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THE EFFECT OF SPREADING DEPRESSION ON THE CONSOLIDATION OF LEARNING

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Abstract—The consolidation of an interhemispherically transferred avoidance response was studied using spreading depression to control transfer and consolidation times. Transmission of the learning required 3 min. The length of time following transfer when consolidation could be disturbed varied with the duration of spreading depression, from 3 min to 2 hr. The effect appears not to be due to disruption of neural firing, but to the cortical potential change caused by spreading depression since a pulsating cathodal current (8.5 μA/mm^2) passed through medial (but not anterior, lateral, or posterior) cortex can abolish retention. It is suggested that the consolidation process may require electrical potential gradients in the cortex and that it is disturbed by agents which decrease or reverse them.

1. INTRODUCTION

PATTERNED neural firing has generally been considered necessary to consolidation. This patterned activity is thought to be organized during acquisition and to continue afterwards in the form of a temporary reverberation in closed neural circuits. Physically, the persisting neural activity is supposed to give rise to a permanent change in the synaptic relations between the active cells and this must occur before activity in the temporary circuit is altered, or retention will be absent or impaired (for a theoretical development of these ideas, see HEBB [15]).

The suggestion that reverberatory activity is necessary to consolidation, though reasonable, seems to be supported primarily by the observation that several agents which interfere with consolidation, such as convulsions and spreading depression, also conspicuously disrupt patterned neural firing. This observation is not conclusive; the additional effects of convulsions and spreading depression on surface potential [7, 19], amount of neural firing [13, 14], neural metabolism [3, 18] and others [19], do not permit a conclusion as to which effect interferes with consolidation.

In contrast, there is considerable evidence that neural firing is not necessary to most of the consolidation process. For example, severe hypothermia (cooling to 2–5°C) blocks neural firing in the cortex and thalamus but does not interfere with retention when induced longer than several minutes following the acquisition trials [27]. Similarly, altering neural activity with anesthetics, tranquilizers, or sleep has little or no negative effect on consolidation [16, 24, 25, 26].

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Present evidence seems to suggest that neural firing may be necessary during the first several minutes following acquisition but not for the entire 1 or 2 hr during which consolidation is known to continue (see ALBERT [1], for a more thorough consideration of the argument). There is a promising technique for further investigation of the process of consolidation in the one-trial interhemispheric transfer of learning discovered by RUSSELL and OCHS [31], and a method of interfering with consolidation in cortical spreading depression [5] which has the advantage of being reasonably well understood physiologically.

The design of the present experiments is first to repeat the findings of RUSSELL and OCHS [31] with an avoidance response. The second step is to show that the interhemispheric transfer is very rapid—an important finding in itself but one which will not be considered further in these experiments. The third step is to show that spreading depression does interfere with consolidation of the transferred learning and that the interval required for consolidation is much longer than that of transfer.

The remaining experiments are concerned with the processes involved in consolidation. In the first, some evidence is presented that consolidation is primarily a cortical process. But the more significant experiments are those which consider the relation between the disturbance of retention and the duration of spreading depression. The results suggest that the interruption of consolidation by spreading depression is complex but that it does not depend on the disorganization of a pattern of neural firing. The final experiments test the possibility that consolidation depends on conditions in the cortex which are disturbed by some aspect of the cortical potential change of spreading depression.

2. METHOD

The experiments all follow a single schedule with minor variations to accommodate the different ways of interfering with consolidation. The subjects were naive male hooded rats weighing 200–250 g from the Quebec Breeding Farm. Cannulas for starting spreading depression were implanted over the medial area of each hemisphere. On the following day (day 1) the animals were trained on an avoidance task while one hemisphere had cortical spreading depression. It is known that spreading depression disturbs the functioning of the affected hemisphere and that with some tasks, the learning that occurs while one hemisphere is depressed is recorded only in the normal hemisphere [4, 28, 31].

On day 2, the animals were given a transfer trial with both hemipheres functional. This causes the learning to transfer from the "trained" to the "untrained" hemisphere. The treatment of the animals, in general, differed only at this point when some agent was used to interfere with consolidation in the hemisphere receiving the transferred learning.

On day 3, the trained hemisphere was depressed and the animal was tested for retention of the transferred learning on the untrained hemisphere. If the disturbance of consolidation abolished retention the animal should take as many trials to learn as with the original training, but if there is retention, the animal should learn in fewer trials. Five to eight animals were usually sufficient to establish a reliable estimate of the amount of retention.

2.1 *Spreading depression technique*

The method of starting spreading depression was to inject potassium chloride (KCl) into a cannula resting on the dura mater over one hemisphere, a method developed by TAPP [32]. The cannula was made from a polyethylene tube (1.90 mm outside diameter; 1.40 mm inside diameter) about 1 cm long. The tube was first flared at one end by heating and the flare was then thinned with a grinding stone. Plugs for the cannulas were also

made from polyethylene tubing (1.52 mm outside diameter) which was heat-sealed at one end and cut to a length of about 3 mm.

The spreading depression cannulas were bilaterally implanted through the antero-lateral parietal plate of each hemisphere while the animal was anesthetized with pento-barbital (Nembutal). A trephine hole in the skull (2.1 mm dia.) was made, leaving the dura mater intact, and the flare of the cannula was gently slipped into this hole between the skull and the dura mater. The bilateral cannulas were held in place with dental cement which itself was held to the skull with screws. The cannulas when not in use were filled with sterile 0.9 per cent saline and capped.

Cortical spreading depression was started by filling the cannula over one cortex with sterile 12 per cent KCl. The KCl was left in the cannula during the entire period that spreading depression was wanted on the cortex and afterwards allowed to dissipate by flushing the cannula with 0.9 per cent saline.

For training or testing during unilateral spreading depression, 12 per cent KCl was injected into the cannula at least 15 min beforehand. The presence of spreading depression was verified by noting a hypesthesia of the body contralateral to the depressed hemisphere. A particularly useful indication was failure to bring the forepaw on the insensitive side back to its normal position when it was moved under the chest. Hypesthesia was also evident from the tendency to allow the forepaw, and occasionally the hind leg, to hang through the grid of the avoidance apparatus.

2.2 *The avoidance task*

The avoidance apparatus was a box $36 \times 10 \times 18$ in. deep with a grid floor. The box was divided into a black and a white half by a sliding partition. The grid floor could be electrified with a 0.33 mA current from a 400-V transformer.

Training in the avoidance task began by putting the animal into the black compartment with the dividing partition in place. After 1 min, the animal was placed in the white side facing the end wall and the partition removed. If the animal moved from the white side to the black side within 5 sec the trial was counted as an avoidance, and the partition was put back in place. If not, the animal was shocked intermittently until it escaped to the black side. There was a 1 min intertrial interval during which the animal remained in the black compartment.

Training continued until the animal reached a criterion of 9 avoidances in 10 successive trials. Animals which reached criterion in 10 or less trials, not counting the criterion trials, were discarded as were animals that did not reach criterion in 30 trials. In the former, unilateral spreading depression had probably not occurred, allowing the animal to learn much faster with both hemispheres functional. Animals that did not reach criterion in 30 trials were discarded because preliminary experiments showed that these animals often would not learn even with additional trials. The procedure was the same during the retention test, but animals being tested for retention were discarded only if they did not reach criterion in 30 trials. About 10 per cent of the animals were discarded for these reasons.

2.3 *Methods of interfering with consolidation*

The technique for using spreading depression to interfere with consolidation was the same as that used for making one hemisphere nonfunctional during avoidance training. Various durations of spreading depression were used to interfere with consolidation. These were not measured directly but were kept constant among animals of the same group by estimating the duration as the time between the first observation of the hypesthesia of spreading depression and the time the KCl was flushed from the cannula.

Subcortical chemical injections [23] were used to disturb separately various parts of the brain that might be involved in consolidation. A chemical that disturbs neural activity was injected into the area through a guide cannula made of 23-gauge stainless-steel tubing which had previously been stereotaxically implanted to end at a point 1 mm above the desired injection site. This cannula was implanted along with the spreading depression cannulas and when not in use was covered with a polyethylene cap. The actual injection was made through a 30-gauge stainless-steel needle which passed through the guide cannula and extended 1 mm further to the injection side. The volume of the injection was controlled with a microsyringe.

Following the experiment, the site of the injection was determined histologically. The rat was perfused and the brain removed, sectioned, and stained. The only change in the general procedure required for the use of this subcortical injection method was to allow several days instead of only one for the animal to recover from the surgical operation.

The slow potential change of spreading depression was considered as a possible cause of the disturbance of consolidation by testing the effect of a potential variation caused by passing a small electric current through the cortex. The current was passed from an electrode above the dura mater, through the animal, and out a silver wire lead on the back. The electrode on the head was held by a polyethylene cannula. The current from this electrode went through the saline in the cannula and then through the dura where the immediate area of polarization was 1.5 mm^2, the area of the bottom of the cannula.

The cortical polarizing electrode was of silver-silver chloride [7] made from 36-gauge silver wire. The current source was a 90-V battery in series with a 10-MΩ potentiometer and a several megohm resistance.

3. RESULTS

For simplicity of communication, the description of these experiments will use the following terminology. The *trained*, or *transmitting*, hemisphere is the one that is normal during the first training; it is the hemisphere that records the learning and transmits it to the other during the transfer trial. The *untrained*, or *receiving*, hemisphere is depressed during the first training and later receives the transferred learning.

The first step in this research was to establish that one-trial interhemispheric transfer is possible with an avoidance task. Two groups of rats were trained with one hemisphere depressed. (In this and subsequent experiments, the number of animals in each group is given in the table or figure containing the results.) Twenty-four hours later, the first was then given a transfer trial with both hemispheres functional. The next day, the originally trained hemisphere was depressed and the animals were tested for retention of the habit. The second group, instead of a transfer trial, was given an additional trial with the untrained hemisphere again depressed and was also tested for the presence of learning in the untrained hemisphere on the following day.

The effect of the transfer trial is clear (Table 1). The group with the transfer trial took an average of 4.9 trials to reach mastery of the habit in retraining, the second group took 13.4 trials ($P<0.01$; all statistics are two-tailed rank tests). To control for the possibility that the good performance of the first group was not due to transfer but to the fact that it had one trial with both hemispheres functional (since the rate of learning with both hemispheres is more rapid than when one is depressed), a third group with no previous training was given one trial with both hemispheres functional and was then tested with

one hemisphere depressed. The number of trials to mastery was 14.9, not as fast as the second group. (There were no significant differences between groups on the original learning in this or succeeding experiments.)

Table 1. The effect of a transfer trial, with both hemispheres functional following unilateral learning, on the rate of learning by the untrained hemisphere

Group	N	Mean trials to learn	
		First learning	Learning with untrained hemisphere
Transfer trial	8	16.3±5.8	4.9±1.1*
No transfer trial	8	14.8±4.1	13.4±6.0
Transfer trial only	7	—	14.9±6.0

* Significantly different from the first learning ($P<0.01$).

These results show that when one hemisphere has been trained, it can transmit some of its information in one trial to the second, untrained, hemisphere.

The next step was to determine how long a period is necessary for the transmission of information and how long a period is necessary for its consolidation in the receiving hemisphere. The time required for transmission of the learning was found by disrupting activity in the transmitting hemisphere with spreading depression at various times following the transfer trial, and about 30 min later testing the animal for retention while the trained, or transmitting, hemisphere was still depressed.

The transfer was found to be complete at 3 min (Fig. 1). When 3 min was allowed for transmission, the relearning took 1.4 trials, and with 15 min the learning was not faster

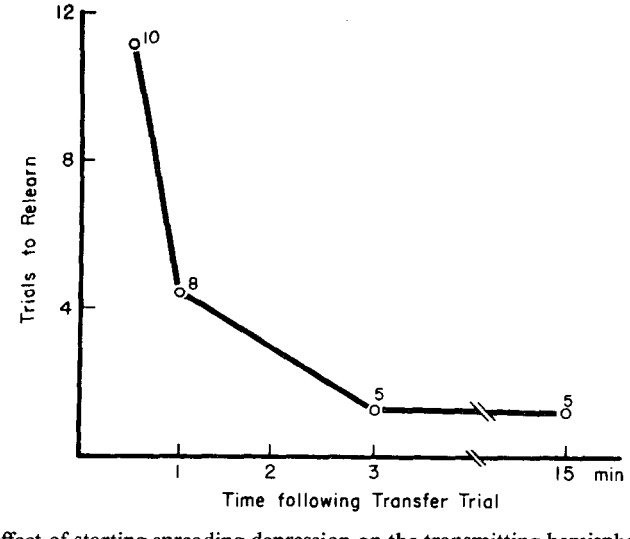

FIG. 1. The effect of starting spreading depression on the transmitting hemisphere at different times after the transfer trial. Transfer is measured in mean trials to relearn with the receiving hemisphere. The number of animals used to determine each point is shown.

(1.4 trials). With shorter times, relearning took longer (1 min, 4.6 trials; 30 sec, 10.9 trials) though in each case there was a significant ($P<0.05$) savings over the original learning. This 3-min transfer time is consistent with the findings of RAY and EMLEY [28] and DORFMAN [10].

With some of the animals in the above groups, the same procedure was followed but retention was not tested until the following day. It seemed possible when the experiment was planned that though spreading depression might interrupt transmission the effect would also be temporary, and transmission might be resumed when the depression had dissipated. However, the performance of these animals was slightly poorer, which seems to show that there was no reactivation of the transfer process after the transmitting hemisphere recovered from spreading depression. The fact that performance was actually poorer suggests a loss of retention instead.

The time course of consolidation in the receiving hemisphere was determined in the same experiment by disrupting the activity of the receiving (instead of the transmitting) hemisphere at varying times following the transfer trial. The spreading depression was maintained on the receiving hemisphere for 30 min. The animals were tested for retention on the following day with the originally trained hemisphere depressed.

The results: spreading depression started at any time within 2 hr after the transfer trial abolished retention by the receiving hemisphere (Fig. 2). Relearning took 14.2 trials for a 1-hr group and 12.0 trials for the 2-hr group; these do not differ significantly and neither represents savings over the original learning. A 3-hr group learned significantly faster (3.4 trials, $P<0.05$) as did a 4-hr group (3.8 trials). The 3- and 4-hr groups did not differ significantly.

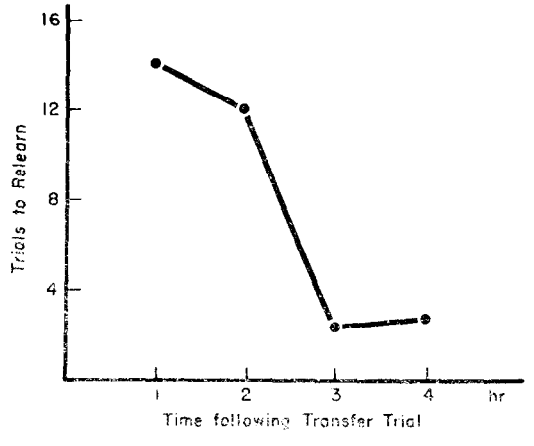

FIG. 2. The effect on retention of starting a 30-min period of spreading depression on the hemisphere receiving the transferred learning. Retention is measured in mean trials to relearn. The scores of 5 animals determine each point.

The one-trial interhemispheric transfer of an avoidance response gives every evidence of being a good preparation with which to study consolidation. The transfer does not take place all at once as would be desirable, but it occurs rapidly and leaves at least a 2-hr period in which to study the process of consolidation.

3.1 *Where consolidation is disturbed*

It is known that the massive firing of cortical spreading depression can invade the amygdala and the caudate nucleus [11] but that in other subcortical structures there is only a change in the pattern of firing [6, 8, 34]. The role of these extracortical disturbances in the disruption of consolidation by cortical spreading depression was explored using chemical injections to create disturbances in three subcortical structures which were thought most likely to be involved in consolidation: the hippocampus, midline thalamus [20], and amygdala [12].

Hippocampal spreading depression was used to disturb the activity of the hippocampus. It was started by injecting 12 per cent KCl into the dorsal hippocampus (5 microliters [μl] at 2 μl/min). The effects are known to spread through the entire structure [4]. The behavioral effect was a contralateral hypesthesia which lasted 5–10 min.

The pattern of firing of the midline thalamus in the region of the paraventricular and rhomboid nuclei was suppressed by 5 per cent procaine hydrochloride (6 μl at 2 μl/min). Interference with normal activity lasted about 20 min as judged by the length of time the placing response was absent.

Neural firing in the amygdala was also suppressed using 5 per cent procaine hydrochloride (6 μl at 2 μl/min). This caused circus movements, ataxia, and slight hyperactivity lasting 10–15 min.

During the first training one hemisphere was depressed. The next day a transfer trial was given and 5 min later a unilateral chemical injection was made into the hemisphere receiving the transferred learning. A control group had cortical spreading depression started by the microinjection method (12 per cent KCl, 5 μl at 2 μl/min). Twenty-four hours later, the trained hemisphere was depressed and the animals were tested for retention of the transferred learning.

The number of trials to relearn for the subcortical injection groups was not statistically different from that of a control group with no physiological disturbance (Table 2). The subcortical groups and the control group had averages between 3 and 5 trials. Each of these groups learned significantly ($P<0.05$) faster than the group with cortical spreading depression, which took 12.3 trials.

Table 2. Retention of the interhemispherically transferred learning when a subcortical structure in the receiving hemisphere is disturbed during consolidation

Structure and disturbance	N	Mean trials to learn	
		First learning	Learning with untrained hemisphere
Hippocampus (hippocampal spreading depression)	6	15.3 ± 2.4	3.5 ± 1.7*
Midline thalamus (procaine synaptic block)	6	12.5 ± 3.0	4.3 ± 2.1*
Amygdala (procaine synaptic block)	6	17.8 ± 4.7	3.5 ± 2.6*
No injection	6	17.3 ± 6.0	4.7 ± 1.9*
Cortex (spreading depression)	6	15.5 ± 3.5	12.3 ± 3.8

* Significantly different from first learning ($P<0.01$).

The failure of the subcortical disturbances to impair retention is not conclusive since other nuclei might be involved, but it does suggest that cortical spreading depression acts on the consolidation of the transferred learning directly, not by disturbing the activity of some other (subcortical) structure. It thus appears that with this learning consolidation is primarily a cortical process.

3.2 *The nature of the cortical effect*

The next experiments considered the possibility that spreading depression disrupts consolidation by disorganizing a pattern of neural firing in the cortex. The first experiment made use of the fact that spreading depression consists of a series of waves of activity, including massive neural firing, which cross the cortex at intervals of several minutes [13, 14, 19]. Since the first wave of activity should completely disrupt the pattern of neural firing, the disturbance of consolidation should be independent of the number of waves, or the duration, of spreading depression.

The animals were trained with one hemisphere depressed and on the following day given a transfer trial. A period of spreading depression of 2, 5, or 30 min was applied to the receiving hemisphere at various times following the transfer trial. On the following day, the animals were tested for retention of the transferred learning.

The results are shown in Fig. 3. It appears clearly that a longer period of spreading depression has a greater effect than a short period, in the sense that it can be applied later and still disrupt consolidation. The 30-min period, for example, significantly ($P<0.05$) disturbs retention when applied as late as 2 hr after the transfer trial; the 2-min period does so only if applied within 1 min of the transfer trial.

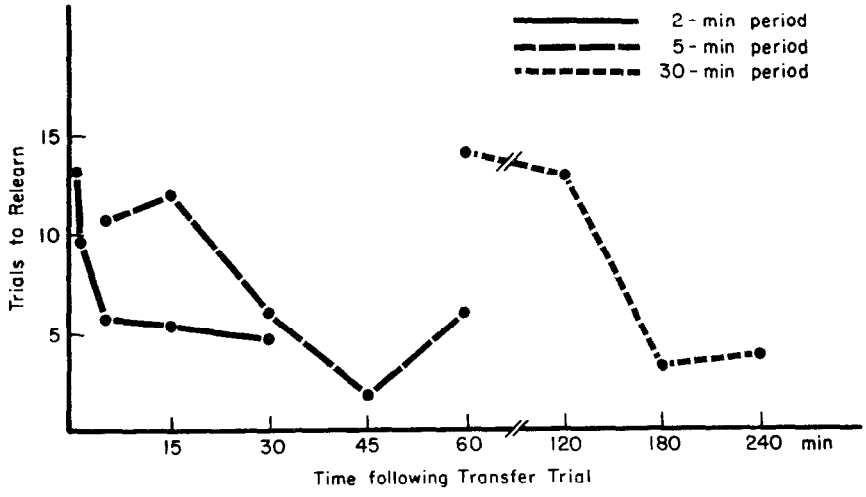

FIG. 3. The effects of 2-, 5-, and 30-min periods of spreading depression applied to the receiving hemisphere at varying intervals following the transfer trial, in terms of the number of trials for relearning. Each point is the average score of 5 animals.

These results show that spreading depression does not have its effect simply by disorganizing a pattern of neural firing, which a short period must do as completely as a long one, but that there is some other way in which it blocks (or reverses) consolidation.

Another way of making the same point is the following. Consider the effect of a 2-min period of spreading depression. If it is applied later than 1 or 2 min following the transfer trial, there is no apparent effect on retention; but there must still be some latent effect on the consolidation process, because if other waves of spreading depression are allowed to follow—in other words, if the spreading depression is left on the cortex longer than 2 min—retention is completely abolished. The nature of this latent effect can be found by starting a second period of spreading depression at varying times after the first 2-min period.

Following the transfer trial the experimental group was given a 2-min period of spreading depression at 5 min after the transfer trial, and then at varying times after that a second period 5 min long was started. The control group was given only the 5-min period of spreading depression at varying times after the transfer trial. Twenty-four hours later the animals were tested for retention with the trained hemisphere depressed.

The results are shown in Fig. 4. The 2-min period of spreading depression clearly lengthened the time when the 5-min period could be started and still disturb retention. The 5-min period alone impaired retention only if started within 15 min of the transfer trial, but when preceded by the 2-min disturbance, it impaired retention when started within 6 hr.

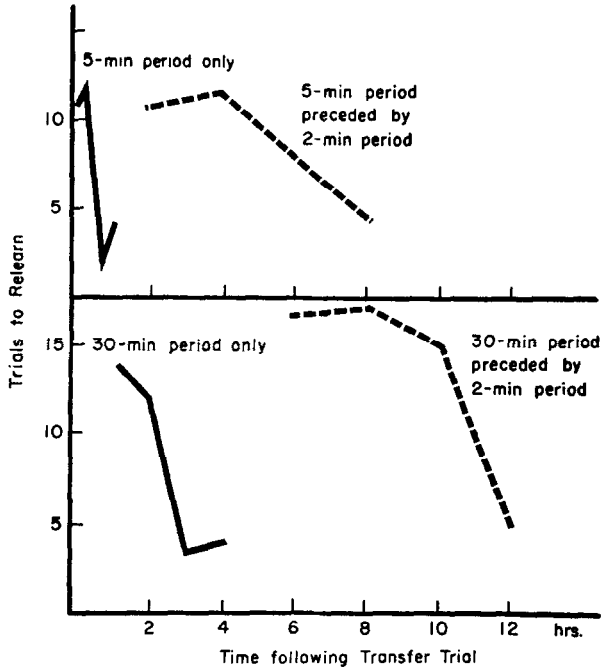

FIG. 4. The effect of a 2-min period of spreading depression beginning at 5 min following the transfer trial on the length of time when a second period, 5 or 30 min long, can be started and still impair retention of the transferred learning. The average scores of 5 animals determine each point.

The experiment was repeated using a 30-min instead of the 5-min period of spreading depression. The experimental group was given the 2-min period of spreading depression

5 min after the transfer trial, and then the second period, 30 min long, at varying times afterward; the control group had only the 30-min period of spreading depression. The animals were tested for retention of the transferred learning 24 hr after the 30-min period ended.

The 2-min period of spreading depression again lengthened the time during which consolidation could be disrupted (Fig. 4). With only the 30-min period, retention was disturbed if the spreading depression began within 2 hr after the transfer trial, but when the 2-min period was given at 5 min after transfer, the 30-min period could be started 10 hr later and still significantly ($P<0.05$) impair retention.

The effect of the 2-min period of spreading depression seems to be to slow consolidation and not to stop it as one would expect if the disorganization of neural firing were critical. It is concluded that there is a slowing rather than a temporary arrest of consolidation since the length of time when the 5- or 30-min period of spreading depression could be applied and still affect retention was not increased by a constant amount corresponding to the time consolidation was stopped.

3.3 *The component that disturbs consolidation*

The massive neural firing of spreading depression does not seem to disturb consolidation by disorganizing a pattern of neural firing but there is evidence that another physiological component, the slow surface-negative potential change which follows the wave of neural firing across the cortex, disturbs consolidation. This potential change, which lasts about a minute at any one point and has a maximum amplitude of 5–10 mV, may be comparable to the d.c. current which has been found by MORRELL and NAITOH [22] to impair acquisition of a conditioned response when imposed during learning.

The effect of the slow potential change was tested using a d.c. current with the negative electrode near the cortical surface. The shape of the spreading depression slow wave was approximated by modulating the current in a 2-min cycle: 15-sec ramp rise to peak current

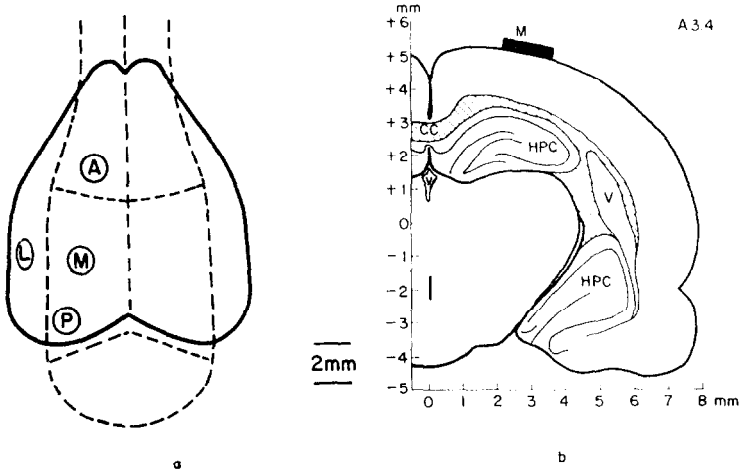

FIG. 5. (a) A dorsal view of the brain (solid line) showing the placement of anterior (A), medial (M), posterior (P) and lateral (L) polarizing cannulas. Placement of the cannulas was guided by the skull markings (dotted line). The diagram is modified from one by KRIEG ([15] p. 203). (b) A coronal section of the brain through the cortical region below the medial polarizing cannula (M). The diagram is from the DE GROOT ATLAS [9a] (HPC, hippocampus; CC, corpus callosum; V, ventricle).

(8.5 $\mu A/mm^2$) and maintained at that level for 45 sec, then a 15-sec ramp decrease to 0 current and maintained for 45 sec. The spread of the wave across the cortex was not simulated. Instead, the experiment depended on the possibility that consolidation was localized in a particular part of the cortex. Only one region was polarized in each animal: anterior, medial, posterior, or lateral cortex (Fig. 5).

During the first training, one hemisphere was depressed and the next day a transfer trial was given. Polarization of a cortical region in the receiving hemisphere began 5–10 min later and was continued for 16 min. The following day, the trained hemisphere was depressed and the animal was tested for retention of the transferred learning.

The surface-negative, or cathodal, polarization abolished retention but only when applied over the medial area (Table 3). There were no significant differences between the relearning scores of a control group with no polarization (4.8 trials) and the anterior and posterior groups (4.8 and 5.4 trials, respectively) but each relearned significantly faster ($P<0.05$) than the medial group (19 trials). Retention in the lateral group was impaired slightly (7.4 trials); relearning was significantly faster than the first learning but not different from either the control group or the medial polarization group. In an additional group the possibility was tested that the poor retention of the medial group was due to some aspect of the polarization or to the current irrespective of polarity. This group was given anodal polarization over the medial area. The relearning took an average of 4.5 trials, not different from the control group with no current.

Table 3. The effect of polarizing different areas of the receiving hemisphere on retention of the transferred learning

Group	N	Polarity of cortical electrode	Mean trials to learn	
			First learning	Learning with untrained hemisphere
Anterior	8	negative	13.3±1.9	4.8±3.8*
Medial	8	negative	15.4±5.6	19.0±8.8
Medial	8	positive	17.5±8.1	4.5±1.5*
Posterior	7	negative	16.0±5.8	5.4±3.4*
Lateral	8	negative	15.8±4.8	7.4±4.7*
No polarization	8	—	18.3±5.6	4.8±1.8*

* Significantly different from first learning ($P<0.05$).

These results show that a surface-negative potential change can interfere with consolidation. They also suggest that the consolidation process for this learning is localized in the medial region of the cortex. The coordinates of the medial cannula are approximately 2.5 mm lateral to the midline and 3.5 mm posterior to bregma (see Fig. 5); a precise placement of the cannula did not appear to be critical.

Another experiment was done to more clearly compare the effects of cathodal polarization and spreading depression on consolidation. This experiment tested the possibility that the disturbing effect of polarization, like spreading depression, depends on the duration for which it is maintained.

The animals were trained and given a transfer trial. At varying times afterward, a 6- or a 16-min period of cathodal polarization was started (same current parameters as in the previous experiment). The animals were tested for retention on the following day.

The results are shown in Fig. 6. Both the 6- and 16-min periods of surface-negative polarization blocked consolidation when started early, but the 6-min period did not impair retention when started at 1 hr after transfer (3.0 trials) while the 16-min period abolished retention when started as long as 2 hr after transfer (15.2 trials).

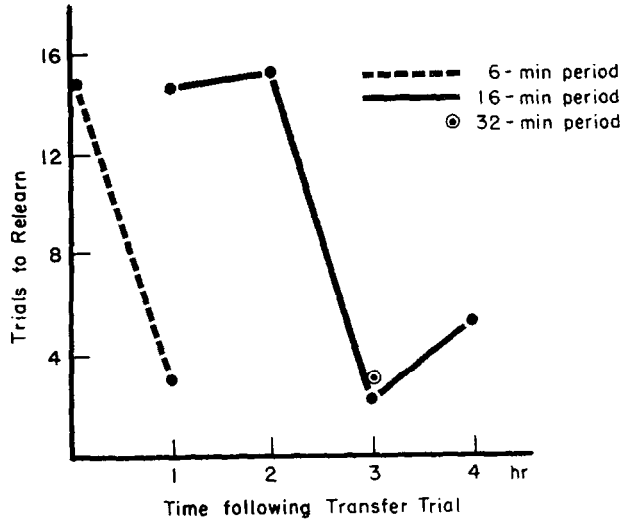

FIG. 6. The effect of a 6-, 16-, or 32-min period of cathodal polarization applied to the medial region of the receiving hemisphere at varying times following the transfer trial in terms of the number of trials for relearning. Each point is the average score of 5 animals.

Surface-negative polarization is clearly similar to spreading depression in the sense that it is also more effective when applied for a longer period. In addition, however, the effectiveness of the 16-min period of polarization corresponds closely to that of the 30-min period of spreading depression (Fig. 3); both impaired retention when started at 2 but not 3 hr after transfer. It seemed likely that this was not a coincidence of having picked the equivalent times for the two agents, but instead, but at about 2 hr after transfer, the part of consolidation which spreading depression and cathodal polarization disturb is complete. This means that polarization started longer than 2 hr after transfer should not interfere with consolidation even if the length of polarization is increased far beyond that which abolished retention at 2 hr. To test this, a group of animals was given a 32-min period of cathodal polarization 3 hr after the transfer trial. When tested the following day, they requred 3.0 trials to relearn, a significant savings over the original learning. This result suggests that cathodal polarization and spreading depression cannot abolish retention when started longer than 2 hr after transfer because the process with which they interefere is complete.

A final pair of experiments examined the importance of two parameters of the cathodal polarizing current for the effect on consolidation. In the first experiment, two wave forms were compared; one was the pulsating current described in the previous experiments and

the other was a constant current of the same intensity (15-sec ramp rise to peak current and maintained for 15.5 min, then a 15-sec ramp decrease to 0 current).

About 5 min following the transfer trial the constant or pulsating cathodal current was applied over the medial area of the receiving hemisphere and maintained for 16 min. Retention was tested the next day.

Table 4 shows that there is a definite effect of the wave form. The group with the constant current learned in an average of 7.9 trials, a significant ($P<0.01$) savings over the original learning, while the group with the pulsating current took 14.8 trials, not faster than the original learning.

Table 4. The effect of two wave forms of the cathodal polarizing current on retention of the transferred learning

		Mean trials to learn	
Wave form	N	First learning	Learning with untrained hemisphere
Pulsating	6	12.7 ± 2.1	14.8 ± 5.4
Constant	8	16.5 ± 3.9	7.9 ± 5.7*

* Significantly different from first learning ($P<0.05$).

This result shows that a pulsating polarizing current is more effective than a constant current in disturbing consolidation. This suggests that a shorter wave length, which is more comparable to those actually occurring in the nervous system, might be even more effective.

In the second experiment, the importance of using a particular current intensity was considered. About 5 min after the transfer trial, a pulsating current of 8.5 or 4.0 $\mu A/mm^2$ was started and maintained for 16 min.

Neither group retained the learning when tested the following day (Table 5). The low intensity group learned in 12.5 trials and the high intensity group in 14.8 trials, not significantly different from one another or the original learning scores.

Table 5. The effect of two current strengths of cathodal polarizing current on retention of the transferred learning

		Mean trials to learn	
Current strength ($\mu A/mm^2$)	N	First learning	Learning with untrained hemisphere
4.0	8	16.5 ± 3.5	12.5 ± 4.1
8.5	6	12.7 ± 2.1	14.8 ± 5.4

The effectiveness of the low intensity current suggests that many current intensities could be used to disturb consolidation, though as with the effect of duration (Fig. 3), higher intensities (within limits) would be more effective than lower ones.

4. DISCUSSION

Surface-negative polarization does disturb consolidation and it is clear that the effect is similar to that of spreading depression. The time following learning when each can be started and still disturb retention seems to be the same and the effectiveness of each is dependent on its duration. These findings suggest that consolidation is disturbed by some aspect of the cortical potential disturbance of spreading depression such as the reversal of the normal surface-positive potential gradient of the cortex, the disturbance of intracellular potentials, or the change in distribution of ions.

The failure to find that neural firing is necessary to consolidation extends the finding of RANSMEIER [27] that neural firing can be blocked at 5 min following an acquisition trial without impairing retention. The present experiments suggest that neural firing is necessary only during the 3 min when the learning is being transmitted from the trained to the untrained hemisphere (Fig. 1) since it is only during this "acquisition" period in the receiving hemisphere that a disturbance of neural firing by a short period of spreading depression impairs retention (Fig. 3). By the end of this 3-min period the information is in a form other than a pattern of neural firing. It is logically possible that there is a short-lasting reverberatory process during the acquisition period which completes the process of making synaptic changes, and that the loss of retention with spreading depression started after this time means that there is a reversal of these changes or an addition of further synaptic connections produced by massive cortical firing. This interpretation, however, is not supported by the other findings. For example, a cathodal current does not cause massive neural firing but does abolish retention while anodal current, which might be expected to increase firing [9], does not interefere with retention (Table 3).

The present results favor a scheme for the consolidation process which involves transient potential changes in the cortex. The basic idea is that the consolidation process may depend on potential relations in the cortex which are established during acquisition. There is evidence to support this scheme from experiments during surface-positive polarization and from the present experiments using surface-negative polarization.

The experiments with surface-positive polarization were done by RUSINOV and co-workers while investigating learning [29, 30]. They have shown that when a surface-positive current is applied to the cortex, some of the polarized cells respond to any strong stimulus that is presented to the animal, and in addition, temporarily (2–3 hr) retain, or "learn", the response to this stimulus so that when the current is turned off, they still respond when the stimulus is presented. That surface-positive potentials are involved in consolidation as well as learning is suggested by the finding that the learning that occurs with anodal polarization is retained longer if a pulsating rather than a constant current is used; the pulsating current lengthens the period of retention from hours to several days and this effect does not seem to be due to a general improvement in learning since acquisition seems to occur just as well with a constant current. This suggests that a pulsating surface-positive current can initiate some process necessary for consolidation.

The effect of the surface-negative current used in the present experiments seems to be the opposite of these surface-positive effects. It blocks consolidation, and like anodal current, it is effective with a pulsating but not a constant wave form. This suggests that the surface-negative current blocks (or reverses) a process that was established by oppositely directed potential changes during learning.

A process of consolidation which depends on potential relations is generally consistent with the evidence for consolidation. One would expect the process to be disturbed by

agents which cause large potential disturbances (except those which are equivalent to a surface-positive current) such as those created in the cortex by convulsions and spreading depression, but not so much by anesthetics and depressants, which do not have this effect. The rate of such a process would be variable, depending on the existing conditions. It might be slowed (Fig. 4) or speeded (as seems to be the effect of extra learning trials [5]) if the conditions are altered appropriately.

The mechanism for storing information and the kind of consolidation process which might be affected by these potentials is not clear at present; this is, however, considered in the succeeding paper which reports additional experiments on the effects of polarizing currents on consolidation [2].

REFERENCES

1. ALBERT, D. J. *The Effect of Spreading Depression on the Consolidation of Learning.* Unpublished doctoral thesis, McGill University, 1964.
2. ALBERT, D. J. The effects of polarizing currents on the consolidation of learning. *Neuropsychologia* **4,** 65–77, 1966.
3. BUREŠ, J. Some metabolic aspects of Leao's spreading depression. *J. Neurochem.* **1,** 153–157, 1956.
4. BUREŠ, J. Reversible decortication and behavior. In *The Central Nervous System and Behavior,* BRAZIER, M. A. B. (Editor) pp. 207–248, Josiah Macy, Jr. Foundation, New York, 1959.
5. BUREŠ, J. and BUREŠOVÁ, O. Cortical spreading depression as a memory disturbing factor. *J. comp. physiol Psychol.* **56,** 268–272, 1963.
6. BUREŠ, J., BUREŠOVÁ, O., WEISS, T. and FIFKOVÁ, E. Excitability changes in non-specific thalamic nuclei during cortical spreading depression in the rat. *Electroenceph. clin. Neurophysiol.* **15,** 73–83, 1963.
7. BUREŠ, J., PETRÁŇ, M. and ZACHAR, J. *Electrophysiological Methods in Biological Research.* Publishing House of the Czechoslovak Acad. Sci., Prague, 1962.
8. BUREŠOVÁ, O., BUREŠ, J. and FIFKOVÁ, E. Analysis of the effect of cortical spreading depression on the activity of reticular neurones. *Physiologia bohemoslov.* **11,** 375–382, 1962.
9. CREUTZFELDT, O. D., FROMM, G. H. and KAPP, H. Influence of trans-cortical d.c. currents on cortical neuronal activity. *Expl Neurol.* **5,** 436–452, 1962.

9a. DE GROOT, J. The rat forebrain in stereotaxic coordinates. *Verh. K. ned. Akad. Wet.* **LII,** No. 4, 1959.

10. DORFMAN, L. J. Interhemispheric transfer of an avoidance response. *Undergrad. Res. Proj. Psychol.,* McGill University **5,** 22–25, 1963.
11. FIFKOVÁ, E. and SYKA, J. Relationship between cortical and striatal spreading depression in the rat. *Expl Neurol.* **9,** 355–366, 1964.
12. GODDARD, G. V. Amygdaloid stimulation and learning in the rat. *J. comp. physiol. Psychol.* **58,** 23–30, 1964.
13. GRAFSTEIN, B. Mechanisms of cortical spreading depression. *J. Neurophysiol.* **19,** 154–171, 1956.
14. GRAFSTEIN, B. Locus of propagation of spreading depression. *J. Neurophysiol.* **19,** 308–316, 1956.
15. HEBB, D. O. *The Organization of Behavior.* Wiley, New York, 1949.
16. HEISTAD, G. T. Effects of chloropromazine and electroconvulsive shock on retention of a modified "one-trial" conditioned avoidance. *J. comp. physiol. Psychol.* **51,** 209–212, 1958.
17. KRIEG, W. J. S. Connections of the cerebral cortex—I: The Albino rat—A: Topography of cortical areas. *J. comp. Neurol.* **84,** 221–275, 1946.
18. KŘIVÁNEK, J. Concerning the dynamics of the metabolic changes accompanying cortical spreading depression. *Physiologia bohemoslov.* **11,** 383–391, 1962.
19. LEÃO, A. A. P. The slow voltage variation of cortical spreading depression of activity. *Electroenceph. clin. Neurophysiol.* **3,** 315–321, 1951.
20. MAHUT, H. Effects of subcortical electrical stimulation on learning in the rat. *J. comp. physiol. Psychol.* **55,** 472–477, 1962.
21. MARSHALL, W. H. Spreading cortical depression of Leao. *Physiol. Rev.* **39,** 239–279, 1959.
22. MORRELL, F. and NAITOH, P. Effect of cortical polarization on a conditioned avoidance response. *Expl Neurol.* **6,** 507–523, 1962.
23. NAKAJIMA, S. Chemical injection into the reticular formation of rats. *J. comp. physiol Psychol.* **58,** 10–15, 1964.
24. OSGOOD, C. E. *Method and Theory in Experimental Psychology,* Oxford, New York, 1958.
25. PARÉ, W. The effects of caffeine and secondal on a visual discrimination task. *J. comp. physiol. Psychol.* **54,** 506–509, 1961.

26. PEARLMAN, C., SHARPLESS, S. K. and JARVIK, M. E. Retrograde ammesia produced by anesthetic and convulsive agents. *J. comp. physiol. Psychol.* **54,** 109–112, 1961.
27. RANSMEIER, R. E. *The Effect of Convulsion, Hypoxia, Hypothermia, and Anesthesia on Retention in the Hamster.* Unpublished doctoral thesis, University of Chicago, 1954.
28. RAY, O. S. and EMLEY, G. Time factors in interhemispheric transfer of learning. *Science, N.Y.* **144,** 76–78, 1964.
29. RUSINOV, V. S. The problem of stationary excitation and changes in the steady potential in the cerebral cortex in conditions of the dominant focus during conditioned reflex formation. *Pavlov* (English translation, Elsevier, Amsterdam) **11,** 6–19, 1961.
30. RUSINOV, V. S. Electrophysiological studies during the formation of a temporary connection. *Proc. Internat. Union Physiol. Sci., XXII Internat. Congress,* Vol. **1,** 882–887, 1962.
31. RUSSELL, I. S. and OCHS, S. Localization of memory traces in one cortical hemisphere and transfer to the other hemisphere. *Brain,* **86,** 37–54, 1963.
32. TAPP, J. T. Reversible cortical depression and avoidance behavior in the rat. *J. comp. physiol. Psychol.* **55,** 306–308, 1962.
33. WEISS, T. Shortlasting desynchronization reaction in the course of the propagation of spreading EEG depression in one hemisphere. *Physiologia bohemoslov.* **10,** 27–35, 1961.
34. WEISS, T. and FIFKOVÁ, E. The use of spreading depression to analyze the mutual relationship between the neocortex and the hippocampus. *Electroenceph. clin. Neurophysiol.* **12,** 841–850, 1960.

Résumé—On a étudié la consolidation d'une réponse d'évitement transférée d'un hémisphere à l'autre en utilisant la dépression extensive pour contrôler les durées de transfert et de consolidation. Il a fallu trois minutes pour transmettre l'apprentissage. La période suivant le transfert pendant laquelle on pouvait troubler la consolidation variait en fonction de la durée de la "dépression extensive" de trois minutes à deux heures. L'effet ne semble pas du à l'interruption de la décharge neurale, mais au changement du potentiel cortical occasionné par la dépression extensive, puisqu'un courant cathodique pulsatoire (8.5 microampères/mm^2) que l'on fait passer à travers le cortex médian (mais non antérieur, latéral ou postérieur) peut faire disparaître la rétention. On suggère que le processus de consolidation nécessite peut être des gradients de potentiel électrique sur le cortex et que ce prosessus peut être perturbé par les facteurs qui les diminuent ou qui les inversent.

Zusammenfassung—Das Lernvermögen für eine Vermeidensreaktion (durch interhemisphärische Reize ausgelöst) wurde während einer diffusen Senkung der Amplitude im Cortex und bei Kontrollfällen untersucht. Die gewonnenen Zeiten für Übertragung eines Reizes und Fixierung eines Lernstoffes wurden verglichen. Die Übertragung des Lernstoffes dauerte 3 Minuten. Die auf eine Übertragung folgende Zeitperiode, in welcher die Aufnahmefähigkeit gestört werden kann, schwankt entsprechend der Dauer einer diffusen corticalen Amplitudensenkung zwischen 3 Minuten und 2 Stunden. Dieser Effekt scheint nicht auf der Unterbrechung neuronaler Funktionsverbindungen zu beruhen, sondern auf eine Potentialänderung in der Hirnrinde zurückzugehen. Eine solche kann sowohl durch eine diffuse Amplitudensenkung als auch durch Kathodenreizströme (8, 5 Mikroamp./mm^2), die am medialen Corticalbereich angreifen, bewirkt werden. Durch beide Verfahren lässt sich das Aufnahmevermögen Lernstoff paralysieren. Es liegt deshalb die Vermutung nahe, dass der Vorgang, welcher die Aufnahmefähigkeit von Lernstoff erlaubt, an eine gewisse Potentialgrösse der Hirnstromtätigkeit gebunden ist. Alle Einflüsse, die das Potential senken oder die Stromrichtung ändern, rufen eine Störung hervor.