cerebral cortices and increased levels of acetylcholinesterase, the enzyme that degrades acetylcholine (ACh) (*5*, *6*). (Measurement of acetylcholinesterase was more feasible at the time than measurement of ACh.) Further experiments revealed that the increase in cortical width involved both increased numbers of glial cells and the growth of dendrites (*7*–*10*).

Both anatomical and chemical lines of inquiry converged in a recent study of the mushroom bodies in the brain of the honeybee, which has proven to be an excellent model system for understanding the neurobiology of learning and memory (*11*). Ismail *et al.* (*12*) exploited the precisely timed change of honeybees from workers to foragers to determine both the effects of extensive foraging experience on the size of the mushroom bodies and the involvement of acetylcholine in the associated structural changes. The findings and their implications are best appreciated within a larger framework.

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Acetylcholine, among all neurotransmitters, has proven particularly intriguing because of its roles in brain plasticity and in learning and memory. For example, cholinergic agonists can facilitate memory, whereas cholinergic antagonists can impair memory (*13*–*17*). Studies of the effects on brain plasticity of cholinergic agents, particularly those engaging muscarinic receptors, have provided a good deal of information about neurochemical substrates underlying learning and related processes. Thus, cholinergic agonists applied directly to sensory cortex produce atropine-sensitive enhancement of responses to environmental stimuli in the auditory (*18*), somatosensory (*19*), and visual (*20*) systems. The major source of ACh to the cerebral cortex is the nucleus basalis of Meynert (NBM) (*21*, *22*). Stimulation of the NBM paired with somatosensory or auditory stimulation induces prolonged facilitation of responses in the somatosensory (*23*) and auditory (*24*) cortices, respectively.

Direct neurophysiological studies have further implicated muscarinic receptors in associative learning. Notably, auditory classical and instrumental (operant) conditioning produce tuning shifts toward and to the frequency of the tone used as the conditioned stimulus (*25*, *26*). Associative learning is also correlated with an expansion of the representation of behaviorally important frequencies in the primary auditory cortex (*27*), such as sounds that predict forthcoming food or water. Additionally, these learning-induced increases in area may serve as a "memory code" for the degree of the behavioral importance of sounds (*28*). The NBM cholinergic system can mimic the effects of associative learning by inducing the same specific shifts in receptive field plasticity as those that develop during classical and instrumental conditioning (*29*), and this plasticity requires the engagement of muscarinic receptors in the auditory cortex (*30*). Lesions of the NBM and blockade of muscarinic receptors impair tuning shifts during classical conditioning procedures (*31*). In humans, specific associative plasticity develops in the auditory cortex, and this is also blocked by cholinergic antagonists (*32*). Finally, pairing a tone with stimulation of the NBM induces actual conditioned stimulus (CS)–specific behavioral memory, although subjects received neither reward nor punishment (*33*). Thus, acetylcholine apparently can mimic the normal effects of experience-dependent memory.

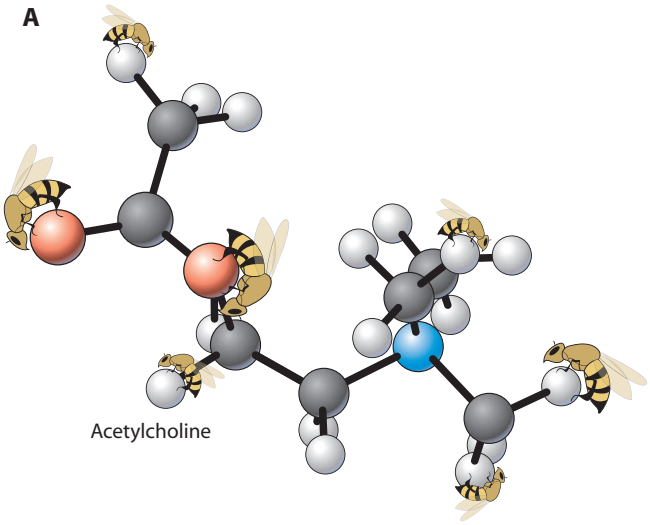
Thus, there is a great deal of evidence that acetylcholine may trigger cellular events that produce memory storage and that enriched experience can produce increases in both neural structures and cholinergic activity. Within this context, the study by Ismail *et al.* can be viewed as one of using enriched experience—foraging—in the honeybee to investigate the involvement of acetylcholine in structural enlargement of the mushroom bodies. However, in contrast to most environmental enrichment studies, the authors used a developmentally timed natural behavior in a natural environment. Honeybees (*Apis mellifera*) typically live about 6 weeks as adults. They first work in the hive and then spend approximately the last 4 weeks of their

lives as foragers. Their mushroom bodies (MBs) are prominent, paired midline structures that receive conspicuous olfactory and visual input. The MBs consist largely of interneuron Kenyon cells and their associated neuropil (the unmyelinated processes that lie between the cell bodies). Previous research indicated that the volume of MB neuropil increases during foraging (*34*). Ismail and co-workers hypothesized that this increase is triggered by the greatly augmented and complex sensory input associated with foraging. Furthermore, as sensory input to the MBs is muscarinic, they hypothesized that the signaling of sensory input by acetylcholine is sufficient (and perhaps necessary) for MB growth in the adult honeybee.

To test these hypotheses, the authors detected and marked new foragers at the entrance to the hives and permitted them to forage for 1 week. The bees were then divided into two groups: those that were permitted to forage for a second week, and those that were tested in the laboratory. The latter were further subdivided and were fed a muscarinic agonist (pilocarpine), a muscarinic antagonist (scopolamine), or nicotine, all in a sucrose solution. The bees lived in the hive for a second week but were prevented from foraging. At the end of the 2-week period, the MBs of all bees were examined. The main findings were that MB size did not increase after 1 week of foraging, but 2-week foragers showed an increase of approximately 14% in volume relative to age-matched caged controls. These findings are essential replications of prior observations and necessary to test the authors' "cholinergic hypothesis."

Bees that could not forage during the second week but were fed pilocarpine developed the same amount of growth of the MBs as those that did forage. This effect of the muscarinic agonist was blocked by scopolamine; nicotine had no effect. Thus, confirming the hypothesis, acetylcholine—probably acting at muscarinic receptors in the mushroom bodies—is sufficient to mimic the effects of extensive foraging experience (Fig. 1).

These findings break new ground in several ways. First, this appears to be the first report that a muscarinic agonist can produce gross structural changes. Previously, Woolf had hypothesized that acetylcholine released from cholinergic axon terminals in the vertebrate brain produces postsynaptic structural reorganization during memory storage. She postulated that acetylcholine release begins a cascade of intracellular signaling events that culminate in the proteolysis of the structural protein MAP-2 (microtubule associated protein–2), destabilizing the postsynaptic cytoskeleton and "thereby favoring dendritic plasticity" (*35*). The present findings are generally consistent with Woolf's formulation, although elucidation of the intracellular mechanisms and detailed structural changes that take place in the neurons of the honeybee MB remain for future investigation. Second, Ismail and co-workers have linked a natural, complex behavior to both large-scale neuroanatomical plasticity and its likely neurochemical signal. This neuroethological approach to attack the problem of the imprint of experience on neural tissue demonstrates how basic neurobiological findings from the laboratory can be used to elucidate "real world" situations and can serve as a paradigm in this field of inquiry. Third, to the extent that natural foraging behavior can be considered "enriched experience"—which is reasonable when considering the marked increase of experiential complexity and behavior outside of the hive—the field has in some sense come full circle from the dawn of such studies. Thus, as noted at the outset, EE was found to increase both markers for ACh activity and cortical



B

Foraging and hive times	Drug treatment	Mushroom body size
1 week foraging, then 1 week in hive	None	No change
2 weeks foraging	None	Increased
1 week foraging, then 1 week in laboratory hive	Pilocarpine	Increased
1 week foraging, then 1 week in laboratory hive	Pilocarpine + scopolamine	No change
1 week foraging, then 1 week in laboratory hive	Nicotine	No change

Fig. 1. (**A**) Whimsical representation of honeybees and acetylcholine, which mediates the structural changes in the honeybee brain that take place in response to foraging. (**B**) Experiments implicating muscarinic acetylcholine receptors in honeybee memory (*12*).

width, the latter reflecting growth of the neuropil. The apparent convergence of mechanisms is striking.

One caveat for the present findings is that, as the authors note, it is unclear which aspects of foraging behavior are linked to growth of the mushroom bodies; controls for increased motor activity (flying) remain for the future. The EE studies in rats showed that the induced dendritic plasticity was not caused by increased activity itself (*36*). Thus, those with a preference for greater universality of learning and memory mechanisms may hope that the same will hold true for the honeybee. However, regardless of the ultimate identity of mechanisms across diverse phyla, each species has its own problems to solve. By learning how they achieve success, we gain a more encompassing understanding of how experience is represented, stored, and used in the service of adaptive behavior.

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