Interactions of ECS and a light-dark cycle on one-way avoidance learning in rats

MICHAEL J. PREWETT and ROGER K. THOMAS
University of Georgia, Athens, Georgia 30601

After 56 days on a light-dark cycle, male Sprague-Dawley rats, 90 to 120 days old, received either ECS or sham ECS 4 or 24 h prior to training during the light or the dark phase of the cycle in one-way active avoidance. These variables interacted significantly to affect both errors and trials to criterion; however, principal consideration is given the errors data. The ECS-4-Dark group had significantly greater mean errors than all other groups (15.5 error $p < .005$), except the Sham-ECS-24-Light group, and the ECS-24-Dark group had significantly fewer mean errors than five of the other seven groups (6.2 errors; $p < .005$). The findings were interpreted in terms of the evidence that ECS and light-dark cycles affect brain acetylcholine and Deutsch's hypothesis (1971) that too much or too little ACh activity may impair retention; this has been shown by Davis (1972) to be applicable to acquisition of one-way avoidance.

The present study combines two variables which have been shown to influence learning, namely, light-dark cycles and the administration of ECS prior to learning. For example, Stroebel (1967) reported more rapid acquisition and extinction of a conditioned emotional response by a group of rats which were trained at the same time each day (within 2 h of the onset of dark in a light-dark cycle) compared to a group trained at random times. Stephens, McGaugh, and Alpern (1967) reported better retention in mice trained and tested on passive avoidance in the dark phase of a light-dark cycle. Further, they reported greater amnesic effects of ECS administered following training in the dark phase than in the light phase.

Gibbs and Mark (1973) reviewed 12 studies in which ECS was given to the animals prior to training. Various levels of performance have been reported, apparently depending on differences in the tasks, numbers of ECS administrations, and times of ECS administration prior to training. A study by Davis (1972; not reviewed by Gibbs and Marks) is most related to the present work. Davis gave one ECS per rat to independent groups either 96, 48, 24, 12, or 4 h prior to one-way active avoidance training. Compared to a non-ECS control group, which had a mean of 21.4 trials to criterion, the ECS groups, in the order listed above, had means of 21.8, 27.2, 17.9, 15.0, and 19.1 trials to criterion. The 12-h group (15.0) and the 48-h group (27.2) differed significantly from controls.

The present work used eight independent groups. A given group was trained either in the dark or in the light phase of an established light-dark cycle, either 4 or 24 h

after receiving either an ECS or a sham ECS (SECS).

METHOD

Animals

The animals were 64 Sprague-Dawley male rats, 90 to 120 days old at the time of the ECS or SECS treatment and subsequent training. They were caged individually and were maintained on an ad-lib food and water schedule throughout the experiment. The animals were established on a light-dark cycle (light on at 0600, light off at 1800 DST) for 8 weeks prior to the beginning of the experimental treatments.

Apparatus

A constant-current ECS device, similar to one described by Hayes (1948), built by the University of Georgia Electronics Design and Maintenance Shop, was used. A 35-mA ECS of .5-sec duration was delivered via bilaterally placed ear clips. SECS animals were treated exactly as the ECS animals except that the ECS was not actually delivered. Acquisition trials were given in the two-compartment avoidance apparatus described by Adams and Lewis (1962). Grason-Stadler electric shock and programming equipment was used to control the CS and UCS variables in training.

Procedure

Either 4 or 24 h prior to one-way avoidance training, half the animals in each time group received ECS and half received SECS. The animals were further divided such that half the animals in each of the above treatment combinations were trained 1½ to 2 h after the onset of dark and half were trained 1½ to 2 h after the onset of light with respect to the light-dark cycle (training, however, was done in a room which was dimly illuminated by ambient light from the room adjacent. To summarize, a three-factor design (ECS vs. SECS by 4 h vs. 24 h by Dark vs. Light) was used with eight rats per group.

Training in the avoidance apparatus began with an animal being placed in the black compartment of the apparatus. When the door separating the black from a white compartment was raised, the animal was allowed 5 sec to move from the black to white compartment. Failure to move to the white side within 5 sec resulted in a 2-mA footshock being delivered to the feet of the animal via the grid floor. The footshock was terminated when the rat crossed to the white compartment. The animals were trained to a criterion of nine successful avoidances in 10

This article was based on the M.S. thesis of the first author submitted to the Graduate Faculty of the University of Georgia. Requests for reprints should go to Michael J. Prewett, now at the Department of Psychology, Western Carolina University, Cullowhee, North Carolina 28723.

Table 1
Mean Number of Errors*

Main Groups	Time of ECS or Sham-ECS Prior to Training	Dark	Light
ECS	4 h	15.5	11.0
	24 h	6.2	8.5
Sham-ECS	4 h	9.0	11.5
	24 h	9.9	12.8

*Any difference as large as 3.6 is significant at $p < .005$.

consecutive trials in one session. The intertrial intervals were from 10 to 15 sec. In addition to trials to criterion, the number of errors, that is failure to avoid footshock, was determined.

RESULTS AND DISCUSSION

Based on mean errors, 12 of the 28 comparisons of mean differences were significant at $p < .005$. All errors comparisons may be reconstructed from Table 1. To be emphasized are the findings that the ECS-4-Dark combination yielded a significantly larger mean errors than the remaining groups except for the SECS-24-Light group, that the SECS-24-Light group had more mean errors than three other groups, and that the ECS-24-Dark group had fewer mean errors than five of the other groups. Significant differences in mean trials to criterion may be determined from Table 2. Such analysis will reveal seven significant differences at the .005 alpha level, with the ECS-4-Dark group requiring more trials to criterion than five of the remaining groups and the ECS-24-Dark group requiring fewer trials to criterion than three groups (including the ECS-4-Dark group).

It is suggested that the errors data are the better indicators of performance and should be given greater emphasis. For example, with a criterion of nine avoidances in 10 consecutive trials, an animal might make 10 errors and reach criterion in 19 trials, while another animal might commit 10 errors and require 59 trials to criterion. Therefore, while the data and statistical analyses for both errors and trials to criterion will be presented, discussion will be limited to the errors data.

The analysis of variance for the errors data indicated a significant hours effect, $F(1,56) = 13.31$, $p < .01$, but there were no significant main effects attributable to the ECS or light-dark conditions. However, the Hours by Light-Dark interaction was significant, $F(1,56) = 7.36$, $p < .01$, the Hours by ECS interaction was significant, $F(1,56) = 27.40$, $p < .01$, the ECS by Light-Dark interaction was significant, $F(1,56) = 8.41$, $p < .01$, and the Hours by Light-Dark by ECS interaction was significant, $F(1,56) = 19.92$, $p < .01$. The mean errors for each group may be seen in Table 1. Using the least significant differences test, a mean errors difference which exceeds 3.6 is significant at $p < .005$.

The analysis of variance for the trials to criterion data

indicated a significant hours effect, $F(1,56) = 15.54$, $p < .01$, but there were no significant main effects attributable to the ECS or light-dark conditions. The Hours by ECS interaction was significant, $F(1,56) = 12.59$, $p < .01$, and the Hours by Light-Dark by ECS interaction was significant, $F(1,56) = 5.91$, $p < .05$. The mean trials to criterion for each group may be seen in Table 2. The least significant difference test indicated that any mean difference which exceeds 5.1 is significant at $p < .005$.

GENERAL DISCUSSION

The present work adds significantly to those studies reporting the effects of ECS (Davis, 1972; Gibbs & Mark, 1973) and light-dark cycles (Stephens et al., 1967; Stroebel, 1967) on learning performances by pointing to the varied interactive effects of these variables on one-way avoidance learning. Using the grand mean errors (10.6) as a basis for comparison, it may be suggested that the ECS-24-Dark combination improves acquisition while the ECS-4-Dark combination impairs acquisition. It is not possible in the present work to define for comparison a "normal" control group, as light-dark was an independent variable and the non-ECS groups received the sham-ECS treatment. The light-dark cycle interacted significantly with the other variables to affect the acquisition of one-way avoidance. This significant light-dark effect together with the related findings for conditioned emotional responses (Stroebel, 1967) and passive avoidance responses (Stephens et al., 1967) suggests a need for assessing the effects of light-dark cycles in the specification of "normal" performances in other experiments; for example, in the present study, the group which might approximate most closely a normal control group is the SECS-24-Light group. Yet, this group had significantly more errors than the SECS-4-Dark, the ECS-24-Dark, and the ECS-24-Light groups as well as significantly fewer errors than the ECS-4-Dark group.

One possible theoretical structure with which the present findings may be interpreted is Deutsch's (e.g., 1971) acetylcholine (ACh) hypothesis. Briefly, this hypothesis suggests that too much or too little ACh activity at the time of retention testing may impair performance. Davis' (1972) research suggests the

Table 2
Mean Trials to Criterion*

Main Groups	Time of ECS or Sham-ECS Prior to Training	Dark	Light
ECS	4 h	27.8	23.9
	24 h	16.6	20.6
Sham-ECS	4 h	20.4	23.1
	24 h	20.6	21.6

*Any difference as great as 5.1 is significant at $p < .005$.

applicability of the ACh hypothesis to the acquisition of one-way avoidance.

Both ECS and light-dark cycles have been shown to affect brain ACh activity. Apparently, direct measures of ACh activity have been made only up to 2 h post-ECS (Essman, 1972), but indirect measures support the suggestion that ACh activity is above normal but declining 4, 12, and 24 h post-ECS (Adams, Hoblit, & Sutker, 1969; Davis, 1972; Davis, Thomas, & Adams, 1971; Wiener, 1970) and has returned to normal 96 h post-ECS (Adams et al., 1969; Davis, 1972). At least two experiments have reported greater ACh activity in the brains of rats during the dark phase of an established light-dark cycle (Hanin, Massarelli, & Costa, 1970; Saito, 1971).

Considering the ECS and light-dark effects on ACh activity, it is reasonable to suggest that among our groups the ECS-4-Dark combination should produce the greatest ACh activity and the SECS-24-Light combination should produce the least ACh activity. Deutsch's hypothesis (1971) suggests that too much or too little ACh activity may impair performance. The ECS-4-Dark group had a significantly greater mean errors than six of the remaining groups. The SECS-24-Light group had the second largest mean errors and differed significantly from three other groups.

While emphasis has been placed on the ACh hypothesis as possibly providing the underlying mechanism to explain the interactive effects on one-way avoidance learning of ECS, light-dark cycles and time of training following ECS or sham-ECS, it must be noted that other mechanisms are probably involved or may be primary. For example, ECS and light-dark cycles affect other neurotransmitters (Essman, 1972; Scheving, Harrison, Gordon, & Pauly, 1968). Furthermore, Essman (1970), who provided much of the data concerning ECS effects on neurotransmitters, has emphasized the role of serotonin in memory. Stroebel (1967), who reported better acquisition of a CER in rats trained in the dark phase of a light-dark cycle, speculated on the role of adrenal corticosteroids in discussing his results. Further research will be necessary before an exact explanation of the mechanisms underlying the interactive effects of ECS and light-dark cycles on learning can be confidently given.

REFERENCES

- ADAMS, H. E., HOBLIT, P. R., & SUTKER, P. B. Electroconvulsive shock, brain acetylcholinesterase activity and memory. *Physiology and Behavior*, 1969, 4, 113-116.
- ADAMS, H. E., & LEWIS, D. J. Electroconvulsive shock, retrograde amnesia, and competing responses. *Journal of Comparative and Physiological Psychology*, 1962, 55, 299-301.
- DAVIS, J. W. Acquisition of one-way avoidance in rats as correlated with experimental alterations of brain acetylcholine activity. (Doctoral dissertation, University of Georgia, 1972.) *Dissertation Abstracts International*, 1972, 33, 3299-B. (University Microfilms No. 72-34, 062.)
- DAVIS, J. W., THOMAS, R. K., JR., & ADAMS, H. E. Interactions of scopolamine and physostigmine with ECS and one trial learning. *Physiology and Behavior*, 1971, 6, 219-222.
- DEUTSCH, J. A. The cholinergic synapse and the site of memory. *Science*, 1971, 174, 788-794.
- ESSMAN, W. B. Some neurochemical correlates of altered memory consolidation. *New York Academy of Sciences Transactions*, 1970, 32, 948-973.
- ESSMAN, W. B. Neurochemical changes in ECS and ECT. *Seminars in Psychiatry*, 1972, 4, 67-79.
- GIBBS, M. E., & MARK, R. F. *Inhibition of memory formation*. New York: Plenum Press, 1973.
- HANIN, I., MASSARELLI, R., & COSTA, E. Acetylcholine concentrations in rat brain: Diurnal oscillation. *Science*, 1970, 170, 341-342.
- HAYES, K. J. Cognitive and emotion effects of electroconvulsive shock on rats. *Journal of Comparative and Physiological Psychology*, 1948, 41, 40-61.
- RICHTER, D., & CROSSLAND, J. Variation in acetylcholine content of the brain with physiological state. *American Journal of Physiology*, 1949, 159, 247-255.
- SAITO, Y. The circadian rhythm of brain acetylcholine levels and motor activity in the rat. *Life Sciences*, 1971, 10, 735-744.
- SCHAVING, L. E., HARRISON, W. H., GORDON, P., & PAULY, J. E. Daily fluctuation (circadian and ultradian) in biogenic amines of the rat brain. *American Journal of Physiology*, 1968, 214, 166-173.
- STEPHENS, G., MCGAUGH, J. L., & ALPERN, H. P. Periodicity and memory in mice. *Psychonomic Science*, 1967, 8, 201-202.
- STROEBEL, C. F. Behavioral aspects of circadian rhythms. In J. Zubin & H. F. Hunt (Eds.), *Comparative psychopathology—Animal and human*. New York: Grune & Stratton, 1967.
- WEINER, N. I. Electroconvulsive shock induced impairment and enhancement of a learned escape response. *Physiology and Behavior*, 1970, 5, 971-974.

(Received for publication January 27, 1975;
revision accepted May 8, 1975.)