Learning and Memory in Bees

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A bee is able to learn quickly and to remember for long periods the color and the odor of flowers that yield nectar or pollen. Now the neural basis of this programmed behavior is being elucidated.

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If you watch a single bee as it moves from flower to flower, you will see that it always chooses the same type of flower and pays no attention to nearby flowers of different color, shape and odor that are attracting other bees. Clearly the bee could not follow this pattern if it were not capable of two things: distinguishing one type of flower from another and learning what flowers offer nectar or pollen and so are worth visiting. In other words, the bee has a memory and an ability to learn.

We and our colleagues at the University of Berlin have been investigating these abilities of the bee in the hope of elucidating the cellular basis of learning and memory. Our studies are both behavioral (we watch bees in various experimental situations in order to determine the extent of their behavioral repertoire) and physiological (we explore the anatomy and function of the bee's brain in an effort to ascertain what parts of it are involved in learning and memory). We find that even though the bee is a tightly programmed behavioral machine, its mechanisms of learning and memory are quite similar to the ones that appear to operate in much more highly evolved organisms, including man.

Although it was obvious to many careful observers in the past (the written comments range from Aristotle to Charles Darwin) that bees can see the colors and smell the odors of flowers and can remember what type of flower they have visited previously, the experimental proof had to wait until 1910, when Karl von Frisch at the University of Munich conducted a simple test that stands as one of the few really crucial and decisive experiments in the study of animal behavior. He attracted foraging bees to a table by offering a honey solution. When enough bees were visiting the place regularly, the dish containing the honey was placed on a piece of blue cardboard, so that the bees saw the blue color as they approached the table and as they sucked the honey.

Now von Frisch was ready for his test. He surrounded the blue cardboard with other pieces of cardboard of the same size but ranging in color from white through various shades of gray to black. On each piece of cardboard, including the blue one, was an empty dish. The bees, which had been conditioned to expect that they would find food at the table, continued looking, and nearly all of them confined their search to the blue cardboard.

The experiment provided strong support for both of the postulates we have mentioned. The bees were distinguishing the blue color from all the other colors, which meant that they could see blue as a color and also that they had learned to take the color (in that situation) as a signal for food. To confirm the second finding it was necessary to show that the bees had not been attracted spontaneously to the color. Von Frisch did so in various ways, as others have after him. Indeed, his experiment has been repeated with a variety of colors, odors and black-and-white patterns.

The behavioral aspects of the bee's performance are therefore well known. Surprisingly, the learning process involved in all these experiments was left unstudied until about 10 years ago. We shall show that the process has several interesting features that make it attractive for an approach toward a neuronal, or nerve-cellular, analysis of memory formation in the bee's brain.

Let us ask a first question. How much precision do bees show in selecting the type of flower from which they collect nectar and pollen? This question can be answered quickly and accurately without the necessity of following a bee on its flight from flower to flower. When a bee makes one of its periodic returns to the hive, it carries a record of its choices in its two pollen lumps (one on each hind leg). By inspecting the contents of the pollen lumps of individual bees one finds that the pollen brought back by a bee from a single excursions is entirely or almost entirely from a single type of flower. Since a bee visits as many as 500 flowers on a single excursion, the degree of precision in the bee's choice is high indeed.

This precision of response, together with the fact that bees can easily be trained to respond to various stimuli, makes the insects particularly suitable for the experiments we had in mind. It is also helpful that all the worker bees from one hive are sisters and therefore are closely related genetically and that bees are highly motivated to search for food, so that each bee makes a large number of decisions (from 1,500 to 2,000 per day).

To prepare for a direct approach to the neuronal events underlying learning and memory we first examined the learning process in detail with behavioral methods. Here we concentrated on experiments in which bees were trained to respond to different colors, but other workers have employed different cues. For example, Martin Lindauer and his colleagues at the University of Würzburg trained bees either to odors or to a daily time schedule, and Rüdiger Wehner at the University of Zurich trained bees to discriminate among black-and-white patterns.

Our arrangement for color training involved an apparatus in which we could employ monochromatic light of adjustable intensity to illuminate two ground-glass disks. Individually marked bees are fed on an unilluminated glass disk in the center of the gray training table. By means of the "waggle dance" elucidated by von Frisch the bees inform their hive mates of this source of food. A newcomer arriving in response to this information is allowed to visit an unilluminated disk three times in order to become familiar with the arrangement. After three visits that yield food a bee is motivated to search for food even when none is offered. During the first three visits of a new bee all other bees are removed from the apparatus and kept in a cage, so that only the experimental bee flies back and forth between the hive and the apparatus.

Our first text evaluates the spontane-
ous-choice preference of the bee. For the first time the experimental bee is confronted with two glass disks of different colors. Neither disk bears food, and each one is equidistant from the center (the former feeding place). The bee approaches the disks, lands on them and searches for food. If the frequency of choice is not equal for both colors, the intensity of one color or the other is adjusted until the bee displays no preference for either one.

After a test period of a few minutes the bee is rewarded with food on one of the two colors. When it comes back, a test is performed in the same way as in the spontaneous-preference trials. Then it is rewarded again, and so on. From this procedure we obtain learning curves for individual bees and therefore are enabled to evaluate quantitatively the factors influencing the learning behavior of bees.

Colors are learned within a few trials. Violet (at wavelengths of from 400 to 420 nanometers) is learned fastest; only one reward is needed to produce a high frequency of correct choices. Bluish-green (490 nanometers) is learned slowest. The characteristic differences of the learning curves are independent of such factors as the intensity of the two colors (if the intensity is at least half a logarithmic unit above the bee's perceptual threshold), the wavelength of the alternative color, the type of pretraining, the age of the test bee, the time (of day or season) and the weather.

One would expect the dependence of learning on wavelength to show a close correlation with the color-vision system of bees. It does not. The bee's color-vision system is trichromatic, with receptors maximally sensitive to ultraviolet radiation (350 nanometers), blue light (440 nanometers) and green light (540 nanometers). Ultraviolet is the brightest and most saturated color to bees, as was shown 20 years ago by Karl Daumer of the University of Munich. As one would expect from such a system, the bee's ability to distinguish colors is best for violet and bluish-green, since the sensitivity of the receptors is

COLOR TRAINING of bees was done by the authors with an apparatus that enabled them to illuminate ground-glass disks (seven centimeters in diameter) with monochromatic light of adjustable intensity. At first individually marked bees are fed (top) on an unilluminated disk in the center of the gray training table. With their "waggle dance" they inform other bees in the hive of the food source. A single responding bee is fed at the central disk, tested for its spontaneous choice of two colors equidistant from the central disk (bottom) and then rewarded with food on the central disk, now illuminated with one of the two colors. Even a brief and small reward causes the bee to return to that color consistently.
COLOR LEARNING by bees is quite rapid for some colors and somewhat slower for others, although a bee can learn any color that is visible to bees. In this set of drawings the flower of the wild rose is represented at the center as the human eye sees it and then, reading clockwise from the top, in order of the bee's ability to associate a color quickly with a reward of food. The color learned fastest is a deeply saturated violet, then blue, ultraviolet (shown as gray), green and orange. Bluish-green, the normal background color for bees, is learned slowest.

most pronounced in that region and they also overlap in sensitivity there. Indeed, this hypothesis was proved to be correct by Otto von Helversen of the University of Freiburg. Such sensitivities are necessary for a precise identification of the predominantly violet and blue flowers, which are specialized in attracting insects for cross-fertilization.

Although bees are most sensitive to ultraviolet radiation, it is not the color that they learn best. The bee ultraviolet radiation is sky radiation, and it seems not to be expected as a pure color at food sources. Mixed with long-wavelength light to form "bee purple" it is learned much faster. Bluish-green is the background color and also is not expected as a food marker. The interactive evolution of flowers and their insect pollinators has led not only to an adaptation of flower colors and bee color perception but also to pre-fixed learning dispositions in bees. This interpretation is supported by Lindauer's studies on the odor learning of bees; naturally occurring odors are learned faster than artificial and uncommon ones.

We note in this context the recent tendency among experimental psychologists to appreciate the value of the ethological viewpoint, which is that behavior is best studied in relation to the animal's natural environment and evolution. Color and odor learning in bees appear to be examples of behavior that can most fruitfully be examined in this way. As an ethologist von Frisch intuitively interpreted his results along this line 40 years ago. Today one can quantitatively separate the design of the bee's perceptual system (the behavioral program for gathering context-specific information) and the neural programs for processing this information into stored memory.

How stable is the bee's memory for food signals? Anecdotal reports from beekeepers suggest that it is highly stable and also durable. One of the first quantitative observations was made in 1963 by Lindauer, who found that bees arrived spontaneously at a feeding place after five months of absence during the winter. To obtain a still more quantitative evaluation we trained bees to blue or yellow with one reward to three, kept them in individual mesh cages in the hive for varying periods of time and then tested their choice behavior. After one reward the frequency of a given choice stayed high for several hours, but it declined on the next day and reached the spontaneous-choice level in from five to seven days. After three rewards, however, we found no reduction in correct choices during the period of about 13 days in which a bee can survive in a cage. It is apparent that bees need only a few rewards to establish a highly stable memory.

The situation is different if the bees are allowed to learn anew. They will switch to a new food source marked with a different color, but the speed of the switch depends on the number of rewards they received at the color that is now unrewarded. In this situation one can observe a curious behavior well known from studies of vertebrates. The animal makes the change more rapidly if it has had a long period of training (25 rewards or more) to the initial color. Another finding is that if two signals carry rewards at different rates, the animal chooses them according to their reward probabilities. (This effect has been studied in detail by Bernhard Schnetter of the University of Würzburg.) Another example is simultaneous training to more than one color. The bee can learn two colors simultaneously and can distinguish them from other colors. If more than two colors are rewarded simultaneously, however, the bees stop distinguishing colors. All of this reveals something of the design logic in their learning programs.

Such complex and long-lasting learning effects are interesting from the viewpoints of ethology and comparative psychology, but they are not suited for an analysis of the neuronal events underlying behavior. We therefore concentrated on analyzing the neuronal processes during and after a single association of a color or an odor signal and a reward. This turned out to be a fortunate decision, because suddenly new perspectives on the learning process appeared.

We first established a most useful result: After a single reward the response level is independent of the quantity of reward, provided that certain time limitations are observed. Even a reward that persists for only 100 milliseconds and has a low concentration of sucrose is sufficient to produce a significant change in response.

Another interesting result appeared when we trained bees with a single short reward (two, five or 10 seconds). Bees tested immediately after the reward displayed a high rate of correct choices. Over the next two minutes, however, the score dropped drastically, reached a minimum at between two and three minutes and then rose again to about the initial level.

This kind of relation between memory formation and time appears in other animals and in man. The physiological mechanisms underlying the association
between a signal and a reward (or a punishment) need time (seconds, minutes and even hours) and most probably go through phases in which the neural substrate for the memory is different. In the typical case the first storage is in a sensory memory, from which the information may quickly disappear if it is not reinforced. On reinforcement the information goes into short-term storage, which is reflected in the high level of correct responses made by a bee immediately after one reward or more. The rise after a subsequent decline represents a phase termed consolidation, wherein the information is transferred to long-term storage.

For the bee, as one might expect, the phases of learning are nicely adapted to the natural learning situation. Most flowers offer such small quantities of nectar that a bee will suck for only a few seconds and will usually approach the next flower within another few seconds. If the flowers are widely distributed, the bee may need up to a minute to find the next similar flower. The initially high rate of correct choices indicates a short-term mechanism that assures the bee of being able to find a similar flower even after very short rewards. The longer-lasting consolidation phase seems to be adapted to the bee’s flight between the food-collecting area and the hive. When the bee comes back from the hive, it again makes a high level of correct choices, indicating that the information is in long-term storage.

How long is a color signal stored and sucked the honey. Next von Frisch surrounded the blue cardboard with other cards of the same size but colored white, black or shades of gray. Each card, including the blue one, bore an empty dish. The bees had been conditioned to expect food at the table, and so they continued to come. Most of them looked only on the blue card.
in the bee's sensory memory? The answer lies in ascertaining the conditions under which a color will still be associated with food even though the color is no longer visible. In our tests of this matter we have found that a color signal perceived by a bee within two seconds before the start of the reward is learned as well as if the signal is present through the entire period of approach and sucking. If a color signal is switched on after the bee starts sucking, it is not associated with food.

Elisabeth Opfinger observed in 1931, when she was a student working with von Frisch, that bees learn a color only if they see it during the approach to a flower; the colors seen during sucking and flying away are not learned. Now we know that a color signal must be present within three seconds before and about .5 second after the start of sucking to be learned. Since a single sucking period of two seconds is enough for the bee to show a highly significant level of learning, the total initial learning period (sensory memory and the association period plus the duration of the reward) appears to be from four to five seconds. This is a convenient situation for exploring the temporal phases of a storage of memory initiated by a single reward.

Do the short-term and long-term phases reflect two physiologically different storage mechanisms? We have treated bees with various procedures that are known to block neural activity in the brain. All of them prevent the formation of short-term memory. (The bees thus treated behave after recovery in exactly the same way as untreated bees. Moreover, control experiments excluded the possibility that the experimental procedures caused a negative reinforcement.)

We have found a correlation between the initial period of declining memory and the period of susceptibility to the blocking procedures. The results tell us that during an initial period of a few minutes the storage of memory is based on physiological mechanisms that are different from the ones involved in later storage. Most probably the early physiological mechanisms are orderly neuronal activities, since our treatments block such activities.

The first step in associative learning obviously is a mechanism of fleeting storage of sensory information, the phase we have called sensory storage. If the information is not reinforced promptly, it disappears. Reinforcement of the visual signal depends on a second sensory system: the sugar receptors on the bee's tongue. If the receptors are stimulated by food, the next phase of the learning program (storage of the information in a memory system) is set in motion.

It is plausible to assume that these initial and rapid processes of channeling and selecting information are based on the electrical activity of particular nerve cells. Electrophysiological recordings might help to identify the cells. But

![Learning Curves](image_url)

**Learning Curves** indicate the speed with which bees learned different colors. The curves have been averaged from the performance of many bees. The average of correct responses rises with the number of food rewards. In these experiments the alternative to the rewarded color was the complementary color: for ultraviolet at 361 nanometers it was bluish-green at 494; for violet at 428 nanometers it was orange at 590, and for blue it was "bee purple," a mixture of 80 percent orange and 20 percent ultraviolet (curve farthest to the right). The learning is quickest for violet at 408 and 418 nanometers and slowest for bluish-green at 494 nanometers. Trained to violet, bees reach an accuracy level of 90 percent after only one reward; for bluish-green they need about five rewards to reach the same level.
where in the bee's brain should one insert a recording electrode? Although the bee's brain is relatively small (about one cubic millimeter in size), it contains something like 850,000 nerve cells—too many for a random searching strategy. We have therefore sought instead to localize the areas of the brain that are involved in the initial storage processes.

Our approach is through the reflex response of the proboscis to sugar-solution stimuli. A bee extends its proboscis as soon as an antenna is touched with sugar solution. We fix a bee in a small metal tube and present a whiff of odor just before touching the antenna with sugar solution and allowing the bee to lick the solution with its proboscis for a few seconds. If the odor is then presented without a sugar solution, the bee will almost invariably extend its proboscis. Only one conditioning trial is needed to establish a high level of responses.

We found that we could expose the brain during this process without unduly affecting the learning behavior. Then we could reversibly block neuronal activity in small areas of the brain by means of a thin cooled needle. The blocked area is as small as 250 micrometers in width and 150 in depth.

Through this procedure our interest came to focus on the corpora pedunculata, also known as the mushroom bodies. There are two of them, each densely packed with neuropils. A mushroom body has four major substructures: the caplike calyx, the stalk, the frontal alpha lobe and the bottom beta lobe. Its main input comes from another structure, the antennal lobe. Historians of the 19th century observed that mushroom bodies are particularly prominent in social insects and inferred that the highest brain functions (learning, memory and complex social behavior) are probably located there.

When we impaired parts of the antennal lobes and mushroom bodies by cooling them, we found a correlation with what we had observed by blocking neuronal activity in freely flying bees. The effect of impairment decreases with increasing time between trial and treatment. The susceptibility to impairment decreases fastest in the antennal lobe and slowest in the calyx.

The results indicate that the antennal lobes and parts of the mushroom bodies are involved in the processing of information into the memory, although to different extents and over different periods of time. The antennal lobes are involved only during a short initial period of about three minutes. The calyx is involved longer. Since the memory can be shown to be impaired even seven minutes after cooling of the calyxes, they are probably the essential structures in the transfer of information from the short-term memory to the long-

STABILITY OF MEMORY in bees is charted. If bees are rewarded three times on a particular color and then kept in the hive for up to two weeks to exclude new learning, they keep a high level of response afterward (upper curve). With only one reward the response level rises at first, a period called the consolidation phase. One day later, however, the response begins to drop; after about six days it reaches the level of spontaneous choice. The reward disk was blue.

CHOICE BEHAVIOR of bees following a one-trial learning period showed this pattern. Bees tested immediately after learning choose the rewarded color with high accuracy. Two minutes later the number of correct responses falls but rises again thereafter in the consolidation phase.
The alpha lobes, which are connected to the calyces by thousands of nerve fibers, are involved too.

The one-trial learning situation in our experiments enabled us to follow the time course of the transcription from short-term memory to long-term. The time courses that we found for various brain structures make it most unlikely that the electrochemical and biochemical processes involved in the transcription follow a simple dynamic within a confined synaptic area. Since blocking neural activity both in freely flying color-trained bees and in fixed odor-trained bees had similar results over time, it appears that the central nervous processes underlying the early storage of memory are similar for both color and odor.

We have identified particular areas of the brain that are involved in the formation of memory of odors. Whether the same areas are correlated with visual learning is an open question. In any event the time courses of learning of both olfactory and visual information are quite similar. Electrophysiological recordings performed on our immobilized odor-conditioned bees should therefore elucidate also the general mechanisms of associative learning that are at work in freely flying bees.

It is not easy to make recordings from single neurons in the bee’s brain because the neurons are usually very thin. Nevertheless, we have found it possible to make such recordings while the insect is free to move its proboscis and can be conditioned to odor. Our electrophysiological studies are only beginning, but a few general findings are available.

The majority of the neurons in the mushroom body receive inputs from more than one sensory system. For example, a neuron might respond to a light flash with inhibition (firing less), to sugar on the antenna with excitation (firing more) and to a mixed odor with excitation. We have observed many different combinations of responses to different stimuli.

Only a few of these neurons change their responsiveness during conditioning. The ones that do were already responding to odor and sugar before conditioning. The change is usually a rise of the spontaneous-discharge rate of the neuron accompanied by an increase in sensitivity to stimuli of odor and sugar water. In some neurons we found a close correlation between the number of conditions and the change in responsiveness. By intracellular staining of neurons we are trying to identify the particular cells that learn. Most of them turn out to be in the area around the mushroom bodies, reinforcing the hypothesis that this part of the brain is highly involved in the processing and storing of olfactory information.

It is apparent from the work that we and others done with bees that associative learning in the bee follows
ODOR CONDITIONING of bees offers another way of testing the formation of memory. The bee is fixed in a tube and its antenna is touched with a drop of sucrose solution. The bee extends its proboscis in an unconditioned reflex. If a whiff of odor is presented shortly before the antenna is touched, and the bee is allowed to taste sugar with its extended proboscis, the odor becomes a conditioned stimulus. After a single conditioning, that is, one taste of sucrose, most bees extend the proboscis even when the whiff of odor is presented without a reward of food.

all the major learning steps that have been established for vertebrate animals. Moreover, bees have sensory, short-term and long-term memories quite similar to those found in vertebrate species. The neural strategies underlying learning and memory storage in the bee also do not differ basically from what is known about vertebrates. With work on the bee, therefore, we should have a good chance of unraveling a few basic rules of associative learning and memory that are also relevant to complex vertebrate neural systems.

BEE'S BRAIN is portrayed schematically in a frontal view. The lamina, medulla and lobula mainly process visual information. The midbrain incorporates the two corpora pedunculata, or mushroom bodies, which are composed of densely packed and highly ordered interneurons. The regions of the mushroom bodies are the calyces, the alpha lobes and the beta lobes. Two other structures, the antennal lobes, are the primary sensory centers for inputs from the antenna. Major nerve tracts handling information flow between mushroom bodies and the antennal lobes are shown in color. Bee's cubic-millimeter brain has some 850,000 nerve cells.
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Bibliography


