METHODS & DESIGNS

Automated discrete-trials techniques of appetitive conditioning in honey bees

J. E. SIGURDSON

Békésy Laboratory of Neurobiology, University of Hawaii, Honolulu, Hawaii 96822

Automated techniques of appetitive conditioning in honey bees are described. The subject is pretrained to come to the laboratory, where it is confined while the training trials are programmed and then is free to return to the hive. The responses recorded are approach to and entering a tube into which sucrose solution is pumped. Illustrative experiments on classical conditioning, simple instrumental conditioning, and choice are reported.

Our knowledge of learning in the honey bee (*Apis mellifera*) has been obtained primarily through the study of unconfined ("free-flying") foragers with variations of a technique developed early by von Frisch (1915) (for reviews of much of this work, see Menzel & Erber, 1978; Wells, 1973). The general procedure is first to permit an animal to feed on a distinctive target containing a drop of high-concentration sucrose solution and then to measure the tendency of the animal to approach or to land on the unbaited target, which may be presented alone or together with other targets differing in such properties as odor and color. This engagingly simple technique makes possible a variety of rather sophisticated learning experiments (Couvillon & Bitterman, 1980; Klosterhalfen, Fischer, & Bitterman, 1978), and yet it has some rather serious disadvantages. It requires the constant effort of the experimenter, who has the responsibility for programming the events of the experiment and recording responses. More important, it surrenders a considerable degree of parametric control to the free-flying subject.

Some progress has been made in the automation of stimulus presentation, reinforcement, and response detection in experiments with free-flying subjects. Pessotti (1972) trained a bee to push a horizontal lever, and other investigators have required the bee to enter a vertical response tube, either depressing a lever in the tube (Núñez, 1970) or simply interrupting a photo-beam (Grossmann, 1973). In all these situations, the response detector controls a syringe-pump that delivers a predetermined amount of high-concentration sucrose to the animal at the proper time without intervention

of the experimenter. Grossmann's situation is perhaps the most successful, and with it he was able to train bees on continuous, interval, and ratio schedules of reinforcement and to observe extinction following such training.

Perhaps the greatest disadvantage of the traditional method is the lack of control over the location of the bee during the presentation of stimuli. Without such control, the bee may even leave the experimental site entirely when confronted with nonreinforcement, as in an intertrial interval or when a negative stimulus is presented. Attempts to control exposure to the experimental situation have been limited for the most part to temporarily shutting the bee out of the laboratory or arranging for the presentation of stimuli within the narrow temporal constraints imposed by the degree of persistence of the nonreinforced bee. A method used by Grossmann and Beller (1971) was to trap the bee in a small plastic cube and hold the cube over the colored stimuli to be sure that the bee would experience them in the intended sequence.

Described here are two fully automated situations that provide better control over the location of the animal during training. The bee voluntarily enters an experimental chamber and is confined there for the duration of a programmed series of conditioning trials—classical or instrumental, free operant or discrete (including the possibility of true nonreinforced trials). At the end of the series, the bee completes the filling of its crop at a free feeder in the chamber and then is permitted to return to the hive. The presentation of stimuli (colored lights) and of reinforcements is handled in much the same way as by Grossmann (1973). The principal response is dipping, which is measured with photodetectors in a "unitary" apparatus with a single tube and in a "choice" apparatus with two response tubes, but a new contact-measuring device also is used. The utility of the "temporary confinement" strategy is demonstrated in a series of representative conditioning experiments.

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CHAMBERS, RESPONSE DETECTORS, AND TRAINING PROCEDURES

Unitary Apparatus

The unitary situation is illustrated in Figure 1, which shows the inside lateral dimensions of the chamber (2 cm in height) and of the entrance tunnel (.9 cm in height) that passes through the wall of the laboratory to the outside. The walls and ceiling of the chamber are constructed of clear Plexiglas and rest as a unit on a Plexiglas floor painted matte black. There is a solenoid-operated door at the outer end of the tunnel, which is normally open, and a solenoid-opening, gravity-closing inner door located midway between two photodetectors. These detectors utilize HEP P2002 IR diode sources and PS 24 photoswitches. Wratten 87B IR filters are mounted just ahead of the sensors to screen out low-wavelength ambient light. The inside of the Plexiglas tunnel is painted matte black to further reduce the effects of extreme fluctuations in intensity of the outside light. A disk of translucent Plexiglas, inset into the black floor of the chamber, can be illuminated from below by one of three GE F4T5 6-in. fluorescent lamps sourced for instant starting. The outputs of the three lamps are filtered to produce three readily discriminable stimuli—nominally, white, green (Wratten No. 58), and blue (No. 47). Rough equation of the lamps' physical intensities is accomplished by blocking different proportions of the surface areas of the lamps. (For an introduction to the special problems encountered in working with the visual system of the honey bee, see Daumer, 1956; Goldsmith, 1963, 1964.)

In the center of the translucent disk is a vertical tube for measuring the dipping response and delivering reinforcement. The tube, diagrammed in Figure 2, is functionally similar to that of Grossmann (1973), but with dimensional modifications designed to improve training efficiency and to permit increased rates of responding when rate is measured. At the bottom of the tube is a 22-gauge nozzle through which a high-concentration sucrose solution (35% by weight) can be delivered by a Harvard Apparatus syringe pump. The pump motor speed and syringe size combine to produce a sucrose flow of approximately 1.2 microliters/sec, the small bore of the nozzle providing for rapid delivery of the fluid to

Figure 1. The unitary apparatus and tunnel. PS = photo-switch.

Figure 2. Cross-section of the vertical response tube in the unitary apparatus.

the tip as the motor begins to turn. The photodetection system used in the tube is the same as that in the tunnel, and its compact size presents minimal obstruction to the light of the stimulus-lamps coming from below. A reservoir of high-concentration sucrose solution with a solenoid-operated cover (the free feeder) is set into the floor of the chamber at the far end. The entire chamber is enclosed in a hinged light-tight shell (not shown) with red Plexiglas ports to permit direct observation of behavior and recording with a high-sensitivity infrared video camera. A small dot of a water-based red paint on the experimental bee is highly visible to the eye and camera. All sensing and control functions are fully automated by means of 24-V relay programming equipment.

In most of the experiments described here, attention is focused on the dipping response; however, mere approach to the tube can be measured as well. A flat metal ring on the stimulus disk is connected to a solid state circuit that detects contact of the bee with the metal ring. The transducer, diagrammed in Figure 3, is an adaptation of a circuit designed by Wise (1977) to measure minute capacitance changes in muscle fiber. The sensor line is connected to the metal ring, and all metal components within 20 cm of the sensor are grounded. In this application, a 555 multivibrator produces a 5-V square-wave output with a frequency of approximately 65 kHz, which varies inversely with the total capacitance on the sensor line. This output (attenuated to 40 mV peak to peak) is taken to Pin 12 of the 560B phase-locked loop. To allow capture and tracking, the frequency of the VCO (in the 560B) must be matched to this frequency by coarse adjustment of the capacitance between Pins 2 and 3 and fine adjustment of the current input to Pin 6. VCO frequency may be measured on Pin 5 if sufficient care is taken to avoid pulling the oscillator. Once set, the VCO and 555 frequencies are continuously phase compared, and an error-signal is generated, filtered, amplified, and detected at Pin 9 as a 90-mV output swing riding on a 12-V offset. This output is sensitive to picofarad changes on the sensor line (as when the bee makes or breaks contact with the

Figure 3. Circuit for capacitive transducer with switching output. All resistors are .25 W, 5% unless otherwise noted, and all capacitor values are in microfarads.

sensing surface), but it is remarkably stable over wide ranges of temperature and source voltage. While the sensitivity of the 555/560B combination to change in capacitance is fixed by circuit components, its output level is adjustable (via current-input changes at Pin 6), as is the switching level of the 339 comparator (via the 50K variable resistor), thus making relay operation contingent upon greater or lesser capacitance change on the sensor line. To provide flexibility in the placement of the 555, which must be close to the sensing surface, the 555 and its components are mounted on a small etched board that is connected to the remaining components with a coaxial lead and a 5-V line.

Unitary Training

A bee is captured in a matchbox at a feeding station several meters from the tunnel entrance. The station provides a large quantity of low-concentration sucrose solution (7%-10% by weight), which is acceptable to the animals before they have been introduced to the high-concentration solution offered in the laboratory, but much less so afterward (Bitterman, 1976). With the light-tight outer shell and the wall-ceiling unit of the chamber removed, the captured bee is released into the response tube as 35% sucrose is being delivered by the syringe pump to the bottom of the tube. While the bee is drinking, a small mark of paint is placed on its abdomen, and the wall-ceiling unit and the shell are replaced. With both tunnel doors open, the bee is allowed to depart for the hive and to enter the chamber again upon its return, at which time the inner door is manually closed. On this first spontaneous visit, the white stimulus lamp is illuminated and the bee is confined until it dips into the response tube, thus starting the pump. The bee is allowed to fill its crop to capacity without interruption and then to leave again for the hive. On the next few visits, now fully automated, the bee typically is magazine trained, which is to say that it is trained to

dip for food in response to the white light, much as a pigeon is trained to take grain from a feeder or a rat to take pellets from a tray at the outset of a conditioning experiment. As the bee enters the tunnel, the outer photoswitch opens the inner door, which closes again behind the bee when the inner photobeam is broken. While confined to the chamber, the bee is exposed to a sequence of white-light presentations separated by intertrial intervals in the dark. A dipping response during the intertrial interval either holds or resets the interval timer, delaying the onset of the next trial; either procedure prevents the start of a trial while the animal is in the tube. Each white-light presentation signals the availability of immediate reinforcement in the tube, the pump being programmed to start when the bee breaks the photobeam. The presence of the bee in the beam is required to keep the pump timer running to the completion of its cycle. If the bee dips and quickly withdraws from the tube, this constraint prevents the accumulation of sucrose in the absence of the bee and the adventitious reinforcement (with a relatively large quantity of sucrose) of other behaviors that may antedate return to the tube. An additional constraint is that the trial terminates only upon completion of the programmed pump cycle. This virtually assures that the bee will take all the food available on that trial.

Once the magazine training has been accomplished, a variety of classical and instrumental experiments may be carried out. For example, a blue light as CS may be followed, after a given interval and independently of the animal's response, by the white light and food; or the reinforcement may be contingent upon response to the blue light. Acquisition and extinction of response to the CS may be measured in terms of latency, probability, or rate.

Both during the magazine training and during the subsequent experimental training, the number of reinforcements and the amount of each reinforcement are

adjusted so that the bee is given less than a full crop-load of the sucrose solution during the programmed series of trials. Capacity is estimated from pilot studies, the results of which agree rather closely with those of Núñez (1970). The purpose is to be sure that even the least hungry bee will continue to be interested in the food throughout the series of trials. After the last trial, the free feeder is uncovered and illuminated with white light, and the bee is allowed to feed to repletion. The tunnel doors are opened 30 sec later, so that the bee is free to leave for the hive when replete. This pattern of entry, training trials, free feeding, and exit may be repeated as often as necessary for a given experimental design.

To take reliable data for a single bee over several hours (or, in some cases, days) requires freedom from interference by other bees that may discover the apparatus. The use of the tunnel entrance and the presence of the low-concentration feeder near the tunnel generally keep the number of intruders to only one or two in the course of a day's work, and these usually appear only during the first few visits of an experimental bee. If a second bee tries to enter the apparatus while one is already inside, the outer photoswitch closes the normally open outer door, trapping the second bee between the inner and outer doors of the tunnel. An alarm is sounded for the experimenter, and the program in progress continues for the first bee, but exit from the chamber is prevented until the experimenter intervenes. If the marked bee is in the chamber, the intruder is removed, and then the marked bee is released as usual. If the marked bee is in the tunnel, the intruder is removed from the chamber, the program for the visit is reset, and the marked bee is allowed to enter the chamber. Whenever the experimental animal leaves the laboratory, an incrementing timer is activated, and its value (the hive-return time) is recorded upon the bee's return. Under good hive and field conditions, these times are consistently under 5 min, and exceptionally long or short periods indicate trouble to the experimenter.

Choice Apparatus

The choice situation, which is illustrated in Figure 4, is in many respects like the unitary situation, but it has two vertically oriented rear-projection screens with a horizontal response tube at the base of each. The design of the response tubes, which contain photodetectors and sucrose-delivery nozzles like those in the unitary device, is shown in Figure 5. The animal responds to a stimulus (colored light projected on a screen) by entering the corresponding tube. A removable Plexiglas chamber (ceiling and walls resting on a black Plexiglas floor) extends from the inside boundary of the entrance tunnel to the screens, its inside vertical height rising from .9 cm at the tunnel to 3.5 cm at the screens. No additional light-tight cover is used with this apparatus because its ceiling and walls are made of transparent red Plexiglas and it is shielded from direct light sources

Figure 4. The choice apparatus and tunnel. PS = photo-switch.

Figure 5. Cross-section of a horizontal response tube in the choice apparatus. PS = photoswitch.

in the room. The stimuli are generated with Industrial Electronics single-plane projectors using GE 313 pilot lamps. Two lamps driven at 35 V are used for each stimulus, which necessitates the use of a simple forced-air cooling system. All of the experiments performed thus far have been done with two colors, nominally orange and blue, that were selected in pilot experiments as readily discriminable but not differentially preferred without differential reinforcement. The orange is produced by a Wratten No. 21 filter together with a .6 neutral-density filter. The blue is produced by a Wratten No. 45 filter.

Choice Training

A bee is carried in from the feeding station and placed on a drop of high-concentration sucrose solution in the center of the apparatus. On its first spontaneous visit, the bee explores the dark chamber until it enters one of the tubes, in which it is fed about 50% of its capacity, after which it must find and enter the opposite tube in order to feed to repletion. On the next visit, the experimental training begins. After a programmed delay to allow for adaptation, the photodetector at the inside end of the tunnel becomes the trial-start switch. The interruption of this beam by the animal initiates a trial

by illuminating both screens and making reinforcement available at the correct one. A response to the correct stimulus produces reinforcement, extinguishes both stimuli, and ends the trial. A response to the incorrect stimulus has no effect, and the bee is permitted to cross over to the correct side and receive reinforcement without penalty. As in the unitary situation, the presence of the bee in the correct photobeam is required to keep the pump timer running to the completion of its cycle. The trial ends only upon the completion of the cycle. The bee is then required to interrupt the trial-start switch again in order to illuminate the screens and to start the next trial. With the scheduled number of trials completed, the bee is given access to the free feeder and permitted to leave when replete. On the third and subsequent visits, a correction procedure with a penalty for incorrect response usually is introduced. In this procedure, an incorrect response extinguishes both stimulus lamps and cancels availability of reinforcement on the correct side. The bee then must retreat to the tunnel and trip the trial-start switch, which sets up the same stimulus and reinforcement conditions that existed at the beginning of the trial. Only a correct response advances the stepping switch and sets up the conditions for the next trial.

ILLUSTRATIVE EXPERIMENTS

Analysis of Classical Conditioning

The dipping response may be conditioned either classically or instrumentally. In the first experiment reported here, a classical procedure was used with a discriminative control for nonassociative effects. In the second experiment, an omission procedure was employed to inquire into the role of adventitious response-reward contiguity in the classical procedure (Sheffield, 1965). Noting that bees trained by the traditional method of von Frisch (1915) must land on the target to take the food, Grossmann (1970) has suggested that landing may function as an "operant," although the change in performance produced by the training typically is attributed to association between target properties and food (Menzel, 1968).

In the first experiment, each of six bees was introduced to the unitary apparatus in the usual manner and then magazine trained with the white light (as described above) for three visits. There were 10 reinforcements per visit with a variable intertrial interval in darkness gradually increasing to a mean duration of 25 sec. Then there were 24 visits with 20 conditioning trials per visit and a variable intertrial interval in darkness, again averaging 25 sec. At the start of each trial, the disk was illuminated either with blue or with green light, 10 trials each in balanced Gellermann orders. For three of the six bees, green was S+, being followed after the 8-sec CS-US interval, whether or not there was a response, by the white light and food; blue was S-, terminating without reinforcement after 8 sec. For the other three bees, blue was S+ and green was S-. The results are

Figure 6. Mean probability of dipping in response to each of the conditioned stimuli in the classical conditioning experiment.

plotted in Figure 6 in terms of mean probability of response to the positive and negative stimuli on each of the 24 visits. The curves show some responding to both colors at the outset of training—generalization, presumably, of the response to white—after which the probability of response to S+ increased sharply and the probability of response to S- declined. The difference in responding is highly reliable: for stimuli, $F(1,4) = 96.80$ ($p < .001$); for visits, $F(23,92) = 4.76$ ($p < .001$); for the Stimuli by Visits interaction, $F(23,92) = 4.91$ ($p < .001$). The asymptotic pattern of behavior on S+ trials, which developed early in training, was to dip into the tube when the stimulus was turned on and to stay there until the white light and food were presented.

In the second experiment, six new bees were trained with a procedure identical to that used in the first study, except that if a dipping response was made to S+ on any trial, the stimulus was turned off without reinforcement at the end of the programmed 8-sec interval. The white light and food followed only if there was no response to S+. As before, S- was turned off without food after 8 sec, whether or not the bee responded. In order to provide a more complete description of the performance under these conditions, two additional measures were taken. The first was contact with the metal ring, an index of approach to the dipping tube during S+ and S- presentations. The schedule for the approach response was, however, purely classical, in that mere approach (without dipping) did not prevent reinforcement. The second additional measure was the latency of dipping in response to the white light whenever it was presented in consequence of the bee's failure to respond to S+.

In the upper portion of Figure 7, the results are plotted in terms of mean probabilities of the approaching and dipping responses to the two stimuli on each of the 24 visits. The curves for dipping show that there was some generalized responding to both colors at the outset, just as there was in the first experiment. Then the level of responding to both colors fell off, although somewhat less rapidly for S+ than for S-, which pro-

Figure 7. (Upper portion) Mean probability of approach and of dipping, DIP(CR), in response to each of the conditioned stimuli in the omission experiment. (Lower portion) Mean latency of dipping, DIP(UR), in response to the reinforcer in the omission experiment.

vides some transient indication of stimulus-reinforcer control. In the end, dipping was suppressed almost completely by the omission contingency, with response to both colors declining to the same low level despite the fact that, at asymptote, S+ was followed by reinforcement on almost every trial. Analysis of variance (based as it must be on the data for all 24 visits) yields the following: for stimuli, $F(1,4) = 7.56$ ($p = .051$); for visits, $F(23,92) = 3.56$ ($p < .001$); and for the Stimuli by Visits interaction, $F(23,92) = 1.42$ ($p = .124$).

In their approach behavior, the bees showed sustained discrimination of the two stimuli. The probability of approach, which was not subjected to the omission contingency, was high at the beginning for both stimuli and then increased somewhat for S+ and declined for S-. Analysis of variance yields the following: for stimuli, $F(1,4) = 109.70$ ($p < .001$); for visits $F(23,92) = 1.29$ ($p = .196$); and for the Stimuli by Visits interaction, $F(23,92) = 2.89$ ($p < .001$). The substantial asymptotic probability of approach to S- reflects the high level of general activity on S- trials, in the course of which the animal tended to contact the detecting ring in an undirected fashion. On S+ trials, in contrast, the animal typically went to the disk and waited until the white light was turned on, whereupon it promptly entered the tube and took the food. The curve in the lower portion of Figure 7 shows the mean latency of the dipping response to the white light on reinforced S+ trials. Established in pretraining, the response was disrupted to some extent at the onset of conditioning (which provided adventitious reinforcement for incompatible behavior in the presence of S+), but it soon recovered.

Experiments with rats show that the relative strengths

of stimulus-reinforcer and response-reinforcer control, which are, of course, directly opposed in omission training, vary with the targeted response (Holland, 1979). The present results for honey bees are strikingly similar to the results of one of the rat experiments, in which response to the food magazine (like dipping in the bees) was eliminated by the omission contingency, and rearing, which (like approach in the bees) was recorded concurrently but had no effect on the occurrence of reinforcement, gave clear evidence of conditioning. Subjected to the omission contingency in a subsequent experiment, rearing in rats gave little indication of response-reinforcer control. It would be interesting to determine whether there are responses in honey bees that are more resistant than dipping to such control.

Partial Reinforcement and Resistance to Extinction in Instrumental Conditioning

In free-operant work with the dipping response, Grossmann (1973) found some indication of greater resistance to extinction after intermittent than after continuous reinforcement, but the comparison was unsystematic, with little control of the number of responses or reinforcements. A recent discrete-trials experiment using the traditional method showed that the tendency of free-flying bees to make contact with a target on which they have previously been fed extinguishes less rapidly after partial delay of reinforcement in training than after training with immediate reinforcement on each trial (Couvillon & Bitterman, 1980). This outcome can be understood from the fact that repeated responding like that measured in extinction is elicited and ultimately rewarded when reinforcement is delayed. The experiment now to be reported shows the promise of the unitary technique for the discrete-trials analysis of partial reinforcement in honeybees.

Each of 10 bees was introduced to the unitary apparatus in the usual way and fed continuously in the tube on the first spontaneous visit. Then there were six "pretraining" visits, on each of which there were 15 trials like the magazine-training trials already described. The reinforcement on each trial was 2 microliters of 35% sucrose solution, the delivery of which ended the trial. The intertrial interval (in darkness) was controlled by a 3-sec timer that was reset by each response during the interval. On the next 14 "training" visits (7-20), there were again 15 trials/visit, all reinforced for five of the subjects, constituting the CRF group. The rest of the animals, the PRF group, were reinforced on only 50% of the trials in balanced quasirandom orders (seven reinforcements on some visits, eight on others). On nonreinforced trials, the white light stayed on for 8 sec, whether or not a dipping response occurred, and then it was turned off. On the next 15 "extinction" visits (21-35), there were 15 nonreinforced trials per visit for both groups. Latency of dipping was measured throughout, and a maximal value of 8 sec was recorded when the animal failed to respond on any trial. Not only

Figure 8. Mean $\ln$ (latency + 1) of dipping in partial (PRF) and continuous (CRF) groups in the three stages of the experiment.

during extinction but also throughout the training, the scheduled sequence of trials on each visit was followed in the usual way by access to the free feeder before returning to the hive.

In Figure 8, the results are plotted in terms of the mean $\ln$ (latency + 1) of response per trial for the 35 visits. The reliability of the method is shown by the similarity of the curves for the two groups in the six pretraining visits, when the groups were treated alike. In the training phase, when the two groups were treated differently, mean latency of responding was greater in the PRF group than in the CRF group [$F(1,8) = 5.55$ ($p = .046$)]. Nevertheless, the PRF group showed greater resistance in the extinction phase. Analysis of the data for Sessions 21-35 yields the following: for groups, $F(1,8) = 5.89$ ($p = .041$); for visits, $F(14,112) = 52.5$ ($p < .001$); and for the Groups by Visits interaction, $F(14,112) = 3.65$ ($p < .001$). A clear partial reinforcement effect remains to be analyzed in the systematic parametric experiments that this technique makes possible.

Positive Behavioral Contrast

In the unitary experiments already described, both latency of dipping and probability of dipping were measured. The experiment now to be described illustrates the use of a rate measure in discriminative training by the discrete-trials/fixed-interval procedure of Woodard and Bitterman (1974). The pretraining procedure was designed to shape repeated dipping. For several visits, the disk was illuminated with white light and each discrete dip into the tube caused the pump to deliver about 1 microliter of 35% sucrose solution (a CRF schedule).

After 30 reinforcements, the light was extinguished and the bee was given access to the free feeder. In the next series of visits, there were six discrete presentations of the white light, separated by intervals in darkness whose duration was controlled by a 3-sec timer that was reset by response. To begin with, each light presentation signaled the immediate availability of five response-contingent reinforcements, but soon, a fixed interval between the start of the trial and the availability of reinforcement was introduced and gradually increased to 15 sec, with the frequency of dipping measured during the fixed interval. In the final stage of pretraining, each visit consisted of 15 15-sec trials, each trial terminating with two response-contingent reinforcements. As usual, access to the free feeder was given after the 15th trial of each visit, and the animal fed to repletion before returning to the hive.

When rate of responding had stabilized, reinforced trials with green (G) and blue (B) lights were substituted for the white light, half with each color in Gellermann orders. This schedule remained in effect for four visits (Stage 1). In Stage 2, 30 trials were given on each visit, 15 with blue reinforced as before and 15 with green, each of which now ended after 15 sec without reinforcement. This training was given on the last four visits of the 1st day of the experiment and throughout the 2nd and 3rd days. On the first 12 visits of the 4th day, both colors were reinforced (Stage 3), after which green was reinforced and blue unreinforced on that day and on the 5th day as well (Stage 4). In Figure 9, the performance of a single bee is plotted in terms of the mean rate of response to each color on each visit for the entire period. The figure gives clear indication of positive

Figure 9. Behavioral contrast in discrete-trials/fixed interval discrimination training. B = blue; G = green.

contrast reminiscent of that found in pigeons (Reynolds, 1961). Sequential analysis shows no local contrast; response to the positive stimulus was the same whether it followed one, two, or three negative trials or one or two positive trials. Quite apart from the demonstration of contrast, the power of the technique for the study of discriminative learning should be noted. The bee in this experiment was generating about 750 data points per day, with no sign of faltering at the time the experiment was terminated. There was wide separation of mean rates of response on positive and negative trials, and there was very little overlap in the rates on positive and negative trials within visits.

Reversal Learning

The first work with the choice situation was done with a purely spatial problem in order to get a clear look at what would be the most salient irrelevant variable in color problems. With both stimulus screens white, first one side was reinforced and then the other, in a series of spatial reversals. There has been no previous work on spatial reversal learning in bees, but visual reversal learning in free-flying subjects has been explored with somewhat unclear results by Meineke (1978) and Menzel (1969). The most extensive studies, by Schmetter (Note 1) of Würtzburg, have not yet been published.

Figure 10 shows the performance of a single bee plotted in terms of initial errors in the original problem (R_0) and in each of 20 subsequent reversals over a 3-day period. There were 10 trials/visit (each visit ending as usual with access to the free feeder) and 8 visits/reversal. The training began on the very first spontaneous return to the apparatus. Both stimulus

screens were illuminated with white light at the start of the visit, but reinforcement was not available on either side. When one of the response tubes was entered, both screens were darkened and the program was adjusted to make the tube first entered incorrect in R_0 . The first trial of R_0 began when the bee tripped the trial-start switch, illuminating the screens and making reinforcement available on the correct side. The correction without-penalty procedure was programmed on this first visit; a penalty for incorrect response was introduced only on the second visit of R_0 (the second spontaneous visit). Correct and incorrect sides were reversed on the 9th visit, again on the 17th, and so forth. The progressive improvement evident in Figure 10

Figure 10. Initial errors per reversal in the original problem (R_0) and in each of 20 spatial reversals.

Figure 11. Within-reversals performance early and late in spatial reversal training.

is analyzed in Figure 11, which is plotted in terms of mean initial errors per visit pooled for the first five (early) and the last five (late) reversals. These within-reversal curves do not show the reduction in negative transfer that is so central a feature of the data for birds and mammals; they show only an increased rate of improvement within reversals, which is familiar from early work with African mouthbreeders (Wodinsky & Bitterman, 1957) and with cockroaches (Longo, 1964).

Visual Probability Learning

Trained to discriminate orange and blue in the choice

apparatus, bees rapidly learn to choose the consistently reinforced color independently of position. In the experiment now to be described, animals were shifted from 100:0 to quasirandom 70:30 and 30:70 ratios. (It should be noted that Schnetter, Note 1, has done a good deal of work on probability learning in free-flying bees, but with nonrandom schedules.) Each bee was introduced to the choice situation and trained for four visits on a 100:0 problem, orange positive for three of the animals and blue positive for the rest. There were 10 correction trials per visit, with no penalty on the first but on all succeeding visits; each visit ended as usual with access to the free feeder. By the fourth visit, all six animals were performing errorlessly. On the fifth visit, the ratio of reinforcement was changed to 70:30, the previous 100% color remaining the majority color but now reinforced on a quasirandom 70% of trials (in modified Gellermann orders), and the previous 0% color reinforced on the remaining trials. Each bee had 40 visits on the 70:30 problem before being shut out for the night, and on the next day each bee had 40 visits on the 30:70 problem—the previous 70% color now reinforced on a quasirandom 30% of trials and the previous 30% color on the remaining trials. In all stages of the experiment, the two colors appeared equally often on each side, and the animals were reinforced equally often on each side.

In Figure 12, the performance of each animal in the

Figure 12. Individual performances in the 70:30 and 30:70 color problems.

70:30 and 30:70 problems is plotted in terms of the percentage of responses to the original majority color in blocks of four visits. The matching in the 70:30 problem is impressive, and, although the animals do not quite match in the subsequent 30:70 problem, there is no tendency toward the maximizing that is characteristic of mammals. An extensive computer analysis of individual performance in each 40-trial block gives no evidence of patterning based on any combination of visual-spatial, win-lose, or shift-stay strategies. The results are reminiscent of those for cockroaches in a spatial problem (Longo, 1964) and for goldfish in a color problem (Woodard & Bitterman, 1973).

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