sacrifice of the animals, the final selection of animals for presentation from a group of ten animals being based on the close similarity of brain amine levels for the two animals at the time of sacrifice. The EEG tracings of Fig. 2*B* present the familiar EEG arousal pattern in rabbits reported by Rinaldi and Himwich (*2*) for reserpine. Similar patterns of EEG arousal were obtained for every animal studied by means of postsurgical reserpine administration after a minimum drug duration of approximately 1 hour. The EEG tracing of Fig. 2*A* reflects the enhancement of the resting rhythms of the brain, which is the expected finding based on the calming action of reserpine. Again, as with etryptamine, the treatment of these two animals was identical except for the administration of drug either before or after surgical preparation for EEG recording.

An obvious conclusion from the results presented is that under some circumstances the type of EEG effect obtained is a direct function of whether the experimental drug has been given before or after the animal has been prepared for EEG recording. The effects reported take on added significance in view of the extensive use made of the rabbit in pharmacological and neurophysiological research. While the mechanism responsible for this difference in effects is unknown, it may relate, in the two drug studies described, to the length of time the animals are in a state of "acute" preparation. In most instances, the phenomenon encountered in these studies would not negate the conclusions drawn from acute preparations but it would seem advisable, in light of these results with reserpine and etryptamine, to utilize presurgical injection procedures if the type of problem requires the use of an acute preparation for long periods of time with postsurgical injection procedures. The presurgical injection method also seems advisable when postsurgical injection methods yield EEG findings at odds with either behavioral or biochemical findings.

Regarding the mechanisms involved in this phenomenon, it is well known that EEG patterns of sleep and arousal are altered under conditions which produce a depression of cortical function such as in the case of traumatic brain injury. This results, in part, from a disturbance in the corticofugal regulatory influences on brain-stem mechanisms (*4*). The effects reported in this paper, however, are of a different kind, since numerous control experiments over a period of several years show that the cortical responsiveness of the rabbit is not materially impaired by the surgical procedures employed in these studies. Electroencephalographic alerting can always be elicited by peripheral stimulation within 10 to 15 minutes after the completion of the electrode implantation. Secondly, in rabbits that had received reserpine for a duration of only 1 hour, EEG activation was apparent by about 45 minutes after either the presurgical or the postsurgical injection procedure, even though the presurgically injected animals had undergone electrode implantation not over 25 minutes prior to the appearance of the reserpine-induced arousal pattern. Consequently, the difference in EEG effects associated with the differing injection procedures cannot be ascribed simply to depression of cortical function resulting from the various procedures involved in electrode implantation.

Whether the anomalous results encountered with reserpine and etryptamine in rabbits are due to a nonspecific deterioration of the "acute" preparation or to some special aspect of the drugs employed is not clear. Studies in progress at this laboratory indicate that the same degree of alteration in brain levels of norepinephrine and serotonin is produced by reserpine in conjunction with either injection procedure, and consequently the phenomenon is probably unrelated to an altered release of brain amines in the case of this pharmacological agent. In any event, both the reserpine and etryptamine studies demonstrate that an alteration in method of drug administration can produce profoundly differing results in terms of EEG sleep and arousal patterns in rabbits.

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20 May 1963

Visual Reinforcement in Siamese Fighting Fish

Abstract. *Male Siamese fighting fish* (Betta splendens) *were conditioned to emit an instrumental response to obtain the visual image of another male of the species. The relative positive reinforcing effects of three visual stimuli capable of eliciting aggressive display were compared.*

Stimuli which evoke unlearned aggressive behavior can act as positive reinforcers for instrumental responses (*1*). The visual image of a mature fighting cock was found to act as a positive reinforcer for a key-pecking response in another fighting cock. The present study is an extension of this line of investigation, in which the relative reinforcing properties of several visual stimuli evoking aggressive display in *Betta splendens* (Siamese fighting fish) are examined. The purpose of this experiment was to establish the positive reinforcing effects of the visual image of one male of the species for the instrumental behavior of another. In addition, the reinforcing properties of three visual stimuli in maintaining the same response were compared.

Four male fish (*B. splendens*), from about 6 to 10 cm long, purchased from a local aquarium supply store, were the experimental subjects. The fish were maintained on a diet of six to 10 tubifex worms, once a day, throughout the course of the experiment. Each fish was housed in a 2-liter pyrex beaker filled with conditioned tap water maintained at 26° to 29°C, when not in the experimental test chamber. The test tank was about 30 by 20 by 25 cm, with transparent Lucite walls (Fig. 1). A Lucite ring about 12½ cm in diameter with a circular aperture of about 7½ cm was suspended in the tank from a translucent Lucite top. A beam of light was focused across the aperture of the ring, falling on a photoelectric cell (Clairex Cl-404) imbedded in the plastic of the opposite side of the ring. A response was recorded when the fish swam through the ring, breaking the beam of light, then continuing through, allowing the light to again fall upon the cell (*2*). The back wall of the tank was used for presentation of visual stimuli.

Three stimuli were sequentially examined for reinforcing properties. In the first procedure a mirror was pre-

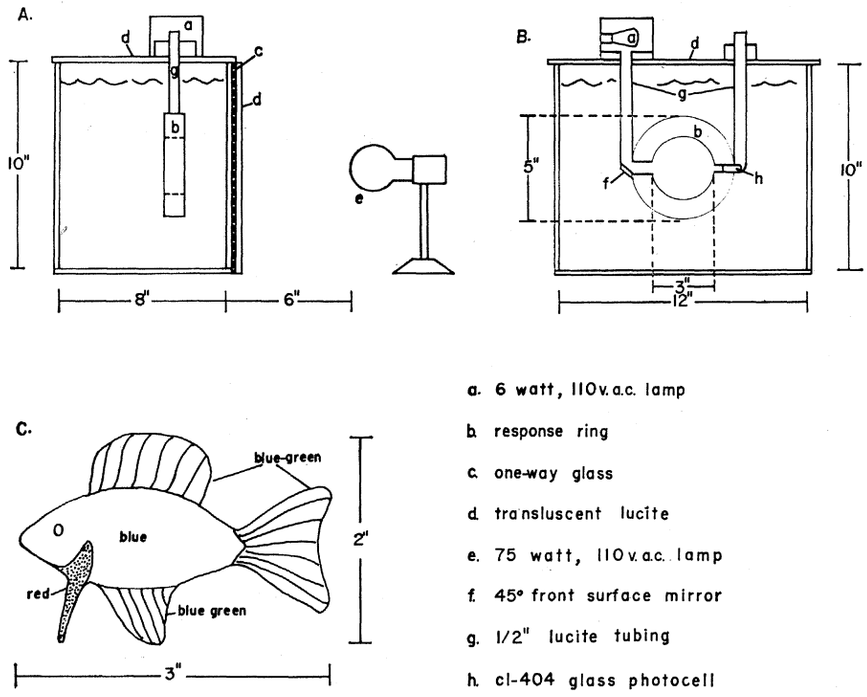

Fig. 1. Apparatus used for studying the reinforcing effects of visual stimuli in *Betta splendens*. All dimensions are in inches. (*A*) Side view of test tank illustrating the relationship of the one-way mirror (*c*) and lamp (*e*) to the response ring (*b*). (*B*) Front view of test tank facing the one-way mirror. The beam of light from the 6-watt bulb (*a*) is directed by a 45° mirror (*f*) across the aperture of the response ring (*b*) falling upon the photo cell (*h*). (*C*) Model of male *B. splendens* used as reinforcer.

sented along the back wall of the tank. Mirror presentation was accomplished by shutting off a 75-watt lamp behind a one-way mirror attached to the wall of the tank for 20 seconds. Subsequently, the fish were tested with the presentation of a model of a male *B. splendens* in agonistic display (Fig. 1) as the reinforcing stimulus. In the initial sessions the model moved across the wall of the tank (30 cm) in 20 seconds. After conditioning and extinction with this stimulus as a reinforcer, the same model was presented in a stationary position along the same wall of the tank for 20 seconds as the reinforcing stimulus.

Figure 2 presents the composite curves of the number of responses per 24-hour session for the four fish throughout the 60 days of this experiment. The operant level (*3*) was determined on the first 2 days of the experiment (10 to 25 responses per session). The acquisition of the response reinforced by mirror presentation, after the first 2 days, is seen in Fig. 2. This behavior approached stabilization after 12 to 14 days at 600 to 700 responses per session.

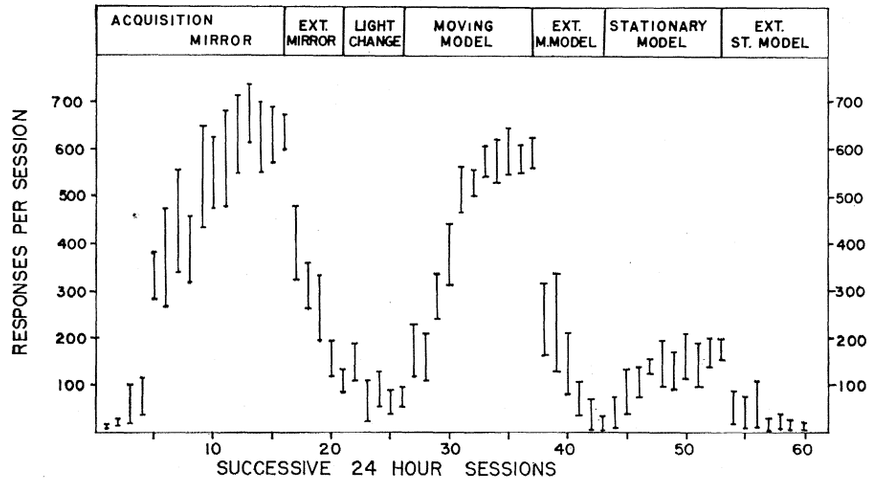

Fig. 2. Total number of responses per 24-hour session for four fish, expressed as a range. After the first two sessions of operant level the sequence of procedures indicated at the top of the graph are described in the text.

Extinction of the response reinforced by mirror presentation began on day 17, followed by a drop from a mean of 650 responses on day 16 to 125 on day 21. For the next 5 days the light behind the mirror was shut off after a response, but the mirror had been removed, thus substituting a change in illumination alone for the illumination change responsible for mirror presentation. The response rate increased slightly on day 22 but returned to 25 to 100 responses for 4 succeeding days, indicating that the mirror presentation, not the change in illumination, was responsible for maintaining the behavior.

Beginning with day 27 each response was followed by the movement of a model of a male *B. splendens* in aggressive display, across the wall of the tank. The number of responses for this reinforcer increased sharply over 10 days, stabilizing at approximately 575 responses per session. Extinction began on day 38, and reached a level of 10 to 50 responses within 6 days.

The final manipulation involved the presentation of the same model held in a stationary position along the wall of the tank for 20 seconds and then removed until another response was made. Responding for this reinforcer reached an asymptotic rate in ten sessions (150 to 200 responses), and was then extinguished to the operant level of 10 to 25 responses in five sessions.

The intrusion of a male *B. splendens* into the established territory of another male of that species has been demonstrated to evoke an unlearned agonistic display pattern (*4*). This stimulus complex frequently precedes combat, with resulting injury, and sometimes death of one of the adversaries. However, as has been demonstrated in the fighting cock (*1*), this same stimulus which precedes aversive consequences in the natural environment can act as a positive reinforcer for an instrumental response. These findings parallel Forselius' (*4*) detailed description of the use of models and mirrors to release a variety of agonistic behaviors in anabantid fishes, including *Betta*. The relative reinforcing properties of the three stimuli used in the present experiment (mirror

presentation maintaining the most responding; moving model, an intermediate number of responses; and the stationary model associated with the lowest response output) are consistent with Forselius' observations on *Betta* and more recent work by Picciolo on *Trichogaster* (*5*). These workers report that agonistic display can most readily be released by a mirror, or by appropriately colored moving models. These findings suggest that the relative positive reinforcing properties of visual stimuli, similar to a male fish in aggressive display, covary with the degree to which stimuli will evoke unlearned display (*6*).

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References and Notes

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2. Difficulties with unfavorable response topographies when using photoelectric devices for measuring responses in fish have been reported [N. Longo and M. E. Bitterman, *Am. J. Psychol.* **72**, 612 (1959)]. Such problems are minimized by programming the apparatus so that the beam of light must be broken and then the photocell reactivated again for 1 second before another response will be effective in procuring reinforcement.
3. "Operant level" is defined as the frequency with which a response occurs prior to reinforcement [B. F. Skinner, *Behavior of Organisms* (Appleton-Century, New York, 1938)].
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6. Supported in part by research grants MY-1604 from the Institute of Mental Health, and NsG 189-61 from the National Aeronautics and Space Administration to the University of Maryland. I am indebted to Larry Potash and Barbara Seldeen for their assistance in conducting the first phase of this research.

13 May 1963

Memory in Mice as Affected by Intracerebral Puromycin

Abstract. *The antibiotic, puromycin, caused loss of memory of avoidance discrimination learning in mice when injected intracerebrally. Bilateral injections of puromycin involving the hippocampi and adjacent temporal cortices caused loss of short-term memory; consistent loss of longer-term memory required injections involving, in addition, most of the remaining cortices. Spread of the effective memory trace from the temporal-hippocampal areas to wide areas of the cortices appears to require 3 to 6 days, depending upon the individual animal. Recent reversal learning was lost while longer-term initial learning was retained after bilateral injections into the hippocampal-temporal areas.*

The suggestion has become increasingly frequent during recent years that nucleic acids or proteins may be concerned with learning and memory. We were led to investigate the effects of the antibiotic, puromycin, on these aspects of behavior by the discovery of Yarmolinsky and de la Haba (*1*) that puromycin produces profound inhibition of protein synthesis in a cell-free system and by the later demonstration that it efficiently suppresses protein synthesis in vivo (*2*). In an earlier paper with de la Haba and Roberts (*3*) we have reported studies on mice which received the maximum amount of puromycin which could be tolerated in a single subcutaneous injection. Although this treatment appeared to suppress the rate of protein synthesis in various parts of the brain to 80 percent of the control value for a period of 6 hours, it was without effect on the learning and retention of simple or discrimination avoidance responses.

The experiments to be reported here have been made with intracerebral injections of puromycin. The amounts injected were smaller than previously used (*3*) so that disorientation of the animal at the time of testing was avoided. With this intracerebral approach, we have found that memory can be consistently destroyed, difference in the effective loci of recent and longer-term memory apparently established, and the time factor concerned in modification of the effective locus determined. Upon recovery, animals were capable of learning again. We cannot now relate these behavioral effects to suppression of protein synthesis, since our biochemical studies are not yet complete.

Adult white mice were trained in a Y-maze with a grid floor through which shock could be applied. The animal was placed in the stem of the Y. To avoid shock the mouse had to move into the correct arm within 5 seconds. If it entered the incorrect arm, it received shock until it moved to the correct arm. Training was continued in one session of about 20 minutes to a criterion of 9 out of 10 correct responses, thus avoiding overtraining. The same procedure was used in testing for memory of the training experience, shock having been given for errors of performance except as noted. In this type of training we have found mice to behave essentially like rats and to retain excellent memory of the training for at least 5 weeks. Intracerebral injections of puromycin, each injection of a volume of 0.012 ml, were made through small holes in the skull, as previously described (*3*).

From one to three injections of puromycin were made into each hemisphere, all at a depth of 2 mm from the surface of the skull. Bilateral injections were made through holes placed (i) just above the angle between the caudal sutures of the parietal bones and the origins of the temporal muscles—these are here designated temporal injections; (ii) 2 mm lateral to the sagittal suture and 2 mm rostral to the caudal sutures of

Table 1. Effects of different sites of injection of puromycin on short- and longer-term memory. T, V, and F refer, respectively, to temporal, ventricular, and frontal injections, all given bilaterally.

Puromycin injections			No. of mice in which memory was:		
Site	Days after learning	Milligrams	Lost*	Impaired*	Retained*
		Short-term memory			
T + V + F	1	0.03 to .06	7	0	0
T	1	.09	8	0	0
T	1	.06	14	3	1
V	1	.09	0	0	5
F	1	.09	0	0	5
V + F	1	.09	0	1	2
		Longer-term memory			
T + V + F	18 to 43	0.03 to .06	7	0	0
T	11 to 35	.06 to .09	0	0	7
V	12 to 38	.06 to .09	0	0	3
F	16 to 27	.06 to .09	0	0	3
V + F	28	.06 to .09	0	2	2
V + T	28 to 43	.09	1	1	2
T + F	28	.09	0	0	3

* For the 37 mice with loss of memory, the means and standard deviations for percentages of savings of trials and of errors were respectively 1 ± 3 and 2 ± 6; for the seven mice with impaired memory, 26 ± 29 and 39 ± 12; and for the 33 mice with retention of memory, 90 ± 14 and 90 ± 9. Negative savings in the group with lost memory have been designated zero so that the mean for this group is an overestimation of savings.