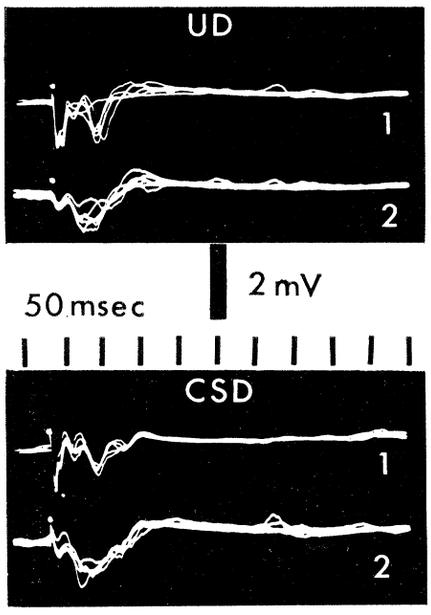

Fig. 2. Evoked responses as in Fig. 1, but in a control animal. Recording sites approximately those of the permanently implanted electrodes. CSD and UD interpreted as in Fig. 1. Chronic brain stimulation was to left suprasylvian gyrus, not paired with foot shock, 1025 times over 69 days. Chronic stimulation as in Fig. 1*C*. Acute stimulation as in Fig. 1*C*, but at 9 volts (about 0.9 ma). Superimposed sweeps.

all animals, were identified as CSD (in the "chronically stimulated direction") or as UD (in the "unstimulated direction"). The CSD responses were evoked by electrical stimulation of the cortical area that had received the chronic electrical stimulation and by peripheral stimuli. The CSD responses were recorded from the surface of the cerebral cortex contralateral to the side that had received the chronic stimulation. The UD responses were evoked by electrical stimulation of the cortex on the side contralateral to that which had received the chronic stimulation, and by peripheral stimulation. The UD responses were recorded from the cortex on the side that had received the chronic stimulation.

Figure 1 shows evoked IDR's recorded from two cats in terminal experiments. In *B* the IDR occurs at all four points (CSD, right) as a single or double positive deflection peaking at 30 to 55 msec; maximum amplitude is 800 μv. The UD responses (left) show an IDR with a much lower amplitude—only about 300 μv. Transcallosal responses are about equal on both sides.

In *C*, for the second cat, a large-amplitude IDR is present (CSD), while no IDR was observed on the chronically stimulated side (UD). The IDR amplitudes, for all seven cats in these experiments, were always greater for CSD. Differences between CSD and UD amplitudes ranged from 80 to 750 μv, with an average of 400 μv. Not only were IDR amplitudes increased, but IDR thresholds were much lower in the "chronically stimulated direction."

These findings of enhancement of the IDR in the "chronically stimulated direction" in the seven cats contrast with the data from the three cats which had received chronic brain stimulation but no foot-shock pairings. At the two cortical locations, left and right suprasylvian gyri, the IDR's were of similar amplitude. One set of records is shown in Fig. 2.

In the one cat tested, the enhanced IDR was persistent. This animal, after 420-percent overtraining, was put aside and received no further training or brain stimulation for 3 weeks prior to the terminal experiment. During this experiment, by the use of computer averaging, a 300 μv IDR was recorded in the "chronically stimulated direction," whereas no IDR could be recorded in the "unstimulated direction" even when 50-percent higher stimulus was used.

It may be argued that the chronic electrical stimulation could produce some unspecific "sensitization" of cortical tissue, which would result in altered evoked responses. No such changes were apparent. In most animals there were no consistent differences, between the chronically stimulated and unstimulated suprasylvian gyri, in potentials evoked to peripheral photic or auditory stimuli. There was likewise no difference in transcallosal response between the two sides.

These experiments seem to demonstrate that facilitation in a multisynaptic pathway can be accomplished by prolonged pairing of direct electrical stimulation of the pathway with a foot shock in a conditioning situation. The IDR enhancement is only in the direction of the chronically stimulated side to the contralateral cortex. Brain stimulation alone, without the reinforcing foot shock, produces no evidence of enhanced conduction in the IDR pathway in either direction.

It has been established by Morrell (*7*) and others that persistent changes in spontaneous activity and in the direct cortical response are observed following continuous epileptiform bombardment from the opposite hemisphere. Similar electrical changes have not, as yet, been related to behavior, and thus no ready comparison with the present experiments is possible.

The enhanced IDR may be more persistent than the electrical changes in evoked potentials and the EEG associated with "learning," as reported by others. Whether any structural changes may be related to the observed evidence for functional changes must await a careful histological study of the cortical and subcortical elements involved.

L. T. RUTLEDGE

Department of Physiology,
University of Michigan Medical School,
Ann Arbor 48104

References and Notes

1. R. Galambos and G. C. Sheatz, *Amer. J. Physiol.* **203**, 173 (1962).
2. E. R. John, D. S. Ruchkin, J. Villegas, *Ann. N.Y. Acad. Sci.* **112**, 362 (1964).
3. L. T. Rutledge, *Federation Proc.* **22**, 457 (1963).
4. ——— and T. T. Kennedy, *J. Neurophysiol.* **23**, 188 (1960).
5. ———, *Exp. Neurol.* **4**, 470 (1961).
6. R. W. Doty, L. T. Rutledge, R. M. Larsen, *J. Neurophysiol.* **19**, 401 (1956).
7. F. Morrell, in *Brain Mechanisms and Learning*, J. F. Delafresnayne, Ed. (Thomas, Springfield, Ill., 1961), p. 375.
8. Work supported by PHS grants NB-1068, NB-02978, and NB-04119. The assistance of Joyce A. Duncan is gratefully acknowledged.

8 February 1965

Retrograde Amnesia from Electroconvulsive Shock in a One-Trial Appetitive Learning Task

Abstract. *Rats deprived of water were placed, for 4-minute sessions, into a chamber containing a hole in one wall. After several sessions the number of times these rats explored the hole markedly decreased. Rats given access to water at the hole for a brief period explored the hole more frequently than controls, when they were tested later. This increase in hole explorations was abolished when the water reinforcement was followed by electroconvulsive shock but not when followed by foot shock.*

One-trial conditioning techniques are useful and at times essential in studying retrograde amnesia and the consolidation hypothesis. In all of these

one-trial techniques, the experimenters have used aversive stimulation as the reinforcer (for example, *1*). It is obvious that retrograde amnesia should be studied with the use of positive reinforcement as well, since different reinforcement (and motivational) systems may respond differently in the retrograde amnesia paradigm. However, most appetitive techniques require several reinforcements before the acquisition of the response can be reliably demonstrated. As such, repeated administrations (after each trial) of a drug or electroconvulsive shock can result in long persisting alterations in the animal which would be operative prior to and *during* a subsequent trial. This would make interpretation of the results difficult. A reliable one-trial technique in which such problems were avoided was used in this experiment.

One hundred and twenty Charles River male albino rats, weighing about 200 g each, were provided continuously with lab chow and given access to water for 30 minutes every 24 hours. This procedure was started about 5 days prior to experimentation. The rats were then placed individually in a chamber for 4 minutes in the morning and another 4 minutes in the afternoon on two consecutive days. The chamber was a plastic box, 12.5 cm high with a 15- by 23-cm grid floor. Centered on one of the 23-cm walls, 3.5 cm above the floor, was a 3.8-cm high and 5-cm long rectangular opening to a tapering tunnel. A photoelectric cell and opposing light bulb were placed on the tunnel wall 2 cm from the opening. Interruption of the light beam operated a counter and timers. On the 2nd day of the experiment they were not given any water, so that on the 3rd day they had been deprived of water for 40 hours. After the four "familiarization" sessions the rats were divided into five groups of 24, matched in terms of the number of times they explored the hole during the fourth session.

On the 3rd day the groups were treated as follows: those in group A, the nonreinforced control group, were placed individually in the box for 1 minute; those in group B, allowed to drink water from a tube in the hole for 10 seconds, removed, ear clips put in place, held for 15 seconds, and returned to the home cage (pseudo-ECS); group C, given access to water as with group B, removed, and given an electroconvulsive shock (ECS) of 150 ma for 0.2 second through ear clips about 12 seconds after the water was given; group D, given access to water as with group B, removed, and given the same ECS 3 hours after the water reward; and group E, given access to water as with group B, removed, and given foot shock about 12 seconds after the water reward. The electrodes for giving foot shock consisted of two flat 7.5-cm square pieces of aluminum which were continuously electrified with 650 v through a 330,000-ohm series resistor. The rat was placed upon the electrodes from which he could immediately escape. These plate electrodes were used to minimize any stimulus generalization there might be to the grid floor of the hole-in-the-wall apparatus.

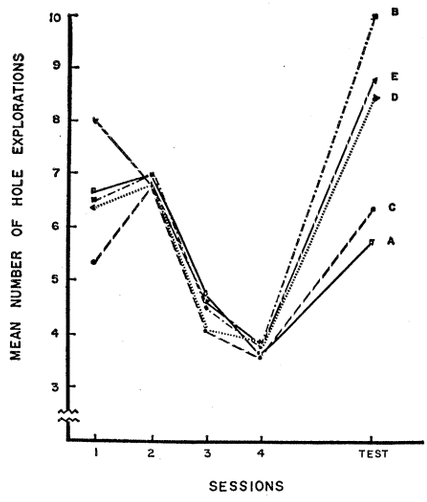

Fig. 1. The mean number of hole explorations for each group for the four familiarization sessions and test session. Group A nonreinforced; group B reinforced, pseudo-ECS; group C reinforced, immediate ECS; group D reinforced, delayed ECS; group E reinforced, immediate foot shock.

On the 6th day of the experiment (3 days after treatment) the rats were again subjected to a 4-minute (test) session while under 40 hours of water deprivation. During this session the rats' overall activity level was rated on a 5-point scale.

Although the time taken by each rat before it explored the hole for the first time and the total amount of time spent exploring the hole were recorded, the results proved too variable for statistical analysis. The most reliable measure was the number of times the hole was explored during the session. Figure 1 shows that such explorations declined uniformly after the second session. On the test day the five groups were aligned in the following manner: group B had the highest mean number of hole explorations, followed closely by groups E and D; the two lowest groups were C and A (*2*). The overall differences among these groups were significant ($F = 4.99$, $p = .001$). For each rat, the score used for statistical analysis was the number of explorations during the test session minus the number of explorations during the fourth session.

The significant difference ($p = .001$) between groups A and B clearly demonstrates the effectiveness of the one 10-second water reinforcement. (Some limited pilot studies suggested that access to water for even less time might also be effective.)

If this procedure were sensitive to agents which cause retrograde amnesia, then the ECS should cause the rats in group C to explore the hole less than those in group B. This expectation was confirmed. Exploration scores for group B were significantly higher than those for group C ($p < .01$). In addition, it might be expected that the scores of group C and group A would be similar. This was also confirmed. Although group C showed slightly more explorations than group A, the difference was not significant ($p = .30$).

Since ECS produced a reduction in exploration, this effect may have been due to aversive, rather than amnestic effects of ECS (*3*). Group E was used to evaluate this possibility. Most of the rats receiving this fairly high level of foot-shock squealed and quickly jumped off the electrode platform. Some of them received more than one shock by making unsuccessful jumps. In spite of this noxious stimulation, this group explored the hole significantly more frequently that group A ($p < .01$) and was not significantly different from group B ($p > .30$). From this it is concluded that, within the parameters used, this one-trial learning cannot be disrupted by a subsequent aversive event, and the reduced exploration observed in group C was caused by the more specific amnestic effects of the ECS.

An alternative possible interpretation of the results is that the ECS caused the animals to become "fearful," or in some way diminished their activity during the subsequent testing

session. Such a proactive effect would, likewise, produce fewer explorations, and thus result in a spurious amnestic effect. If this interpretation were correct, delayed presentation of ECS (group D) could be expected to elicit a similar effect. This did not occur. In contrast to group C, group D explored the hole significantly ($p = .01$) more than the nonreinforced group A. Again, in contrast to group C, group D was not significantly ($p > .20$) different from the reinforced pseudo-ECS group B. Moreover, observations of overall activity showed that there were no significant differences between the ECS and non-ECS (excluding group E) groups. Thus, the interpretation that the one ECS produced diminished activity and a spurious amnestic effect is not supported.

However, the greater number of hole explorations of the delayed-ECS group over the immediate-ECS group did not reach significance ($p = .10$). Thus, it seems possible that ECS might exert some limited retrograde amnestic effects even 3 hours after reinforcement (*4*). A longer reinforcement-ECS interval might have produced a significant difference making the findings more conclusive.

S. S. TENEN

Medical Research Laboratories,
Chas. Pfizer & Co., Inc.,
Groton, Connecticut

References and Notes

1. J. Burěs and O. Burešová, *J. Comp. Physiol. Psychol.* **56**, 268 (1963); M. C. Madsen and J. L. McGaugh, *ibid.* **54**, 522 (1961); C. A. Pearlman, S. K. Sharpless, M. E. Jarvik, *ibid.*, p. 109.
2. The increase in hole explorations from the 4th to test session of group A can possibly be attributed to either of two variables or both. Three days had lapsed between the 4th and test sessions, whereas only about 4 hours had passed between the 3rd and 4th sessions. The prolonged absence from the apparatus could have resulted in a reawakening of "curiosity." The second factor is probably more important. Whereas the animals were only deprived of water for about 22 hours on the 4th session, they were deprived for about 40 hours during the test session. This increase in drive level could easily have resulted in increased activity.
3. E. E. Coons and N. E. Miller, *J. Comp. Physiol. Psychol.* **53**, 524 (1960); W. J. Hudspeth, J. L. McGaugh, C. W. Thomson, *ibid.* **57**, 61 (1964).
4. J. Burěs and O. Burešová, *ibid.* **56**, 268 (1963).
5. Probably any discretely measurable, "naturally" occurring response that is of a moderately high frequency (and which will diminish with repeated trials) is reliably responsive to the effect of one reinforcement. This hole-in-the-wall procedure was suggested by some unpublished experiments of Dr. J. R. Trotter (Australian National University, Canberra). He had used the response of the rat placing its head into the food cup as an operant, and aperiodically reinforced it with food pellets.
6. The technical assistance of Miss Gale Geist is gratefully acknowledged.

26 March 1965

Reptilian Thermoregulation

J. E. Heath [*Science* **146**, 784 (1964)] takes "many workers" to task for poor scientific practices in the study of reptilian thermoregulation. Since I [*Copeia* **1963**, 107 (1963)] am the only one of the workers mentioned, and since Heath's conclusions are controverted by a reanalysis of his data, I feel obliged to indicate some of the probable sources of confusion and error.

Heath's remarks are largely a protest against a progressive simplification of approach. He complains that "many workers have discarded the several categories of thermal responses proposed by Cowles and Bogert [*Bull. Am. Museum Nat. Hist.* **85**, 265 (1944)] in favor of determining the body temperatures of reptiles surprised in the field." (These categories are: the lethal minimum, the critical minimum or cold narcosis, the voluntary minimum, the basking range, the normal activity range, the maximum voluntary tolerance, the critical maximum, and the lethal.) The wording of Heath's complaint suggests a possible confusion between the *schema*, that is, the response categories of Cowles and Bogert, and the *methodology* practiced by field workers. If his point is that the characteristics of certain thermal responses cannot be elucidated by the collection of body temperatures in the field, I agree.

However, his main complaint seems to be that this attrition of tradition in the analysis of reptilian thermal relations has reached such proportions that those of us who take temperatures of lizards in the field can no longer be said to be studying thermoregulation. Heath says that "only two of [Cowles and Bogert's] categories, the maximum voluntary tolerance and minimum voluntary tolerance, contain behavior which alters the heat load upon the animal" and that the other categories, including the basking range, "are not directly related to active regulation." This is perplexing. It is well documented that basking and foraging lizards assume postures and choose microhabitats that depend in varying degrees on their body temperatures, the position of the sun, and the time of day. Do not these activities alter the radiational and conductive heat loads on lizards?

Heath also criticizes workers for purposely ignoring data: "In some cases body temperatures below an arbitrary level are ignored because they lie in the so-called 'basking range' of the animal." In my report I deleted three low temperature records from a total of 297 because of the marginal thermal conditions at the times of collection. Heath's justifiable criticism is the motivation for an experiment on beer cans from which he concludes that the deletion of such lower records markedly changes the results and interpretations.

Heath exposed 11 water-filled beer cans to the sun during July 1963, and monitored the temperatures of the cans and the nearby air temperatures hourly from 1030 to 1830 P.S.T. Assuming that recording began at 1130, there should be 110 pairs of observations. However, in the legend of his Fig. 1, a histogram of the beer-can temperature data, N is given as 97 (although there appear to be 100 entries in the histogram itself). In Fig. 2, a scatter diagram of beer-can and the corresponding air-temperature records, there are 96 entries. (The two figures are irreconcilable in other ways as well.)

Heath reports that "can temperature is loosely correlated with air temperature ($r = +.41$; $P < .005$)." The same statistics recalculated from his Fig. 2 ($N = 96$) are $r = +.68$; $P < .001$. Heath goes on, "Following the precedent of others, all can temperatures below an arbitrary level, in this case 30°C, were ignored . . ." (about one-third of the data). In the relevant part of Fig. 2, Heath actually omits all can temperatures below 30.5°C plus two of the highest records. He found the correlation between can and air temperatures in this amputated scatter distribution to be $-.09$; a probability value of less than .05 is given for this insignificant r value. By inspection it is clear that the association is positive rather than negative. The recalculated correlation in this case, including the omitted records, is $+ .45$; $P < .001$.

The magnitude of these errors casts doubt on the validity of Heath's conclusions. First, the omission of the lower records turns out not to have an appreciable effect on the results; all the recalculated r values are positive and highly significant. Second, his conclusion that can temperature is inde-