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Small Brains, Bright Minds

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Learning, memory, and social behavior are innate properties of the honeybee that are essential for the survival of each individual as well as for the survival of the hive. The small, accessible brain of the honeybee and the availability of the complete sequence of its genome make this social insect an ideal model for studying the connection between learning, memory, and social behavior.

Insect brains are structurally very different from mammalian brains, and yet the basic demands of life are quite similar in both groups of animals. What, where, and how should experience be stored for effective use in the future? How should innate information be combined with acquired information? How do the motivational and evaluating neural systems interact? How does the brain choose among similar behavioral options? All of these questions require analyzing neural circuits in animals as they undertake different behaviors. Neural circuits are composed of a large number of single neurons, each of which has its own specific gestalt, connectivity, and history. Ideally, one would like to track neural events within a network of fully characterized neurons. Insect brains provide us with such an option. Many neurons can be identified at the single-cell level according to their structure, enabling us to trace network properties to single-neuron functions and to understand the working of the network according to the composition of participating neurons. One insect that provides a valuable model system for examining neural pathways and their connection to learning and memory and social behavior is the honeybee (*Apis mellifera*).

Honeybees are social animals and of all insects have the most sophisticated community structure. Like other social animals, they require sophisticated cognitive faculties: They do not survive in isolation, they need to communicate intensively with each other, and they depend on a safe return to their community housing. Navigation during exploratory behavior, mating, and foraging is a result of innate knowledge about celestial compass properties, but these guiding structures need to be related to the environmental features of the home range through learning. In this sense, the honeybee offers an excellent model system for the study of cognition at an intermediate level of complexity. Given that its brain is rather small—only 1 mm^3 in size, containing 950,000 neurons (see Figure 1)—and is accessible to recording and manipulation, the neural and cellular underpinnings of the honeybee brain may provide us with unique information (Menzel and Giurfa, 2001). Recordings from single identified neurons and the imaging of intracellular Ca^{2+} concentration in defined subsets of neurons, performed as the animal learns, can provide us with substantial information about the where and what of the neural correlates of learning. Localized manipulation of signaling cascades may lead to an understanding of the molecular components required for the formation of sequential and parallel memory phases.

The honeybee's social system is characterized by the division of labor among worker honeybees, an age-

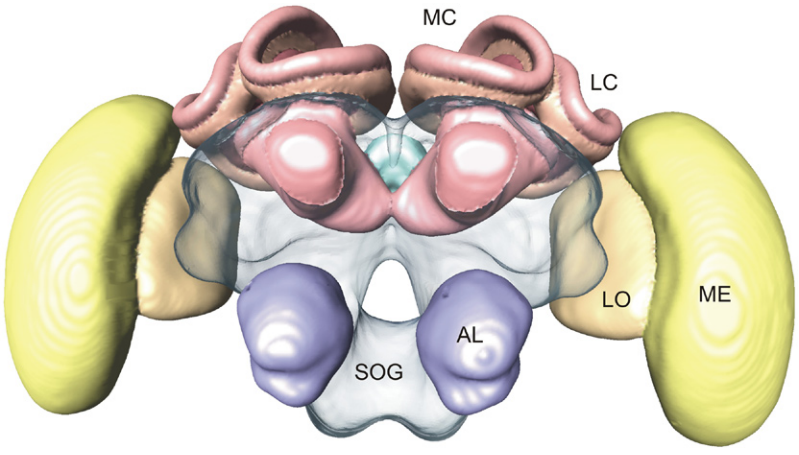

Figure 1. Three-Dimensional Reconstruction of the Honeybee Brain
The visual ganglia medulla (ME) and lobula (LO) are shown in yellow, the primary olfactory neuropil (antennal lobe AL) in blue, and the paired mushroom bodies in pink. The primary sensory neuropils send projections to the input sides of the mushroom body, the lateral (LC) and medial (MC) calyces. Other neuropils present in the brain (shown in transparent blue) are the protocerebral lobe and the suboesophageal ganglion (SOG).

based but highly regulated process. Workers accomplish defined tasks in response to pre-established genetic programs that can be adapted at any moment to changes in the colony's needs. Several physiological systems are involved in controlling behavior. Production of vitellogenin, a protein involved in reproductive physiology that is expressed by young worker bees, correlates with the colony's foraging strategy (Amdam et al., 2004). Neuronal factors also regulate the division of labor. *Amfor*, a gene encoding a cGMP-dependent protein kinase, is involved in the regulation of foraging (Robinson et al., 2005). Nevertheless, a correlation between gene expression and social behavior does not qualify these genes as "social." Indeed, the cGMP effector system is also known to control feeding in solitary insects. Thus, conserved signaling pathways can be adapted to new requirements in a social system.

Understanding the honeybee social system also requires describing how the animal's cognitive capabilities influence social organization. As described below, the bee performs complex learning tasks rather well. What are the neural correlates of these complex learning capabilities? To answer this question, it will be necessary to relate the brain's structural organization to the social tasks performed. The first attempts to do this involved tracking brain growth during aging and the transition to different tasks (Farris et al., 2001). The volume and structure of the mushroom bodies—multisensory integration centers involved in memory formation—were found to change with task transitions (see Figure 1).

From Free-Flying Bees to Molecules

Honeybees communicate distance and direction toward a food source or a nest site using a ritualized movement, the waggle dance (von Frisch, 1967). The debate about whether the dance does indeed transmit this information to the recruited bees has recently been conclusively settled by continuously tracking the flight path of bees out in the open (Riley et al., 2005, Menzel et al., 2005). Learning at the food site transcends elementary associative events. Bees learn to generalize symmetrical versus nonsymmetrical patterns and even switch very quickly from one kind of pattern to the other, indicating some form of categorization (Menzel and Giurfa, 2001). A large range of established associative learning tests were studied in food-trained free-flying bees or in the conditioning of a simple response, proboscis extension (PER). Overall, no differences were found in these tests between free-flying bees and those tested in PER learning. These tests included those that ask whether configural rather than elementary associations are formed by bees. Taken together, the findings enable us to extract general rules of learning and to test whether they apply equally well to vertebrate and insect model systems. This not only inspires further studies and is mutually complementary but also provides a basis for analyzing the common neuronal and molecular underpinnings of learning and memory. One example in the honeybee is that of the memory reconsolidation process initiated by extinction learning, which leads to the well-known behavioral phenomenon of spontaneous recovery. Spontaneous recovery is the reappearance of a memory that had been extinguished (Stollhoff et al., 2005). This finding offers an explanation for a characteristic phenomenon of extinction learning and needs to be tested in vertebrate systems.

The search for the neural substrate of associative olfactory learning in the bee brain has gained enormously from the identification of a single neuron, the ventral unpaired median neuron of the maxillary neuromere in the suboesophageal ganglion (VUM_{mx1} neuron), which mediates reinforcement in reward learning (Hammer, 1993). The axodendrons of this neuron branch into the antennal lobe and the mushroom-body calyx, both of which are involved in forming an olfactory memory trace. Downregulating octopamine receptors—the receptors for the putative neurotransmitter released by the VUM_{mx1} neuron—using RNA interference leads to impaired learning (Farooqui et al., 2003). An essential component of long-term memory formation in the antennal lobe is the cAMP/PKA signaling pathway, whereas the maintenance of mid-term memory depends on a second kinase, PKC, which is constitutively activated by the Ca^{2+}-dependent protease calpain (Müller, 2002). Imaging intracellular changes in Ca^{2+} concentration in the glomerular substructures of the antennal lobe reveals specific enhancement of responses to a reward-paired odor compared with an unpaired odor (Menzel, 2001). It will be important to discover the targets of learning-induced PKA and PKC activity to further unravel the molecular mechanisms of mid- and long-term memory.

The mushroom body's involvement in the consolidation of memory was discovered 30 years ago using localized cooling experiments (Menzel, 2001). Neurons leaving the mushroom body change their response properties specifically with olfactory learning. For example, a single neuron called PE1 was recently found to undergo associative long-term potentiation after pairing electrical stimulation of the mushroom-body neurons with intracellular depolarization of PE1 (Menzel and Manz, 2005). At the input side of the mushroom body, Ca^{2+}-imaging experiments showed strengthening of the postsynaptic responses of mushroom-body neurons that were specific for a learned odor stimulus (P. Szyszka and R.M., unpublished data). These observations indicate associative plasticity at both the input and the output sides of the mushroom body (see Figure 1). The next step will be to ask how these forms of plasticity are related, whether they encode different aspects of the learned stimulus, and how they are involved in different forms of learning and memory. In addition, it will be important to ask how the memory traces in the antennal lobe and the mushroom body are related.

From Molecules to Behavior

The honeybee genome sequence has been recently completed (Human Genome Sequencing Consortium, http://www.hgsc.bcm.tmc.edu/projects/honeybee/). This provides an outstanding opportunity for studying the molecular mechanisms of social behavior and learning and memory.

The analysis of gene expression correlated with physiological processes will gain more accuracy by generating DNA chips based on the genome. The fast access to sequence data is enabling researchers to study the functional role of genes and their products without first having to identify and characterize them. Thus, analysis of gene expression, its correlation with physiology, and studies of protein-protein interactions will be facilitated.

A huge obstacle in honeybee research has been the absence of techniques to specifically interfere with gene expression and protein function. Pharmacological tools are under development for application in vertebrates; their specificity in the honeybee has not always been clear. An alternative has been the use of antisense and RNAi techniques (Fiala et al., 1999, Farooqui et al., 2003). However, such techniques only allow the blocking of gene expression, and their efficiency must be determined for every gene, which is tedious and time consuming. Transgenic bees might be the solution to these problems. However, engineering transgenic bees that are not eliminated from the colony due to the strict social control of their comrades in the hive may not be possible with the tools available now (Robinson et al., 2000). In any case, these transgenic animals may be problematic given the restriction that such animals may not be released into the environment, where the most interesting behavioral experiments could be carried out. An alternative to transgenic animals might be the transfection of particular genes into selected structures (such as the mushroom body) in single bees. The feasibility of such manipulation has been demonstrated by in vivo electroporation of the honeybee brain (Kunieda and Kubo, 2004). Nevertheless, new vectors (transposable elements and viral vectors) and new insights into tissue-specific regulation of gene expression will be necessary to gain the required efficiency and selectivity.

Molecules Are the Bridge

Two closely related topics are intensively studied in the honeybee: social behavior and learning and memory. Both reflect innate properties of social insects that are simultaneously relevant for each animal's survival. This, in combination with its small and accessible brain, predetermines the honeybee as an ideal model system.

Sequencing the honeybee genome represents a huge step forward in honeybee research. It will most likely focus our view on the molecular mechanisms underlying both social behavior and learning and memory. This focus will be crucial in the future to bridge the gap between the honeybee invertebrate model system and vertebrate model systems because it will allow us to differentiate between species-specific adaptations and general mechanisms. It will provide us with insights into the exciting question of why invertebrate and vertebrate brains are structurally very different even though the basic demands of life are quite similar in both groups of animals.

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