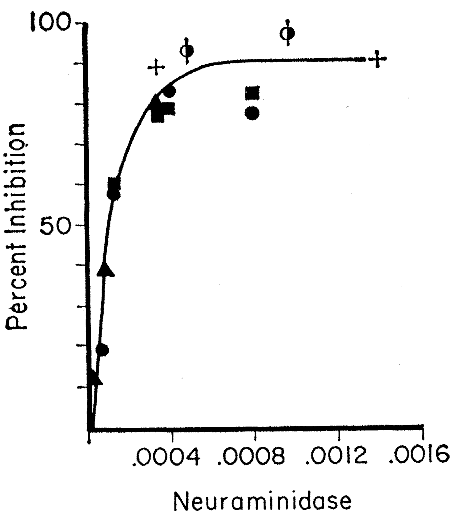

Fig. 2. Protective effect of bacterial neuraminidases (units per egg) against Lee-B virus. *Clostridium perfringens*, solid triangle, cross; *Vibrio cholerae*, half-solid circle, solid square, solid circle.

photungstic acid in $0.1M$ HCl, and the sialic acid liberated was determined on a portion as described by Warren (*11*). The results are expressed as units of enzymatic activity; 1 unit is the amount of enzyme that liberates 1 μmole of sialic acid in 1 minute under the conditions stated.

Eleven-day-old embryonated eggs were used. One-tenth milliliter of the appropriate neuraminidase was injected into the allantoic cavity, and the eggs were returned to the incubator for 2 hours before they were injected with 0.1 ml of diluted infective allantoic fluid containing Lee-B or PR-8 virus. The fluid infected with Lee-B virus had a hemagglutination titer of 640 units per milliliter—$10^{7.2}$ EID$_{50}$/ml (egg infectious doses, 50 percent effective)—and was diluted 1 to 1000 before administration. The PR-8 infective fluid had a hemagglutination titer of 1280 units per milliliter ($10^{8.6}$ EID$_{50}$/ml) and was diluted 1 to 100,000 before administration. After 48 hours the infected chorioallantoic fluid was harvested from each egg, and its hemagglutination activity determined by the method of Salk (*12*). In each test group, 10 to 20 eggs were used and the hemagglutination titers were averaged. The percentage protection is expressed as the difference in averages of control and test groups divided by the control times 100.

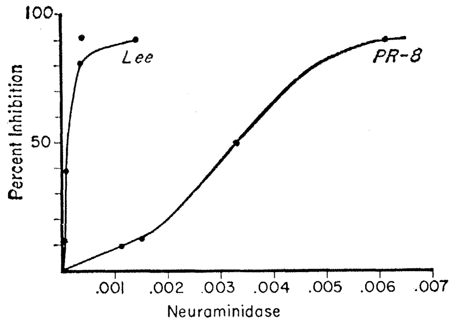

Fig. 3. Comparison of the protective effect of neuraminidase (units per egg) from *Clostridium perfringens* against PR-8 and Lee-B viruses.

The "protective" effect of several concentrations of neuraminidase isolated from Asian influenza virus on infection by Lee-B virus in four typical experiments is shown in Fig. 1. The effect becomes maximal at about 0.0004 units of neuraminidase per egg. Similar results with bacterial neuraminidases are shown in Fig. 2. Again, maximal protection occurs at about 0.0004 units per egg.

It was shown by Stone (*1*) that the protective effect of neuraminidase from *V. cholerae* varied with different viruses. Figure 3 shows that the same is true with the neuraminidase from *C. perfringens* which is more effective against Lee-B than against PR-8 virus.

These results extend previous observations with the neuraminidase of *V. cholerae*, and show that neuraminidase from Asian influenza virus and from *C. perfringens* can also protect cells of the chick embryo against infection by certain strains of influenza virus. The results are in accord with the proposition that specific receptor sites containing sialic acid on infectible cells are required for attachment and attack by influenza viruses, and that different viruses vary in the extent to which they depend on the "intactness" of these receptor sites.

Our experiments clearly show that the protective action is due to neuraminidase since very similar results are obtained with highly purified neuraminidase from several sources. The effect is a temporary one, since after 72 hours the titers of control and protected groups are comparable. This indicates that the receptor sites tend to regenerate over a period of time as previously reported by Stone (*1*).

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6 January 1964

Visual Problem-Solving in a Bottlenose Dolphin

Abstract. *A captive 8-year-old dolphin, well adapted to contact with human beings, was tested by the discrimination method for underwater perception of visual forms or patterns. The animal successfully discriminated 21 of the 25 pairs of stimuli presented. After having learned a particular combination, the dolphin was immediately able to respond to different but related pairs which had been modified in various ways. The memory of the animal for discriminations previously made was excellent.*

Although a good deal of speculation exists on the problem-solving ability of the bottlenose dolphin, *Tursiops truncatus* (Montagu), there is little scientific information on the subject. Many observers, impressed by the striking performances and playful antics of captive specimens, have been quite willing to assign a high order of achievement to this marine mammal. More than 15 years ago McBride and Hebb (*1*) observed that *Tursiops* is a very superior animal indeed, and they ranked it somewhere between the dog and the chimpanzee with respect at least to emotional and motivational behavior. Others have pointed to the size and complexity of the cerebral cortex and higher brain centers (*2*). Since degree of cerebral complexity is often thought to imply behavioral capability, we have here again indirect—but only indirect—support for the notion that the dolphin is capable of a high level of problem-solving performance (*3*). Clearly what are needed are some first-hand measures of this ability.

It was the object of the research reported here, carried out at Florida State University, Tallahassee, to examine the problem-solving processes of *Tursiops truncatus* that involve the optical instead of the auditory or acoustical receptor. We hoped to find out something about the dolphin's ability in test situations without reference to the well-known echo-ranging or sonar method of perception used by this animal (*3*).

An 8-year-old bottlenose dolphin served as a subject in the tests. It had been in captivity for 7 years and was unusually well adapted to contact with human beings. It was 2.3 m long and weighed about 135 kg.

The animal was kept in a rectangular concrete tank or pool filled with clear, filtered seawater. The surface area of the tank was 6 × 10 m, and the water depth was 2.4 m. The entire aquarium was enclosed and roofed over; thus there was protection from the weather and considerable control of ambient illumination. The stimuli were presented beneath the water by the two-choice discrimination method.

Any movement on the part of the experimenter, which might have served as an uncontrolled cue, was concealed by a vertical plywood screen. The screen extended upward from about 5 cm below the surface of the water to a point 110 cm above the surface. A stimulus-panel or stimulus-board, 60 cm on a side, could be lowered into the water from behind the screen. The patterns to be discriminated were viewed through two transparent Lucite windows, 30 cm apart, in this stimulus-panel. A perpendicular divider, also of of transparent Lucite, projected downward and outward from between the stimuli, thus preventing the animal from moving sideways from one pattern to the other. Figure 1 is a diagram of the apparatus.

The stimulus figures were in the form of stencils. They were made from thin shimming brass which was painted a flat black. A white background behind the cut-out portion of each stencil caused its shape to stand out. Common geometric forms were used, such as a circle, a triangle, a diamond, a star, and a heart. With two exceptions, each of the patterns had an area of 13 cm^2. When tested (underwater) in the illumination of the experimental environment, the white reflecting surfaces of the figures gave light intensity readings of 8 to 10 meter-candles.

In arranging the forms into pairs to be discriminated, a figure was often used in several different contexts. Thus, in successive combinations the heart was paired with a cross, with an angle bar, with an X, and with an inverted heart. In some cases a given pattern was positive, and in others the same pattern was negative (see Table 1). Such complications were deliberately introduced in order to test the animal's perceptual abilities. As is customary with the discrimination method, the patterns were randomly rotated from side to side according to a chance sequence.

A trial began with lowering of the stimulus-board into position and ended when the subject had indicated a choice by pressing the tip of its "chin" against one or the other of the forms. The minimum intertrial interval was 30 seconds, and during this interval the dolphin returned to a fixed starting point about 6 m in front of the apparatus. There it waited for the sound produced by lowering the stimulus-board on the next trial. If the dolphin was slow in returning to the starting place (as it was on occasion), the intertrial interval was necessarily lengthened.

All successful responses were reinforced. This was accomplished by dropping a small piece of thread herring (*Opisthonema oglinum*) through a feeding slot above the water on the side of the positive stimulus (see Fig. 2). Since each piece of herring was cut so as to weigh about 15 g, an entire session of approximately 100 trials could be conducted with 1½ kilograms of fish. The periods of experimentation and of testing (including rest intervals) lasted from 8:30 A.M. to about noon.

In all, the animal had more than 7000 choices or test trials in the course of this study, but in the early stages most of these were exploratory and were necessary to develop a standardized and workable procedure. Some of these pretraining trials were made with the stimuli in air above the water line (*4*). A typical underwater response is illustrated in Fig. 2.

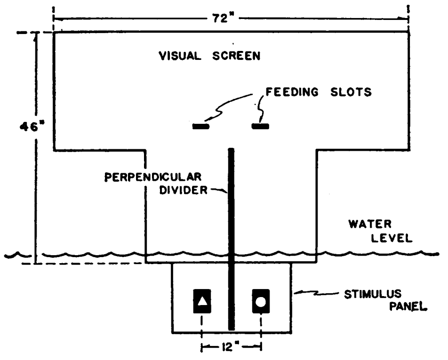


Fig. 1. Diagram of underwater discrimination apparatus, showing principal dimensions. The perpendicular divider, which protruded 30 cm from the screen, was constructed of clear Plexiglass beneath the water line.

The most effective procedure for training the animal to learn a new discrimination, it was found, was a special version of the method of approximation. One of the first problems mastered by the dolphin had been that of distinguishing between a light and a dark area—as, for example, between a sheet of brass painted black and a similar sheet in natural brass color. To make use of this already familiar intensity differential to guide the dolphin in learning to discriminate between forms or shapes which it had never before seen, the experimenters first showed the positive stencil of the new pair, completely black. At the same time, the negative pattern was presented with a brass border around it. This served as a frame around the white area of the

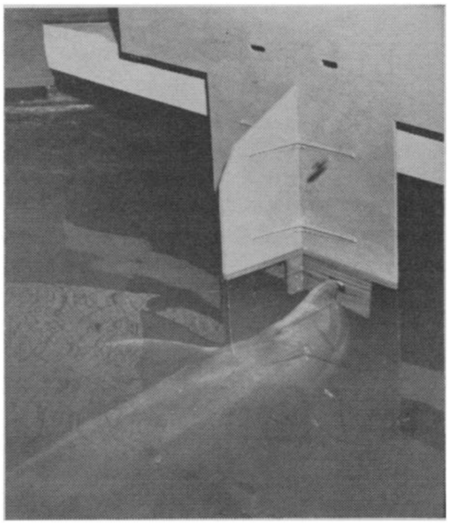


Fig. 2. A successful underwater response in the test situation. The dolphin has just touched the positive stimulus with the tip of its "chin" and is being rewarded with a small piece of fish, dropped from a feeding slot in the visual screen. The fish may be seen falling toward the water.

Table 1. Stimuli which were reversed in different combinations.

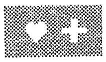
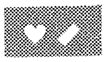
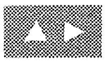
Stimulus pattern	Positive when paired with:	Negative when paired with:
Circle	Horizontal dumbbell	Triangle
Square	Inverted	Horizontal diamond
Cross	Angle bar	Heart

negative stimulus, making it appear brighter as well as larger than the positive stimulus.

The width of the brass border encircling the negative shape was then progressively decreased. For approximately the first 20 trials the border was 1.25 cm wide. For the next ten trials it was 0.9 cm. For the third group of ten trials it was 0.6 cm, and for the fourth group, 0.3 cm. When the border around the negative form had been removed entirely, the discrimination was then made solely on the basis of form instead of on the basis of brightness or intensity. The criterion of mastery in these learning situations was 20 errorless discriminations in succession. A similar technique has recently been described by Terrace (*5*) in training pigeons to distinguish vertical from horizontal lines.

Of the 25 different stimulus pairs which were shown the dolphin, it successfully discriminated 21, or 84 percent. For 11 of these pairs, discrimination was learned by the method of approximation just described. For the remaining ten pairs, discriminations were made immediately in special tests, without any further training. For these special tests, a well-learned (or previously learned) pair was shown for ten additional (or post criterion) trials, and these trials were immediately followed by ten further trials with an entirely new (or test) pair without any interval or interruption. A pair of test stimuli presented in this way varied from the originally learned pair which preceded it in having either one or both of its patterns altered. In some instances the positive pattern of a test pair was changed, in some instances the negative pattern was changed, and in some instances both stimuli of the new pair were different. All such tests are actually instances of transfer of training, or of transposition of a discrimination.

Table 2. Tests in which the negative stimulus was changed. (Here the positive stimulus for each pair is at left, the negative stimulus at right.)

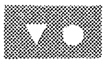
Stimulus		Subject's response
Original pair	Test pair	
		Successful
		Successful
		Successful

Of special significance in this connection is the use of the triangle-circle and triangle-triangle combinations. An equilateral triangle with its apex pointing upward (positive stimulus) was paired with a circle (negative stimulus). The dolphin learned this combination through practice. The animal was then immediately tested with a triangle with apex downward and a circle; a triangle with apex to the left and a circle; and a triangle with apex to the right and a circle. It solved all of these problems at once, giving ten perfect trials in each case. It went to the triangle in each case, regardless of the fact that the triangle had been rotated.

Subsequently the animal was trained to discriminate a triangle with apex upward (positive) from a triangle with apex downward (negative). Upon reaching criterion in this new learning, it was tested with a triangle with apex upward against a triangle with apex to the left, and with a triangle with apex upward against a triangle with apex to the right. In these test trials the dolphin now went without error to the triangle with apex upward and avoided the other triangles (see Table 2).

With problems such as these, complicated relationships exist. When paired with the circle, all four triangles are positive shapes and are consequently to be approached. Yet when paired with an apex-upward triangle, the other three triangles are negative shapes and are to be avoided. Three of the triangles, in other words, are negative when paired with a fourth triangle but positive when paired with the circle. Such reversals can be confusing. In some cases even the experimenters found it hard to keep them straight. In spite of such difficulties the dolphin had little or no trouble responding to, and retaining, these combinations and could respond to one after another within a single experimental session without making a mistake. It made two discriminations without error after an interval of 7 months.

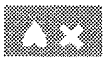
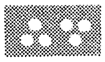
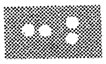
Table 3. Tests in which the positive stimulus was changed. (Here the positive stimulus for each pair is at left, the negative stimulus at right.)

Stimulus		Subject's response
Original pair	Test pair	
		Successful
		Successful
		Successful
		Successful
		Successful

The various relationships between the originally learned discriminations and the corresponding test problems are shown in Tables 2–4 (*6*). "Successful" means that the dolphin immediately made ten correct responses in the test situation. "Unsuccessful" responses are chance or near-chance performance in response to the test patterns. There were no real intergrades. The dolphin either passed the ten-trial test or failed it. Of 14 such tests illustrated in Tables 2–4, the dolphin passed ten and was unsuccessful in four.

In Table 2 are represented three tests in which only the negative stimu-

Table 4. Tests in which both positive and negative stimuli were changed. (Here the positive stimulus for each pair is at left, the negative stimulus at right.)

Stimulus		Subject's response
Original pair	Test pair	
		Successful
		Successful
		Unsuccessful
		Unsuccessful
		Unsuccessful
		Unsuccessful

lus was changed (*7*). It can be argued in such instances that the subject was simply approaching the positive pattern and ignoring the negative. Against this interpretation, however, are the responses to the tests of Table 3, in which the positive form was changed but the negative remained constant. In these cases, one may say, the animal was only avoiding the negative stimulus in going to the various positive stimuli. Yet neither of these arguments holds for the forms of Table 4, where *both* of the test stimuli differed from those in the originally learned pair.

The dolphin passed two of the tests of Table 4 but failed four of them. Those which it failed, it appears, required stretching of an association or transposition beyond the animal's capability. Probably the dolphin could have *learned* to make the discriminations which it failed to make in these tests had it been given regular training in doing so, but in the "unsuccessful" responses of Table 4 the stimuli were not recognized as being related to the learned pairs.

Although the literature on the problem-solving abilities of other animals is extensive, few studies have been made under conditions sufficiently like those described here to permit valid comparison. The work of Klüver (*8*) with monkeys and that of Robinson (*9*) on chimpanzees is indirectly related to our studies. Each of these investigators trained his subjects with one set of visual stimuli and tested them with others. Klüver's tests were based on discrimination of size differential between paired visual designs, and Robinson's, on discrimination of sameness and differences between three-dimensional objects. After a subject had mastered the common principle involved, it could perform test problems by applying the principle to them. On the other hand, the dolphin in our tests had no single rule or principle by which to make the transfer from the originally learned pairs to the corresponding test pairs; the arrangement was more complex than one in which the same rule is followed in multiple situations.

The studies by Rensch (*10*) on a 5-year-old Indian elephant are probably closest to our studies with the dolphin. When tested in a two-choice situation, Rensch's elephant learned to discriminate 20 different pairs of figures. Rensch also tried altering some of the learned pairs (the method employed in our tests) and found that the elephant could transpose the learning to test pairs which were changed in various ways. Rensch believes (as we believe in the case of the dolphin) that his subject could, with additional training, have learned the correct response to many more pairs.

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6. Tables 2–4 include only those patterns which were used in the test situations. Other pairs of stimuli (not included in the tests) which the animal also learned to differentiate were as follows: a star versus a reversed L; a vertically oriented diamond versus a chevron; a cross versus an inclined bar; a horizontal diamond versus a square; a square 13 cm^2 in area versus a square 19.5 cm^2 in area.
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11. This research was supported by National Science Foundation grant 20354. We are indebted particularly to John H. Hurlbut of the Johns Pass Aquarium at St. Petersburg, Fla., for permitting us to use his remarkable dolphin, Paddy, in these experiments. Cooperation and assistance were also given by Dr. Howard D. Baker and Karl Maier of Florida State University and by Dean John M. Bevan, Tom Dillon, Bill LaBrant, and Larry Southard of Florida Presbyterian College. The research was carried out during the summer of 1962 and the spring of 1963.

12 November 1963

Drug Administration to Neonatal Rats: Effects on Later Emotionality and Learning

Abstract. *The effects of extra stimulation during postnatal days 2 to 4 in rats are mitigated by the injection of either norepinephrine or chlorpromazine prior to the stimulation. Behavioral changes in locomotion and defecation occurred in the open field, and there were changes in speed and accuracy of learning a simple maze problem.*

There is a substantial amount of evidence to indicate that extra stimulation, when experienced during infancy, has profound effects on adult behavior. In many of the studies from which significant results have been obtained, the neonate was stimulated physically (by handling, mechanical rotation, loud sounds, or temperature variations, and so forth) and the animals became less emotional and more resistant to stress. The mechanisms by which these effects are mediated have remained unexplored.

It has been generally accepted that when an organism encounters a sudden or marked environmental change, or an emergency situation, there results a massive autonomic response (*1*). The organism is mobilized to a "flight or fright" response by the sympathetic division of the autonomic nervous system. Extra stimulation in early infancy may represent such a situation.

The pituitary-adrenal axis appears to be essential for the adult stress response; little is known of the biological basis of the infantile stress response. Pituitary and adrenal responsiveness to stimulation has been demonstrated in infant rats. However, there is still some question as to the age at which there is a functional unity of the axis (*2*). Regardless of whether or not there is complete maturation of the axis, it is possible that even partial activation of this system may alter enzymatic processes which could produce long-term impairment of the physiological mechanisms upon which the adult animal depends for stress reactivity and emotional behavior.

One approach to the study of this problem is to inject the neonate with hormones which initiate the stress syndrome while systematically manipulating the environment. As a second approach, which we devised for exploring the mediation of early experience on later behavior, we injected chlorpromazine, following Killam's suggestion (*3*) that chlorpromazine may enhance the central filtering-out of afferent impulses. Our experiment was designed specifically to examine the effects of infantile extra stimulation, the injection procedure, the pharmacological treatment, and the possible interactions between the extra stimulation and pharmacological treatment on later emotional and learning behaviors.