Effects of ribonuclease on acquisition and retention of escape-avoidance behavior in a selectively bred rat strain*

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Before and during the acquisition of escape-avoidance behavior, ribonuclease was administered to rats of a strain selectively bred for high rates of avoidance conditioning. In two replications of the experiment, it was found that the animals injected with 0.01-gm/kg ribonuclease in saline for 7 days before the behavioral testing was undertaken did not show any appreciable acquisition of the conditioned response, whereas the animals injected with the same dosage 7 days before testing as well as during 7 days of training acquired the response, starting the fourth day of training; on the seventh day, these animals were not any different from the control groups, which went through the same procedure except that only saline injections were administered.

The enzyme ribonuclease (RNase), which, according to Grossman (1967, p. 860), "splits the linkage between ribonucleotides by selectively hydrolyzing the phosphate esters which attach pyrimidine nucleotides to purine nucleotides," when applied through cortically implanted cannulas has been reported to bring about amnesia for conditioned responses in pigeons and mice. This amnesia lasted anywhere from 4 to 11 days (Mei Chen-tung, 1964; Krylov, Danylova, & Tongur, 1965a, b). Hendrickson & Kimble (1968) presented evidence that a solution of RNase applied to an exposed portion of the cortex resulted in the loss of ability of rats to run a T-maze previously learned to a criterion of 9/10 trials. Krylov et al (1965b) also report that when mice are given single pretraining intercranial injections of RNase or a single interperitoneal (IP) injection of RNase during training, the period of learning is greatly extended. However, details were not provided in this study, and interpretation of the results is difficult.

The present experiment was undertaken to investigate the effects of RNase on the escape-avoidance behavior of a selectively bred strain of rat. This strain was developed originally by Bignami (1965) for high rates of avoidance conditioning and designated as Roman high avoidance (RHA) by Broadhurst & Bignami (1965) and has been redesignated as RHA/Lu by Satinder (1971).

EXPERIMENT 1

Subjects

Thirty-two naive males of RHA/Lu strain from the 16th generation were used as Ss. The animals were 301

(SD = 7) days of age on the first day of experiment. All the animals were bred and reared in the laboratory and weaned at 23 days of age. Before experimentation, they were housed in groups of two or three of the same sex in cages measuring 7 in. wide, 10 in. deep, and 7 in. high. During the course of experimentation, the animals were code numbered and housed in individual cages on the same rack. Food (Purina Rat Chow) and tap water were available ad lib. The laboratory temperature was thermostatically controlled within the range of $72^{\circ} \pm 2^{\circ} \text{F}$, and the humidity level was maintained at 40%. Fluorescent lights were on from 9:00 a.m. to 9:00 p.m.

Apparatus

The apparatus was an automated shuttlebox measuring 18 x 7 x 6 in. with no barrier. The box was pivoted on its short axis. When the animal crossed from one side to the other, the box tilted slightly and activated a microswitch. Only a small shift of the weight across the axis was needed. The conditioned stimulus, a tone, was delivered through 2-in. speakers, one each, on the center of two of the opposite walls of the box. The unconditioned stimulus, an electric shock was presented through the grid floor with neon bulbs arranged to maintain a constant voltage drop between adjacent grids. The shuttlebox was enclosed in a soundproof ventilated chest.

A synchronous digital computer with a single timing device accurate to ± 0.05 sec was programmed to control: the number of trials, the intertrial interval, duration and intensity of the conditioned and unconditioned stimuli. It also recorded and displayed the number of trials completed, the number of avoidances made, the accumulated avoidance and escape

latencies, as well as the number of intertrial crossings.

Procedure

The animals were divided randomly into four groups of eight, one group being assigned to each of the following treatments: RNase injections, placebo injections, needle prick, and handling. All animals were assigned a code number independent of their treatment group. This code number was the only mark of identification on each animal cage so that a double blind procedure for injection and training could be employed. Treatments alone were administered for 7 consecutive days, beginning at the same time each day. The RNase group received an intraperitoneal (IP) injection of .01 gm/kg of RNase in a .85% saline solution; the placebo group received IP injections of .85% saline at the same dosage level as RNase; the needle-prick group was pricked IP with a 26-ga 3/8-in. hypodermic needle, and the handling group were held in an identical manner and for a similar length of time to those animals actually being injected. Each animal was weighed daily at the time of treatment.

Following treatment on the seventh day, animals from each group were assigned randomly to one of two treatment subgroups, each having four animals, for the 7-day escape-avoidance training period.

The two RNase subgroups were: RNase plus avoidance training (R-RA) and avoidance training only (R-A). The R-RA subgroup continued to receive the same dosage of RNase daily while undergoing training in an escape-avoidance conditioning situation; the R-A subgroup received only the training. One placebo subgroup received placebo with avoidance training (P-PA), while the other received RNase injections with training (P-RA). The first needle-prick subgroup received needle pricks and training (N-NA), the second received RNase injections and training (N-RA). One handled subgroup received only training (H-HA), while the other was given RNase injections and training (H-RA).

During the 7-day training period, 45 min after the administration of treatments, the animals were placed in the shuttlebox and given one practice trial, followed by 10 training trials. On each trial a tone of 22 kHz, with an intensity of 83 dB (against a background noise of 63 dB) was sounded for 5 sec and was followed without delay by a 5-sec electric shock. The shock had a pulse duration of 10 msec, a peak pulse amplitude of 9×10^3 V, and peak pulse current of 3×10^{-3} A. The pulse repeated at the rate of 15/sec. The intertrial interval

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Fig. 1. Mean number of avoidances (upper left) and intertrial crossings (upper right), and mean avoidance (lower left) and escape (lower right) latencies for the R-RA, R-A, P-PA, and P-RA groups over 7 days of acquisition and 4 days of retention.

averaged 20 sec. The animals could avoid being shocked by moving from whichever end of the shuttlebox they occupied at the onset of the tone to the other end of the shuttlebox before the tone went off. For each 10 trials the cumulative number of avoidances, avoidance and escape latencies, and the intertrial crossings (ITC) were recorded.

Following the 7 days of training, treatments for each subgroup were discontinued and animals were given 10 test trials per day for 4 consecutive days.

EXPERIMENT 2

Subjects

Sixteen naive males of RHA/Lu strain from the 16th generation were used as Ss. The animals were 262 (SD = 13) days of age on the first day of experiment. The animals were bred and reared in exactly the same manner as described in Experiment 1.

Apparatus

The apparatus was the same as in Experiment 1.

Procedure

The same procedure as described in Experiment 1 was used except that the needle-prick and handled groups were not included, because these groups did not differ significantly from the saline-injected groups.

RESULTS

In Experiment 1 no significant differences were found between any of the six subgroups not given RNase injections during the 7-day pretraining period. The P-PA and P-RA subgroups were selected to represent these

control groups. And, inasmuch as Experiment 2 was essentially a replication of Experiment 1 and an analysis of variance revealed no significant differences between the experiments, data from the two experiments were combined.

Mean Number of Avoidances

During the 7 days of acquisition, differences were found between groups ($p < .005$) and between groups over trials ($p < .005$). Animals given RNase injections for 7 days prior to but not during the training period (R-A) made significantly fewer avoidance ($p < .001$) than any of the subgroups (e.g., P-PA, P-RA) not injected with RNase before training. The R-RA subgroups made significantly fewer avoidances than any of the placebo subgroups ($p < .01$) for the first 3 days of training only. No significant difference could be shown between the R-RA and the R-A subgroups on the first 3 days of training, but from Day 4 onwards differences were significant ($p < .005$). On Days 8-11 the only significant effect was the difference in mean number of avoidances between the R-A group and all other subgroups.

Mean Number of Intertrial Crossings

There was no significant difference in the number of intertrial crossings made by any of the groups in either of the experiments.

Mean Avoidance Latency

Avoidance latency over days decreased for all subgroups ($p < .005$) except the R-A subgroups whose

latency actually increased, leaving it significantly higher than all the other ($p < .01$) groups.

Mean Escape Latency

Animals in all subgroups except R-A decreased their escape latency over trials ($p < .005$). Up to the third day of training, both RNase subgroups (R-A and R-RA) took longer to escape than the placebo subgroups. From Day 4 to Day 11 only the R-A subgroup showed no decrease in escape latency ($p < .01$).

DISCUSSION

The findings clearly demonstrated that the prior treatment with RNase inhibited escape-avoidance learning in the R-A group. There was some indication of a slight inhibitory effect on learning in the R-RA subgroup, but the deficit is probably due to a slight lag at the onset of training and not an overall decrement. After the third day of training, the placebo groups and R-RA subgroup gradients were similar.

On the surface at least, there appears to be a contradiction inherent in the present results. Chronic administration of RNase during a 7-day pretraining period seems to have a long-lasting inhibitory effect upon the learning of the avoidance task, whereas continuance of this treatment for 7 days more, during a period of training as just mentioned, appears to have minimal effect on the acquisition of the avoidance response. It is possible that the onset of training would bring about an increase in RNA synthesis, sufficient to nullify the effect of the RNase. Pevzner (1966), in a review of the RNA literature, cites examples where neural excitation was followed by an increase in RNA synthesis of as much as 81.66%. This might be relevant to the findings on the R-RA subgroup, but it does not help to explain the lack of learning in the R-A subgroup.

It may be suggested that once into the body, the foreign RNase would hydrolyze any RNA with which it came in contact and during this time some form of antibody system which gains strength daily is formed to combat the intruding RNase. It is known that the intrusion into the body by a foreign protein that threatens the metabolic balance is countered by a subsequent build-up of antibodies specific to, and bent on the destruction of, the intruding protein. This "immune" reaction is unimodal in humans and can first be noticed about 10 days after the body is infiltrated. An indication that some type of "immunity" process might be at work can be seen from the shape of the learning curves of the R-RA subgroup (Fig. 1) as compared with those of the placebo groups. A delay of approximately 3 days is apparent

before the R-RA curve begins to climb up, i.e., on the 10th day of treatment, 3rd day of training.

Further, if injections of RNase were discontinued, the antibody system would be left in a state of full production with two probable alternatives. It might attack the whole cell structure, whose ultimate destruction would account for the long-term loss of ability to learn, or it could concentrate on the native cell RNase, which is perhaps a more logical probability. If this were the case, the synthesis of RNA would be inhibited. Hadjiolov (1967) feels that one of the roles of cellular RNase is to split the newly formed RNA from its DNA template. Destruction of the RNase, then, would leave the RNA locked to its template, blocking further synthesis.

An "immunity" system could also account for the lack of effect of the RNase administration which began at the same time as training. If protein is involved in short-term memory, as Flexner, Flexner, & Roberts (1967) proposed, then, by the time the existing supply of protein would have expired, the "immunity" system would be in control and more protein would be being synthesized (cf. Fig. 1, P-RA avoidances). The possibility also exists, as Pevzner (1966) has pointed out, that increased neural stimulation would have facilitated RNA synthesis, possibly to the point where the RNase was not bringing about total destruction of RNA. This latter point is probable in light of Dingman & Sporn's (1961) findings that active RNA must be present for learning to take place.

Effects of two FR sizes on the value of information about time to FR*

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Two pigeons were exposed to a situation in which information about time until the onset of a fixed ratio schedule could be obtained by an observing response. Two S— durations were programmed, each following the FR schedule half of the time. An observing response during S— produced a color correlated with the duration of S— currently programmed. Observing responses were maintained when the FR size was FR 30, but were greatly reduced at FR 75.

A previous experiment by the author (Kendall, in press) demonstrated that stimuli differentially associated with periods

- REFERENCES
- BIGNAMI, G. Selection of high rates and low rates of avoidance conditioning in the rat. *Animal Behaviour*, 1965, 13, 221-227.
- BROADHURST, P. L., & BIGNAMI, G. Correlative effects of psychogenetic selection: A study of the Roman high and low avoidance strains of rats. *Behavior Research & Therapy*, 1965, 2, 273-280.
- DINGMAN, W., & SPORN, M. B. The incorporation of 8-azaguanine into rat brain RNA and its effects on maze learning by the rat: An inquiry into the biochemical basis of memory. *Journal of Psychiatric Research*, 1961, 1, 1-11.
- FLEXNER, L. B., FLEXNER, J. B., & ROBERTS, R. B. Memory in mice analyzed with antibiotics. *Science*, 1967, 155, 1377-1383.
- GROSSMAN, S. P. *Textbook of physiological psychology*. New York: Wiley, 1967.
- HADJIOLOV, A. A. Ribonucleic acids and information transfer in animal cells. *Progress in Nucleic Acid Research*, 1967, 7, 195-239.
- HENDRICKSON, C. W., & KIMBLE, R. J. Cortical application of ribonuclease and amnesia for a visual discrimination. *Psychonomic Science*, 1968, 13, 149-150.
- KRYLOV, O. A., DANYLOVA, R. A., & TONGUR, V. S. The influence of ribonuclease on conditioned and unconditioned reflexes of white mice. *Journal of Higher Nervous Activity (Pavlov)*, 1965a, 15, 78-85.
- KRYLOV, O. A., DANLOVA, R. A., & TONGUR, V. S. On the participation of RNA in reflector activity of white mice. *Life Sciences*, 1965b, 4, 1313-1317.
- MEI, C.-T. Effect of cortical application of ribonuclease upon higher nervous activity. *Acta Psychologica Sinica*, 1964, 27, 292-297.
- PEVZNER, L. Z. Nucleic acid changes during behavioral events. In J. Gaito (Ed.), *Macromolecules and behavior*. New York: Appleton-Century-Crofts, 1966.
- SATINDER, K. P. Genotype dependent effects of d-amphetamine sulphate and caffeine on escape-avoidance behavior in rats. *Journal of Comparative & Physiological Psychology*, 1971, in press.

appeared automatically at the end of an S— period. The results were interpreted as being favorable to the information theory of conditioned reinforcement, which says that stimuli that allow the animal to predict the onset of an important event become conditioned reinforcers (Hendry, 1969). In the present experiment, two values of the fixed ratio (FR) schedule were studied to determine if the FR value affected the reinforcement value of informative stimuli.

SUBJECTS

The Ss were two experimentally naive female White Carneaux pigeons.

APPARATUS

A commercial (Lehigh Valley Electronics) pigeon chamber with two keys was located in a room provided with white masking noise. Electromechanical programming and recording equipment was located in an adjacent room. Only one of the keys was used in the present experiment.

PROCEDURE

The pigeons were trained by successive approximation to peck the key, which was transilluminated green. Three seconds of access to grain served as the reinforcing event. When keypecking was established, the number of responses required for reinforcement was gradually increased to 30 (FR 30). The birds were trained on FR 30 in the presence of the green key light for four sessions of approximately 30 reinforcements each. The houselight in the box was illuminated at all times, except during the feeder cycle.

Following the above preliminary training, a period of nonreinforcement (S—) was instituted after each completion of FR 30. During each S— period, the key remained dark unless pecked. Half of the FRs were followed by a 30-sec S— period and the other half by a 90-sec S— period. The two S— durations alternated in an unpredictable order, each being preceded and followed by FR 30. The stimulus for FR 30 appeared automatically at the end of an S— period. A keypeck during S— produced one of two colors, depending on which S— duration was programmed. If the 30-sec duration was programmed, a peck produced a 1-sec flash of white light on the key. During a 90-sec S—, a 1-sec flash of red was produced. Keypecks during S— were designated "observing responses," since their only programmed consequence was to produce stimuli differentially associated with S— duration. This condition of the experiment is called the "informative" condition and abbreviated "I."

Following the informative condition at FR 30, a control procedure was

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