in that it points to a resolution of the apparent contradiction between the fact that perceptual capacities undoubtedly do change with age, and the fact that many local functions make their appearance very early in life.

T. G. R. BOWER

*Department of Psychology, William James Hall, Harvard University, 33 Kirkland Street, Cambridge, Massachusetts 02138*

### References and Notes

1. T. G. R. Bower, *Psychon. Sci.* **1**, 365 (1964); *Science* **149**, 88 (1965).
2. H. Helmholtz, *Physiological Optics III* (Optical Society of America, 1935).
3. For example, J. Piaget, *Les Mécanismes Perceptifs* (Presses universitaires de France, Paris, 1962); S. Klimpfinger, *Arch. Ges. Psychol.* **88**, 599 (1933). My discussion and experiments are aimed only at those theories of shape perception that assume that projective shape and perceived orientation precede shape constancy in perceptual development, and that projective shape, orientation, and real shape are separately registrable attributes of an object in space. Some projective shape-slant correlation models are not committed to the position of separate registration.
4. B. Stavrianos, *Arch. Psychol.* **61**, 5 (1944).
5. To ensure that the trapezoids used were pictorially equivalent to the rectangle in its various orientations, the latter was photographed in all four positions, and matte enlargements were pasted on the visible surfaces of the corresponding trapezoids.
6. The sequential testing methods that I used decreased total numbers of responses elicited on successive days without affecting the pattern of responding on any given condition. Therefore numbers of responses elicited by the unrewarded stimuli are expressed as proportions of the numbers elicited by the rewarded stimulus, thus cancelling absolute differences and allowing the pattern to appear clearly.
7. K. Eissler, *Archiv. Ges. Psychol.* **88**, 487 (1933).
8. Supported by the Milton Fund through Harvard University. I thank E. J. Gibson, J. J. Gibson, and H. N. Ricciuti for help and advice.

27 December 1965

## An Examination of "Transfer of Learning" by Nucleic Acid

Abstract. *Nucleic acid extracted from brains of trained animals and injected intraperitoneally into naive animals produced no "transfer of learning" effect on several tasks under many conditions. $P^{32}$-Labeled RNA was not found in the brain after intraperitoneal administration. Even intraventricular injections of nucleic acid produced no "transfer" effect.*

A recent article (*1*) clearly indicates the excitement currently generated in neurobiology by the discoveries in the field of molecular genetics. As pointed out, the application of such findings to an understanding of some of the long-existing problems of biological memory seems very promising. The research activities of many individuals attest to the current efforts directed toward a grasp of the relationships between nucleic acids, proteins, and biological memory (*2*). There are reports (*3*) that "transfer of learning" effects have been accomplished through the extraction of nucleic acid from the brains of trained animals and its subsequent injection into naive animals. Such a finding would, indeed, seem to furnish the first direct relation between nucleic acids and biological memory. We have used many different testing devices over a wide range of conditions and now report our unsuccessful attempts to find "transfer of learning" effects.

In the first study 20 mice (male Swiss-Webster albino, approximately 60 days of age) were trained on a two-choice brightness discrimination task to swim to the nonpreferred, darker alley. Ten additional animals were trained always to turn left regardless of the light's position. A raised platform in the correct alley allowed the mice to escape from the water (18°C). Each trial began when the platform was submerged, so that the mice were forced to swim to the appropriate one of the two remaining alleys. An error was recorded whenever the "dark-trained" animals entered the lighted alley or the "left-turn" animals entered the right alley. Every animal was given six training trials each day for 11 days. The intertrial interval was 30 seconds. After the training, the dark-trained animals were divided into two groups: group I consisted of ten animals that made no more than one error on the last 18 training trials; and group II consisted of ten animals that made as many as four errors out of the last 18 training trials.

Two groups of dark-trained animals, the left-turn group, and a group of untrained mice were killed, and their brains were quickly removed. The brains from each group were placed in cold phenol containing 0.15*M* NaCl (1:1) and homogenized with a tissue grinder. The aqueous phase was removed after centrifugation, and the nucleic acids were precipitated with two volumes of ethanol. The precipitate was collected by centrifugation and dried in a stream of air. The total nucleic acid collected for each group was then resuspended in 2.0 ml of 0.15*M* NaCl (*4*). A group of ten test animals served as recipients of each extract; each animal received intraperitoneal (I.P.) injections of 0.2 ml of the appropriate preparation. Ten mice were injected with 0.15*M* NaCl to serve as additional controls. In this and subsequent studies all animals were coded at the time of injection, and testing was completed without knowledge of the animals' prior treatments.

Eighteen hours after injection, the spontaneous activity of each animal was determined automatically for a 2-minute test interval. The activity was tested in a rectangular apparatus with a floor of metal plates that, when bridged by a mouse, activated an electromagnetic counter. Two hours later, the experimental animals were given six trials in the water maze with the same procedures described. The activity measures and the discrimination tests are presented in Table 1*A*. There were no significant differences between any of the experimental groups on either of the two behavioral measures.

Since the preceding study failed to produce any evidence of "transfer of learning," the extraction procedure was altered slightly to follow more closely one procedure reported to have given "transfer of learning" effects (*3*). This involved the addition of $MgCl_2$ to the nucleic acid before precipitation (*5*). Male Swiss-Webster mice were trained in the water "Y" maze. The procedures were the same as described except that all 20 mice were given six trials on a light-dark discrimination daily for 7 days. By the end of training, none of the trained animals had committed more than one error out of the last 12 trials. The mice were divided into two matched performance groups of ten animals each. A group of ten naive mice was also used. The brains were removed and processed in the usual manner, except that for the extraction of the brains of the naive group and one trained group, ethanol plus 0.1*M* $MgCl_2$ was used for precipitation (*3*). All of the resulting precipitates were resuspended in 0.15*M* NaCl to a final volume of 5 ml. The ten animals in each of the three groups injected (I.P.) with nucleic acid and the group injected with 0.15*M* NaCl were tested for a 2-minute spontaneous-activity sample 15 hours after injections. At 16 hours after the injections, all animals were

Table 1. Spontaneous activity scores and correct choices made in the first block of six test trials in the water "Y" maze. Additional blocks of six test trials each were given at 24-hour intervals (*A*) or at 12-hour intervals (*B*) but neither produced additional information. There were ten animals in each group. Results are given as means ± S.D. NA, nucleic acid; S, saline.

| Injection | Training | Activity measures | Correct* trials |
|---|---|---|---|
| | *A, 24-hour interval* | | |
| S | | 79.1 ± 15.9 | 2.2 ± 1.4 |
| NA | Naive | 75.9 ± 19.2 | 2.1 ± 1.4 |
| NA | Left-turn | 69.9± 23.1 | 2.7 ± 1.4 |
| NA | Dark trained (group I) | 75.8 ± 19.4 | 2.8 ± 1.8 |
| NA | Dark trained (group II) | 74.7 ± 16.9 | 2.5 ± 1.4 |
| | *B, 12-hour interval* | | |
| S | | 86.1 ± 13.1 | 2.1 ± 1.4 |
| NA | Naive ($MgCl_2$) | 77.3 ± 23.1 | 2.1 ± 1.1 |
| NA | Dark trained ($MgCl_2$) | 74.4 ± 23.9 | 3.0 ± 2.4 |
| NA | Dark trained | 86.9 ± 22.2 | 2.4 ± 1.3 |

* Statistically, an analysis of variance yielded scores which did not approach critical values of "F."

given six trials in the water "Y" maze. Subsequently, six test trials were given at 12-hour intervals (Table 1*B*). The addition of $MgCl_2$ before precipitation of the nucleic acids affected neither the activity nor the discrimination test scores.

In a further attempt to test the ability of brain nucleic acids to "transfer learning" to naive animals by way of I.P. injections, male rats (Sprague-Dawley derived, approximately 150 days of age) were trained on a light-dark discrimination in an automated shuttle-box discrimination apparatus. Two chambers were separated by a central partition with two plexiglass doors. On each trial the rats were required to push open a lighted door and shuttle to the other compartment in order to escape from a grid-delivered foot shock (1 ma). Selection of the dark, locked door resulted in a foot shock delivered by a small grid in front of that door and in the automatic recording of an error. The 30-second intertrial intervals and changing of the correct door positions were automatically controlled by electronic programming. Trials and errors were also automatically recorded. Ten male rats were trained daily to a criterion of nine correct choices out of ten consecutive trials. After 10 consecutive days of criterion performance the animals attained the performance criterion within the first ten training trials on each day. The ten trained and ten naive rats were killed with chloroform, and their brains were immediately homogenized in a mixture of phenol and 0.15*M* NaCl. The nucleic acid was prepared as described and was suspended in a solution of 0.15*M* NaCl containing 0.1*M* $MgCl_2$. The test animals were injected I.P. with the appropriate nucleic acid preparation. Approximately 18 and then 42 hours after injection, each rat was given 20 test trials in the discrimination apparatus. The two test groups did not differ significantly on performance on the test trials (Table 2*A*).

In a second experiment with rats the same techniques were used except that the "donor" animals received twice as much training (20 consecutive days of criterion performance). The recipient animals were tested at 8, 16, and 40 hours after they had been injected I.P. with 0.6 ml of the appropriate nucleic acid preparation (Table 2*B*). The changes in procedure were not effective in producing differential effects.

Since earlier reports of successful "transfer of learning" were based upon "donor" animals which had been trained in an appetitive situation (*3, 6*), an appetitive task was used in the next experiment. Male mice (60 days old, Swiss-Webster) that were deprived of water (that is, maintained at 80 percent of body weight) were first trained for 8 days to run a straight alley for a water reward. The mice were given a single daily trial in a modified Lashley III maze. Errors were scored whenever a mouse entered blind alleys. After 18 days of training, eight mice that had exhibited no more than one error throughout the last 3 days of training and eight naive animals were killed, and their brains were removed. The extraction procedures were the same as described. The resulting precipitate was resuspended in 0.15*M* NaCl containing 0.1*M* $MgCl_2$. The solutions were injected I.P. (0.5 ml) into two groups of eight mice that had received only "straight-alley" training to water. A control group that also received this prior training was injected with the 0.15*M* NaCl containing 0.1*M* $MgCl_2$ solution. After injections, the mice were given single test trials in the Lashley III maze every 8 hours. The three groups did not differ in performance on the test trials (Table 3).

Since the studies just described used a stringent performance criterion, a less complex task was employed subsequently. Fifty mice (60 days old, male Swiss-Webster) were trained in a shuttle box to approach either the light (Group A) or the dark (Group B) in order to avoid a grid foot shock (1 ma). Two groups of 25 mice each were given ten trials daily for 8 days. When the stimulus light switched from one side of the apparatus to the other (every 30 seconds), the animals could avoid a foot shock by moving to the appropriately lighted side of the apparatus within 5 seconds. By the 8th day of training, animals successfully avoided foot shock on 60 percent or more of the

Table 2. Error scores of rats in the automated light-dark discrimination apparatus following I.P. injections of nucleic acid solutions. Results are given as means ± S.D. of the number of errors.

| Training | Trials | | |
|---|---|---|---|
| | 1–20 | 21–40 | 41–60 |
| *A, test 18 and 42 hours after injection* | | | |
| Naive | 12.9 ± 5.6 | 12.0 ± 6.5 | |
| Light | 11.8 ± 3.4 | 11.1 ± 5.0 | |
| *B, test 8, 16, and 24 hours after injection* | | | |
| Naive | 11.9 ± 4.0 | 8.1 ± 4.3 | 5.8 ± 2.8 |
| Light | 9.7 ± 3.2 | 7.2 ± 3.6 | 4.7 ± 3.4 |

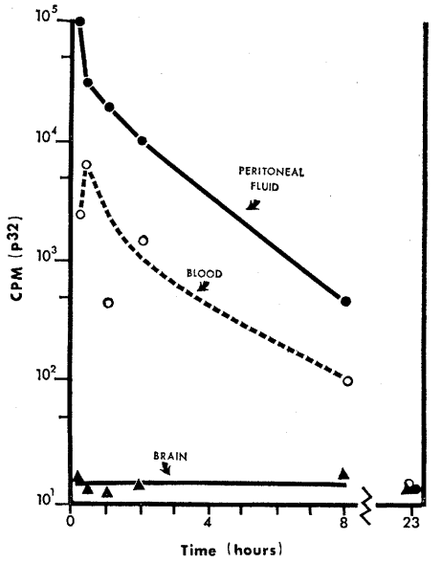


Fig. 1. Fate of $P^{32}$-labeled RNA after I.P. injection into rats. RNA from rat brain and mouse L cells was injected I.P. ($10^6$ count/min) and portions of peritoneal fluid, blood, and brain were removed at the times indicated. The amount of radioactivity in each sample after phenol extraction was determined with a Nuclear Chicago gas-flow counter. The results are a composite of three experiments and are presented as the number of counts per minute recovered in 1 ml of peritoneal fluid, 1 ml of blood, and the total brain (cerebrum and cerebellum).

Table 3. Initial errors in the Lashley III maze at 8, 16, and 24 hours after injection of nucleic acid (NA) solutions into water-deprived mice. Eight animals in each group; results are means ± S.D. S, saline solution; N, naive; T, trained.

| Injection | Training | Initial errors | | |
|---|---|---|---|---|
| | | 8-hr | 16-hr | 24-hr |
| S | | 5.6 ± 3.4 | 3.6 ± 2.6 | 2.6 ± 2.2 |
| NA | N | 4.4 ± 2.5 | 4.1 ± 2.9 | 2.6 ± 2.0 |
| NA | T | 4.8 ± 2.8 | 3.4 ± 2.5 | 2.1 ± 2.4 |

trials. On the remaining trials, escape responses were very rapid and efficient. After the training period, the brains were removed from the two groups of trained mice and from a control group of 25 naive mice.

The nucleic acid precipitates were prepared in the usual manner except that the $MgCl_2$ was omitted in resuspension with 0.15*M* NaCl. The final preparations were injected I.P. into eight experimental mice per "donor" group. Thus, the test mice were injected with the equivalence of brain nucleic acid collected from three "donor" animals. A control group of eight mice received an injection of 0.15*M* NaCl. Four hours after injections, each animal was tested in the shuttle box. The testing began with the animals on the lighted side, and stimulus lights changed sides every 30 seconds. No foot shock was given. An activity floor consisting of electrically conductive square sections was placed over the grid floor. During the 5-minute, 10-test trial session, the amount of time spent in the lighted side of the apparatus was measured. Spontaneous activity exhibited by each animal under both the light and the dark conditions was measured. Finally, the number and direction of shut- the responses on each trial were recorded (Table 4. Even with the modest performance demands of this task and the enhanced likelihood of transfer with the larger amount of nucleic acid injected, no evidence of "transfer of learning" was observed.

Since we could not detect a transfer of learning when the crude nucleic acid preparations described above were injected I.P., we thought it imperative to determine whether the RNA contained in these preparations could cross the blood-brain barrier and be detected in the brain. Accordingly, RNA in brain was labeled in vivo by the injection of $P^{32}$-orthophosphate directly into the intraventricular cavity. In addition, $P^{32}$-labeled RNA was prepared in mouse L cells propagated in tissue culture. The RNA was extracted as from the tissue described except that the resulting material was reprecipitated, in the presence of 0.001*M* ethylenediaminetetraacetate (EDTA), at least ten times to remove the bulk of ethanol-soluble $P^{32}$. Sprague-Dawley rats were then injected I.P. with $10^6$ count/min of labeled RNA (approximately one-half brain per recipient animal). Animals were killed at various times, and the radioactivity was measured in the animal's peritoneal fluid, blood, and brain (Fig. 1). The early high concentrations of radioactivity were rapidly cleared from the peritoneum and blood, and no significant amounts of radioactivity could be found in the brain. However, most of the radioactivity was excreted in the feces and urine.

In view of the finding that little, if any, nucleic acid passes the blood-brain barrier after I.P. injection, a final attempt to induce "transfer of learning" was made by directly injecting the nucleic acid into the ventricles of the brain. Initially, 60 Swiss-Webster male mice approximately 55 days of age were prepared as "donors." Thirty mice were given inhibitory (or "passive") avoidance training on a "step-through" apparatus. The mice were trained to remain on a small platform mounted in front of a hole leading to a darkened box. Entries were punished with a momentary 4-ma foot shock. Each mouse was given massed training trials with a 30-second intertrial interval until it remained on the platform for 30 seconds. All animals were retrained to the same criterion every 12 hours for a total of seven training periods. At the time of training, each of the other 30 animals was paired with a trained animal in such a way that it received comparable foot shock in a small lucite box with a grid floor each time an experimental mouse was shocked.

After training, 12 trained and foot-shock control pairs of animals were randomly selected from the two groups. A group of 12 naive mice was also used for controls. The mice were then killed, and their brains were removed and placed into ice-cold Hanks balanced salt solution. The brains of each group were then placed in a solution of phenol, 0.15*M* NaCl, and 1 percent sodium dodecyl sulfate (SDS) at 60°C and homogenized with a tissue grinder. After two successive precipitations, the nucleic acid was dried and resuspended in 0.15*M* NaCl to a total volume of 0.5 ml. Five groups of 12 test animals each were used either as nucleic acid "recipients" or as control groups. Bilateral intraventricular injections of 20 $\mu$l of solution were made with 27-gauge hypodermic needles while the mice were lightly anesthetized. One control group received intraventricular 0.15*M* NaCl, and the other received no injection but was etherized. The re-

Table 4. Illumination preferences, activity measures, and shuttle responses collected from mice tested in the shuttle box. Eight animals in each group; NA, nucleic acid; S, saline.

| Injection | Training | Mean time in light (sec) | Activity | | Shuttle responses (No.) | |
|---|---|---|---|---|---|---|
| | | | In light (mean) | Total (mean) | Light to dark | Dark to light |
| S | | 172.0 | 130.7 | 212.4 | 24 | 41 |
| NA | Naive | 158.3 | 123.9 | 204.1 | 26 | 40 |
| NA | Dark | 184.0 | 140.6 | 209.8 | 16 | 50 |
| NA | Light | 166.3 | 129.3 | 203.1 | 23 | 42 |

Table 5. Response latencies of test animals on the step-through apparatus after intraventricular injections of the appropriate nucleic acid preparations. NA, nucleic acid; S, saline. Results are means ± S.D.

| Injection | Training | Number of animals | Response latencies (in seconds) | |
|---|---|---|---|---|
| | | | 1st trial | 2nd trial |
| S | | 12 | 13.2±5.7 | 17.8± 6.1 |
| None | (Ether control) | 12 | 5.7±2.7 | 17.2± 5.7 |
| NA | Naive | 12 | 7.3±8.9 | 17.3± 7.7 |
| NA | Foot-shock | 12 | 6.8±6.4 | 13.5± 7.2 |
| NA | Trained | 12 | 9.0±6.8 | 17.9± 8.7 |
| None* | "Donor" trained | 18 | 29.9±0.4 | 30.0± 0.0 |
| None* | "Donor" foot-shock | 18 | 10.3±7.5 | 22.6±11.2 |

* These animals were from the same groups of animals that were prepared as "donor" animals. They were retested at the time of testing of the injected experimental animals.

maining 18 animals in each of the "donor" groups were used to provide comparisons for the five experimental groups. At 18 hours after injections, all animals were given inhibitory avoidance training as described. The amount of time each animal stayed on the small platform before entering the darkened hole was recorded on each of two test trials. The latencies of the recipients of "trained" RNA did not differ significantly from those of animals in the other groups (Table 5). In contrast, it should be noted that with the exception of but one animal, those animals which were originally trained demonstrated criterion performance on the two retention tests.

In conclusion, the numerous variables which provided unsuccessful attempts to demonstrate "transfer of learning" should be summarized. First, several training and testing tasks have been used in both the preparation of the "donor" animals and the subsequent assay for "transfer of learning" effects. As may be judged from the number of trials required for original learning by "donor" animals, these tasks cover a wide range of relative difficulty. Spontaneous activity measures and response latency scores were included to provide an assay of more general transfer effects that might not necessarily be associated with learning. Motivational variables manipulated included foot shock, water deprivation, and cold-water immersion. Additional variables included the use of two species of animals, the degree of "donor" training, and the inclusion of testing intervals longer than those previously reported (*3, 6*). Under these diverse conditions, the results provided no evidence of "transfer of learning." The nucleic acid extraction and administration procedures were selected to minimize the possibility of losing any fraction that might be responsible for the "transfer of learning." Cold phenol extractions and precipitations with and without $MgCl_2$, as well as hot phenol extraction with SDS, failed to yield an "active" fraction of nucleic acid. Because of the lack of evidence that nucleic acid crosses the blood-brain barrier after I.P. administration, the nucleic acids were introduced directly into the brain by intraventricular injections. Yet, even under these conditions, there was no evidence for a "transfer" effect. Finally, the amount of total nucleic acid injected into the test animals was varied from the equivalence of nucleic acid from one brain to the equivalence from three brains. Still no "transfer" effect was found.

Although the training schedules and devices used have proven very effective in other studies of conditions affecting learning and memory, findings of "transfer of learning" via RNA reported by others (*3, 6*) were not corroborated in our laboratories. Rather detailed replications of those procedures originally reported as successful (*3*) have also failed to produce "transfer of learning" effects (*7*). Such negative findings suggest that the reported "transfer" effect, if it exists, is either a very limited phenomenon or a very difficult one to reproduce.

MARVIN LUTTGES, TERRY JOHNSON
CLAYTON BUCK, JOHN HOLLAND
JAMES MCGAUGH
*Department of Psychobiology*, and
*Department of Molecular and Cell Biology, University of California, Irvine*

**References and Notes**

1. Francis O. Schmitt, *Science* **149**, 931 (1965).
2. W. Dingman and M. B. Sporn, *J. Psychiat. Res.* **1**, 1 (1961); R. W. Gerard, T. J. Chamberlain, G. H. Rothschild, *Science* **140**, 381 (1963); H. Hydén and E. Egyhazi, *Proc. Nat. Acad. Sci. U.S.* **49**, 618 (1963); D. E. Cameron and L. Solyom, *Geriatrics* **16**, 74 (1961); B. W. Agranoff and P. D. Klinger, *Science* **146**, 952 (1964); J. B. Flexner, L. B. Flexner, E. Stellar, *ibid.* **141**, 57 (1963).
3. F. R. Babich, A. L. Jacobson, S. Bubash, A. Jacobson, *Science* **149**, 656 (1965); A. L. Jacobson, F. R. Babich, S. Bubash, A. Jacobson, *Science* **150**, 636 (1965).
4. The average wet weight for a mouse brain was 310 mg, and the average nucleic acid yield was 0.24 mg. For rats the average wet weight was 1430 mg, and the average nucleic acid yield was 1.20 mg. The amount of nucleic acid was determined by spectrophotometer measurements at 260 mμ.
5. The addition of $MgCl_2$ during ethanol precipitation rendered the nucleic acid precipitate nearly insoluble in 0.15*M* NaCl.
6. E. J. Fjerdingstad, Th. Nissen, H. H. Roigaard-Petersen, *Scand. J. Psychol.* **6**, 1 (1965).
7. C. G. Groves and F. M. Cory, *Science* **150**, 1749 (1965).
8. Supported by research grant GB 2301 from NSF and MH 10261 from NIH and postdoctoral fellowships (1-F2-A1-23-167-01, 1-F2-A1-13,382-01) from the NIAID. We thank H. Alpern, D. Lerner, and W. Sparks for technical assistance.

10 November 1965

## Imagery: Effect of a Concealed Figure in a Stimulus

Abstract. *A concealed figure formed by the contours of a perceptually dominant figure influenced the content of viewers' subsequent imagery, although in describing the stimulus they showed no awareness of the concealed figure even after several exposures.*

In a common picture puzzle, the contours of a perceptually dominant figure conceal a recessive but independently identifiable shape. The concealed form is rarely spontaneously perceived. Normally, not until the viewer is told what to look for and where to look does he perceive the hidden figure.

What is the psychological status of such a concealed figure? According to the Gestalt point of view (*1*), it should have no independent status as a percept within the total configuration; only when the field is reorganized so that the concealed shape becomes figural ought it attain perceptual effectiveness.

Another view is that the stimulus potency of the concealed figure as meaningful content is not ruled out by its being experientially weak or never consciously perceived (*2*). One possibility is that some response evoked by a picture which contains a concealed figure will include content provoked by that figure. According to this view, the actually reportable percepts are only a segment, although the dominant one, of an ensemble of responses and associations activated by the entire configuration. The ensemble includes reported and unreported connotations of the dominant percept, and unperceived but nevertheless registered aspects of the picture array as well. These recessive particulars of the configuration are not likely to be evident in a direct report of perception but may emerge indirectly in freer, more open-ended modes of response, such as imagery. The present experiment was concerned with the question whether an unreported concealed figure will influence subsequent imagery.

The experimental stimulus, taken from Elkind *et al.* (*3*), was a picture containing two forms, a perceptually dominant tree and a perceptually recessive duck shaped by the branches of the tree; the control stimulus showed only the tree modified so as to eliminate the outlined duck (see Fig. 1). All subjects were instructed as follows: "A picture will flash on the screen three times. When the picture goes off, I want you to sit back, relax, close your eyes, and wait for an image of a *nature scene* to come to your mind's eye.