

Table 3. Incorporation of  $C^{14}$ -algal protein hydrolysate into adrenal protein. Holtzmann rats (200 g) were either untreated, hypophysectomized, or hypophysectomized and immediately given actinomycin D or 5-fluorouracil intraperitoneally. At the time indicated after treatment, each animal was given 4  $\mu$ c of  $C^{14}$ -algal protein hydrolysate (1.8 mc/mg) in the femoral vein, and the adrenals were removed 10 minutes later for measurement of  $C^{14}$  incorporation in adrenal protein as described (1). Results are means  $\pm$  S.E. of each mean; number of animals in each group is in parentheses. Abbreviations: H, hypophysectomy; A, actinomycin D; F, 5-fluorouracil.

Treatment		Incorporation in adrenal protein	
Type	Injection (mg)	Time (hr)	Amount (dpm/100 mg)
None			4644 $\pm$ 482 (17)
H		8	3987 $\pm$ 314 (6)
A	1	2	3194 $\pm$ 352 (7)
A	1	4	2197 $\pm$ 66 (8)
A	1	6	2512 $\pm$ 514 (8)
A	1	8	2370 $\pm$ 352 (4)
F	30	4	3693 $\pm$ 138 (4)
F	30	8	3373 $\pm$ 204 (4)

corticosterone of  $3.02 \pm 0.21 \mu\text{g}/3$  minutes.

The acute increase in corticosterone secretion in response to ACTH was therefore inhibited 24 hours after the administration of actinomycin D. No animal mortality was observed during the experiment. One should note that there is no evidence that the adrenocortical response to given quantities of ACTH is inhibited by stress per se (8); thus it is unlikely that the inhibitory effect of actinomycin D reflected non-specific toxicity of the drug. A decrease in adrenal-corticosterone content (9) and a small decrease in peripheral plasma corticosterone (10) in response to ACTH have also been noted 24 hours after the administration of actinomycin D. It is not clear whether single or multiple steps in corticosterone biosynthesis are inhibited by the antibiotic. It is therefore possible that the RNA involved in certain steps of biosynthesis of corticosterone are stable for more than 24 hours.

We next sought to determine whether overall protein synthesis is regulated by RNA of comparable stability. Rats were again hypophysectomized and immediately given actinomycin D (1 mg). Protein synthesis in vivo was determined by measurement of incorporation of intravenously injected  $C^{14}$ -algal protein hydrolysate into adrenal protein. Hypophysectomy itself resulted in only a slight decrease in protein synthesis over the 8-hour period (Table 3), although marked decreases have been observed over longer periods after hy-

pophysectomy (11). Treatment with actinomycin D resulted in a rapid decrease in protein synthesis within 4 hours, with no further decrease between 4 and 8 hours after its administration.

Thus it appears that the RNA templates regulating overall synthesis of adrenal protein are heterogeneous in turnover rate. The RNA that regulates synthesis of a large fraction of adrenal protein has a turnover time of less than 4 hours, but the synthesis of the remaining adrenal protein is dependent on RNA that is stable for at least 8 hours. Fluorouracil only slightly decreased synthesis of adrenal protein (Table 3), suggesting that under the conditions of our experiments the drug is relatively ineffective in modifying the observed activity of template RNA.

Although our study does not entirely rule out the possibility that specific moieties of template RNA were not inhibited by actinomycin D, the large concentrations of the antibiotic used and the long period of observation after its administration mitigate against this explanation. Under certain experimental conditions actinomycin D inhibits protein synthesis by mechanisms other than its inhibition of RNA synthesis (12); thus there is the possibility that the turnover times of the RNA templates inferred from these experiments were even longer than the data indicate.

Template RNA in bacterial cells may be heterogeneous in stability (13). Heterogeneity in turnover of RNA that

regulates synthesis of proteins in the rat thyroid has also been reported (14). Thus the template RNA for the major physiological function of the thyroid (thyroglobulin biosynthesis) and of the adrenal (steroidogenesis) appears to be relatively stable.

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## Retrograde Amnesia: Effects of Handling and Microwave Radiation

**Abstract.** Rats that were irradiated with microwaves immediately after the training trial in a one-trial shock-avoidance learning task retained the conditioned avoidance response 24 hours later. However, rats that were handled a few minutes each day for 3 days before the experiment did not retain the response, although they were capable of learning in a later test.

Electroconvulsive shock, anoxia, anesthesia, brain stimulation, and changes in body temperature can produce retrograde amnesia (1). Theoretically these treatments interfere with the perseverating neural activity required for consolidation of a memory trace. Microwave radiation should also interfere with recent memory. Heat production is the main effect of microwave radiation, although there are other effects (displacement and conduction currents, forces on dipoles, and intracellular orientation effects) which could alter neural activity (2). Heat produced a retrograde amnesia in goldfish, abolished the

electroencephalogram (EEG) in rats, and desynchronized the EEG in rabbits (3). Microwave radiation abolished the action potential of a dog's sciatic nerve by direct heating to 50°C (4). I expected microwave radiation to interfere with the electrical activity of the nervous system and produce a retrograde amnesia for a conditioned avoidance response. I trained rats in one trial to avoid stepping off a small platform, by shocking them as they did so. Fifteen seconds after the training trial I irradiated the animals with 12-cm microwaves which quickly raised their body temperatures by 6°C. A test trial

Table 1. Change in response latency in a one-trial shock-avoidance learning task. (Md = median, Q = semi-interquartile range, N = sample size, C = control, H = heated, S = shocked, SH = shocked and heated.)

Item	Time (sec)			
	C	H	S	SH
<i>Unhandled</i>				
Md	— 2.0	— 3.2	10.4	15.4
Q	3.0	2.0	29.4	16.5
N	10	7	10	8
<i>Handled</i>				
Md	— 1.4	0	8.0	0
Q	1.95	2.2	26.5	3.3
N	10	9	10	9

24 hours later showed that the heated animals retained the conditioned avoidance response even better than unheated controls. I repeated the experiment with one modification; I handled the rats a few minutes each day for 3 days before the experiment. The heated rats in this experiment did not acquire the conditioned avoidance response.

The subjects were 80 male albino rats purchased from Holtzman Laboratories, Madison, Wisconsin. All the animals were 50 to 70 days old at the beginning of an experiment which lasted 1 week. The animals' previous histories were unknown. They were housed in group cages, five rats per cage, and they were not handled prior to the experiment except on the day they arrived at the laboratory and as described below. Food and water were continuously available in their home cages. Forty rats were used in the first experiment without any previous handling. The other 40 rats were handled a few minutes each day for 3 days before the second experiment. Handling consisted of transferring one cage of rats at a time from their home cage to a table via a cardboard box with

sawdust on the bottom. During their stay on the table, which lasted about 3 minutes, I petted them, put them in my pockets, and let them run up my arms. They were then returned to their home cage. All animals were placed in the training apparatus and the microwave oven. The 40 unhandled rats were divided into four groups of ten animals each. The control group (C) received neither shock nor heat. Another group (H) received only heat. A third group (S) received only shock, and the fourth group (SH) received both shock and heat. The 40 handled rats were grouped in the same way.

The training procedure was intended to teach the rats in one trial to avoid stepping off a 6- by 3- by  $\frac{3}{4}$ -inch ( $15\frac{1}{4}$ - by  $7\frac{3}{4}$ - by 2-cm) wooden platform situated 1 inch ( $2\frac{1}{2}$  cm) above the center of the floor of a 20-inch (50-cm) cubical box. The box, which was made of  $\frac{1}{4}$ -inch ( $\frac{2}{3}$ -cm) plywood, was open at the top, and the floor was an electric grid made of No. 14 AWG plated copper wire spaced at  $\frac{1}{2}$ -inch ( $1\frac{1}{4}$ -cm) intervals. The grid could be connected to a 50-volt, 60-cy/sec current. The interior of the box was illuminated by a 60-watt lamp in an upper corner. A rat on the platform could be observed through a 6- by 2-inch ( $15\frac{1}{4}$ - by 5-cm) one-way window. A 5-inch ( $12\frac{3}{8}$ -cm) high hardware cloth cage, fitted over the platform, was used to restrain the subject until the trial began. The cage was raised and lowered remotely with a pulley system.

A Tappan microwave oven (model R3L, 2450 Mc) was used to irradiate the animals. The oven was modified so the output could be varied by rheostat control of the current to the magnetic field of the magnetron. A 1-quart (0.9-liter) jar of water was kept in a

corner of the oven to act as a power sink, and the water in it was replaced with cold water after every treatment. A pilot group of animals of the same age, sex, and strain as the experimental animals was used to determine the optimum irradiation procedure. Their rectal temperatures were measured with a Tele-thermometer (Yellow Springs Instrument Co.) and probe. The rats' normal rectal temperatures were approximately 36°C. A rat was placed in an empty 1-gal (3.8-liter) glass jar which was placed in the oven. A 15-second burst of radiation with a field current of 125 ma raised the animal's temperature to approximately 41.5°C. The field current was then reduced to approximately 25 ma and maintained there for 5 minutes. When they were first exposed to the microwave radiation, the rats made violent attempts to get out of the jar. Later they flattened themselves against the bottom of the jar. Their ears, feet, and tails became pink. They salivated considerably, and their ventral surfaces became wet. Similar signs were observed in mice heated with 2- to 3-m radio waves (5). Irradiation produced convulsions in six experimental animals, and they were eliminated because it was presumed that convulsions would in themselves produce a confounding amnesia. After the treatment the animals were returned to their home cages.

On the day of an experiment an animal was removed from his home cage and restrained on the platform. When the rat had all his feet on the platform, the cage was quietly and rapidly lifted. The response latency ( $t_1$ ), that is, the number of seconds required for the rat to step to the grid, was recorded. Twenty-four hours later the procedure was repeated, and a second latency ( $t_2$ ) was obtained. An increase in latency ( $t_2 - t_1$ ) for animals that were shocked indicated that a conditioned avoidance response was established. Immediately after he stepped off the platform during the training trial an animal was placed in the 1-gal glass jar in the oven. Rats in groups H and SH were irradiated, and the others were kept in the oven an equivalent length of time. One animal stayed on the platform over 2 minutes on the training trial, and he was eliminated. Most of the rats explored the restraining cage with their forepaws. If the cage was raised while they were grasping it, they were carried up with it and could not make an appropriate response. Since I knew which animal

Table 2. Probabilities associated with the occurrence under the null hypothesis of values as small as an observed U of the Mann-Whitney U test (2-tailed). Multiple comparisons among all eight treatment combinations were made on  $t_2 - t_1$ , the change in response latency. (C = control, H = heated, S = shocked, SH = shocked and heated.)

Groups	Groups							
	Unhandled				Handled			
	C	H	S	SH	C	H	S	SH
Unhandled								
C	—		.05	< .002			< .01	
H		—	.05	.000			< .01	
S			—					
SH				—	< .002	< .002		.01
Handled								
C					—		< .05	
H						—	< .05	
S							—	.05
SH								—

I was testing on the second day, I attempted to avoid bias in a judgment of when an animal had stopped exploring by raising the cage as soon as the rat released it. This interval never exceeded 30 seconds.

The median changes in latencies are shown in Table 1. The median was preferred over other measures of central tendency for two reasons. Variabilities were large and unequal (see the semi-interquartile ranges in Table 1); and the distributions of scores of shocked animals were highly skewed because the animals were removed from the platform after 60 seconds on the test trial. I handled the animals in the second experiment because I expected this treatment to reduce the variability in their latency scores (6). Handling did seem to reduce variability in group SH. Group H was included to test for transfer of fear of the oven to the box and to control for any effects due to heating alone. Multiple comparisons were made with a two-tailed Mann-Whitney U test (7). First, the  $t_1$  scores of all the unhandled rats were compared with the  $t_1$  scores of all the handled rats to determine if there was an overall bias introduced by handling. I expected handled rats to have a shorter latency because of decreased timidity (6), but there was no difference between the two groups. The median of all  $t_1$  scores was 4.5 seconds. Next, multiple comparisons were made among the eight treatment combinations on  $t_1$  to determine if there was a bias that might affect the change in latency. There were no differences. Finally, multiple comparisons were made among the eight treatment combinations on  $t_2 - t_1$ , the change in response latency. Probabilities of .05 or less, associated with the appropriate U's, are shown in Table 2; only U's with probabilities of .01 or less are considered significant. Heating alone had no effect on response latency. Shocked rats tended to acquire a conditioned avoidance response, but the large variation in their scores prevented the data from reaching the desired level of confidence. Contrary to expectation, unhandled rats that were heated after receiving shock-avoidance training acquired a conditioned avoidance response. This acquisition is apparent whether the animals are compared with their own controls ( $p < .002$ ) and heated group ( $p = .000$ ) or handled controls ( $p < .002$ ) and heated group ( $p < .002$ ). On the other hand, handled

rats that were heated after receiving shock-avoidance training did not acquire a conditioned avoidance response when compared with unhandled SH rats ( $p = .01$ ) or the control groups.

To determine if the radiation impaired the rats' ability to learn a complex task, I evaluated the performance of the handled animals in a 14-unit T maze. Thirty days after the one-trial avoidance learning, the animals from the second experiment were put on a 24-hour food deprivation schedule over a 5-day period. They were then pre-trained for 5 days to run for food in an L maze, receiving one trial a day. Finally, they received one trial a day for 20 days in the T maze. They were allowed to eat wet mash for 20 minutes in the goal box after every trial. An error was recorded each time an animal moved into a blind as far as the base of his tail. A plot of mean errors in the maze versus days for heated and unheated animals showed no differences. Justesen, Pendleton, and Porter (8) showed that heating by 7-cm microwave radiation impaired the ability of rats to learn a similar maze. However, their animals were weanlings when they were heated, they received six treatments instead of one, and they were tested in a water maze. Their results are not comparable to the present study.

It is not clear why handling should predispose an animal to a retrograde amnesia from microwave radiation; however, it is clear that handling is an important variable in studies of retrograde amnesia.

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## Speech Duration Effects in the Kennedy News Conferences

Abstract. Transcripts of the 61 regular Kennedy news conferences were examined in an attempt to provide a replication of the "speech duration effect" previously found in two-person interviews and during manned space flight. A positive relation was found between the length of the reporters' questions and the length of the President's answers.

The relation between the lengths of astronaut and ground communicator statements reported by Matarazzo *et al.* (1) supports approximately a decade of research on the subject of speech durations during interviews.

Matarazzo has found, almost uniformly, that the length of response by the interviewed individual is positively associated (that is, the longer the question the longer the answer) with the duration of the interviewer's speech "unit." This positive relation was demonstrated in medical interviews, psychotherapeutic interviews, civil service and department store job interviews, and in "free" conversation between two persons (2).

The astronaut-ground communicator study, an ingenious extension of the aforementioned series of replications, involved examination of speech durations during the two three-orbit manned space flights of the U.S. Mercury program. The rank order of orbits, on the basis of mean speech duration, was identical for ground communicators and astronauts in both space flights.

The study of speech duration can be extended to another situation—the presidential press conference. In comparison to the situations studied earlier, even that of the astronaut, the press conference is a situation in which the typical relation of speech lengths would not be expected to occur. It is less a "free," nondirective situation, with many of the reporters' questions having been prepared in advance. The President too has been briefed on the questions likely to be asked.

Matarazzo used an interaction chronograph to study the length of the speeches. In our study, duration was inferred from a count of lines in the transcripts of the 61 regular Kennedy news conferences held in Washington (3). Although the line-count method does not take the speed of talking and the pauses directly into account, Mata-