INSECT LEARNING

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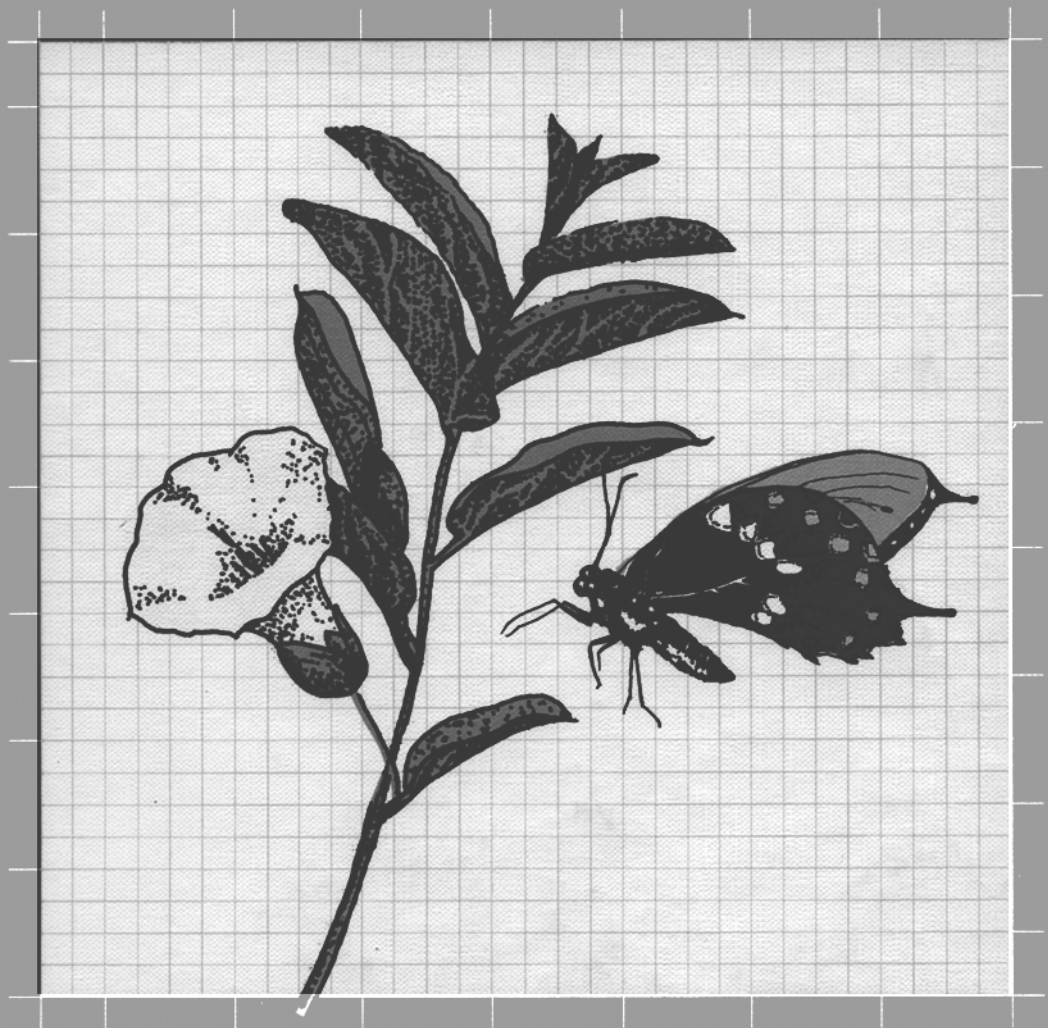

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Functional Organization of Appetitive Learning and Memory in a Generalist Pollinator, the Honey Bee

Randolf Menzel, Uwe Greggers, and Martin Hammer

Individual experience with environmental stimuli leaves multiple traces of neuronal plasticities in the nervous system. Receptors adapt to prolonged stimulation; neural circuits habituate to repeated stimuli and dishabituate or sensitize to arousing stimuli; and new functional connections are formed or existing ones abolished by associative and latent learning. What are the rules of neural plasticity and how do they relate to the biological constraints under which they have evolved? The neuroethological approach taken in the study of honey bee learning and memory tries to understand the neuronal mechanisms of the multiple memory traces as adaptations to the particular demands of foraging by a generalist pollinating insect. The study of the functional dynamics of memory thus serves two goals: to unravel the informational sources which guide the sequences and time dependencies of the animal's choice behavior, and to better understand the neural correlates of the various forms of memory.

In the case of a social insect like the honey bee, motivational states, activation of alternative behavioral sets (like resting, food collecting, searching, nest keeping, feeding, etc.), and decisions within any one set depend on innate and acquired experiences, both of the individual animal and of the whole society. Individual behavior and social phenomena as sources of information are equally important (Frisch, 1967; Seeley, 1985; Lindauer, 1959, 1955). The memories from recent and remote experiences, from multiple or single learning events, from mere exposure to stimuli or from contingent pairing of stimuli with other significant stimuli differ in their informational content, in the balance between innate and acquired behavioral routines, in their time dependencies, and in their sensitivities to new experience. This paper will focus on the implications that multiple memory traces have for choice behavior of individual honey bees in the context of appetitive learning. We will argue that, at any moment, time- and event-dependent processes inherent in the memory traces provide the

animal with expectations about the consequences of its responses to stimuli. Most importantly, these expectations are heavily dependent on intrinsic, automatic memory processes and not just on exposure to external events. This perspective differs fundamentally from those developed in the framework of behaviorism (Hull, 1943; Bitterman, 1988) and traditional Pavlovian reflexology (see Pavlov, 1967). Both of the latter approaches focus on stimulus-response properties and neglect the autonomous contributions of intrinsic functional elements of the nervous system, which reflect both constraints on the cellular machinery and evolutionary adaptations designed to satisfy an animal's needs. Our own approach embraces concepts in both ethology and cognitive psychology. Cognitive terms such as expectation, prediction, attention, decision, orientation in time and space, and communication between members of a society will be applied here to learning and memory in honey bees. However, notions about consciousness or mental operations, even at a rudimentary level, will not be entertained here. Aspects of this fascinating and controversal issue are discussed by Griffin (1984) and Menzel (1990).

Functional Properties of Unconditioned and Conditioned Stimuli

Most stimuli inform the animal and are thus meaningful even to naïve, unexperienced animals. Sign stimuli release whole sets of innate behaviors while arousing stimuli alter the status and direction of attention. Few (if any) stimuli are strictly neutral in the sense that their appearance is irrelevant to the animal. Learning is a property of the nervous system in which informational status of stimuli is changed as a consequence of being passively or actively exposed to stimuli and their combinations. Repeated exposure to a stimulus without any relevant consequences for the animal leads to habituation of the response initially evoked. The sudden, unexpected appearance of a strong and meaningful stimulus arouses the animal, dishabituates habituated responses, and may transfer its informational capacity to less effective and more neutral stimuli which are temporally closely related to its appearance. These strong and meaningful stimuli are usually called unconditioned stimuli (US), while stimuli with more neutral and less obvious significance to a naive animal are called conditioned stimuli (CS). Although unsatisfactory in many respects, these terms (introduced by Pavlov, 1967) have become accepted as technical abbreviations for two extremes in the informational content of stimuli, and will be used here. Insects appear to differ from other animals with rich behavioral repertoires (such as mammals) by exhibiting more instinctive behavior, assigning more innate meaning to stimuli, and being more "prepared to learn" particular stimuli.

Insects should therefore be ideal for learning studies because changes in the informational content of stimuli should be particularly dramatic and selective (Gould and Marler, 1984). Greater preparedness for selective associations among insects may in turn be reflected in lower complexities in the neural substrate of learning and memory, more automatic intrinsic properties for the formation of the memory trace, and a closer relationship between the plasticities of the neural network and the behavioral consequences.

Training freely flying honey bees to a colored or scented target is a very useful and powerful method with which to study visual or olfactory abilities and numerous other aspects of honey bee behavior (Frisch, 1967). The natural context for these training experiments is the fidelity of individual bees to a particular flower species as a food source, a fact well known for more than 100 years (Fabre, 1879; Forel, 1910). An example of this fidelity is illustrated by the behavior of three honey bees observed in a patch offering flowers of four different species (Fig. 4.1). Each honey bee is perfectly tuned to one of the plant species and does not collect nectar or pollen from any other species. The learning underlying this choice behavior is very fast and establishes a long-lasting memory (review Menzel, 1990). Examples of the acquisition functions of two colors are given in Figure 4.2. In a dual-choice situation a violet target is preferred at a very high level after a single learning trial, while a similar preference for a green target is acquired only after several trials. Odorants are learned even faster than color (Koltermann, 1969). A single learning trial on a floral odor results in nearly 100% preference for that odor.

The memory for the CS depends on the number of learning trials. As Figure 4.3 shows, honey bees remember a blue-color target for several days, even after a single learning trial if they are prevented from learning new signals by enclosing them in the colony until the test (Menzel, 1968). If honey bees are rewarded three times on the blue target, they do not forget it for their lifetime. Interestingly, learned performance improves during an initial period, indicating that the memory controlling choice behavior may be strengthened with time since the last experience. This aspect of the memory was examined further in experiments in which shorter time intervals were chosen (Fig. 4.4). It was found that immediately after the first trial, the probability of choosing the rewarded color target was very high, then decreased to a minimum ca. 3 minutes after the learning trial, and then increased again. Such a dual-phase time course indicates that the memory trace may consist of different functional components and that an early form may be consolidated into a later form (Menzel, 1987). Below, we will use this observation as a basis for analysis of the underlying mechanisms.

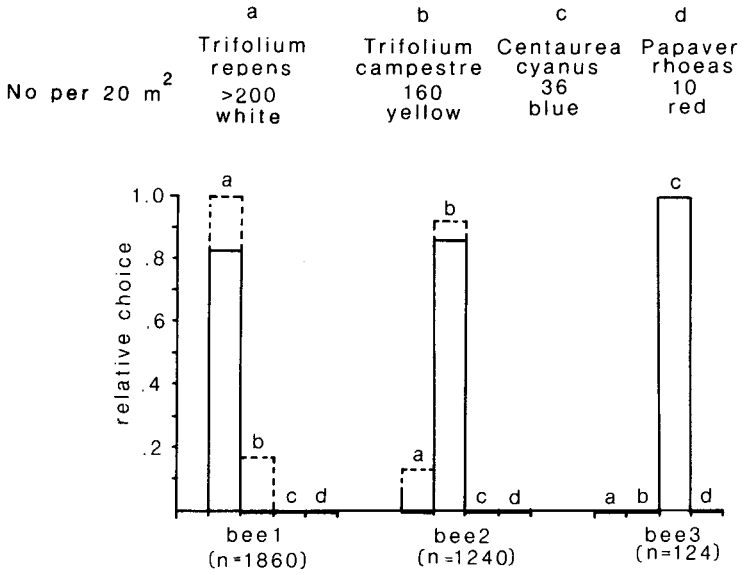

Figure 4.1. Choice behavior of three individually marked bees (Nos. 1, 2, 3, abscissa) in an area of 20 m² with four species of simultaneously blooming plants (a: *Trifolium repens*, > 200 flowers, white; b: *Trifolium campestre*, 160 flowers, yellow; c: *Centaurea cyanus*, 36 flowers, blue; d: *Papaver rhoeas*, 10 flowers, red (= bee UV-violet). Nectar is provided by the two *Trifolium* species, pollen by the poppy, and pollen and nectar by the cornflower. The choices are counted during several foraging bouts of each bee (n gives the number of choices). The dotted line gives the relative frequency of approaches without landing and the bars with the solid line, the relative frequency of landings. The cornflower was also visited, but only by unmarked bees.

The search for functional mechanisms requires more manageable experimental conditions. A very suitable paradigm is olfactory conditioning of the proboscis extension reflex (PER), a paradigm which was developed by Kuwabara (1957) and used successfully for odor discrimination tests by Vareschi (1972) (Fig. 4.5). Honey bees learn quickly to associate an odor with a sucrose reward. The sucrose stimulus delivered to contact chemoreceptors at the antennae and subsequently to the extended proboscis is a strong appetitive, unconditioned stimulus. Sucrose functions to (1) release reflexes; (2) modulate ongoing activities, enhancing the probability or the strength of responses to other stimuli; and (3) reinforce a conditioned stimulus. These properties (termed "releaser," "modulator," and "reinforcer" properties, respectively) are discussed in turn below (Fig. 4.6):

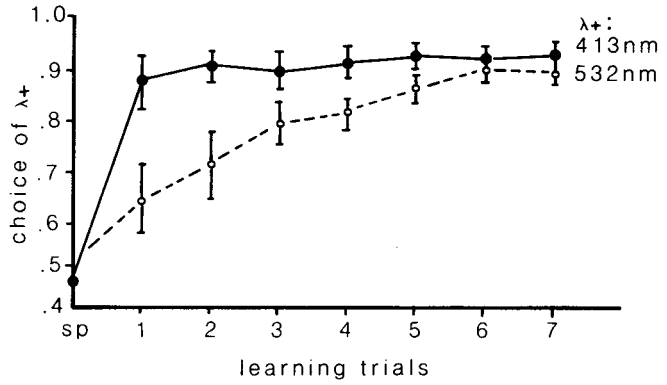

Figure 4.2. Average acquisition functions for two different spectral lights (413 nm = violet, 532 nm = green) in a dual-choice test with two color targets as alternatives. The spontaneous-choice test (sp) was performed after three initial rewarded trials on unilluminated ground glass. After the spontaneous-choice test, the single bee was either rewarded on the violet (upper curve) or the green target (lower curve). The test bee flew back to the colony after each reward and was tested for its choice behavior after it came back to the experimental setup. The tests lasted for 4 minutes, and direct approaches in flight were counted as choices. (Number of test bees: 14 for 413 nm, 34 for 532 nm; number of choices: 1532 for 413 nm, 3,872 for 532 nm, from Menzel, 1967, redrawn).

1. *Releaser function.* Several responses are released by sucrose stimulation: movement of both antennae toward the sucrose solution, extension of the proboscis with sideward and upward searching movements, rhythmic licking movements of the glossa even without contact to the solution. Several additional reflexes are released (e.g., increase in body temperature, ventilation movements of the abdomen, and leg movements), but we will focus on proboscis extension.
2. *Modulator function.* Other responses such as the weak response to an odor are enhanced and ongoing behavior is modulated. For example, the probability that the animal will respond with an extension of the proboscis to pure water or to mechanical stimuli at the antennae is facilitated by sucrose stimulation.
3. *Reinforcer function.* The effects of sucrose on behavior can be transferred to stimuli whose own presentation is contingent upon presentation of sucrose (e.g., during classical conditioning).

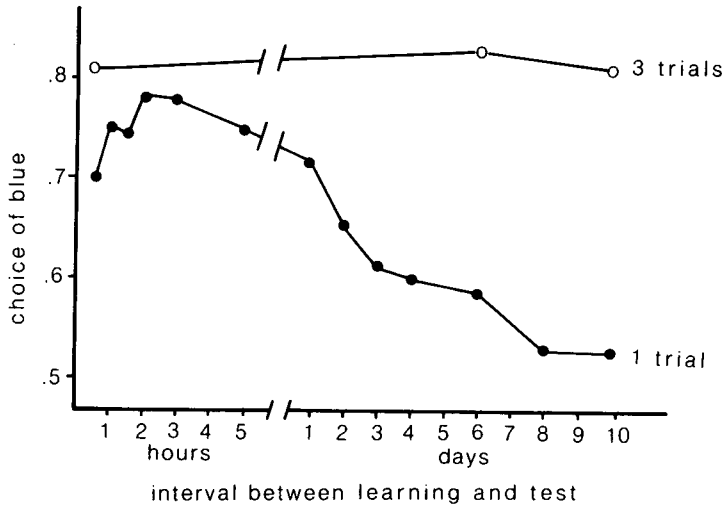

Figure 4.3. Time course of the memory for a blue target after a single learning trial and after three learning trials; from Menzel, 1968, redrawn). The freely flying bees were introduced into the dual-choice test as described for Figure 4.2 and rewarded for 20 seconds, either once or three times on the blue target. The alternative target was always yellow. The spontaneous choice between the two colors was balanced (close to 50% each). The bees were caged within the hive for various time periods and released shortly before the test (abscissa). Each bee was tested only once for about 4 minutes. Similar memory functions were found for a yellow target.

Both the modulatory and reinforcing function of the US will be discussed at length below. All three functional properties—reflex releaser, arousing modulator, appetitive reinforcer (Fig. 4.6)—act together in classical conditioning, and it is important to analyze the different memories initiated by each of these US properties.

A certain low proportion of honey bees respond to an odor stimulus with the PER even before conditioning. We usually term this a "spontaneous response," although it is not clear whether learning prior to the experiment induces the response or whether there is a weak response tendency even without prior learning. The distinction is of general importance, and it is unfortunate that the critical experiments (i.e., experiments which would integrate odor deprivation during larval and pupal development with adult conditioning) have yet to be performed in a satisfactory way.

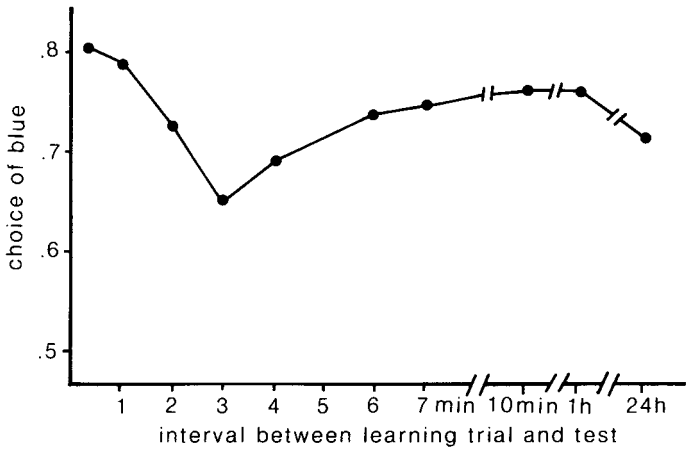

Figure 4.4. Time course of the memory after a single learning trial on a blue-color target (freely flying bees, same experimental arrangement as in Figure 4.2 and 4.3; redrawn from Menzel, 1968; and Erber, 1972, 1975a,b). Each experimental bee was tested only once for four minutes after the time interval indicated at the abscissa. Bees tested up to an interval of 10 minutes were kept flying freely; those for tests after longer intervals (1 hour to 24 hours) were caged in the colony (see Figure 4.3). The minimum around 3 minutes is highly significantly different from the initial and later high levels of choice behavior (χ^2 test, $p \leq 0.01$).

Some definitions of learning require that associative learning lead to a new behavioral response, the β-response, although in many learning paradigms the result of a CS/US pairing is just an increase in the probability or strength of a preexisting response to the CS (α-response). Hull's (1934) distinction between α- and β-response is taken by certain authors (e.g., Schreurs, 1989; Gormezano, 1984) to imply two classes of associative learning, whereas others see a continuum from pairing-specific sensitization, through protection from habituation of a weak α-response to the emergence of a new conditioned response (β-response) (Carew et al., 1984; Hawkins et al. 1989; Colwill and Rescorla, 1988).

The discussion may appear at first glance to be an academic quarrel over semantics. However, the discussion takes on a greater significance when it is considered in light of evolutionary arguments that lower animals with their greater development of instinctive behavior may be capable only of α-conditioning and that the development of a totally new behavior as a result of CS/US pairing is a property of more highly evolved nervous

Figure 4.5. Experimental arrangement for olfactory conditioning of the proboscis extension reflex (PER). Bees are harnessed in metal tubes by a stripe of sticky tape in the neck region. Sucrose stimulation of the antennae releases the PER and arouses the animal. The US used in conditioning experiments is a compound of sucrose stimulation first of the antennae and then of the proboscis (see text).

systems. Indeed, conditioning of an α-response, pairing-specific sensitization, and protection from habituation refer to mechanisms of neural plasticity which are restricted to specific stimuli and their combinations and lead to a behavioral change in a few or even a single pairing trial. Such prepared associations have been defined as a unique form of learning, "instinctive learning," (Gould and Marler, 1984). The concept is a most useful one, stressing as it does species-specific adaptations in learning abilities. However, the concept also tends to downplay the innovative power of associative learning. In the case of olfactory conditioning in the honey bee, for example, a whole range of chemosensory CS can be conditioned. Some stimuli (e.g., pheromones like citral, geraniol, floral odorants) release an α-response, while others (e.g., propionic acid) appear neutral or slightly aversive, and still others (e.g., fatty acids or the sting pheromone isoamylacetate) are strongly repellent. Honey bees can easily be trained to any of these odorants both in instrumental conditioning paradigms using freely flying bees and in classical PER conditioning paradigms using tethered ones (Menzel, 1990). The acquisition function of these various odors is often the same, though odors may differ in the number of extinction

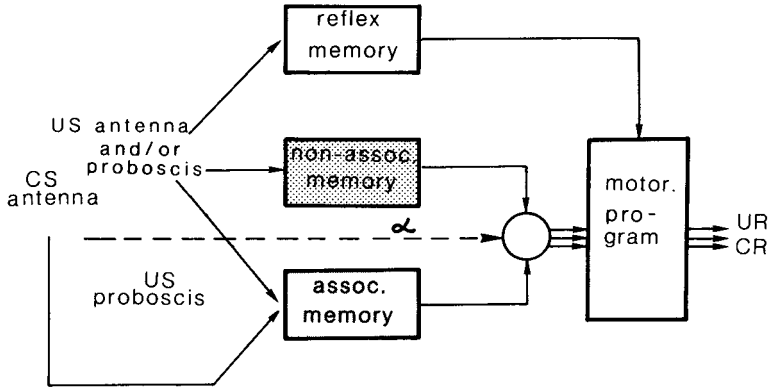

Figure 4.6. Functional organization of the PER-conditioning paradigm. The US (sucrose stimulation of the antenna and/or proboscis) has three properties: reflex releaser, response modulator, and CS reinforcer in CS/US pairing experiments (see text). The CS (olfactory stimulus) is not a completely neutral stimulus but causes a PER in a small proportion of the test animals before conditioning. This response is called α-response according to the nomenclature in animal learning studies (see text).

trials necessary to reach a conditioned response (CR) criterion and in their potential to act as a conditioned US (a phenomenon known as "second-order conditioning"). Other stimuli such as mechanical stimulation of the antennae, which are obviously aversive, can still become a CS after a few CS/US pairings. These observations illustrate two important concepts: (1) an α-response to a CS may facilitate associative learning of the CS, but does not appear to be a prerequisite for it, and (2) the honey bee is fully able to adopt completely new responses to a CS as a result of conditioning. We thus favor the view that α- and β-conditioning represent two extremes of a continuum.

Requirements for Optimal CS/US Pairing

Contiguity between CS and US is the most important factor in the conditioning process (Rescorla, 1967, 1988; Rescorla and Holland, 1982). In the case of PER-conditioning with a single conditioning trial, the optimal CS/US interval is +5 to 0 seconds with the CS preceding the US (so-called "trace or forward conditioning") (Fig. 4.7). Multiple conditioning trials

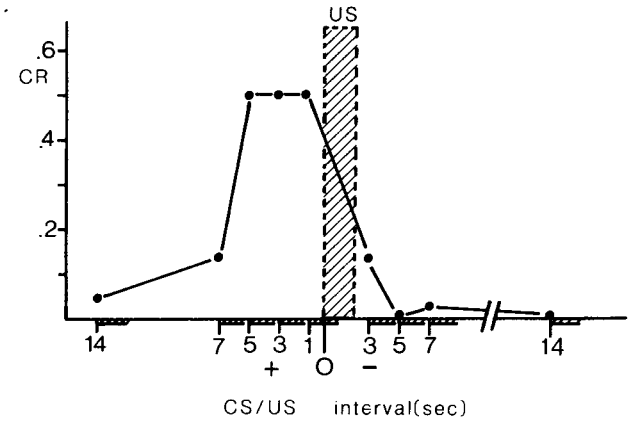

Figure 4.7. Optimal CS/US interval in single-trial olfactory PER-conditioning. The CS (carnation) was presented for 2 seconds at one out of nine different times before or after the US. After this single pairing, the response probability of CR (conditioned response) was tested 20 minutes later. Each point gives the CR probability of a group of animals (10–18 animals in each group). The US lasting for 2 seconds is marked with a striped bar, the CS pulses (abscissa, striped lines) occurring before the US appear at the left side (+), and those after the US appear at the right side (−) of the US. The CR is successfully established if the CS precedes the US by up to +5 seconds. Because the CS lasts only for 2 seconds, a CS trace lasting over 3 seconds can be associated with the US. Backward conditioning is ineffective.

indicate that the animal learns to respond to the CS also at CS-US intervals of +10 seconds, but not at intervals of +30 seconds (Fig. 4.8). The CS must be stored in a kind of sensory memory, which outlasts stimulation by several seconds.

To characterize sensory memory further, an experiment was performed in which two different odors were presented in succession, and the US was applied immediately after the second CS (Fig. 4.9). If the two CSs (denoted O_1 and O_2) are separated by 30 seconds, only O_2 is associated with the US. No effect on O_1 or from O_1 onto O_2 is found, because the response probabilities to O_1 and O_2 alone are the same as in tests when both odors were presented in succession (either in the order presented during con-

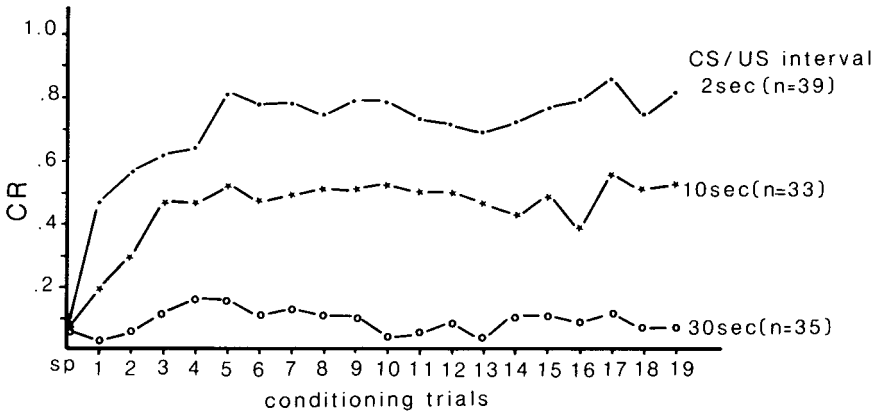

Figure 4.8. Three acquisition functions for different CS/US intervals in olfactory PER-conditioning. The CS (carnation, 2 second) precedes the US (40% sucrose solution, 2 seconds) by either 2 seconds (upper curve), 10 seconds (middle curve) or 30 seconds (lower curve). Multiple-trial conditioning reveals that a CS trace of up to 8 seconds is still successfully associated with the US, but much longer intervals are ineffective. Sp gives the response probability to the CS before conditioning (spontaneous response). Number of animals: $n = 39$ for 2 seconds CS/US interval, $n = 33$ for 10 seconds, $n = 35$ for 30 seconds.

ditioning or in the reverse order). If the two CSs follow each other quickly (1-second interval), however, the animals associate both odors with the US, although O_2 somewhat more than O_1. Interestingly, the animals appear to learn the sequential order of the CSs, because O_2 elicits significantly higher response if the sequence during the test is the same as during the learning trials (O_1, O_2) than if the order is reversed (O_2, O_1).

These kinds of experiments were undertaken to determine whether CS/US contiguity is a fixed, stereotyped property or is flexible and under the control of sensory events or behavioral conditions. In our earlier work with variable CS/US intervals in single conditioning trials, we were impressed to find similar optimal CS/US intervals in instrumental odor learning and olfactory PER-conditioning, and concluded that the optimal CS/US interval is a fixed property of associative learning in honey bees (Menzel, 1990). However, we already know from Grossmann's (1971) experiments with freely flying honey bees that the CS/US interval can be extended considerably by so-called cued delay procedures. Now we find in the multiple-trial conditioning experiment (Fig. 4.8) that the CS/US interval can be extended also in multiple-trial PER-conditioning. Furthermore, we see in

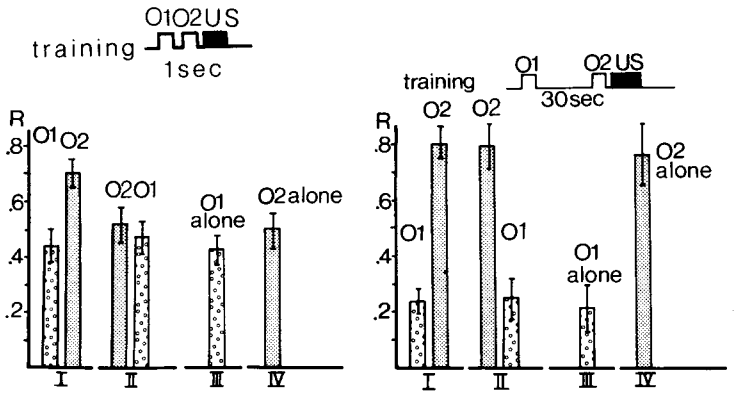

Figure 4.9. The CS trace in olfactory PER-conditioning is characterized by a double CS/single US experiment with two different intervals between the two CSs (1 second and 30 seconds). The two CSs were carnation (C) and propionic acid (P) presented each for 2 seconds, and the US is the usual sucrose solution (40 %, 2 seconds). C and P are two odorants which bees learn to distinguish within a few trials if differentially conditioned. The acquisition functions are similar with C acquired somewhat faster than P. Both series of experiments were run in a balanced fashion, with C being the first CS for some animals and P being the first for the other animals.

The sequence of stimulus presentation and pairing was the following (O_1 and O_2 correspond to either P or C depending on the experimental group; O_1O_2 indicates that the two odors are presented in sequence at the interval of 1 second or 30 seconds depending on the experimental group; + marks US reinforcement; the semicolon indicates an interval of 15–30 minutes): O_1; O_2; O_1O_2+; O_1O_2+; O_1O_2+; O_1O_2+; O_1O_2+; O_1O_2+; O_1; O_1O_2+; O_2O_1; O_1; (*)O_1O_2+; O_2; O_1; O_1O_2+; O_2O_1; O_1O_2+; O_2; O_2O_1; O_1O_2+; O_1; O_2O_1; O_1O_2+; O_2; O_1O_2+; O_2; O_1; O_2O_1; O_1O_2+; O_2; O_1; O_1O_2+; O_1; O_1O_2+; O_2; O_2O_1. The bars in the figure give the response probability to O_1 or O_2 for the different test conditions as cumulative responses after the 12th stimulation (13th–34th from *) during the respective olfactory (O_1, O_2) stimulation.

The four different test conditions are represented by the four groups I to IV. **I**: Sequence of odors during the tests as during conditioning: O_1O_2. **II**: Sequence of odors during the tests reversed to that during conditioning: O_2O_1. **III**: Response to O_1 alone. **IV**: Response to O_2 alone. Two bars are given for the test conditions I and II, because the PER could occur after O_1 and/or O_2. The number of tests for each test condition is: **I**: $n=925$, **II**: $n=523$, **III**: $n=595$, **IV**: $n=514$. Since each animal was tested several times in each test condition, the relative response rate R could be calculated for each animal, and thus the standard deviation of the average could be calculated. Number of test animals; upper graph: $N=97$, lower graph: $N=100$.

the dual CS/single US experiment (Fig. 4.7) that two CSs with different time relationships to the US and their temporal sequence can also be conditioned. We tentatively conclude that contiguity is a flexible component of honey bee learning.

The Intrinsic Dynamics of Memory as Determined by the Single Learning Paradigm

The drastic change in behavior as the consequence of a single trial indicates a strong innate preparedness to learn odors. Aversive learning has often been found to be extremely fast in vertebrates [e.g., food avoidance conditioning (Kamin, 1969; Garcia and Koelling, 1966)], but one-trial appetitive learning as in the honey bee is unusual and most convenient for learning studies which aim to analyze the intrinsic components of memory processing. As described above, free-flying honey bees need only one reward on a scented target (Koltermann, 1969) or on a target with violet color (Menzel, 1967) to choose the target afterward with very high probability. Since the learning trial in olfactory PER-conditioning lasts only a few seconds and the amount of reward can be as little as fractions of a microliter, time-dependent processes following the learning trial can be isolated effectively and distinguished readily from event- or experience-dependent processes. Such intrinsic time-dependent memory processes are evidence of a neural machinery which establishes a preordained memory trace.

The Non-associative and Associative Memory Trace

The modulatory action of a single US exposure leads to an increase in the olfactory α-response immediately after the US and a fast decay within the following 3 minutes (Fig. 4.10). By comparison, an associative learning trial with the optimal CS/US interval for PER-conditioning produces a biphasic time course of the conditioned response which is very much like the time course of conditioned choice behavior in freely flying, color-trained honey bees (Fig. 4.4). The first phase is characterized by fast decay of the CR probability which, although beginning at a higher level, parallels the time course observed after a single sensitization trial. The CR probability in the second phase, 3–10 minutes after the single CS/US pairing, rises slowly over time. One can conclude from these patterns that non-associative memory initiated by the sensitization trial contributes considerably to the high response level within the first minutes and that a specific associative memory component develops slowly over several minutes. During this process of consolidation, responses become con-

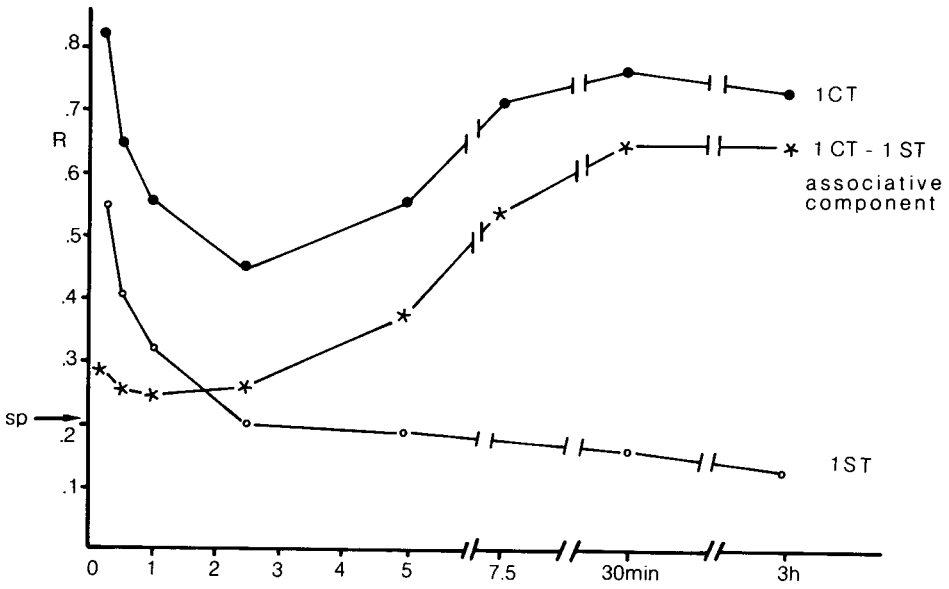

Figure 4.10. Time course of the proboscis extension response to an odor stimulus either as a sensitized α-response after a single US to the antennae and proboscis (lower trace, 1 ST) or as a CR after a single pairing (upper trace, 1 CT). The tests were performed at various intervals (abscissa). Each point represents the response probability of an independent group of animals. The number of animals tested in five series of 1 ST experiments is $n = 2{,}057$ and in three series of 1 CT experiments is $n = 921$. The middle line is the difference between the two functions and is interpreted to represent the associative component of the memory after a single conditioning trial. The arrow marked with "sp" gives the spontaneous response rate for all eight series of experiments.

trolled more and more by associative memory alone. Associative memory lasts longer than 24 hours (even after a single learning trial) in both PER-conditioned harnessed honey bees as well as in color-trained free-flying ones (Fig. 4.3).

Assuming that the response to the CS at any time after a single CS/US pairing is a simple joined function of the US sensitization effect and the CS/US pairing effect, the biphasic time course can easily be understood, because the nonassociative memory fades faster than the associative memory resulting from the consolidation process strengthens. An important implication of this interpretation is that, immediately after the learning trial, a weak associative memory already exists which is strengthened over time (see Fig. 4.10).

Stability of the Memory Trace

Consolidation of the memory trace after a single learning trial leads to changes in memory not only with respect to its control over choice or response behavior but also with respect to its resistance to change by new information. Consolidation thus influences the extinction of memory (due, for example, to exposure to unreinforced CS presentations), the outcome of reversal learning (i.e., the outcome of reinforcement with a new CS), the resistance of memory to certain experimental treatments which cause retrograde amnestic effects, and the tendency for the learned stimulus to be distinguished from more-or-less similar stimuli (cf. Smith, this volume, for a discussion of stimulus generalization). These phenomena have been well known in vertebrate learning ever since Ebbinghaus's (1885) famous experiments on human memory (Müller and Pilzecker, 1900; Weiskrantz, 1970; Squire and Cohen, 1982). They emphasize the time- and event-dependent character of a memory processor in the nervous system which, after its initiation by a learning trial, proceeds through phases. The properties of these phases are likely to reflect species-specific adaptations to biologically relevant learning processes under natural conditions. In the case of an insect collecting nectar and pollen (which provide only minute amounts of food and exist in a large number of competing plant species), we should expect to find a functional match between properties of these phases and the temporal dynamics of foraging. We shall return to this point after a detailed characterization of the memory processing in the honey bee.

Unreinforced experience with a formerly rewarded CS (i.e., extinction) has little effect on response level in olfactory PER-conditioning and on color-training experiments (Menzel, 1967, 1968, 1990). The conditioned response decreases only slowly after many repetitions of the CS without US. (If, however, the CS is paired with an aversive US such as water under conditions where sucrose solution is expected, the CR decreases much faster.) However, reward duration affects sensitivity to extinction even after a single trial. Figure 4.11 shows the resistance to repeated extinction trials in instrumentally color-trained animals. Resistance is weak after a short reward (2–5 seconds) and strong after a long reward (7–15 seconds). Furthermore, if the time interval between a single PER-conditioning trial and an extinction trial is varied, it becomes clear that the memory trace is more sensitive to extinction during the first 3 minutes after conditioning than later (Fig. 4.12).

The stability of the early memory trace after a single learning trial can also be tested in a dual-reversal learning experiment where the intertrial interval (ITI) between the initial learning trial and the reversal trial is varied between several seconds and 10 minutes (Fig. 4.13). Again, two

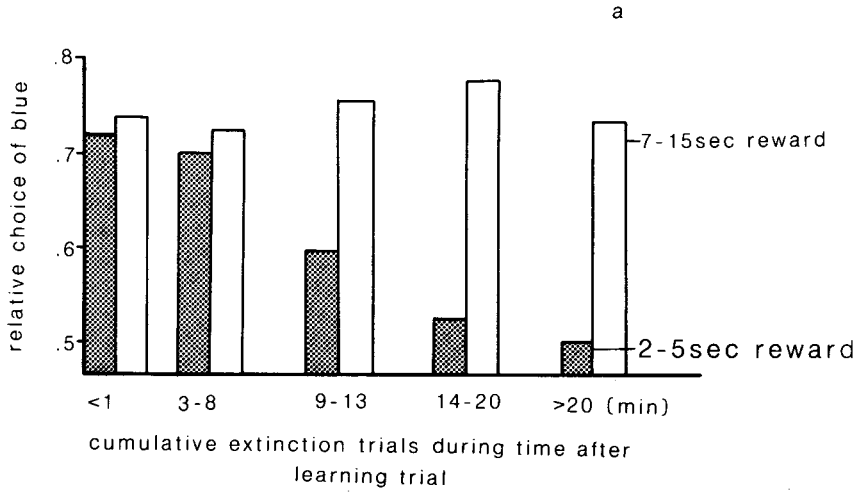

Figure 4.11. Resistance to extinction in freely flying, color-trained bees after a single short (2–5 seconds, dotted bars) or a longer (7–15 seconds, open bars) reward. The instrumental learning trial was given at time zero on a blue target. The alternative color yellow is chosen equally strongly in the dual-choice test before conditioning. Bees are chased from the target after a certain time ranging between 2 and 5 or 7 and 15 seconds. When they return to the experimental setup, they are presented with the two colored alternatives without any reward continuously during the next 30 minutes. Extinction trials accumulate for both groups of animals at about equal frequency. Number of animals tested: 74, number of choices: 2,072 (redrawn from Menzel, 1968).

groups of freely flying honey bees were examined which differed in the strengths of the US (5 seconds vs. 15 seconds during the initial trial). The honey bees were tested for their response to the two color targets in a forced dual simultaneous choice at a time $\geq$ 10 minutes after the reversal trial. Honey bees are prepared to reverse their learning to the new color target at ca. 3–4 minutes ITI, i.e., when the conditioned response after a single learning trial is minimal (compare with Fig. 4.4). This result suggests that new information is acquired best at the time when the joined action of early (nonassociative) memory and that of consolidated (associative) memory is weakest (Menzel, 1979)

A similar effect was found for olfactory PER-conditioning (Fig. 4.14). The odor conditioned first (O_1) establishes a stronger memory than the odor conditioned next (O_2) if the ITI is either very short (30 seconds) or long (10 minutes), but memories of the two odors O_1 and O_2 are equally strong if the ITI is 3 minutes. The two odors used in this experiment

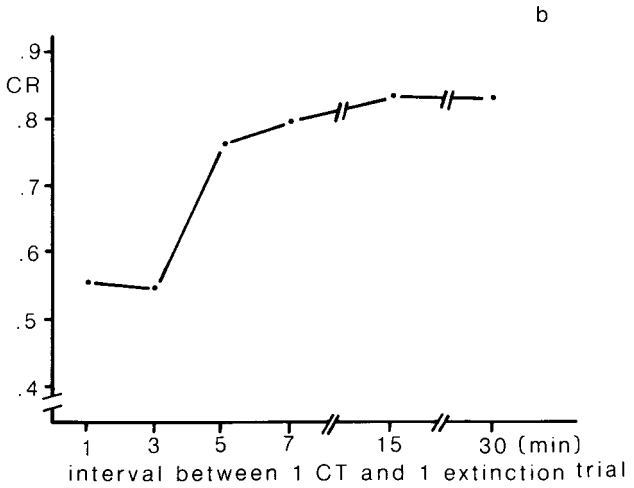

Figure 4.12. Sensitivity to extinction after a single PER-conditioning trial. The time interval between the conditioning trial (1 CT) and the extinction trial was varied between 1 and 30 minutes (abscissa). The test trial was given 1 hour after the CT. Six independent groups of animals were tested at the intervals 1, 3, 5, 7, 15, and 30 minutes respectively (20–30 animals in each group). The difference between the two first and the four later groups is highly significant ($p \leq 0.01$, χ^2 test).

(geraniol, propionic acid) were selected because honey bees are unlikely to generalize between them. This precaution presumably reduced any tendency for the second conditioning trial to reinforce partially the first conditioned odor. Geraniol is acquired faster (CS: 0.57) than propionic acid (CS: 0.34, see Fig. 4.14 control groups). The α-response is considerable for geraniol (spontaneous response probability = 0.18) and nil for propionic acid. Furthermore, proprionic acid releases aversive responses (backward movements of the antennae), while geraniol does not. Nevertheless, the response probability to the first conditioned odor is always higher than in a control group which was conditioned only to that odor. The CR to the second conditioned odor for an ITI of 30 seconds is equal to the CR after conditioning only that particular odor, whereas it is significantly higher for an ITI of 3 minutes. Since a repetition of the US alone at an ITI of 3 minutes does not enhance the CR (see control groups in Fig. 4.14), the second associative learning trial strengthens memory of the first conditioned odor even in the absence of generalization between the

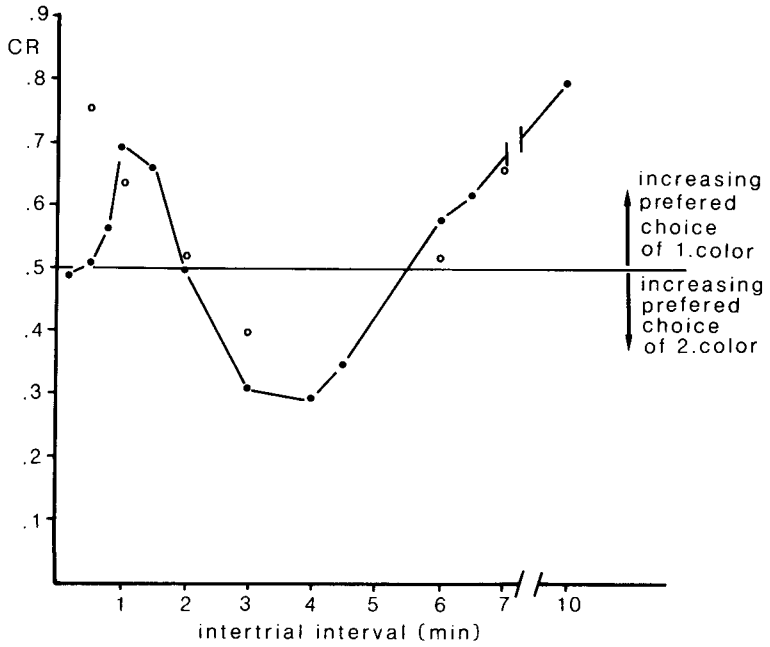

Figure 4.13. Two-trial reversal learning of freely flying bees with varying intertrial intervals (ITI, abscissa) between the initial trial on blue (1. color) and the second trial on yellow (2. color). The bees were tested for the choice between the two colors in a dual forced choice more than 10 minutes after the second learning trial (redrawn from Menzel, 1979). Two groups of bees were distinguished—those rewarded for 5 seconds (●) and those rewarded for 15 sec (○). The ordinate gives increasing choice proportions for the color learned first upward and for the color learned second downwards.

CSs. This strengthening of the first established memory trace appears to be independent of the ITI. However, the second memory trace is enhanced only for the ITI of 3 minutes. We conclude from these results that pro- and retroactive facilitatory processes are strongest at the time when the joined action of the nonassociative and the associative memory on the CR is weakest (ITI = 3 minutes), whereas only retroactive processes are effective when the nonassociative memory is strongest (ITI = 30 seconds).

Results for the two US strengths used in the experiment with the freely flying honey bees differ in the first minute (Fig. 4.13). A long US during the initial trial induces stiffer resistance to reversal learning than does a short US. This result corroborates those of resistance to extinction reported

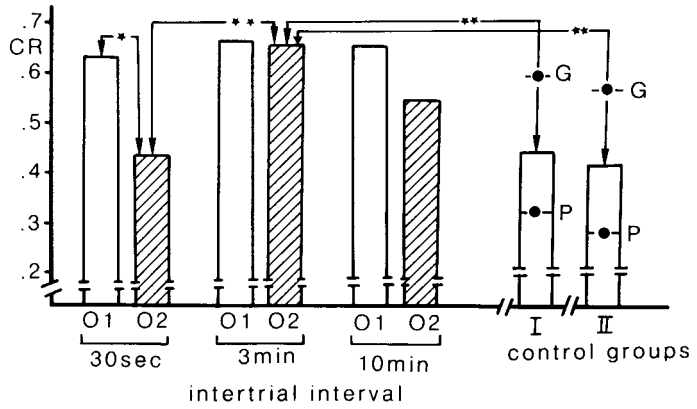

Figure 4.14. Two-trial-reversal conditioning of the olfactory PER. The two odors used were geraniol (G) and propionic acid (P). In one group of animals, geraniol was the first conditioned odor (O_1) and propionic acid the second (O_2); in a second group, propionic acid was O_1 and geraniol O_2. The results are pooled and the probability of the CR to either O_1 or O_2 is given for three intertrial intervals (30 seconds, 3 minutes, 10 minutes). CR was tested 30–60 minutes after the second trial; 141 animals were tested. Control group I gives the CR after one conditioning trial with propionic acid (P) or geraniol (G) alone (the bar corresponds to the average of both); animals of control group II received also only one conditioning trial with geraniol (G) or propionic acid (P) (bar: average), but received an additional US 3 minutes after the conditioning trial.

in Fig. 4.11, if we assume that the balance between the non-associative and the associative memory immediately after the first trial depends on US strength. In particular, a longer and stronger US enhances short-lasting non-associative memory more than it facilitates the consolidation process of associative memory.

The temporal dynamics of the memory trace after a single learning trial are also revealed by retrograde amnestic procedures. Experimental treatments such as narcosis, cooling and weak electric brain stimulation induce amnesia if applied within the first few minutes after the learning trial, but cause no effect if applied at intervals longer than 5 min (Menzel et al. 1974; Erber, 1975a,b; Erber et al. 1980) As Figure 4.15 shows, the amnestic gradient is quite independent of both learning conditions and sensory system (e.g., it is the same for classical olfactory PER-conditioning of har-

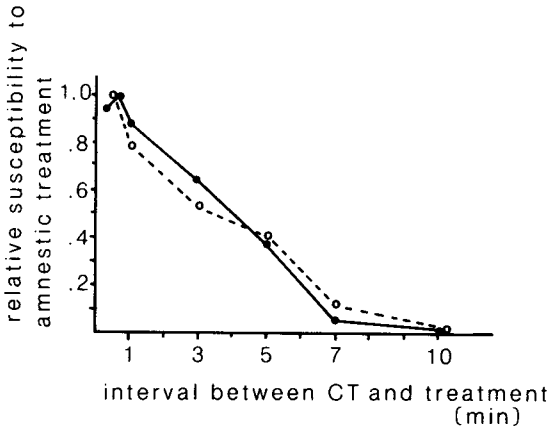

Figure 4.15. Time course of retrograde amnesia induced by weak electric brain stimulation (EBS) after a single instrumental odor-training trial (○; after Erber, 1975a,b, redrawn) or after a single olfactory PER-conditioning trial (●; after Erber et al. 1980, redrawn). The abscissa gives the time interval between the conditioning trial and the EBS. The ordinate expresses the amount of retrograde amnesia as the relative change in the conditioned performance (dual choice or CR probability) as tested more than 30 minutes after the EBS treatment.

nessed honey bees and instrumental color conditioning of freely flying ones). To some extent, the gradient depends on the experimental procedure. For example, cooling and narcosis appear less effective than electric brain stimulation (EBS), perhaps because EBS acts immediately to interfere with ongoing neural activity, whereas cooling to a few degrees above 0°C or narcosis with N_2 or CO_2 require up to 1 minute to become effective (Menzel, 1984, 1987, 1990). Significantly less amnesia is observed if the animal has been trained or conditioned for more than two trials and if the EBS is applied immediately after the last trial. The extent of amnesia corresponds to the contribution to memory of the last learning trial (Erber, 1975a,b; Menzel, 1984).

It is concluded that multiple learning trials help to establish a stable memory much faster than just a single trial. We next examined whether the additional learning trial promotes transfer of the susceptible memory trace (resulting from the first learning trial) into a stable memory or whether

a new memory is immediately transferred into an unsusceptible form (as, for example, if short-term memory is occupied as a consequence of prior learning trials). If transfer is accelerated by an additional trial, we might ask which of the components of the second trial (CS or US alone or CS/US pairing) is responsible for this effect (Menzel and Sugawa, 1986). It appears (Fig. 4.16) that transfer is promoted by the second trial, because the content of the second learning trial is erased by EBS but not that of the first trial.

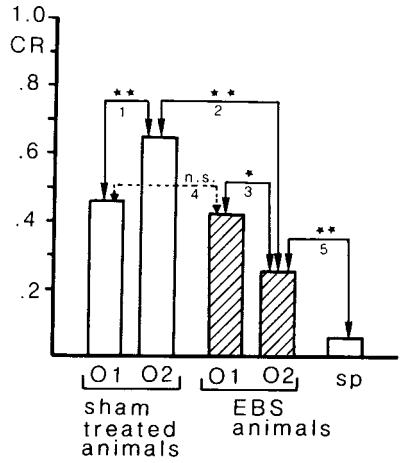

Figure 4.16. Facilitation of the consolidation process in a dual-olfactory PER-conditioning experiment. Groups of bees were conditioned to two odors (geraniol, propionic acid) in quick succession (within 30 seconds). For one group geraniol was the first odor (O_1) and propionic acid the second (O_2); for the other group the order was reversed. The data from both groups are pooled. EBS was applied immediately after the second conditioning trial, thus well within the time period which causes full retrograde amnesia after a single learning trial (see Fig. 4.15). CR tests were performed 1 and 2 hours later. The result of both CR tests are pooled; 100 animals were tested in each of the four groups (temporal order of geraniol and propionic acid, sham treated and EBS). Sp gives the spontaneous response level pooled for both odors and for all four groups.

The results from the sham-treated animals (open bars, left) indicate that response to the second conditioned odor (O_2) is higher than that to the first conditioned odor (O_1) (χ^2 test, $p \leq 0.01$, line 1). EBS has no effect on the response to O_1 (dotted line 4), but induces retrograde amnesia to the second learning trial (line 2). The CR to O_2 is also lower than to O_1 in the EBS group (line 3, $p \leq 0.05$), further indicating that the EBS is selectively acting on the memory for O_2. However, memory for O_2 is not completely erased, because the spontaneous response to either odor before conditioning is less than the CR to O_2 (open bar sp; line 5) (from Menzel and Sugawa, 1986, redrawn).

Together with the additional control experiments in Menzel and Sugawa (1986), these results prove that only a second associative learning trial is able to induce the faster transfer to an unsusceptible memory and not an additional exposure to the CS or US alone.

Although each of the experiments presented in Figs. 4.14 and 4.16 contains the control group necessary to reach the conclusions presented above, there is still an unresolved contradiction between the results for the 30-second interval groups. In one case (Fig. 4.14) the odor conditioned first (O_1) provokes a stronger response than the odor conditioned second (O_2). In the other case (Fig. 4.16) the effect is reversed. We know from the results with freely flying bees (Fig. 4.13) that short-term reversal learning (ITI < 1 minute) is highly sensitive to the strength of the US. A longer lasting US strengthens the stimulus learned first more than a stimulus learned second (*open circles* in Fig. 4.13). It is unknown whether this dependence might explain the discrepancy between the results in Figs. 4.14 and 4.16, and how associative and non-associative memories interact in olfactory conditioning as a function of US-strength and temporal dynamics. These dependencies appear as the most effective components in the control of the choice processes of freely flying bees (Figs. 4.21 and 4.26).

The consolidation process, which establishes a stable memory after a single olfactory learning trial, can be roughly localized in the brain by reversibly blocking neural activity in selected brain areas. This is done through the use of thin, cooled needles inserted into the brain at successive time intervals after the conditioning trial (Menzel et al., 1974; Erber et al., 1980) (Fig. 4.17). Cooling the antennal lobes induces retrograde amnesia only when treatment is applied immediately (i.e., < 2 minutes after a learning trial. By contrast, the mushroom bodies are prone to cold-induced amnesia for a longer time after a learning trial. The output regions of the mushroom bodies (termed α-lobes) are associated with a faster time course than the input regions (termed calyces).

Dynamics of the Memory Content

Consolidation of memory is most likely an active internal process that incorporates the new memory into existing memories. It is possible, for example, that a recent memory may change in content during consolidation as a consequence of interactions between old and new memories. We attempted to evaluate this possibility by looking for stimulus generalization to odorants, either immediately after a conditioning trial or at an interval of 15 minutes (Smith and Menzel, unpublished data) (Fig. 4.18). Responses to four odors (hexanol, citral, geraniol, 2-hexanol) were assayed in a PER-conditioning paradigm which employed a single conditioning trial. These

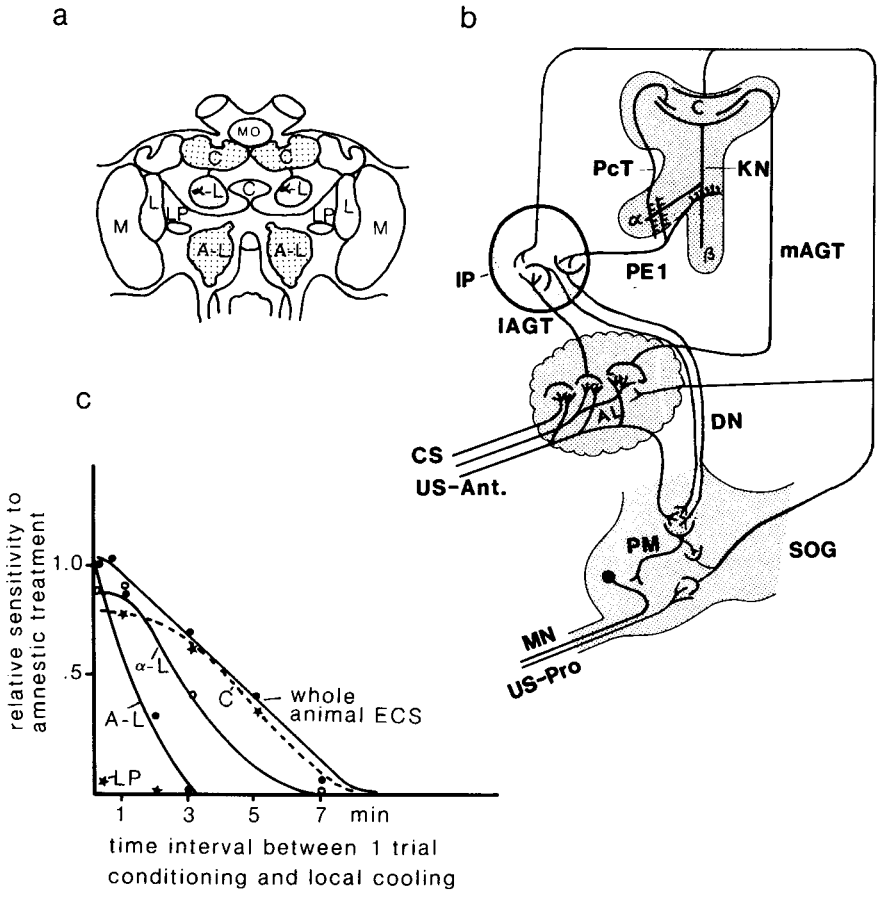

Figure 4.17. *a.* A diagram of the bee brain as it is relevant for the neural circuits and neuropils underlying the olfactory PER-conditioning. The major neuropils in the supraesophageal ganglion (= brain) are M (medulla) and L (lobula), the two inner visual ganglia; A-L (antennal lobe), the primary olfactory neuropils; C (calyx) and α-L (α-lobe) belong to the mushroom bodies; LP (lateral protocerebrum) is an unstructured neuropil ventrolateral to the mushroom bodies; C (central body); Mo (median ocellus). *b.* Major pathways that are involved in PER-conditioning. SN = antennal sensory nerve; MN = motor neurons controlling the mouthparts; mAGT = median antenno-glomerularis tract, a major relay pathway to the chemosensory input region of

odors were selected because honey bees discriminated readily between any pair of them (Smith and Menzel, 1989; Vareschi, 1971). Results of two experimental series are illustrated in Figure 4.18. In one series (trained odor = citral), the response profile to the four odors is quite similar at both test periods (Fig. 4.18). However, the response to the conditioned odor becomes relatively stronger over time, indicating that the degree to which bees will generalize from a conditioned odor to novel odors has been reduced during consolidation. Memory content has thus changed over time so as to favor even more the conditioned odor. This result was also found for hexanol and 2-hexanol. For geraniol, however, the response profile changes drastically during consolidation (Fig. 4.18). Response to the trained odor is highest in the short term, but response to an untrained odor (i.e., citral) is highest in the long term. Analysis of the time course of CR after the conditioning trial with citral or geraniol reveals that the CR to citral follows the usual biphasic time course with a rise at intervals longer than 3 minutes, whereas the time course of the CR to geraniol lacks such a prominent rise.

These results illustrate a very important property of the intrinsic memory processor—namely, its dependence on the nature of the stimulus. Certain CSs evoke stronger changes in response during consolidation than others. For salient stimuli, consolidation does indeed change the content of memory such that an animal is able to discriminate more precisely between conditioned and novel stimuli. For other stimuli, content changes not quantitatively but qualitatively; the bee remembers something other than the actual stimulus/reward pairing. We may summarize by remarking that a stimulus may often be recorded in memory as something different from what was actually experienced. This obviously reflects the influence on

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Figure 4.17. (Continued) the mushroom bodies; KN = Kenyon cells, the intrinsic neurons of the mushroom body; α and β are the two output lobes of the mushroom body; Pct = proto-cerebro-calycal tract, a feedback tract between the α-lobe and calyx; SOG = subesophageal ganglion; PM, DN = premotor and interneurons that relay the descending commands to the motorneurons. *c.* Time-courses of local cooling which leads to amnestic effects in olfactory PER-conditioning. In all cases, both antennae were exposed to the CS, and the animals were conditioned by one trial. The indicated paired structures were cooled to 1°C for 10 seconds (for experimental details see Erber et al. 1980). The abscissa gives the time interval between the conditioning trial and the onset of cooling. The ordinate gives the sensivity to the amnestic treatment. The latter is the inverse of the proportion of animals responding to the CS 20 minutes after the amnestic treatment, at a time when the animals

memory formation of preexisting information stored in the nervous system, information which may be innate or itself acquired through experience.

Time Course of Choice Behavior Under Natural Conditions

A foraging bout of a honey bee is characterized by two obvious temporal periods: (1) the interval between successive foraging bouts during which the honey bee returns to the colony, unloads the collected nectar and/or pollen, and returns to the food source, and (2) the interval between successive landings within the patch of distributed food sources. A few examples of frequency distributions associated with these periods are given in Figures 4.19 and 4.20. Obviously, the frequency distribution for successive flower choices will depend very much on the spatial distribution of flowers, the availability and quantity of nectar and pollen, and on the proportion of flowers containing a reward. The data in Figure 4.19 were recorded for bees foraging in patches in which flowers were relatively dense and evenly distributed. Thus far, interval distributions have not been examined in which flowers are separated by longer distances and distributed in subdivided patches, e.g., where plants bearing several to many blossoms occur together as discrete units. The frequency distribution of bout intervals (Fig. 4.20) was calculated for training experiments using artificial feeding stations, because no data are available for natural food sources.

Where many flowers are visited during a bout, these two temporal periods are easily distinguished, being in the range of seconds for intervals between successive approaches or landings within a patch, and in the range of minutes to hours for intervals between foraging bouts. Each landing on a flower corresponds to a single learning experience even for very short rewards (Menzel, 1968; Menzel and Erber, 1972). Moreover, unrewarded landings contribute an inhibitory learning component (i.e., contribute to extinction), but the effect on the behavior is much less than that of a rewarded experience (see above discussion on resistance to extinction). The quick succession of visits within the patch ensures that immediate or short-term memory following one choice controls the next choice to a significant degree (the precise rules underlying choice behavior will be discussed below). Only in the next foraging bout are choices controlled by long-term memory. It is this memory which is almost exclusively tested in training experiments, even though sensitization effects of the US and short-term memory clearly contribute to flower choice as well.

In many plant species, a large number of flowers are borne on a single plant and represent a subpatch of food items. Bees are known to apply particular search strategies when exploiting such a subpatch, e.g., they often start collecting from the lower flowers and gradually work upward (review Heinrich, 1984). Consequently, the flight paths between visits within

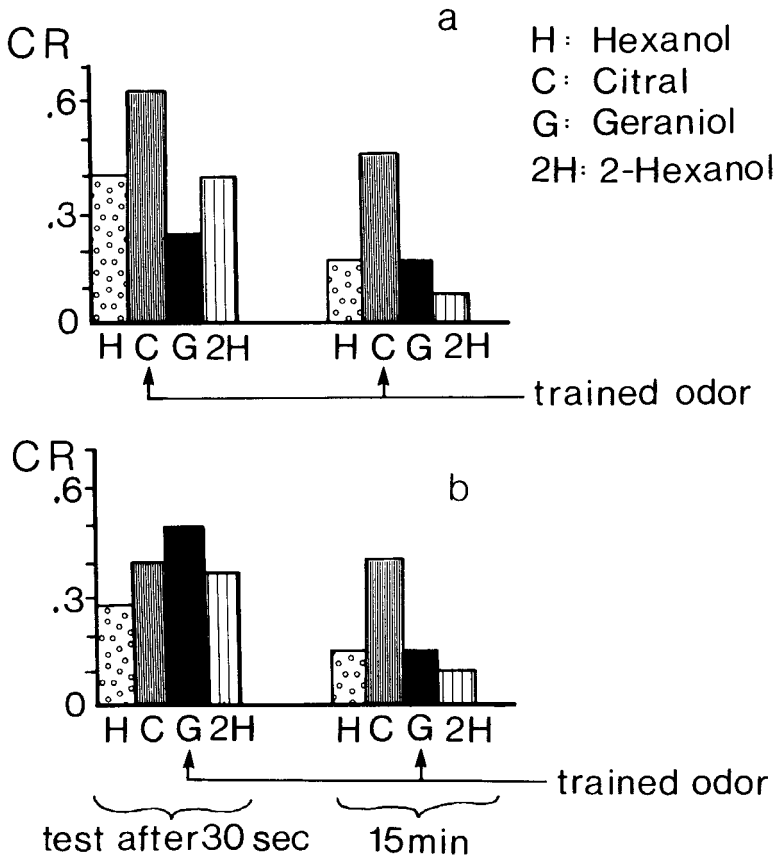


Figure 4.18. The content of short-term and intermediate-term memory. The PER was conditioned to citral (in a) or geraniol (in b) in a single trial. Each group was divided into two subgroups, one of which was tested 30 seconds after conditioning (short-term memory) and the other 15 minutes after conditioning (intermediate-term memory). Each subgroup was again divided into four groups with respect to tests using one of four odorants. If citral is trained (a) consolidation of short-term into intermediate-term memory sharpens the generalization profile in favor of the trained stimulus, whereas after training to geraniol, the generalization profile changes drastically, indicating a reevaluation of the memory during consolidation. Statistics: the χ^2 test reveals that differences in CR exceeding 18% are significant with $p \leq 0.01$ (540 animals in all 16 test groups).

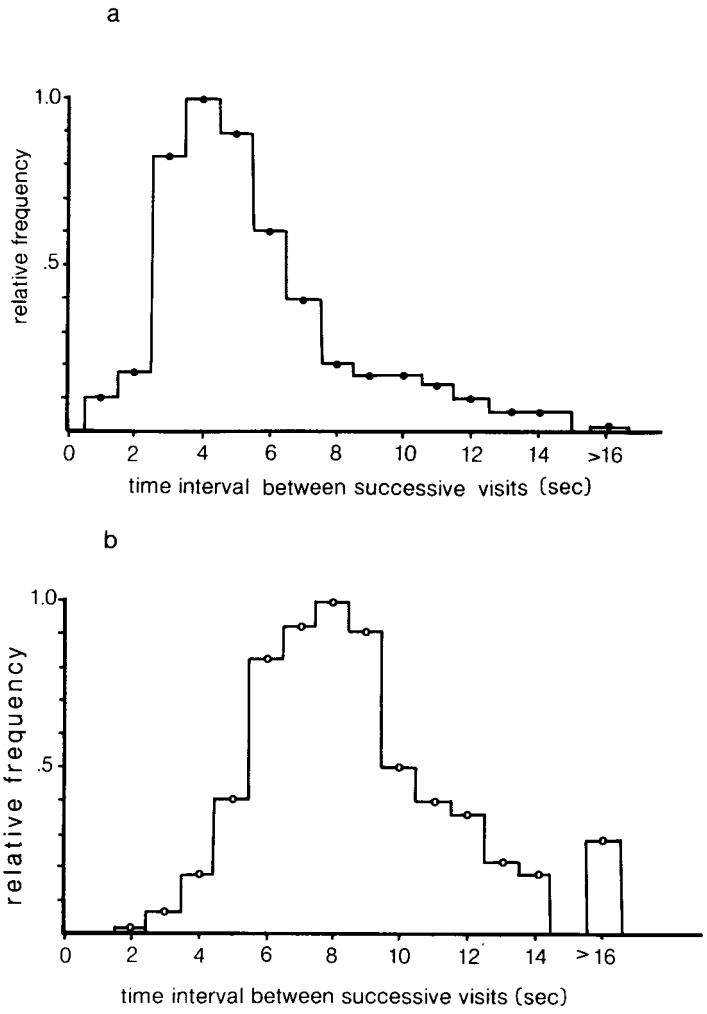


Figure 4.19. Frequency distribution of the time interval between successive landings of individually recognized honey bees on flowers of four different plant species. The time interval (abscissa) includes the handling time on the respective flower. The ordinate gives the frequency for the 1-second bin width in relative proportions of the maximum. a: Pollen-collecting bees on *Doronicum* sp. (n = 514). b: Nectar-collecting bees on citrus flowers (n = 1,180). c: Nectar-collecting bees on *Corydalis carva*, an early-spring-blooming Papaveraceae (n = 182). d: Pollen- and nectar-collecting bees on *Salix* (n = 161).

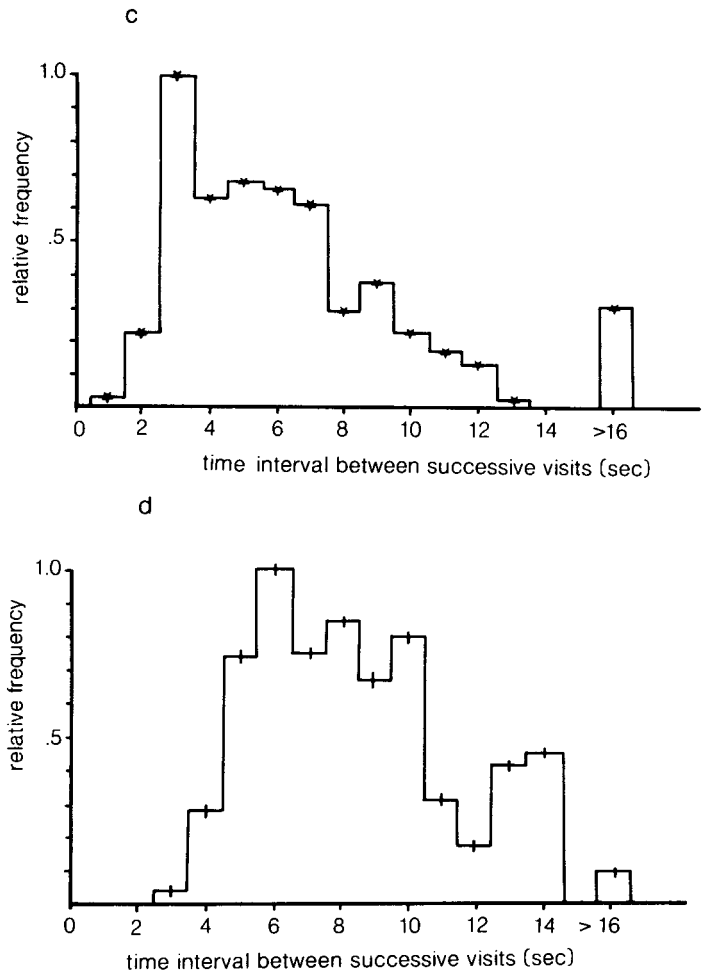


Figure 4.19. (*Continued*)

a subpatch are more directed and shorter than those between subpatches. Although flower distributions on a single plant and on neighboring plants of the same species differ greatly among species, it still must usually be the case that flights between flowers of the same plant species (subpatch) are shorter than flights between flowers of different plant species. These conditions have an important consequence for flower choice. Where successive choices follow each other quickly, the honey bee is probably landing on flowers of the same plant species. Where more time elapses between landings, the honey bee may well be landing on flowers of different species.

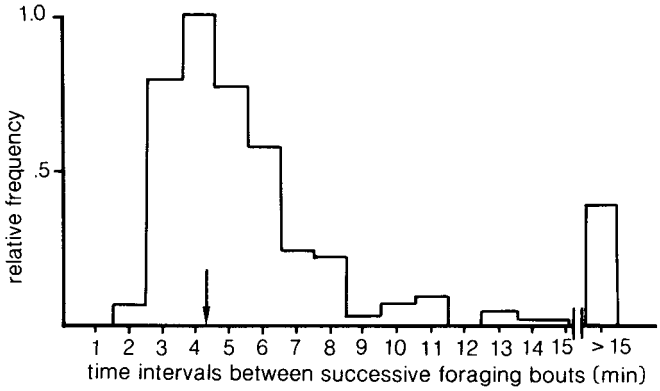


Figure 4.20. Frequency distribution of time intervals between successive bouts. The data were collected during training sessions on an artificial feeding place 100 m away from the hive. Ambient temperature was between 20 and 26°C, and the individually trained bees were fed on a 1.5-m sucrose solution without interruption. The time interval (abscissa) includes the time for unloading the crop within the colony.

It will be shown next that such temporal patterns have a significant impact on choice behavior in an experimental arrangement which resembles quite closely the natural conditions but which permits the behavior of a single honey bee to be monitored continuously.

Consider an experimental setup in which a honey bee collects sucrose solution from four computer-controlled feeders (Greggers, 1989; Menzel and Greggers, 1982; Greggers and Menzel, in press). The four feeders are arranged at distances of between 30 cm and 2 m depending on experimental conditions, are marked with the same or different colors, and offer sucrose solution at a constant flow rate which differs among the four feeders (e.g., 0.062 μl/minute, 0.125 μl/minute, 0.25 μl/minute, and 0.5 μl/minute). Since the feeders differ in reward quantity in a 1:2:4:8 ratio, we shall label the four feeders 1, 2, 4, and 8 respectively. In such a patch the honey bee forages for 20–50 minutes after arrival from the hive, until it has filled its crop and flies back to the hive. During this time each feeder is visited an average of 30 visits during one bout.

The choice frequency on each of the feeders partially matches the reward proportions. Let us first focus our attention on the time dependence of the choice frequency after a visit on any of the four feeders (Fig. 4.21). After leaving a feeder, the bee makes a choice either to return to the same feeder (termed a "return visit," RV) or to visit any of the three other feeders

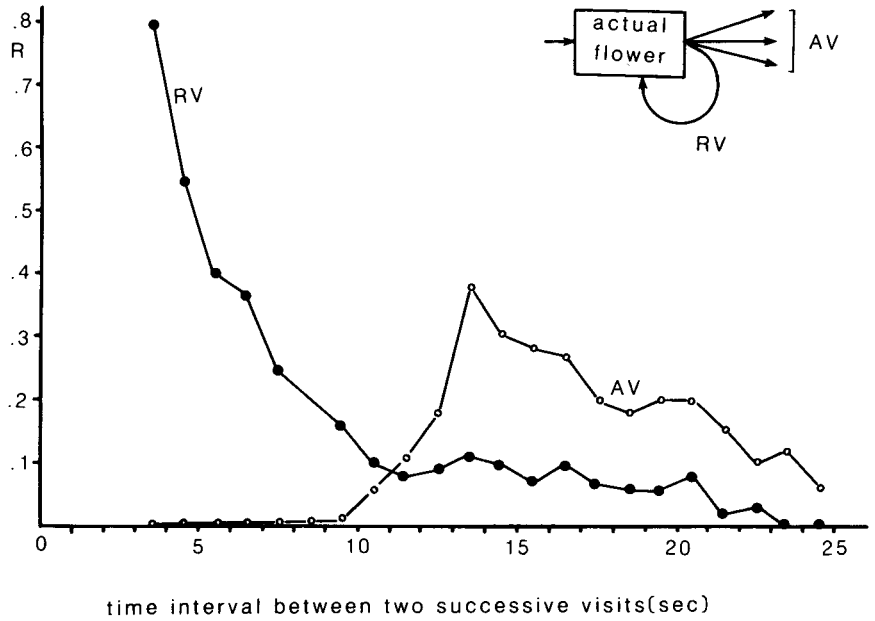


Figure 4.21. Temporal dynamics of the choice behavior of bees collecting nectar from four feeders (see text). Two categories of choices are distinguished; return visits (RV, the bee returns to the same feeder), alternate visits (AV, the bee choses one out of the three alternative feeders). The time interval between the moment when the bee stops sucking on the actual feeder (time zero) and the next arrival is plotted on the abscissa. The ordinate gives the relative frequency. The amount of sucrose solution provided by all four feeders together is 0.83 µl/minute (Greggers, 1989). Number of evaluated visits: $n = 579$.

(termed an "alternate visit," AV). As noted below, the probability of an RV or an AV depends on several parameters (e.g., the amount of reward experienced during the last visit). With respect to the time dependence of RV and AV flights, it is obvious from Figure 4.21 that the probability of an RV is very high immediately after the last visit. With time, the RV probability falls steeply and the AV probability increases. The high probability of RVs at short time intervals is not a consequence of a shorter distance to the just-visited feeder, because a honey bee flying with a speed of approx. 2 m/second can easily reach any feeder within less than 3 seconds.

The temporal dynamics observed for the RV and AV choices indicate that honey bees tend to return to the subpatch if they decide quickly but shift to alternative subpatches if more time elapses. In terms of memory processes, fast RV choices should be dominated strongly by the immediate,

short-term memory phase with its strong nonassociative component (i.e., by sensitization). This interpretation is supported by the observation that, immediately after a large reward, RV flights are much more frequent than AV flights. A strong US (i.e., one exceeding the average reward from all feeders) causes strong sensitization and thus induces a strong immediate memory. Our experiments show that such a strong US arouses the animal, which leads to faster movements within the patch, more intensive probing for reward inside the feeder, and higher flight speed after leaving the feeder. Besides these more general effects, the stronger US initiates also a tendency to return to the same food source (RV flights). This indicates clearly that the immediate memory includes CS-specific, associative components.

The paths of bees were reported to be more tortuous after larger rewards than after small or even null ones (Pyke, 1978a; Heinrich, 1979; Schmid-Hempel, 1984, 1985a,b). Our analysis indicates that such movement is much more specific, highly dynamic, and goal-directed than previously thought. The temporal dynamics of the behavior is related to memory processes, particularly the sequence of a strongly US-dependent immediate memory and a later consolidated memory.

Continuous Updating of the Memories in Multiple Learning Conditions

Learning is a continuous process and thus usually involves a large number of successive experiences. Multiple experiences continuously update the memory trace, shape its content, and make it less susceptible to random variation in the environment. Multiple-trial learning in honey bees has been described in several reviews (Bitterman, 1988; Menzel, 1985, 1990; see also Gould, this volume). Here we select examples of our functional approach to honey bee learning which emphasize the plasticity of the memory trace and the intrinsic components of the updating process.

Reversal Learning

Under natural conditions, a generalist pollinator is exposed to changes in food availability over its lifetime and must be able to switch to new food sources when appropriate. Indeed, reversal learning has often been observed in the honey bee (von Frisch, 1967; Menzel, 1969, 1990; Seeley, 1985). The process of reversal learning has been studied in dual-choice experiments with freely flying, color- or odor-trained bees and in olfactory PER-conditioning. These studies reveal a few general features which are also known from the vertebrate-learning literature. For example, Meineke

(1978) performed an experiment in which he trained a bee over 18 days in a multiple blue/yellow reversal task. Each reversal session was continued, until the animal chose the new color at the same high level (i.e., a "criterion level"). He found a reduction in the number of reversal-learning trials necessary to reach criterion after a few reversals when retrained to blue, but variable results and a large number of reversal cycles when retrained to yellow (Fig. 4.22). During the first 2 days, reversals to blue were relatively slow to take place, but performance improved later and reached a low and stable level. By contrast, reversals to yellow were very variable over most of the training time and showed improvement only after 13 days of training with more than 40 reversals.

A higher preparedness to learn blue as a food signal is known from several experiments (Menzel, 1990) and also appears in another reversal-learning experiment in which the initial learning trials were varied (Fig. 4.23). Again, blue and yellow (presented as spectral lights of wavelengths 444 nm and 590 nm, respectively) were trained in a dual-choice experiment and the number of initial trials on either color differed for different animals (Menzel, 1969). Initially reversal to the new color was retarded by increasing the number of learning trials on the first color, and reversal from blue to yellow was slower than from yellow to blue. However, after more than ten initial learning trials, reversal became easier for both colors and the animals switched to the new color as easily as after weak initial training. It thus appears that after an extended experience with a food source, honey

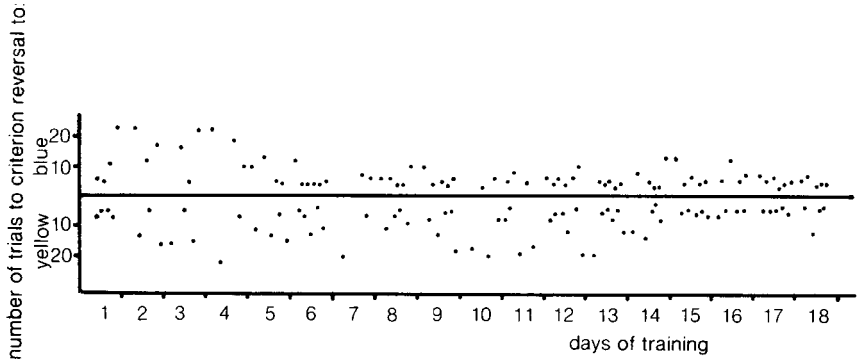


Figure 4.22. Multiple-reversal experiment with a freely flying bee trained in a dual forced choice experiment with the color targets blue and yellow. The number of learning trials on either of the two color targets needed to reach a criterion of correct choices after the reversal is plotted at the ordinate, upward for blue, downward for yellow. The animals were trained over 18 days with a total of 75 reversals to yellow and 76 to blue. The points indicate the number of learning trials needed to reach the criterion at the particular color (after Meineke, 1978, redrawn).

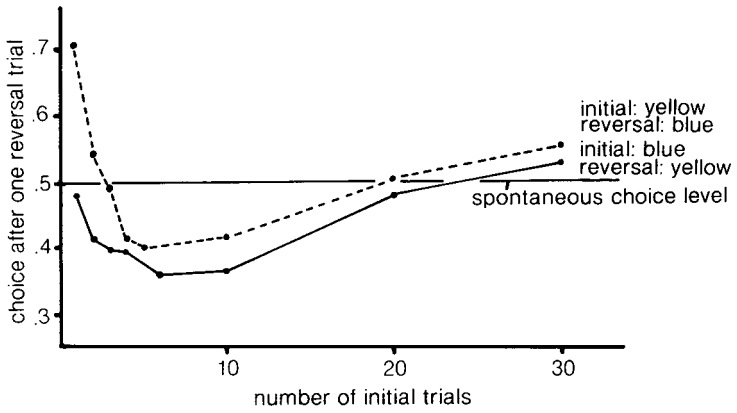


Figure 4.23. The overlearning-reversal effect in freely flying honey bees trained to yellow and blue targets in a dual forced choice situation. Before training the bees chose the yellow (spectral light 590 nm) and the blue (spectral light 444 nm) equally frequently (spontaneous choice level = 0.5). The choice performance was tested after a single reversal trial which followed a variable number of initial learning trials (abscissa) on the other color. The ordinate gives the ratio of choices for the reversal color divided by those for the initially trained color (after Menzel, 1969, redrawn).

bees are prepared to switch to a new one, even though neither the strength of the US nor any other physical parameter has changed. In the vertebrate-learning literature such a phenomenon is known as the overlearning-reversal effect. The overlearning-reversal effect might indicate that a US loses its power as a reinforcer after extended training (Rescorla, 1967, 1988). Proper control experiments (e.g., partial-reinforcement schedules followed by reversals) have yet to be performed for honey bees.

Memory-Based Choice Allocation in Patches With Variable Food Sources

Under natural conditions, the food sources of pollinating insects (i.e., the flowers) compete for pollinators and, in an evolutionary sense, tend to optimize the ratio of investment (=nectar and/or pollen) to profit (=fertilization) in part by minimizing the amount of nectar and/or pollen offered to the pollinator. Small amounts of food force the pollinator to visit many flowers, but the plant runs the risk that it may lose in the competition with other plants because the pollinator seeks the highest net profit. The actual amount of food discovered by the pollinator depends on many factors (e.g., physiological conditions of the plant and the flower, number and species of pollinators working simultaneously in the field, and

weather conditions) and thus is highly variable over time and space. Optimization criteria both in the proximate mechanistic context and the ultimate evolutionary context control the choice behavior of the pollinator. For the pollinator, one of several goals is to gain as much food with as little investment and risk as possible by choosing food sources accordingly (MacArthur and Pianka, 1966; Heinrich, 1983; Waddington et al., 1981; Pyke, 1978b). Optimal-decision theories (Maynard Smith, 1978; Krebs et al., 1978; Pyke, 1984) have been applied with limited success to explain the choice behavior of a foraging bee (Waddington and Holden, 1979; Waddington, 1985; Pleasants, 1981; Heinrich 1983). The models developed thus far are inadequate in their focus on the energy budget of the foraging animals and also ignore informational components and mechanisms of memory formation and retrieval. Experimental design thus far has also been unsatisfactory, because only two alternative feeding places are generally used and the animal's behavior is not resolved continuously over time.

The general experimental design underlying our efforts to address these issues was described above. A single bee works on four feeders (33% sucrose solution; relative flow rate in the four feeders in a ratio of 1:2:4:8, total flow rate in all four feeders = 0.94 μl/minute). The bee imbibes all of the sucrose solution available during each visit. Since its potential rate of uptake (ca. 1 μl/second) exceeds the flow rate of any feeder, the bee visits all four feeders at an average frequency of about one visit per minute.

Under these conditions, the bee partially matches its choice behavior (and other parameters such as licking time, flight speed toward the feeder, and the inverse of handling time before licking) with the average amount of reward. The expected amount of reward must be stored in a kind of long-term or reference memory, because this partial matching is found not only during the course of continuous foraging within the patch during a given bout but also during the first approaches after returning from the hive to begin the next bout. The same results were found for conditions in which flowers were set to zero flow rate, a classical test situation in learning experiments.

Under natural conditions, the animal controls the amount of reward in each flower by coordinating its own action with the productivity of the flower. The animal experiences a certain reward during each visit, and its next choice might depend on both an expectancy as a consequence of its immediate experience and long-term memory. The line in Figure 4.24 (all choices) shows the overall-choice frequencies under the conditions of our experiment, conditions which quite closely resemble natural conditions. Compared to a perfect matching between choice proportions and the flow rate of the reward, it is obvious that the low-reward feeders (Nos. 1 and 2) are more frequently visited, and the highest reward feeder (No. 8) is

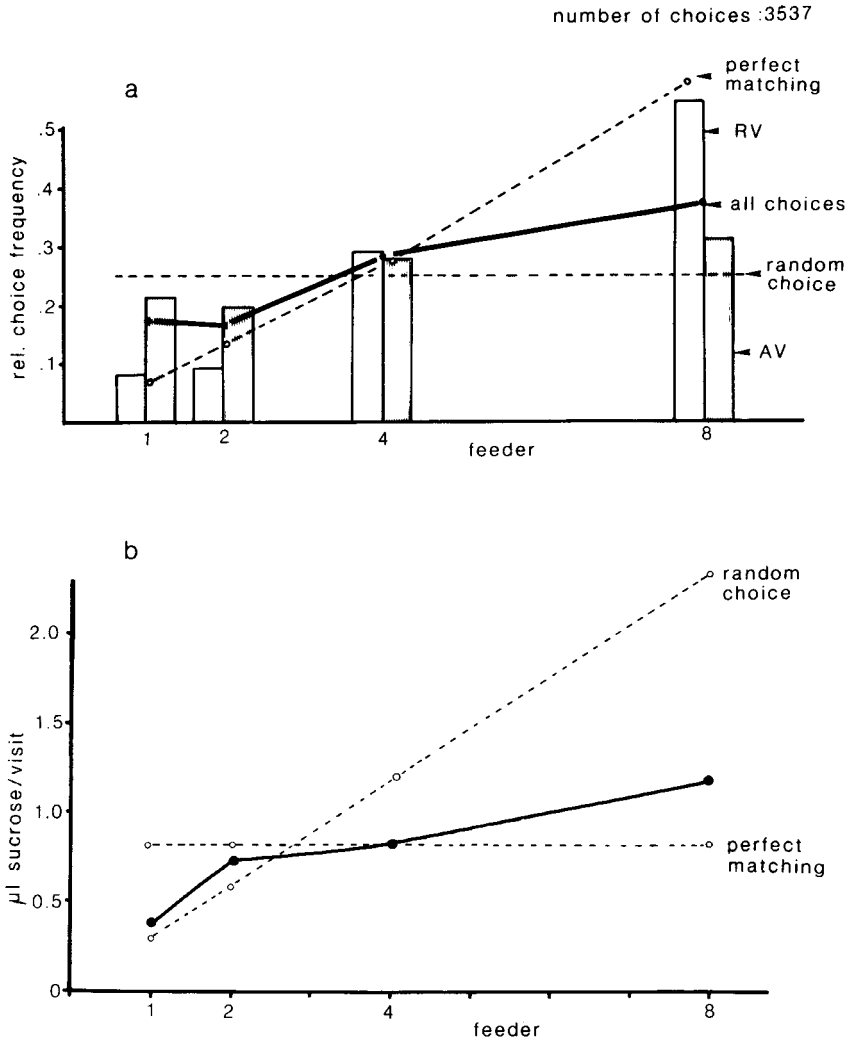


Figure 4.24. *a.* Choice matching under the conditions of a constant but different flow of sucrose solution in four feeders. The four feeders are numbered according to their relative flow rates (see text). The thick line indicates the choice matching for all choices. The dotted lines depict two extreme choice strategies discussed in the text, perfect matching and random choice. The latter is equivalent to a regular visit at any temporal pattern which leads to an average of equal choices at each feeder. The bars mark the choice behavior during return visits (RV) to the same feeder and alternate visits (AV) to one of the three remaining feeders. *b.* Average gain of reward (µl sucrose solution) per visit at the four feeders (see text). Dotted lines mark the two extreme strategies, perfect matching and random choice.

less frequently visited. Consequently, the reward gained per visit (Fig. 4.24b) is less for the low-reward feeders and higher for the highest-reward feeder than expected under perfect matching. From an informational point of view, the bee continuously collects information at the expense of imperfect matching. Perfect matching would provide the bee with a constant reward per visit but with no information about the differences among the four feeders. Only if the bee kept track of each choice over a long period of time would it be able to calculate the productivity of the feeder by dividing the total reward gain through the number of visits at each particular feeder. Obviously, the honey bee's memory content is insufficient for such a demanding job and so it adopts a different strategy.

Random choice, in contrast to perfect matching, would maximize information about the differences. However, in that case, the bee would either reduce its energy budget by visiting the four feeders at a frequency set by the flow rate of the high-reward feeders or it would leave excess amounts of reward in the high-reward feeders by visiting them at a frequency set by the low-reward feeders. In the latter case, it would risk other bees discovering the high-reward feeders. Given constraints on memory, the compromise between maximizing net energy gain and informational gain is manifested in suboptimal matching.

The experiment described in Figure 4.24 was repeated under several different conditions: vertically- vs.-horizontally-arranged feeders, shorter (30 cm) and longer (1.5 m) distances between neighboring feeders, equal or strongly different color signals around the tube entrance, other reward ratios (1:2:4:8), and different sucrose concentrations. Results appear to be independent of these parameters (Greggers and Menzel, in press). Interestingly, feeder No. 1 with the lowest reward was always chosen a bit more frequently than feeder No. 2. Within any given experiment, this difference was never significant but was consistent over all test conditions. This result suggests that honey bees are programmed to invest a certain amount of energy and time for probing, regardless of whether or not the low-reward conditions in less frequently visited feeders have changed. Information collection is thus as important as optimization of energy gain.

An analysis of behavioral sequences on a real-time scale gives us some hints about mechanisms of choice performance and the action of different forms of memory involved in the decision process. The bars in Figure 4.24 indicate that the total number of all return flights to the just-visited feeder (RV) match the reward distribution better than the total number of all AV flights (see also Figs. 4.21 and 4.26b). Since, on average, feeder No. 8 provides more reward per visit than feeders No. 1 and No. 2 (see Fig. 4.24a), the probability of an RV should depend on the amount of reward

acquired during the last visit. Indeed, the probability of an RV increases with the amount of the last reward.

Correspondingly, the probability of AV is inversely related to the amount of reward acquired during the last visit. The choice during AVs depends strongly on the feeder at which the bee begins its AV flight (Fig. 4.25). Very good matching is found for AVs following a visit at feeders No. 1 and No. 2, while very poor matching after visits at flowers 4 and 8. This means that honey bees starting from low-reward feeders recruit a memory about reward distributions which contains more reliable information about the long-term experience in the patch than that of bees starting from a high-reward feeder. This "reference memory" is overridden shortly after

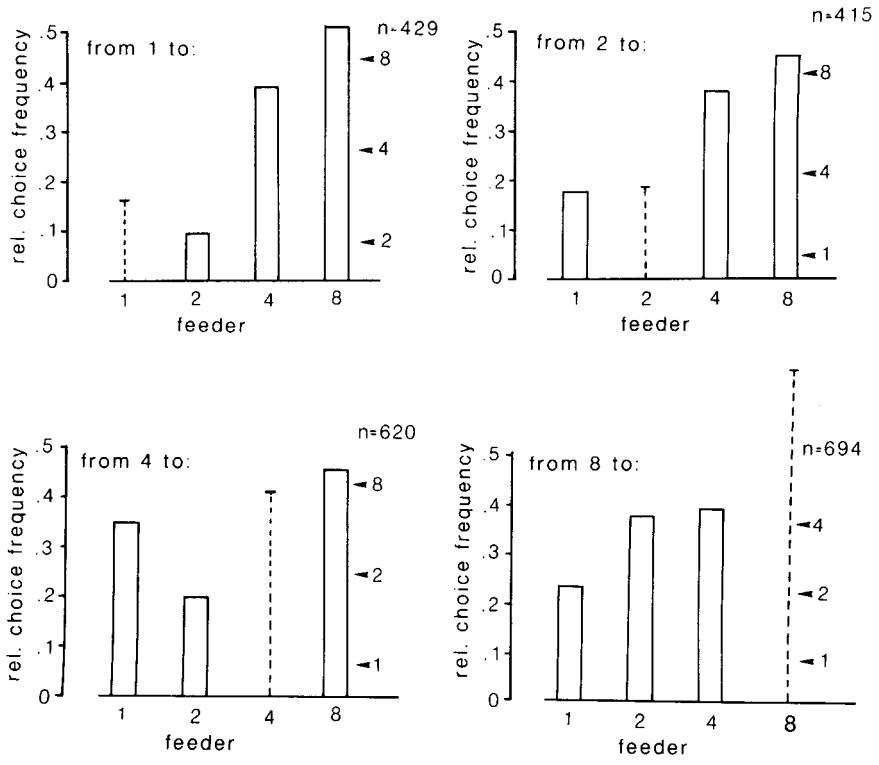


Figure 4.25. Choice frequency during alternating visits (AV), from feeder No. 1 (left upper graph), from feeder No. 2 (right upper), from feeder No. 4 (left lower), from feeder No. 8 (right lower). The dotted line gives the relative choice frequency of the corresponding RV flights. The arrowheads on the right side mark the relative choice proportions for perfect matching. n gives the number of choices of eight bees.

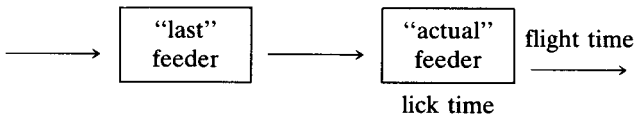
a high reward, irrespective of where the bee receives it. The sensitizing effect of a strong reward lasts in turn for only a short period of time (see Fig. 4.21). After that time (specifically, between 5 and 10 seconds after takeoff from the last-visited feeder, depending on average reward rate in the entire patch and thus on flight speed; see Greggers and Menzel, in press), choice is controlled primarily by the reference memory.

How is the information collected during a visit used to update memory? Theories on classical and instrumental conditioning favor the conclusion that the difference between the expected and the actually experienced US strength is the most important factor in learning (Rescorla, 1967, 1988; Rescorla and Wagner, 1972). Since flight time between visits correlates both with the average amount of reward in the whole patch and the amount of reward during the last visit, we can use flight time as an indicator of the expected next US and the flight time after that reward as a measure of the deviation between expected and experienced US. Furthermore, licking time depends primarily on the expected reward because the minute amount of sucrose solution is imbibed in less than 5 seconds of the average licking time. Table 4.1 gives the results for two categories of US strength: low reward (<0.4 µl sucrose solution), high reward (>0.4 µl). It is obvious that the US experienced at the "last" flower determines an expectancy for the US at the "actual" feeder, because a high "actual" reward induces a longer licking time after a high "last" reward than after a low "last" reward. The flight time after the "actual" reward for RV flights is short for the transition from low to high reward, long for the transition from high to low reward, and not different for successive rewards of the same amount (low-low, high-high). AV flights do not depend on those transitions (not shown).

It is obvious from these results that the dynamics of memory processes and the limited capacity of the bees' short-term memory have to be taken into account in explaining its foraging behavior. Bees, like all foraging animals, are not omniscient at any stage of their foraging cycle and are programmed to keep track of changes in food availability. The honey bee's foraging behavior reflects a compromise between food collection and information collection. Bees are hardly playing with a two-armed bandit for which they first would have to discover the probabilities of success in order to apply optimal rules (Houston et al., 1982; Krebs et al., 1978). Rather honey bees, like other animals, continuously collect and retrieve information in the process of making choices. The temporal dynamics of foraging, the structure of their memories, and the limited capacity of certain memory stages appear to be the framework in which proximate mechanisms account for ultimate goals.

The dynamic model developed on the basis of these results formulates the following two rules: (1) the informational capacity for updating the

Table 4.1. Results for two categories of US strength



Lick time at the actual feeder: (low < 0.4 µl, high > 0.4 µl):

		Reward "actual" feeder	
		Low	High
Reward "last" feeder	Low	7.1 sec ± 0.8 n = 693	14.9 sec ± 0.9 n = 1076
	High	7.0 sec ± 0.6 n = 721	18.5 sec ± 0.8 n = 769

Flight time of RV-flights after visiting the actual feeder:
(low < 0.4 µl, high > 0.4 µl):

		Reward "actual" feeder	
		Low	High
Reward "last" feeder	Low	6.0 sec ± 0.7 n = 231	4.3 sec ± 0.6 n = 253
	High	6.4 sec ± 0.8 n = 264	5.0 sec ± 0.7 n = 186

memory is proportional to the difference between the expected and the experienced amount of reward (i.e., a "difference rule") and (2) retrieval of the expected amount of reward activates either a short-lasting working memory or a long-term reference memory. Retrieval from working memory overrides retrieval from reference memory and dominates over short time periods immediately after a reward. Retrieval from working memory depends strongly on the amount of reward. Reference memory does not appear limited in time or capacity and stores the sum of all informational components resulting from the difference rule.

These rules predict certain peculiarities which were actually confirmed by the results. Figure 4.26 gives an example. One implication of the two rules is that the choice of feeders depends on the time interval between the last reward and the next choice. At very short intervals, the most choices are allocated to the high-reward feeders. At longer intervals, the highest-reward feeder, No. 8, loses its attractiveness, whereas feeders No. 1 and No. 4 become more attractive. This pattern is an outcome of the first rule,

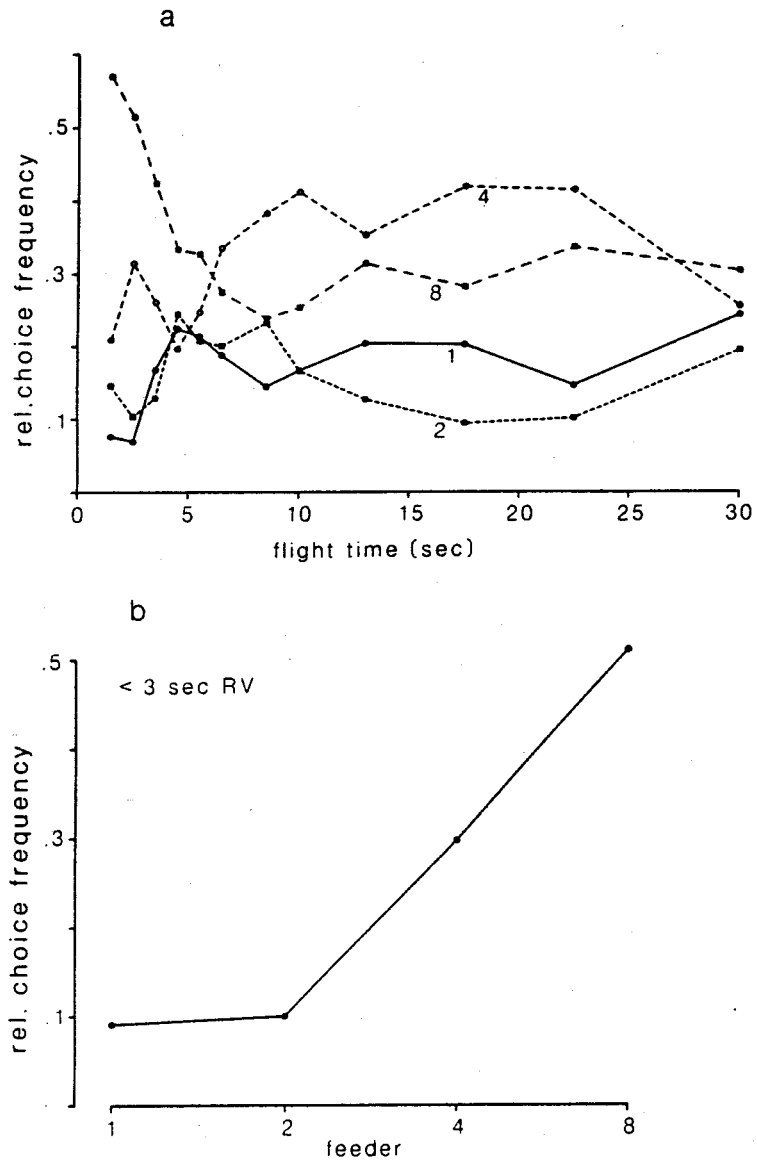


Figure 4.26. Temporal dynamics of the choice behavior for four feeders with the relative rates of sucrose flow 1, 2, 4, and 8 (see Figure 4.25 and 4.26). Part *a* gives the time dependence of the choice for intervals of 2–30 seconds between two successive visits for both RV and AV flights together. The numbers 1, 2, 4, and 8

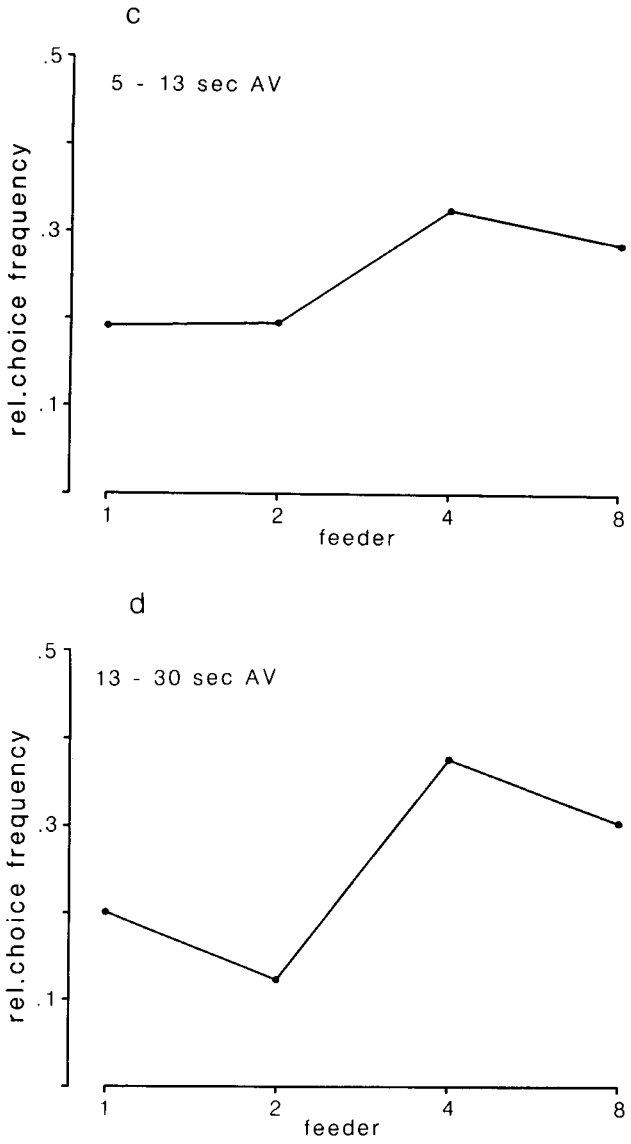


Figure 4.26. (Continued) beneath the curves indicate the choice for the respective feeder. Parts *b–d* plot the same results for three time horizons (*b*, < 3 seconds; *c*, 5–13 seconds; *d*, 17–30 seconds) and for the four different feeders (Nos. 1, 2, 4, 8 at the abscissa). Note the reduction in the choice of 8 and 2 and the enhancement of the choice of 1 and 4 at longer time intervals.

which leads to a relatively stronger long-term memory for feeders No. 1 and No. 4 and a relatively weaker memory for Nos. 2 and 8 (see Greggers and Menzel, in press).

Conclusion

Memory formation and retrieval is as highly dynamic and multiphasic a process in the honey bee as it is in animals with large brains. To the extent that memory dynamics reflect cellular and network properties underlying the different forms of plasticity in the nervous system, they are indicative of the existence of a series of information-storage mechanisms. Memory dynamics are also adapted to the particular needs of the animal in nature. Our results favor the conclusion that, for a honey bee working in a floral patch, the tight match between the expected sequence of food encounters and the programmed transitions between memory phases simplifies the decision-making task in a continuously fluctuating and highly unpredictable world. The honey bee's memory records neither the number nor the sequence of rewarded and unrewarded landings. It calculates neither an average of any sort nor a probability of positive or negative encounters. The decision rules applied by the bee are obviously different from that of a player at a two-armed bandit machine, yet optimization criteria are met both in the short term and the long term. The honey bee meets these criteria through the application of certain rules of thumb which relate the amount of reward, the time to next encounter, and the precision with which the most recent or the more remote memory is activated. These memory-retrieval mechanisms appear to produce expectations which may differ considerably among the different memories and permit the honey bee to adapt quickly to changes in environmental conditions. The signal for the next step of memory formation is the deviation between the expected and the experienced US or reward. The dynamics of memory processes protect the animal from being caught in a suboptimal patch of food distribution. At the same time, the limited capacity and duration of short-term memory prevent honey bees from accumulating information about the environment indefinitely. Lack of "knowledge," however, does not lead the bee to a probabilistic relationship between its actions and their consequences. The reason for this lies in the very nature of memory dynamics. If an individual honey bee experiences a higher reward than expected by retrieval from the long-term memory, short-term memory is triggered and keeps the individual within the patch, but only during the active status of the short-term memory which now carries an updated, higher expectation of reward. If a positive encounter is added within a short period of time, the long-term memory is not changed and the animal

regulates its choice behavior according to the previous status of the long-term memory. If an even higher reward is experienced during the lifetime of the short-term memory, then the consolidation process updates long-term memory and maintains high expectations in short-term memory as well. It is obvious that such a system will work only if the temporal dynamics and the transfer properties between forms of memory match very well conditions of flower-foraging in nature. Since these can change quite drastically over the course of the year, one might expect additional long-term adaptations which account for the changing circumstances of the honey bee colony.

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