of a motor, metabolic, arousal, or other deficit, these animals could be considered to be in a chronic state of deprivation. In that food motivation appears to bear a fairly direct relation to body weight (*20*), it could be hypothesized that the strong food motivated behavior exhibited by animals that have recovered from lateral hypothalamic lesions is due to their chronic state of "lesion-imposed" deprivation. They readily approach and begin eating food, yet they may be unable to consume sufficient amounts and metabolize it efficiently (*21*).

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22. We thank Andrea Murray for collaboration. John Kelsey provided some of the septal animals. Supported by NIH grant MH-14596 to S.B.

16 November 1970; revised 4 January 1971 ■

Amnesia Produced by Spreading Depression and ECS: Evidence for Time-Dependent Memory Trace Localization

Abstract. *Rats were given electroconvulsive shock and bilateral cortical spreading depression, either alone or in combination, at various times after a single passive avoidance training trial. Assessment of retention deficits, 24 hours after training, revealed a U-shaped amnesic function for cortical spreading depression as compared with the short linear function consistently obtained with electroconvulsive shock in this situation. Induction of cortical spreading depression immediately after training resulted in an extension of the amnesic gradient produced by electroconvulsive shock, presumably by disruption of the subcortically confined memory trace. In addition to indicating a subcortical locus of action for the amnesic effects of electroconvulsive shock, these results are interpreted as favoring a hypothesis of time-dependent memory trace localization in short-term memory processing, which involves an initial subcortical localization of the trace followed by a phase involving either direct or indirect cortical participation in a multistage memory fixation process.*

Past research has demonstrated that electroconvulsive shock (ECS) administered shortly after training produces behavioral performance deficits on a subsequent 24-hour retention test. This phenomenon, commonly referred to as retrograde amnesia, is generally attributed to a disruption of memory consolidation (*1*). A number of recent studies have attempted to localize areas within the brain specifically responsible for this amnesia in the belief that such findings will lead to a better understanding of the neurobiological basis of the memory storage process (*2*).

Our purpose in the experiments reported here was to investigate the temporal and sequential role of the cortex and subcortex in the memory fixation process as well as the amnesic action of ECS. This was done by studying the memory disruptive effects of ECS and cortical spreading depression (CSD), alone and in combination, on learning of a single-trial passive avoidance task in rats. The results of these experiments may be summarized as follows: (i) confirmation of a previously reported U-shaped function between degree of amnesia and time elapsed between training and initiation of CSD; (ii) delay of the period of vulnerability for memory disruption to CSD by induction of CSD immediately after training; (iii) extension of the ECS-induced temporal gradient of amnesia as a result of functional decortication by induction of CSD immediately after training. In addition to indicating a subcortical locus of action for the amnesic effects of ECS, these data are interpreted as favoring a hypothesis of time-dependent memory trace localization in short-term memory processing, which involves an initial subcortical localization of the trace followed by a phase that involves either direct or indirect cortical participation in a multistage memory fixation process.

Subjects were 194 male rats (*3*), 90 to 100 days old; they were maintained in individual stainless steel cages with free access to food and water.

The one-trial passive avoidance apparatus (*4*) consisted of a small compartment separated from a larger compartment by a guillotine door. Animals were given a single training trial followed by a single retention trial 24 hours later. During training each animal was placed in the small compartment, the guillotine door was opened, and the latency to enter the large compartment was measured to the nearest 0.1 second. After entry, the guillotine door was closed and the various treatment procedures were initiated. For animals receiving aversive training, closing of the door automatically triggered the presentation of a 60-cycle a-c electric shock of 1.0-ma intensity

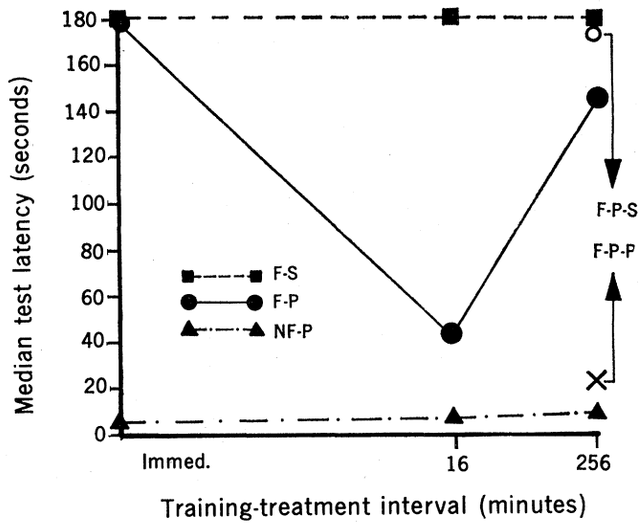

Fig. 1 (right). Median retention test latencies as a function of the time elapsed between termination of foot shock (F) and application of either 25 percent potassium chloride (P) or 25 percent sodium chloride (S) on the dura. In all cases the reversal procedure was initiated 4 hours after application. For the F-P-S and F-P-P groups, potassium chloride was applied immediately upon offset of the 2-second foot shock. Sixteen minutes after reversal, the F-P-P group received a second application of potassium chloride and the F-P-S group received sodium chloride. Aversiveness of potassium chloride per se was ascertained in the three NF-P control groups by allowing animals to make the behavioral response on the training trial without the presentation of foot shock and then applying potassium chloride to the dura at one of the appropriate intervals. A maximum latency of 180 seconds was allowed on the retention test trial.

through the grid floor of the large compartment for 2.0 seconds. The retention test procedure was similar to that for training but without the presentation of either punishing foot shock or memory disruptive treatment. A maximum latency of 180 seconds was allowed for the retention test.

The CSD was initiated and maintained by application of potassium chloride solution directly onto the dura by means of bilateral cannula assemblies (*5*). Potassium chloride was applied immediately after training without physical disruption to the free-moving animal within the training compartment. After initiation of CSD, the cannulas were unscrewed from the cannula bases and were replaced with cotton pledgets soaked with potassium chloride. Animals that received potassium chloride at times other than the immediate interval were returned to their home cages after training. Treatment was initiated at these later intervals by applying pledgets soaked with potassium chloride to the dura at the appropriate interval. Loss of the tactile placing response was used as the behavioral manifestation of potassium chloride action on the brain (*6*). Any animal that did not show loss of the response after potassium chloride treatment was discarded. Four hours after initiation, CSD was reversed by removing the pledgets; the holes were swabbed with pledgets soaked in physiological saline and were dried with cotton, and nylon plugs were screwed into the bases.

In animals that received ECS, a segment of 26-gauge Chromel-A wire was inserted subcutaneously just behind and at the base of each ear at the time of cannula implantation. The wires were twisted into loops 1 cm in diameter. A 100-ma, 0.5-second ECS was administered through alligator clips attached to these loops. Animals that received ECS at intervals longer than 4 seconds were returned to their home cages after training. At the appropriate interval, ECS was administered to these animals while they were being held by the experimenter. All animals were returned to their home cages immediately after seizure induction. Animals that did not exhibit either tonic flexor or extensor seizures were discarded.

In the first experiment we investigated the memory disruptive effects of bilateral CSD induced at various times after training. Our purposes were (i) to replicate the U-shaped function of amnesia reported by Rabedeau (*7*), with inclusion of additional groups of animals, and (ii) to determine if initiation of CSD immediately after training results in a subcortical confinement of the memory trace in a labile form which is released to the cortex after the CSD reversal.

Degree of amnesia was investigated for three training-treatment intervals: immediate, 16 minutes, or 256 minutes. The experimental groups received foot shock followed by application of 25 percent potassium chloride solution (F-P, $N = 10$ per point); the learning control groups received the aversive stimulation followed by application of 25 percent sodium chloride solution (F-S, $N = 10$ per point). Three groups of animals were tested for possible aversiveness toward potassium chloride solution per se by having potassium chloride applied to the dura at the various intervals after they had entered the large compartment without presentation of foot shock (NF-P, $N = 5$ per point). Nineteen additional animals were given aversive training with potassium chloride administered via the cannula system immediately upon termination of foot shock, after which pledgets soaked in potassium chloride were left on the dura for 4 hours before the reversal procedure was initiated. Sixteen minutes after the reversal procedure, ten of the animals received a second potassium chloride application (F-P-P), while the other nine rats received sodium chloride (F-P-S).

Differences in response latencies for all groups were analyzed by the Kruskal-Wallis one-way analysis of variance and were found not to be significant for training but significant for retention ($P < .001$). The median latency for each group to enter the large compartment on the 24-hour retention test is shown in Fig. 1. The U-shaped function of retention deficit that resulted from CSD induced at the three intervals may be clearly seen in this figure. Between-group comparisons were made with the Mann-Whitney U test. Complete retention in the groups that received potassium chloride either immediately or 256 minutes after training was indicated by the fact that latencies for these two groups did not differ significantly from their respective F-S learning controls ($U = 46$ and $U = 44.5$) but did differ

significantly from the immediate and 256-minute NF-P controls ($P < .002$ for both, with $U = 0$ and $U = 1$, respectively). Retention deficits in animals that received potassium chloride 16 minutes after training were significant (16 minutes for F-P versus 16 minutes for F-S; $P < .02$, $U = 14$), with latencies being statistically indistinguishable from control animals that did not receive aversive training. The lack of aversiveness of potassium chloride applied to the dura is also indicated by the similar retention latencies for groups of animals that did not receive shock but were given potassium chloride at the immediate, 16-minute, and 256-minute intervals (NF-P).

The latencies for the F-P-P group were significantly lower than those for the F-P-S group ($P < .02$, $U = 10$). This retention deficit was not significantly different from that produced by a single potassium chloride application 16 minutes after foot shock ($U = 33$). Furthermore, the F-P-S group was not significantly different from animals that received sodium chloride 16 minutes after training ($U = 42$).

The data of this study confirm the finding of a U-shaped function of amnesia for the disruptive effects of CSD induced by potassium chloride (*7*). Rabedeau (*7*) interpreted this function as indicating an initial subcortical memory trace localization, which is followed within a few minutes by a release of the trace for eventual permanent storage involving cortical participation. We have chosen to interpret our data in a similar manner. Additionally, Rabedeau suggested (though without support of experimental data) that depression of the cortex during the initial phase of subcortical localization resulted in a confinement of the trace at the subcortical level until the return of normal cortical functioning. However, an alternative explanation is that immediate induction of CSD results in subcortical confinement and subsequent fixation of the trace without the involvement of the cortex. The amnesia observed in the F-P-P group in the present experiment (that is, the group that received a second potassium chloride treatment 16 minutes after reversal) supports the original hypothesis. That is, immediate induction of CSD appears to result in a temporary confinement rather than in permanent fixation of the trace at the subcortical levels, which indicates a delay or slowing down of the normal sequence rather than reorganization of the memory fixation process.

The purpose of the second experiment was to investigate the roles of the cortex and subcortex in the production of amnesia with ECS. Our reasoning for this experiment was as follows. If indeed (i) immediate CSD does confine the memory trace at the subcortical level in a labile form and (ii) ECS produces memory impairment

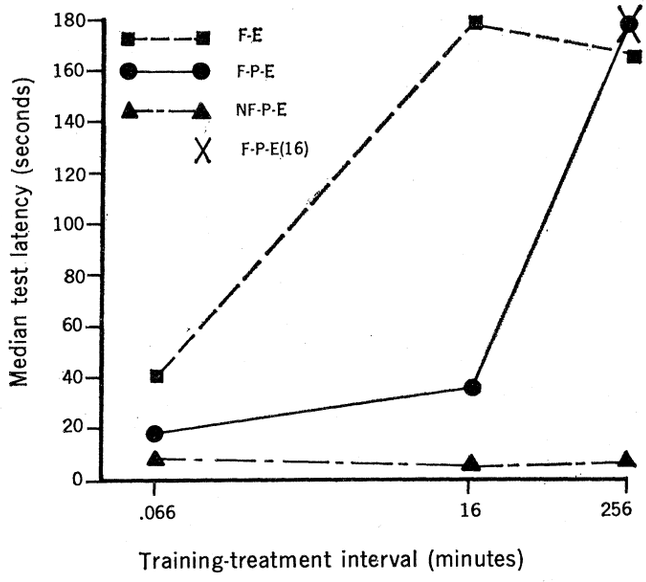

Fig. 2. Median retention test latencies as a function of the time elapsed between termination of foot shock (F) and electroconvulsive shock (E) administration in normal animals (F-E) and animals made functionally decorticate by application of potassium chloride directly to the dura immediately upon foot-shock offset (F-P-E). Animals in the NF-P-E groups received the same training procedure but without presentation of foot shock (NF). This was immediately followed by application of potassium chloride to the cortex with electroshock presented at one of the appropriate intervals. The reversal procedure (that is, removal of potassium chloride from cortex) was initiated 4 hours after chloride application except for animals in the F-P-E and NF-P-E groups that were convulsed at the 256-minute interval. In the last two groups, animals were convulsed first, and then the reversal procedure was undertaken. In the F-P-E (16) group, electroconvulsive shock was presented 16 minutes after the reversal procedure. Rats were allowed a maximum response latency of 180 seconds.

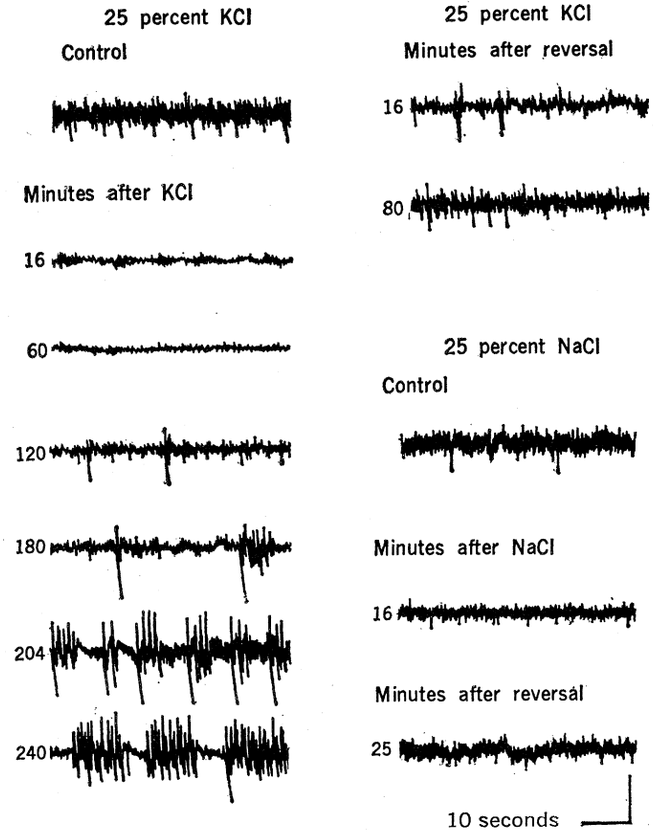

Fig. 3. Sample tracings showing the time course of electrocorticographic changes produced by application of 25 percent potassium chloride and 25 percent sodium chloride directly to the dura of the rat. The bipolar recordings were obtained from two separate animals by use of an eight-channel Offner Dynograph. Calibration: horizontal axis, 10 sec/cm; vertical axis, 100 μv and 200 μv for KCl and NaCl, respectively.

by disruption of this labile trace at the subcortical level, then it should be possible to extend the short temporal gradient of retrograde amnesia produced with ECS by using the memory trace confinement technique discussed above.

Ten groups of animals were used, with ECS delivered either 4 seconds, 16 minutes, or 256 minutes after training. In three of the groups, spreading depression was initiated immediately after foot-shock offset and ECS was delivered at one of the intervals (F-P-E). Four hours after initiation CSD was reversed for the first two groups ($N = 10$ each), and it was reversed immediately after ECS delivery in the 256-minute group ($N = 13$). Comparison of retention latencies was made with groups receiving ECS at each of the corresponding intervals after no foot shock and immediate potassium chloride treatment (NF-P-E, $N = 10$ per group) or after aversive foot shock alone (F-E, $N = 7$ per group). An additional group of ten animals received aversive training followed by immediate application of potassium chloride. The CSD was reversed 4 hours later, which was followed in 16 minutes by ECS [F-P-E (16)].

Initial latencies did not differ significantly between groups, but retention latencies did ($P < .001$). Median latency on the 24-hour retention test is shown in Fig. 2 as a function of the training-ECS interval under the various conditions. Delivery of ECS after aversive training and CSD resulted in a significantly longer amnesic gradient than did ECS after aversive training alone.

There were no significant differences between controls, which did not receive foot shock, and the two groups of trained animals that received ECS at the 4-second interval or the group of animals that received ECS at the 16-minute interval under CSD. As was anticipated from our previous experiments, which consistently demonstrated a short retrograde amnesia gradient for this task (*8, 9*), ECS was without effect when delivered 16 or 256 minutes after training alone or 16 minutes after CSD reversal. What was not anticipated, however, was the lack of memory disruption when ECS was applied 256 minutes after training but before CSD reversal [the difference between this group and the control group with no training was significant ($P < .02$, $U = 6$)].

In an attempt to clarify this finding, we decided to look at the electrocorticographic changes produced by potassium chloride applied to the dura in six additional rats, which had been previously prepared with bilateral cannula bases. Animals were lightly anesthetized with ether on the day after surgery and were placed in a stereotaxic apparatus. The scalp incision was reopened, and a local anesthetic was applied to the area. To obtain recordings in the conscious animal without movement artifacts, rats were immobilized with an intraperitoneal dose of *d*-tubocurarine (3 mg/kg). Artificial respiration was maintained by means of a Phipps and Bird respirator pump, with air being administered through a piece of polyethylene tubing (No. 50) inserted into one of the nostrils. Bilateral monopolar and bipolar electrocorticograms of both cerebral hemispheres were obtained from 0–80 stainless steel screws located approximately 8 mm anterior and 3 mm posterior to the centers of each trephined hole.

A marked attenuation of electrical activity was noted in all animals within 2 minutes after application of potassium chloride (the shortest possible delay between application of the cotton pledgets and switching the recorder to the record position). This attentuation remained essentially constant up until the second hour, during which time periodic spiking and bursts of activity began to appear. This activity increased in both frequency and intensity up to the time of initiating the reversal procedure. Administration of 25 percent sodium chloride produced a slight, transient decrease in electrocorticogram amplitude. Sample electrocorticographic recordings are presented in Fig. 3.

It has been demonstrated that the repetitive, periodic electroencephalographic (EEG) activity noted during CSD is accompanied by concomitant recovery of cortical functioning (*10*). This finding, coupled with the EEG recordings in the present study which show increased cortical activity within 120 minutes after induction of CSD, suggests that the lack of amnesia noted with the 256-minute ECS group prior to initiation of the reversal procedure was the result of at least a partial premature release of the subcortically confined trace with subsequent cortical interaction.

In summary, the data of the second experiment are consistent with the prediction of an extension of the ECS-induced retrograde amnesia gradient by subcortical confinement of the labile memory trace. On the basis of differences in short retrograde amnesia gradients obtained in the same task with use of ECS and CO_2 anesthesia, Paolino *et al.* (*8*) have suggested that memory trace consolidation may be a multistage process in which the stages are differentially susceptible to disruption by different treatments. We interpret the present data as being consistent with such a hypothesis and further suggest that these stages may operate in a sequential manner; that is, they may be connected in series instead of being parallel. Furthermore, the locus of action distinction for ECS and CSD may indicate that these stages are involved with different brain regions at different times after training. It is suggested that CSD induced immediately after training results in a slowing down of the fixation process by delaying cortical participation and thus prolongs the period of susceptibility to ECS disruption at the subcortical level. However, an alternative interpretation for this phenomenon is that CSD enhances the disruptive effectiveness of ECS by altering the pathway of the externally applied current through brain.

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7 January 1971; revised 18 February 1971