Learning of instrumental avoidance responses under conditions of bilateral spreading depression in rats*

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Twelve hooded rats were trained to make an active avoidance response. Six of them learned under conditions of cortical spreading depression (CSD), and six learned without CSD induction. Days to criterion were compared, and the CSD animals required significantly more days to learn the response. The final levels of accuracy reached by the CSD-trained rats and the non-CSD-trained animals were not significantly different. Animals trained CSD and then tested non-CSD showed no significant decrements between conditions. The decrement from final non-CSD training to CSD training was significant, although the CSD performance was significantly superior to the initial performance of the CSD-trained group. The above results were discussed in the context of the roles of cortical and subcortical memory mechanisms.

It has been known for some time that topical application of potassium chloride (KCl) to the cerebral cortex elicits a state of spreading depression (CSD) of the electrical activity of the cortex and a cessation of normal cortical functioning (Leão, 1944). Lower centers do not seem to be affected. This state is assumed to induce functional cortical ablation that is reversible within a few minutes to several hours, depending upon the concentration of KCl applied. Preparations may be trained or tested without cortical function on one day and with cortical function on the next. Such preparations are useful in studying cortical and subcortical encoding and readout relationships and transfer of memory between cortical and subcortical structures.

Using such preparations, Russell, Plotkin, & Kleinman (1970) have concluded that the emotional elements present in avoidance tasks are conditioned classically and subcortically, while instrumental habits are conditioned cortically. Russell, Kleinman, Plotkin, & Ross (1969) reported that CSD rats can only be conditioned classically (as CER), and Plotkin (1970) has stated that any apparent active avoidance learning under CSD is only a sensitization effect. With overtraining, rats trained whole-brain do show some evidence of retention when tested CSD (Plotkin, 1970), suggesting the possibility of time-dependent adaptation variables. While Plotkin (1970) had concluded that his animals showed no evidence of operant learning when trained under CSD, he

also reported some improvement on the second (and final) day of training, further suggesting that the learning effects of CSD may be a function of the time rats are under the influence of CSD.

The present study was designed to test the hypothesis that, with enough time, true instrumental active avoidance learning would be evidenced in animals trained under CSD and that transfer would occur from a whole-brain (WB) training state to a CSD testing state. Positive results might indicate either time-dependent subcortical adaptation to a lack of cortical dominance in mediating operant learning or suggest the need to reevaluate the equivalence of the CSD-induced "functional" lesion to surgical cortical ablations.

SUBJECTS

Twelve hooded rats, six male and six female (distributed equally between the training groups) from the Occidental College colony, were used. Mean age was 190 days.

APPARATUS

A three-chamber shock box (84 cm long x 20 cm wide x 33 cm high) was used. One end chamber was painted black, the center chamber gray, and the other end chamber white. The two end chambers had shock-grid floors connected by a relay switching system to a LeHigh Valley Electronics (LVE) 113-33 solid state shocker-scrambler (ac output). The center chamber was also equipped with a shock-grid floor connected to a nonscrambled LVE ac shock source. Partitions with 10 x 10 cm openings divided the chambers. Each chamber was furnished with a hinged clear Plexiglas lid. Closing the center lid depressed a microswitch connected to a time delay relay,

which, in turn, initiated shock to the center chamber after 3 sec had passed. A stopwatch was used to time the interval between placement of the rat in the center chamber and the animal's whole-body entry into the safe end chamber.

SURGICAL PROCEDURE AND ELICITATION OF SPREADING DEPRESSION

Under Nembutal anesthesia, 4-mm trephine openings were bored in the skull above the parietal-occipital regions of the left and right hemispheres. Perforation of the dura was carefully avoided. One tight suture was made at the rostral end of the incision and another loose suture secured the scalp over a small wad of cotton soaked in chloromycetin. (In a previous unpublished study, no significant differences in learning or memory had been found between 18 rats injected daily for 6 weeks with chloromycetin and a dextrose-injected control group.) Animals were allowed 1-day recovery time before training.

Before training or testing, the rats were lightly anesthetized with ether, the loose suture and cotton removed, the trephine openings cleaned with cotton swabs and saline, and 2-mm square filter paper pledgets soaked in physiological saline (control animals) or 23% KCl (CSD animals) placed in the center of the openings. Completeness of the depression was measured by the loss of the placing reflex (Bureš & Burešová, 1960). In order to insure the animals against inflammation, fresh chloromycetin-soaked cotton was placed over the openings on each day after training or testing and the scalp was sutured closed. With this procedure, animals can be used for up to 14 successive days before tissue healing makes elicitation of CSD unreliable.

PROCEDURE

Animals were placed in the gray center chamber of the shock box facing the front or back of the chamber on a random schedule to control for E bias. Center shock came 3 sec after center lid closure. The stopwatch was started as the animal was placed on the center grid and stopped when the animal's body had entered the safe end chamber. Animals were run in four daily blocks of 10 trials each, with 1-min rest periods between blocks and 15-sec rest periods between trials. During each interblock rest period and 30 min after the conclusion of trials, the placing reflex was tested. In all cases, the reflex was absent, indicating that the animals had been depressed during the entire testing period.

Six animals were trained CSD and six WB. Three CSD and three WB animals were trained with the white

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Fig. 1. Percent of correct choices per block of 10 trials averaged within CSD-trained and WB-trained groups. Taller vertical slashes on the abscissa indicate days. One CSD-trained rat reached criterion on Day 2 and hence Day 3 of CSD training has $N = 5$. Its postcriterion day scores have been averaged with the scores obtained by the other CSD-trained rats on their first postcriterion day (Day 4).

chamber always wired to a shock source and the black chamber always safe. The other six animals received shock in the black chamber.

As soon as any animal met the criterion of nine correct choices in each of the two last successive blocks of a day's trials, training was concluded. On the following day, these rats were given another 40 trials to test same-condition transfer. On the second posttraining day, animals trained WB were tested CSD and animals trained CSD were tested WB.

RESULTS

All WB rats reached criteria on the first day, while one CSD-trained animal required 2 days and the other five required 3 days (the t value of 7.44 is significant at the .01 level). While WB rats learned to choose the unshocked chamber in many fewer trials than did the CSD-trained rats, terminal performance of the two groups was almost identical. As can be seen in Fig. 1, WB-trained animals tested CSD exhibited larger performance decrements ($t = 8.2$, significant at .01 for $df = 5$) than did CSD-trained rats tested WB ($t = 1.67$, n.s.). However, in comparing the means of the first 40 trials (four-trial blocks) of the CSD-trained animals with the means of the first 40 CSD testing trials of the WB-trained rats, the WB-trained rats were superior ($t = 3.02$, significant at .05 for $df = 5$), indicating positive transfer from WB training to CSD testing.

It was possible to make a perfect discrimination score by making only escape responses (running after the center shock came on). Plotkin (1970) referred specifically to the inability of CSD animals to make active avoidance responses (which are considered more difficult than escape responses). As the shock came on for the center grid

3 sec after the center door was closed, response times of more than 3 sec were counted as escapes and response times of less than 3 sec as active avoidances. The results of this analysis were similar to the results reported above for correct choices. During the first day of training, CSD rats averaged 32% avoidances, compared to 71% avoidances for the control rats ($t = 5.62$, significant at .01 for $df = 5$). After all the rats had met criterion during training, they were run an additional day in the training condition. The CSD rats averaged 84% avoidances and the WB rats 89% avoidances ($t < 1$). When WB rats were tested under CSD, they dropped to 48% avoidance for the first 10 trials and improved to 86% avoidance for the last 10 (mean of 40 trials = 71%). A comparison of the overall means of the WB-trained rats with the first day means of the CSD-trained animals was significant at .01 ($t = 7.43$, $df = 5$), again indicating positive transfer from the WB training to CSD testing conditions. Rats trained under CSD dropped to 78% avoidance on the first 10 WB testing trials and reached 91% avoidance on the last 10 WB testing trials (overall mean = 85%), indicating no decrement from the last day of CSD testing (overall mean = 84%).

DISCUSSION

Given enough time, rats can learn instrumental avoidance habits when trained under conditions of CSD. This suggests that at least some of the functions that have been assumed to depend upon an intact cortex may be mediated by subcortical structures if enough time is allowed for the subcortical areas to adapt to a loss of cortical dominance or that subcortical areas may mediate instrumental learning but subcortical instrumental learning is a slower process than

cortical instrumental learning. As an alternative to the above model of brain functioning, in which subcortical areas are postulated to be less efficient but parallel systems for the learning and performance of simple instrumental responses, a reexamination of the analogy between the CSD state and surgical cortical ablations may have to be made. It is possible that the reported improvements over time of the discriminated active avoidance response may have represented cortical adaptation to the CSD state rather than subcortical encoding and readout. Fertziger & Ranck (1969) have found that interstitial potassium ion concentrations increase during electroconvulsive shock (ECS). As CSD involves artificially increasing interstitial potassium ion concentrations, the above result suggests a common mode of action between ECS (also used to produce memory disruptions) and CSD. Kesner, McDonough, & Doty (1970) found that with continued application of ECS, disruptions of complex memories gradually diminished. As ECS involves more than just cortical areas, a general recovery of the brain from disrupting conditions rather than just a shifting of functions becomes a possibility.

To summarize, in the present study, it was found that instrumental avoidance responses may be learned by rats under conditions of CSD and that WB training shows subsequent positive transfer to CSD testing. While the second reported result agrees with the findings of Plotkin (1970), the first does not. This variance may be a function of the present surgical procedure, which produces animals that may be depressed and trained for a long enough time to adapt out the more debilitating short-term effects of CSD. A more general conclusion might be that conclusions drawn on short-term studies may require modification when behavior is observed for a longer period of time.

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