lenge]. Thus maximum mean lesion scores were 1.3 in animals vaccinated with the alum-phosphate vaccine formulation and 0.9 in the alum-hydroxide formulation, compared to a maximum mean score of 3.3 for control animals (Table 1). Studies comparing different formulations and dosages of gD-1t are needed to determine whether the high level of protection achieved with CFA can be duplicated with an adjuvant acceptable for parenteral administration in humans.

Our results demonstrate that the clinical manifestations of primary genital HSV-2 infection can be significantly reduced by vaccination with recombinant gD-1t. It is not known whether these preparations were completely effective in preventing virus replication in the protected animals. Further studies will be required to determine whether vaccination against HSV with gD-1t prevents latent infection by the viruses or whether this type of vaccination merely diminishes the clinical symptoms of HSV infection. However, it is clear that a single HSV-1–derived glycoprotein can provide protection from the clinical symptoms of genital HSV-2 infection in the guinea pig when administered in conjunction with a potent adjuvant. Other studies will be necessary to determine whether HSV-2 gD or other HSV glycoproteins affect the potency of this vaccine. These considerations notwithstanding, we believe that our data justify further consideration of gD-1t as a subunit vaccine for prevention of HSV-1 and HSV-2 infections in humans.

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11. Truncated HSV-1 glycoprotein D was harvested from serum-free medium conditioned by growth of gD10.2 cells (*9*). The cell culture medium was clarified by filtration, concentrated by ultrafiltration, and fractionated by ammonium sulfate precipitation. The gD-1t was then purified to near homogeneity by immunoaffinity chromatography (T. Gregory, D. Vettlerlein, R. D. Hershberg, in preparation). The immunoaffinity column was prepared by coupling a monoclonal antibody produced against HSV-1 to cross-linked Sepharose CL-4B (Pharmacia Fine Chemicals) and eluted by a method similar to that described by R. Axén, J. Porath, and S. Ernback, [*Nature (London)* **214**, 1302 (1967)].
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13. Female Hartley guinea pigs 2 months of age and weighing approximately 250 g were purchased (Charles River). All the animals were immunized and scored in accordance with a double-blind protocol. For experiments involving Freund's adjuvant, the primary immunization consisted of gD-1t (30 μg) emulsified in 50 percent CFA, with 0.5 ml being injected subcutaneously into the loose skin above the neck and 0.5 ml injected intramuscularly into the thigh. After 31 days the animals were given the same amount of antigen incorporated in IFA. Control animals were injected by the same protocol as the experimentals, except that adjuvant alone was injected. Experimental and control animals were challenged intravaginally with HSV-2 19 days after the booster injections (see legend to Fig. 2). For experiments involving alum adjuvant, 30 μg of gD-1t incorporated in alum-phosphate or alum-hydroxide (0.15 ml) was used for both the primary and the booster immunizations. Protein in alum adjuvant was injected by intramuscular injection into the hind legs. Animals were given booster injections 25 and 51 days after the primary immunization and were challenged with live virus 27 days after the last booser injection.
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How Bees Remember Flower Shapes

Abstract. *Bees are able to learn to distinguish between flowers with different shapes or patterns. Some studies have suggested that bees remember only isolated features such as spatial frequency and line angles, rather than the photographic search images that are characteristic of vertebrates. New data indicate that this presumptive vertebrate-invertebrate dichotomy is false; bees can store flower patterns as a low-resolution eidetic image or photograph.*

One of the most interesting questions regarding learning is how information is processed and stored. Animals do not remember everything about a food source, for example, but focus on a particular and characteristic subset of cues. In many species, the same animal will concentrate on different constellations of cues in different behavioral contexts (*1*). A honey bee, for instance, can learn and remember a pattern of polarized light that it has seen in the sky and use the pattern in subsequent orientation, but the bee cannot learn the same pattern when it is offered as a cue for a food source (*2*). Animals frequently display strong spontaneous preferences within a sensory modality. Honey bees, for instance, prefer to land on and learn to recognize most quickly violet-colored food sources (*2*). Storage of what is remembered is not free of such biases. Honey bees store information about food sources in time-linked sets and must forget everything about a flower in order to learn a single change—that is, if only a flower's odor is altered, the bee must relearn its color, shape, and other characteristics even though these have not changed (*3*).

How honey bees remember a flower's shape—its outline and the pattern of colors on its petals is not known. Hertz (*4*) concluded that the bees' spontaneous preference for highly dissected patterns (shapes with a high ratio of edge to area, or high spatial frequency) was so great that only the crudest sort of learned discrimination was possible. Later, investigators (*5*) found that shape learning could be much more subtle, but concluded that spatial frequency and other, yet undefined and less important, characteristics were remembered rather than a photograph-like (eidetic) image. This is an attractive model: great neural economy is achieved (*3*), and the learning, which may resemble alpha-conditioning, can be rapid and reliable (*1*). However, Wehner (*6*), who showed that bees could learn the angle of a set of parallel lines on a vertical food source, initially concluded that an eidetic image was involved but later said that the bees might instead have been remembering the parameter of line angle as an isolated feature.

The main argument in favor of the proposal that bees remember a set of isolated features such as spatial frequency and line angle is their inability to generalize. Hence, bees that have been trained to feed on a black triangle do not select a triangular-shaped checkerboard pattern over, for instance, a square

checkerboard pattern of the same area. This is not, however, an entirely compelling argument since even an eidetic image is likely to be highly colored by sensory processing. It could be, for example, that bees do remember eidetic images and that edges are the strongest feature stored in that picture. Hence, alteration of edge patterns to create a more general shape may be interpreted quite differently by bees and by human beings (*3*). It is also possible that bees could rely on "part figures" as rats do; although able to remember patterns photographically when necessary, rats will instead sometimes focus on one or more simple but diagnostic features when this strategy will work (*1*). The report of Collett and Cartwright (*7*) that bees probably store eidetic images of landmarks adds a certain plausibility to the possibility that bees remember shapes "photographically."

The essential difference between the isolated-feature or parameter hypothesis and the eidetic image or picture hypothesis is that only a picture necessarily preserves the spatial relation between components in the flower. I designed experiments to try to distinguish between these two alternatives.

Individually marked foragers from a hive of *Apis mellifera ligustica* were trained to a testing apparatus consisting of a green box that could rotate. Each of the four vertical faces of a box had two artificial flowers with different patterns: either flat disks 6.4 cm in diameter or funnels 6.4 cm in diameter and 5.0 cm deep. Only one face of the box was available to the foragers at a time. During training, one pattern (S^+) provided 1.5*M* unscented sucrose solution from a reservoir in the neck and the other (S^-) had none. On one face of the box, S^+ was on the right and S^- on the left; on a second face, S^+ was on the left and S^- on the right. By rotating the box, S^+ was on the left during half of the training visits and on the right during the other half. Bees made ten training visits before testing began. For testing, the third and fourth faces were used, where both the S^+ and S^- patterns were without food. In half the tests, S^+ was on the same side as the S^+ on which the foragers were last reinforced, and in the other half it was on the opposite side (*8*). Bees were tested singly and allowed to make five choices (*9*); then the apparatus was rotated to a training side and the forager was allowed to land and feed on the S^+ pattern. Another five choices were recorded on the next visit, and so on until the bee had either made 25 choices or, after 10, 15, or 20 choices, the bee had shown a strong preference (binomial test, $P < 0.01$) for the S^+ pattern (*10*).

As Fig. 1 shows, bees under these conditions were able to learn to distinguish between a variety of patterns that differed primarily or exclusively in the spatial relations among the elements. Consider the patterns at the upper left in Fig. 1 (A_1), for example: both S^+ and S^- have the same spatial frequency; both have equal areas of blue and yellow; both have equal lengths of vertical, blue-on-left and yellow-on-right boundaries, horizontal blue-on-top and yellow-on-bottom boundaries, and so on. Nevertheless, bees quickly and reliably learned to distinguish S^+ from S^-; the somewhat unlikely possibility that no learning had taken place and the bees were simply displaying a strong spontaneous preference can be ruled out by the complementary experiment (A_2, Fig. 1) in which S^+ and S^- were reversed. Perhaps the bees could distinguish S^+ from S^- without relying on an eidetic image by including among their memorized parameters the characteristics of the edges between the "flower" and the green background color. This possibility can be ruled out on the basis of experiments C, D, E, F, and G (Fig. 1); in each case, the features of the flower and background boundaries are identical between S^+ and S^-.

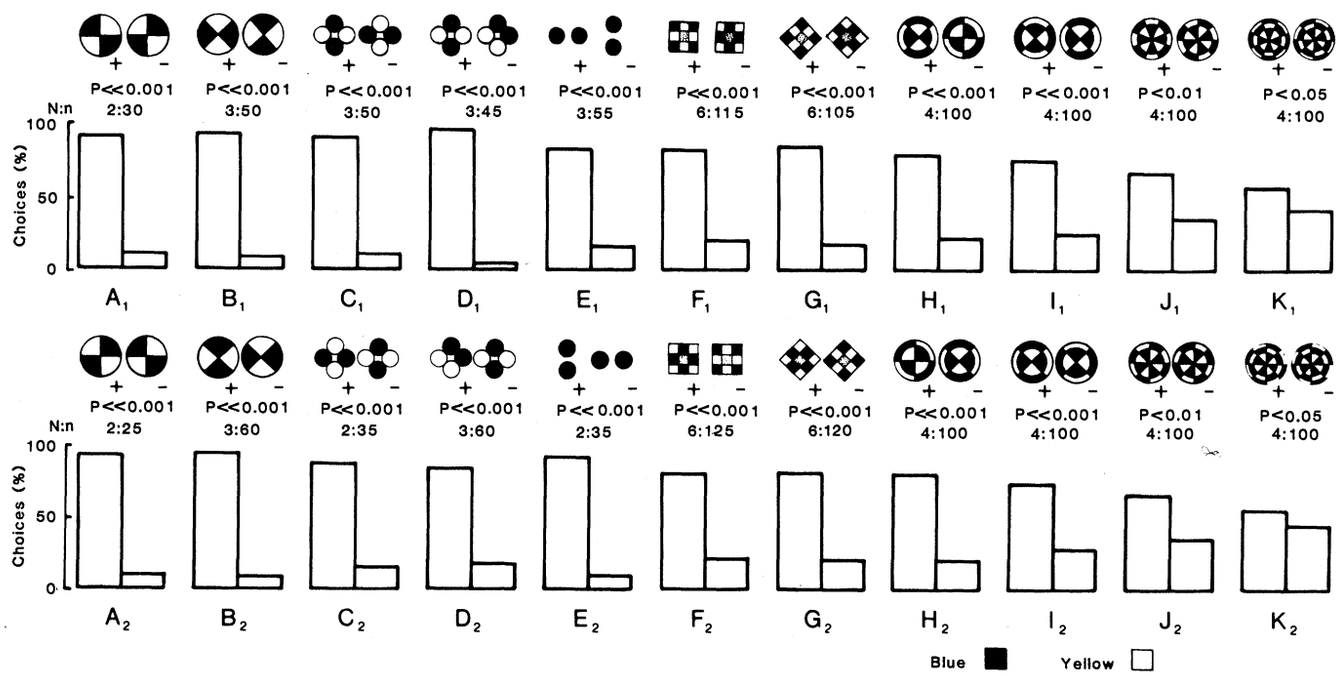

Fig. 1. Bees were trained to a pattern offering 1.5*M* sucrose (indicated by "+") ten times. During testing, bees were offered two unrewarded patterns and their choices recorded: *N*, number of bees tested; *n*, number of choices. Except for experiments H, I, J, and K, all bees individually displayed a preference significant at the 0.01 level or better. In experiment H, all bees individually displayed preference significant at 0.02 or better. The results of all bees tested were pooled; pooling appears justified because variance between individuals was small and because when sample sizes between bees were not equal, the pooled results are weighted toward the less accurate individuals and so underestimate the true average. The significance of the pooled results of each test are shown. The results of experiment K are not significant at $P < 0.05$. Patterns used in experiments C, D, E, F, and G were on discs; the rest were on funnels. The lightly shaded squares in the center of the patterns used in experiments F and G were painted green, the same color as the background.

These data suggest that the bees were remembering the spatial relationships of the elements in each pattern. This alternative is consistent with the results of experiments H_1 and H_2, in which bees had essentially the same degree of difficulty learning to distinguish two pairs of spatially complex patterns, even though prominent parameters of the H_1 pair (vertical and horizontal versus diagonal boundaries) were quite different, whereas in H_2 they were the same. The difficulty that bees experience by learning to distinguish spatially complex patterns is not likely to be a consequence of their poor visual resolution: measurements from the distance at which bees appear to "study" and choose between the patterns in these experiments indicate that a minimum of 50 ommatidia in each eye would be directed at each segment of the most complex pattern (J) that bees were able to learn; even for patterns that bees cannot distinguish after 25 trials (those in experiment K, for instance), half that number would be involved. More likely, perhaps, is that the limiting factor is the resolution of the eidetic storage in the bee's brain.

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8. The training procedure used here is different in two important ways from that used in most other studies: the patterns were displayed vertically, and the S^- was presented simultaneously. In nature, of course, few flowers are horizontal, and S^- abounds.
9. A "choice" consisted of a bee flying into a funnel and landing. When a trained forager found no food, it almost always flew out (the normal behavior observed on nectar-depleted blossoms), inspected both flowers by hovering in front of them, and then landed again. When, as occasionally happened, a bee took off, hovered briefly in the same flower, and then landed again, the second landing was not counted as a choice since the bee had not examined the alternative pattern.
10. The effect of an early stopping rule like the one used here in experiments A through G is to increase the overall chance of a false positive. By means of a 20-million event Monte Carlo simulation, I determined that the net effect was an increase of 2.37. The summed probabilities in Fig. 1 were revised to compensate for this effect. The summed probabilities in all the experiments are based on the assumption, usual to work on bee learning, that each landing is an independent choice. The validity of this assumption was tested in two ways. First, four of the experiments (B_2, H_2, I_1, and J_1) were repeated with a testing protocol allowing bees to land only once on each return. The data from these tests were compared to the data obtained from the blocks-of-five protocol used here; no difference was noticeable. (For experiment B_2, the summed single-visit-protocol data were 58 S^+/2 S^- versus 56 S^+/4 S^- for the five-block-protocol data; for H_2 the results were 79 S^+/21 S^- versus 79 S^+/21 S^-; for I_1 the results were 77 S^+/23 S^- versus 74 S^+/26 S^-; for J_1 the results were 62 S^+/38 S^- versus 65 S^+/35 S^-.) Second, I compared the choice patterns within and between blocks of test for all 22 experiments reported here as well as for 66 similar experiments. I asked whether sequential choices of the same pattern within a block were either more or less common than would be expected on the basis of the overall choice ratio, whether the first choice in each block was more or less likely to be correct than the second, third, fourth, or fifth, and whether within-block variance was either larger or smaller than between-block variance. Each of these comparisons indicated that choices within a block were indistinguishable for choices on separate visits, and thus can be treated as essentially independent.
11. I thank C. G. Gould, K. M. Schenck, and A. Weisenberger for building the testing apparatus and G. Watson for statistical advice. Supported by NSF grant BNS 82-01004.

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Regional Myocardial Substrate Uptake in Hypertensive Rats: A Quantitative Autoradiographic Measurement

Abstract. *Severe hypertension causes global and regional changes in myocardial perfusion and substrate utilization. Regional perfusion and fatty acid utilization were evaluated by dual-tracer autoradiography in normotensive and hypertensive rats of the Dahl strain. The regional distributions of perfusion and fatty acid utilization were homogeneous in normotensive rats. Severe hypertension was associated with a homogeneous pattern of regional perfusion, but fatty acid utilization was focally decreased in the free wall of the left ventricle. The decrease in fatty acid uptake was associated with a concomitant increase in glucose utilization. These findings suggest that severe hypertension is associated with uniform myocardial perfusion and focal alterations in the substrates used for the performance of myocardial work.*

Increased myocardial work caused by severe hypertension leads initially to myocardial hypertrophy and, if untreated, to heart failure (*1*). Although the hypertrophic myocardium has a proportional increase in myocardial perfusion, capillary density is decreased and the myocardial perfusion reserve is abnormal (*2*, *3*). The energy requirements of the normal heart in the basal state are usually met by catabolism of free fatty acids (*4*), with minimal contributions by lactate and glucose. The substrates used to provide energy during persistent increased myocardial work, such as occurs in prolonged hypertension or aortic stenosis, are less well defined. When the left ventricle is called upon to perform increased pressure work, the myocardium may not respond uniformly. In this circumstance, the effects on endocardium versus epicardium and on the free wall versus the septum of the left ventricle may differ (*5*, *6*). We investigated the possibility that persistent increases in pressure work are associated with alterations in the catabolism of metabolic substrates in hypertensive rats. Using quantitative dual-tracer autoradiography, we evaluated the regional distribution of glucose and free fatty acid analogs and correlated the results with changes in regional perfusion.

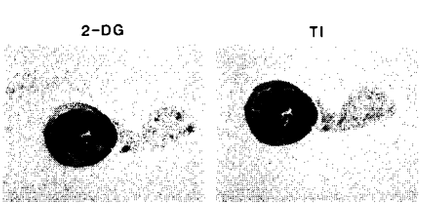

Fig. 1. Dual-tracer autoradiograph of a selected midventricular slice of myocardium from a hypertensive rat given injections of thallium-201 (Tl) and 2-deoxyglucose (2-DG) labeled with carbon-14. The thallium distribution is uniform, whereas glucose uptake is increased in the endocardium.

The Dahl strain of rats, derived from common Sprague-Dawley ancestors, have differing genetic predispositions to experimental hypertension (*7*). Originally, genetic separation was achieved on the basis of divergent blood pressure responses to ingested salt (NaCl). The hypertension-prone animals develop fatal hypertension from salt intakes to which hypertension-resistant animals respond mildly, if at all. Rats used in our study were weaned when they were 3 weeks old. Normotensive rats received 0.4 percent NaCl and hypertensive rats received 8 percent NaCl by weight in their food for 5 weeks. At age 11 weeks, the blood pressure of the rats was measured by tail cuff; the animals were weighed; and the regional myocardial utilization of substrate was determined. The mean systolic blood pressure of the hypertensive rats was 209 ± 13 mmHg, and their mean body weight was 228 ± 11 g. Normotensive rats had a mean