ASSOCIATIVE LEARNING AND MEMORY
PROCESSING IN BEES

The social life of the honeybee colony forms the ecological framework for the individual animal’s behavior and is crucial for each bee’s survival, because an individual bee cannot exist on its own (Frisch, 1967; Lindauer, 1967).

Associative Learning

The study of learning and memory formation in bees under natural conditions has focused on latent learning during navigation and on operant learning in the context of food collection. In the laboratory it has focused on appetitive classical conditioning. Bees departing from the hive perform observatory learning flights (Capaldi et al., 2000), and establish a map-like spatial memory for their colony’s location relative to landmarks within the framework of their sun compass system (Menzel et al., 2000). When a searching bee discovers a nectar- or pollen-producing flower, it quickly learns to associate the surrounding visual and olfactory signals with the reward. It learns olfactory stimuli (e.g., floral odors) and colors very quickly (within one or a few learning trials). Patterns need more learning trials. Whereas latent learning during navigational tasks may not require a rewarding stimulus, reward learning is a forward-associative process because signals perceived before the reward are associated, whereas those perceived during the reward or during the departure flight are associated less effectively or not at all.

Research on various operant learning phenomena (e.g., reversal and multireversal learning, overlearning, inhibitory learning, context-dependent learning, and reward schedule learning) has found performances similar to those in mammals (Couvillon and Bitterman, 1988; Menzel, 1990). Multiple experience with varying signals but one constant feature (e.g., different kinds of symmetrical patterns) leads to the formation of a concept (the concept of symmetry) that allows the bee to choose new patterns with the same feature as learned targets (Giurfa, Eichmann, and Menzel, 1996). Bees also develop a concept of sameness and difference when they are trained in delayed matching-to-sample tasks, in which they are required to respond to a matching stimulus or a non-matching stimulus (Giurfa et al., 2001). They also transfer the learned rules to new stimuli of the same or a different sensory modality. Thus, not only can bees learn specific objects and their physical parameters, but they also extract rules and apply them to novel situations.
Classical Conditioning

Classical conditioning of reflexes is a convenient way to study the behavioral and neural mechanisms of associative learning. In the honeybee, the proboscis-extension reflex (PER) to a sucrose stimulus at the antennae is a reliable reflex in the context of feeding. A hungry bee will reflexively extend its proboscis (tongue) when the antennae are touched with a drop of sucrose solution. An odor (conditioned stimulus, CS) presented shortly before the sucrose reward (unconditioned stimulus, US) will be associated with the reward, even under conditions in which the animal is harnessed in a tube or is being prepared for physiological studies (Menzel and Müller, 1996). The associative nature of PER conditioning to odors has been established by demonstrating that only forward-pairing of CS-US sequences is effective. Unpaired CS and US presentations or CS- or US-only presentations do not lead to learning, and in differential conditioning (one odor CS+ paired with US, the other CS- unpaired), bees respond only to the CS+ and not to CS (Menzel, 1990). The predictive value of the CS depends on the reliability with which it is causally related to the US. In differential conditioning, the reversal to the initially unpaired stimulus CS is slower after more frequent unreinforced preexposures than after fewer preexposures. The same applies for US-only preexposures in an otherwise reinforced context, indicating that the absence of an expected US leads to inhibitory learning. If naive animals are stimulated with a compound of two odors and one of them is later associated with sucrose reward, the animals will also respond the second odor of the compound, even though this odor was not explicitly experienced during a learning trial (Müller et al., 2000). This form of learning (sensory preconditioning) indicates stimulus associations between equally evaluated stimuli and thus transcends the classical associative paradigm.

The role of reinforcement in the formation of an association is an essential question in learning theories. Are associations formed only by close contiguity between the CS and US? The blocking phenomenon indicates that this is not the case: If a novel CS appears together with a learned stimulus, this novel stimulus will be learned to a lesser extent or not at all (Kamin, 1968). The blocking paradigm is central to most current models of associative learning, and the phenomenon is explained either by the assumption of a competition between the two CSs (the already learned one and the novel one) for attention (Mackintosh, 1975) or for a limitation of reinforcing function that depends on the expectation or prediction of reinforcement (Rescorla and Wagner, 1972). Since attention, expectation and prediction are cognitive faculties, it is argued that cognitive capacities need to be introduced in theories about associative learning. It is thus interesting from a comparative point of view whether the bee with its tiny brain shows the blocking phenomenon. This question cannot yet be definitively answered. Blocking is found in some studies (Smith and Cobey, 1994; Thorn and Smith, 1997), but not in others (Gerber and Ullrich, 1999). Blocking across sensory modalities was also not seen in training free-flying bees (Bitterman, 1996; Funayama, Couvillon, and Bitterman, 1996). Second-order conditioning is another procedure that tests whether associative learning requires contiguity between CS and US. In a positive outcome of second-order conditioning one argues that a CS can acquire the potential of a US. This has been demonstrated for olfactory PER conditioning (Menzel, 1990).

Rules of elementary associative learning assume that in learning a compound stimulus, animals learn the associations between the reinforcer and the compound elements separately (Rescorla and Wagner, 1972). Contrary to this assumption, configural learning theories assume that, in learning a compound, animals build a new entity made from the conjunction of compound elements and that a connection is made between this new configuration and the reinforcer (Rudy and Sutherland, 1992). The different processing strategies underlying elementary and configural olfactory learning were studied by the negative patterning discrimination. In negative patterning two single stimuli are reinforced (A+, B+), while the compound is not (AB). Solving this problem—responding less to the compound than to the single elements—can be explained only by taking configural associations into account. Otherwise, summation of the elementary associative strengths in the compound should result in stronger response to the compound than to the elements. Honeybees can solve negative patterning discrimination in olfactory conditioning of the PER (Deising, Lachnit, Hellstern, and Giurfa, 2001). The fact that bees solve negative patterning discrimination in olfactory conditioning and in odor/odor tasks (Couvillon and Bitterman, 1988) shows that linear associations between single stimuli and the reinforcer are not the only ones underlying associative learning in honeybees (Giurfa et al., 2001).

Memory Dynamics and Memory Localization

Memory is an animal's capacity to retain acquired information and to use it for future behavior. In the context of association theory, memory is the potential of a conditioned stimulus to activate an established associative link. Some researchers, however, view learning as acquiring information rather than responses, in which case memory would be a dynamic and self-
A single learning trial leads to an early form of short-term memory (eSTM) that is accompanied by a short enhancement of PKA and PKC activity. Consolidation to mid-term memory after a single trial (MTM) is a time-dependent process lasting several minutes. The molecular and cellular events related to the transition from eSTM to MTMs are unknown (see ?). MTMs decays after several hours but retention is still significant after one day, indicating that even a single trial can induce longer-lasting forms of memory to a low degree. Multiple learning trials lead to a succession of four memory phases that are arranged partially sequentially and partially in parallel. Early and late STM (e/LSTM) are not separable, because consolidation is strongly facilitated by trial repetition, high retention rates within the acquisition process, and strong resistance to extinction and reversal trials even immediately after conditioning; eSTM is accompanied by stronger and longer-lasting PKA activation and an activation of NO synthase (NOS). Both cellular responses are required for the transition to LTM, but may not be necessary for MTM formation. Transition to MTM after multiple trials (MTMm) is accompanied by constitutive activation of PKM via a proteolytic pathway that is essential for MTMm formation. Blocking proteolysis, however, does not inhibit the transition to the two forms of long-term memory (LTM), indicating parallel pathways from STM to MTM and LTM. Inhibition of protein synthesis interferes only with the formation of LTM. Massed conditioning leads predominantly to eLTM, spaced conditioning to LTM.

organizing process of information storage. Support for such a cognitive interpretation of memory in the honeybee comes from the fact that olfactory memory formation is not identical to the process of acquisition. Memory needs time to develop and proceeds through phases that differ in their susceptibility to in-
tering events, their content, and their neural and cellular substrates (Menzel, 1999; Menzel and Müller, 1996; see Figure 1).

The memory trace for olfactory cues is distributed and involves two of the three convergence sites between the olfactory pathway and the reward pathway. The reward pathway was identified by Hammer (1999) and assigned to a single identified neuron, the VUMAx neuron. Two of the three convergence sites—antennal lobes and mushroom bodies—are, respectively, the primary and secondary processing regions in the olfactory pathway, and each of these neuropils establishes its own memory trace independently of the other (Hammer and Menzel, 1998). The two traces are, however, different at least with respect to their dynamics, and are likely to store different information.

Researchers have made progress in unraveling the neural correlates of memory for the antennal lobe by visualizing the changes in odor coding as a consequence of olfactory conditioning (Faber, Joerges, and Menzel, 1999). The antennal lobe is organized into glomeruli; odors are coded as specific spatial-activation patterns of the glomeruli. These patterns can be imaged using calcium-sensitive fluorescent dyes. As a result of conditioning, the neural representation of a trained odor becomes more pronounced and more distinct from nonrewarded odors, but its general features do not change, indicating that learning at this level intensifies the neural code of the learned signal but does not create a new representation. It is unclear what specifies the odor-induced activity patterns as those of a learned odor, because a stronger stimulus also induces a more pronounced and distinct activity pattern, but bees have no trouble distinguishing between a strong nonlearned odor and a weak learned odor.

A stable, lifelong memory is formed even after only a few learning trials as the result of sequential steps of memory processing. Five memory stages are distinguishable on the basis of their respective temporal dynamics and their physiological and biochemical properties. The cellular and neuronal machinery underlying the memory stages is basically similar to those known for other model systems (Aplysia: Botzer, Markovich, and Suswien, 1998; Müller and Carew, 1998; Drosophila: Dubnau and Tully, 1998; Chick: Rose, 1991), although each model system has its own temporal dynamics. This indicates that the cellular and molecular machinery underlying the processing of associative memory follows general rules but is flexible enough to adapt to the particular timing required under natural conditions. Some researchers argue that the timing of memory stages in the honeybee reflects an adaptation to the requirements during foraging at distributed and unreliable food sources (e.g., flowers; Menzel, 1999; Giurfa et al., 2001).

Conclusion

The honeybee provides a model system for the study of neural substrates of low and intermediate levels of cognitive faculties. Neural analysis is supported by robust forms of associative learning that occur even under conditions when intracellular recordings or optophysiological measurements of single or multiple neuron activities are performed. The functional organization of the brain with a considerable number of uniquely identifiable neurons is also advantageous for relating cognitive functions to neural events in circumscribed circuits. Biochemical analyses of the role of protein kinases (e.g., PKA, PKC) and enzymes (e.g., NO synthase) relate directly to behavioral phenomena such as memory stages. Associative processes in the bee brain are not restricted to elementary forms but reflect configural processing and context-dependent associations. Thus the bee brain may serve as a model for the study of cognitive processes at an intermediate level of complexity.

See also: CONDITIONING, CELLULAR AND NETWORK SCHEMES FOR HIGHER-ORDER FEATURES OF; CONDITIONING, CLASSICAL AND INSTRUMENTAL; INSECT LEARNING; KAMIN'S BLOCKING EFFECT; NEURONAL SUBSTRATES; SECOND MESSENGER SYSTEMS

Bibliography


Rudolf Menzel

ASSOCIATIVE LEARNING IN HERMISSEND A

Few features of conscious experience have captured the human imagination more than the proclivity of animals to learn and to retain the consequences of experience in memory. Learning not only provides for the adaptation of organisms to changing environmental demands, but also, and more important, for the persistence of learning—that is, long-term memory—which provides us with a history of human experience. In spite of the widespread interest in learning and memory, their basic mechanisms remain among the least thoroughly understood areas of physiology.

An attractive experimental approach to this problem at a fundamental level is the analysis of learning and memory in the less-complex central nervous system of invertebrates. One animal that has contributed to the physiology of learning and memory is the nudibranch mollusk *Hermisenda crassicornis*, whose behavior can be modified by a Pavlovian conditioning procedure. The *Hermisenda* central nervous system is relatively simple, consisting of many identifiable neurons that can be studied in detail using biochemical, biophysical, and molecular techniques. An additional advantage is that the two sensory structures and their central pathways supporting the conditioned stimulus (CS) and unconditioned stimulus (US) are totally intact in the isolated central nervous system. This attractive feature facilitates the search for cellular correlates of learning that have been identified and have been the focus of biochemical and molecular analyses.

Pavlovian Conditioning

Pavlovian conditioning of *Hermisenda* involves changes in light-elicted locomotion and foot length (conditioned responses, CRs) produced by stimulation of the visual and vestibular systems with their adequate stimuli (Crow and Alkon, 1978; Lederhendler, Gart, and Alkon, 1986). The Pavlovian conditioning procedure consists of pairing light, the conditioned stimulus (CS), with high-speed rotation, the unconditioned stimulus (US). As shown in Figure 1, after conditioning, the CS suppresses normal light-elicted locomotion and elicits foot shortening. Retention of conditioned behavior persists for several days to weeks depending upon the number of conditioning trials used in initial acquisition (Alkon, 1989; Crow and Alkon, 1978). Pavlovian conditioning in *Hermisenda* exhibits CS specificity and is dependent upon the association of the two sensory stimuli involving both contiguity and contingency. Crow and Offenbach (1983) showed that conditioned animals exhibit suppressed locomotor behavior in the presence of the CS; however, their locomotor behavior in the dark was not significantly changed. Nonassociative contributions to behavior are expressed in the initial trials of the conditioning session and the decrement rapidly following the termination of multtrial conditioning.