Interactions of Scopolamine and Physostigmine with ECS and One Trial Learning

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DAVIS, J. W., R. K. THOMAS, JR. AND H. E. ADAMS. *Interactions of scopolamine and physostigmine with ECS and one trial learning.* PHYSIOL. BEHAV. **6** (3) 219–222, 1971.—Twelve groups of 10 rats were trained on a one-trial, passive avoidance task and were tested for retention four hr later. Six groups received electroconvulsive shock (ECS) immediately following the learning trial, and the remaining six groups were in a non-ECS condition. The six groups in both the ECS and non-ECS conditions were divided into three groups which received either saline, scopolamine or physostigmine injections before the learning trial and three groups which received the injections before the retention trial. The results suggested that physostigmine prior to the learning trial or scopolamine prior to the retention trial protected memory from the normally disruptive effects of ECS. In addition, scopolamine alone before the learning trial or physostigmine alone before the retention trial had a disruptive effect on retention. Results are discussed in terms of influences of the drugs and/or ECS on ACh activity.

Memory   Learning   Electroconvulsive shock   Physostigmine   Scopolamine   One-trial learning
Brain acetylcholine

WOLFE and Elliott [8] reviewed the relationships between acetylcholine (ACh) and convulsive activity. The evidence suggested, in part, that the administration of ACh to the brain results in epileptic-like electrical activity, that the administration of acetylcholine-esterase (AChE) inhibitors results in epileptiform brain waves and that ACh activity in the brain is increased following the commencement of convulsions. Adams, Hoblit and Sutker [1] studied AChE levels at several time intervals following electro-convulsive shock (ECS). They reported an increase in AChE immediately following ECS and normal values of AChE were not seen until 96 hr post-ECS.

On the assumption that AChE and ACh show a correlated increase following ECS and in view of the well known relationship between ECS and retrograde amnesia [2, 4] Adams *et al.* [1] investigated the degree of ECS-induced amnesia as a function of the pharmacological manipulation of ACh. Following 20 active avoidance training trials, one group of rats received ECS and a second group was given a sham ECS treatment. Retention trials which were given four hr later were preceded in some rats by injections of physostigmine (anti-AChE) or scopolamine (anti-ACh). The results suggested that scopolamine partially eliminated the retention deficits usually seen following ECS while physostigmine in association with ECS resulted in a greater deficit in retention than that usually seen following ECS.

The present work examined the interactions of ECS and physostigmine or scopolamine on the acquisition and retention of a one-trial passive avoidance task.

METHOD

Animals.

The animals were 120 naive Sprague–Dawley male rats approximately 90–120 days old. The rats were maintained on an ad lib food and water schedule throughout the experiment.

Apparatus.

The apparatus included a one-way avoidance box with a door in the middle dividing a black, dark side and a white side exposed to normal room illumination. Grason–Stadler control, shocking, and timing devices were used. A method for delivering ECS reliably and immediately following the learning was achieved by soldering short snap leads to surgical wound clips which were clamped behind the animal's ears (this was done under ether anesthesia three days before the trials were run). The short snap leads were then connected to longer leads going to the programing equipment at the time the trials were run. A 35 mA constant current of 0.5 sec duration was used to produce the convulsion.

Procedure

The rats were given one learning trial in a passive avoidance task. The training began when the rat was placed in the white, lighted side of the box and the door to the black, darkened side was opened. The rats rapidly entered the black, darkened side. Upon entering the dark side they received a 3 sec 2 mA

foot shock. A second trial (designated the retention trial) was given four hr later to assess whether they had learned to avoid the shock by remaining on the white side. Response latency was measured on both trials from the time the door was opened until the rat moved into the dark side. If the rat did not respond within one min, the trial was terminated.

The rats were divided into 12 groups of 10 rats. Six groups received ECS immediately following the learning trial. The remaining six groups did not receive ECS (designated hereafter as the NECS groups). Three of the six groups from each of the ECS and NECS conditions recieved equal volume injections of saline (1 mg/kg body weight), scopolamine (1 mg/kg body weight), or physostigmine (0.3 mg/kg body weight) 30 min prior to the learning trial. The remaining three of six groups from each of the ECS and NECS conditions received the saline, scopolamine or physostigmine injections 30 min prior to the retention trial.

RESULTS

There were no significant differences in response latencies between any of the subgroups on the learning trial. A three-way analysis of variance of the response latencies on the retention trial showed significant interactions ($p < 0.001$) between drugs (saline, physostigmine and scopolamine) and time of injection (prior to learning trial or prior to retention trial) and between drugs and ECS or NECS.

Figure 1 shows the mean response latencies of the 12 subgroups on the retention trial. Analyses of the simple effects seen in the present study indicate that any mean difference in response latency between subgroups as great as 19.3 sec was significant ($p < 0.01$). Based on these analyses, the following results in Fig. 1 are emphasized: (1) The saline control groups regardless of when injected, showed little evidence of retention if ECS followed the learning trial, but retention was seen when no ECS was given. The following statements about the results of scopolamine injections or physostigmine injections are relative to the appropriate saline control group. (2)

Scopolamine significantly increased response latency following ECS if scopolamine was given prior to the retention trial, but scopolamine injections prior to the learning trial significantly reduced response latency on the retention trial if no ECS was given. (3) Physostigmine injections prior to the learning trial in the ECS condition significantly increased response latency on the retention trial, however, in the NECS condition physostigmine injections significantly reduced response latencies on the retention trial.

DISCUSSION

The results seen in the saline control groups in the present work are consistent with the well known finding that ECS disrupts performance on a retention trial if ECS is given immediately following a learning trial. Such a disruption has been interpreted as interfering with memory consolidation [2, 4]. Data from the present experiment suggest that protection from the disruptive effects of ECS may be seen if physostigmine is given prior to the learning trial or if scopolamine is given prior to the retention trial.

There are at least two hypotheses to explain the present data. One of these maintains that there is an optimal range within which ACh activity must occur for learning/memory to be seen. The second hypothesis suggests that the state of ACh activity during learning must be matched during the retention test in order for memory to be seen. Both hypotheses depend on the assumptions that physostigmine facilitates the action of ACh and that scopolamine inhibits the action of ACh. It will be useful to consider the effects of ECS on ACh in order to attempt an explanation of the significant interaction of these drugs with ECS.

The most relevant study of the effects of ECS on ACh activity was by Richter and Crossland [6]. These authors determined the ACh content of rat brain following ECS prior to convulsions in some rats and during convulsions in other rats. It should be noted that ACh is so rapidly hydrolyzed by AChE that brain content of ACh at a given time is thought to
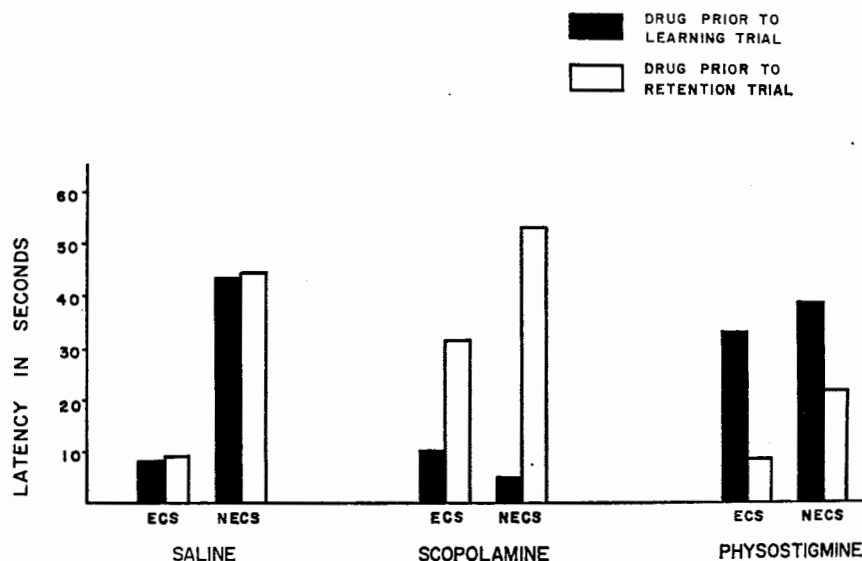

FIG. 1. Response latencies on the retention trial as a function of ECS vs. NECS, solution injected, and time of injection.

be correlated negatively with ACh activity. Compared to control animals, Richter and Crossland's post-ECS, pre-convulsive rats showed less ACh content immediately after ECS, but ACh content returned to normal just prior to convulsions. The onset of convulsions was associated with a second decrease in ACh content apparently due to a second massive release of ACh. The authors suggested that the convulsions ended when ACh content fell below that which resynthesis could keep pace. By 100 sec after the onset of convulsions, the convulsions had ceased and ACh content again rose to normal values.

It is not known at present what the effects of ECS are on ACh content for intervals such as the four hr one used in the present work. Based on Adams et al. [1] determinations of AChE four hr after ECS and assuming that ACh and AChE are correlated, it is reasonable to suggest that ACh activity at this time is greater than normal. In addition both the optimal range hypothesis and the state-dependent hypothesis are consistent with the suggestion that ACh activity is increased by ECS for as long as four hr. The data in the present work are not consistent with the view that ACh activity is normal or less than normal four hr after ECS.

The optimal range hypothesis is consistent with the following interpretations of the data in the present experiment. The results seen in the saline, NECS groups are considered to be consistent with ACh's activity being withing the optimal range during learning and retention. The data from the saline, ECS groups are thought to be due to elevation above the optimal range by the ECS so that retention is not seen. In the scopolamine, ECS group which received the injection prior to retention testing, it is suggested that scopolamine brought the elevated ACh activity back within the optimal range so that retention was seen. The results seen when scopolamine was given prior to the learning trial which was followed by ECS may be due to either too little ACh activity at the time of learning or too much ACh activity at the time of retention testing due to the ECS. The former suggestion is supported by the pre-learning, scopolamine injected group in the NECS condition. In the physostigmine, ECS groups, physostigmine prior to learning apparently protected that learning from the interference of ECS. It is suggested that physostigmine elevated ACh activity but within the optimal range, and this elevated ACh permitted better consolidation prior to ECS than is seen in the appropriate saline control group. It is suggested that the the better consolidated learning was able to withstand the effects of ECS. The effect of physostigmine injected prior to retention was probably to summate with the ECS resulting in ACh activity above the optimal range at the time of retention testing.

The state-dependent hypothesis maintains that the level of ACh activity at the time of learning must be matched at the time of retention testing for evidence of retention to be seen. When scopolamine was given prior to retention testing in the ECS condition, the retention seen according to this hypothesis would be due to the normal ACh activity at the time of learning being approximated at the time of retention testing. It is suggested that the ACh elevating effect of ECS was coun-

tered by the scopolamine so that near normal ACh activity occurred at the time of retention testing. The poor retention seen when scopolamine preceded the learning trial was the result of a mismatch of ACh with the retention trial, namely, low ACh at the time of learning due to scopolamine and high ACh at the time of retention due to ECS. The results seen when physostigmine preceded the learning trial might be explained by the match of high ACh at the time of learning due to the drug and high ACh at the time of retention due to ECS. The poor retention seen when physostigmine preceded the retention trial might be due to normal ACh at the time of learning and high ACh at the time of retention due to the effects of ECS and physostigmine.

It is noted that the optimal range hypothesis and the state-dependent hypothesis encounter difficulties when one considers the effects of the drugs in the NECS conditions. While it is consistent with the optimal range hypothesis to explain the poor retention seen when scopolamine preceded the learning trial (low ACh at the time of learning resulting in poor consolidation), it is difficult to explain the good retention seen when scopolamine preceded the retention trial. Similar difficulties arise with the disparity of results seen in the physostigmine-NECS groups. The state-dependent hypothesis is consistent with the poor retention seen in the pre-learning scopolamine or preretention physostigmine NECS groups, but the hypothesis would appear to be at odds with the good retention seen in the preretention scopolamine or the pre-learning physostigmine NECS groups. Both hypotheses can be supported by the NECS data if one concluded that drug induced (at the doses used in the present work) decreases of ACh activity are more detrimental to learning, whereas, drug induced increases are more detrimental to retention. In terms of the optimal range hypothesis, one would need to determine where within the optimal range the drugs were having their effect, or in terms of the state-dependent hypothesis, one would need to determine what degree of mismatch can be tolerated for learning/memory to be seen and whether the learning trial and retention trial are differentially sensitive to mismatches. Choosing between the hypotheses should be possible when precise indications of the ACh activity in the various experimental conditions of the present work are available.

The findings of the present work are considered to be consistent with the suggestions of Deutsch and his co-workers [3, 7] that there is an optimal level of ACh required following learning in order for memory of that learning to be demonstrated. Deutsch has argued that too little or too much ACh blocks memory. The time relationships studied in Deutsch's work suggested that ACh levels decreased one day after training, increased over normal levels seven days after training, and then declined thereafter ultimately leading to forgetting. The optimal range hypothesis presented in the present work was based on Deutsch's earlier suggestions about the role of ACh in memory. If this hypothesis is correct, the present work suggests that the effects of changes in ACh level such as those seen over the time periods studied by Deutsch may also be studied in shorter periods of time if ACh activity is manipulated by ECS, scopolamine and physostigmine.

REFERENCES

1. Adams, H. E., P. R. Hoblit and P. B. Sutker. Electroconvulsive shock, brain acetylcholinesterase, and memory. *Physiol. Behav.* **4:** 113–116, 1969.
2. Chorover, S. T. and P. H. Schiller. Short-term retrograde amnesia in rats. *J. comp. physiol. Psychol.* **59:** 73–78, 1965.
3. Deutsch, J. A. and D. Deutsch. *Physiological Psychology.* Homewood, Ill.: The Dorsey Press, 1966, pp. 84–88.
4. Duncan, C. P. The retroactive effect of electro-shock on learning. *J. comp. physiol. Psychol.* **42:** 32–44, 1949.

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5. Goodman, L. S. and A. Gilman. *The Pharmacological Basis of Therapeutics.* New York: MacMillan, 1965.
6. Richter, D. and J. Crossland. Variation in acetylcholine content of the brain with physiological state. *Am. J. Physiol.* **159**: 247–255, 1949.
7. Weiner, N. and J. A. Deutsch. Temporal aspects of anticholinergic and anticholinesterase-induced amnesia for an appetitive habit. *J. comp. physiol. Psychol.* **66**: 613–617, 1968.
8. Wolfe, L. S. and K. A. C. Elliott. Chemical studies in relation to convulsive conditions. In *Neurochemistry*, edited by K. A. C. Elliott, I. H. Page and J. H. Quastel. Springfield, Ill.: C. C. Thomas, 1962, pp. 694–727.