Proactive and retroactive effects of diethyl ether on spatial discrimination learning in inbred mouse strains DBA/2J and C57BL/6J

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Diethyl ether was given before or after a single daily spatial discrimination trial in a water maze. Both C57BL/6J and DBA/2J mice made more errors than controls of these same strains when they were etherized after a trial, although the decremental effect on performance was more notable in the former strain. Pretrial etherization had no effect on errors, but mice etherized prior to each trial showed faster swimming times than control Ss. These data were discussed in terms of strain differences in ether-stimulated arousal or activity level.

A perseveration-consolidation theory of memory suggests that a neurophysiological consolidation process maintains habit acquisition from trial to trial by fixing the practice effect of a trial after it is over. Among other anesthetics, diethyl ether has been administered at varying intervals after a learning trial to determine the point at which CNS depressing effects of anesthesia will interfere with this hypothetical consolidation process. Es have reported either no effect of ether on learning (McGaugh, 1966) or a decremental effect (Abt, Essman, & Jarvik, 1961; Jarvik, 1964; Herz, Peeke, & Wyers, 1966; Pearlman, 1966; Alpern & Kimble, 1967). More recently, Wimer (1968) and Wimer, Symington, Farmer, & Schwartzkroin (1968) reported that ether administered immediately after learning trials actually facilitated shock escape and passive avoidance learning in the DBA/2J mouse strain, although it had no effect on the C57BL/6J strain. Cherkin (1968a) has suggested that Wimer and Wimer et al's procedure of administering light doses of ether for short durations served to produce an ether-stimulated facilitation in learning. The present experiments were designed to determine (1) if Wimer et al's etherization procedure would result in improved learning in Strain DBA/2J and have no effect on Strain C57BL/6J for a more complex learning task, i.e., spatial discrimination learning, and (2) if

ether presented prior to a trial would have the same effect on learning as ether presented after a trial. Effects of pretrial etherization on learning do not appear to have been considered in previous experiments. A finding of enhanced learning with pretrial etherization as well as posttrial etherization would suggest that the stimulating effects of ether anesthesia affect habit formation as well as memory consolidation.

METHOD

Ninety-six male 6-week-old mice of Strains DBA/2J and C57BL/6J served as Ss. They were given 30 discrimination trials in a gray sheet-metal T-maze (Waller, Waller, & Brewster, 1960) with rounded arms and no top or bottom. The maze was immersed 15 to 20 cm in a tub of water ($24^{\circ} \pm 2^{\circ} \text{C}$). Trials were presented 24 h apart and consisted of swimming from the start point of the maze to a ladder located at the end of the correct arm. If S swam to the incorrect arm, it was permitted to find the correct arm and escape. The correct arm was defined as that chosen on less than half of the 10 habituation trials prior to testing in which S could escape from either arm of the maze. To control possible olfactory and visual cues, the top-bottom, front-back, and right-left orientation of the maze in the water tank were varied between trials according to a random schedule.

Wimer (1968) and Wimer et al's (1968) etherization procedure was followed exactly. Etherized Ss were placed in a 50-ml glass beaker containing cotton saturated with anesthesia grade diethyl ether. They were removed from the beaker as soon as the righting reflex was lost and breathing became deep and irregular. Consistent with Wimer (1968) and Wimer et al's (1968) observations, loss

of righting reflex occurred in approximately 35 sec. Control Ss were placed in an identical beaker with no ether. Twenty-four Ss of each strain were assigned randomly to Experiment 1 and 24 were assigned to Experiment 2. Half the Ss in Experiment 1 received ether immediately after a trial and half received control handling. The Ss in the ether and the control groups were matched as closely as possible for swimming speed. The same design was used for Experiment 2, except that half of the Ss of each strain received ether 8-10 min prior to a daily trial and half received control handling. The 8- to 10-min interval was chosen because obvious effects of recovery from anesthesia were not observed after this period.

RESULTS

Performance measures were (1) errors and (2) swimming time from the start point of the maze until the S's tail cleared the choice point. These data met assumptions underlying analyses of variance, and they were arranged in a 2 by 2 by 3 factorial format within each experiment for this purpose. There were two strains, two treatments (ether vs control), and three blocks of 10 trials each.

For Experiment 1 there was neither a significant difference in errors between strains ($F = 2.86$, $df = 1/44$, $p > .05$) nor a significant Treatment by Strain interaction ($F = 2.85$, $df = 1/44$, $p > .05$). However, averaging over all other conditions, Ss that received ether after a trial (EA) made significantly ($F = 10.12$, $df = 2/88$, $p < .01$) more errors than those that received control handling (CA). Further, there was a significant Strain by Treatment by Blocks interaction ($F = 5.3$, $df = 2/88$, $p < .05$). The nature of this interaction may be seen in Fig. 1. While the EA and CA groups of Strain DBA/2J and the CA group of Strain C57BL/6J showed a decrease in errors on Block 3 relative to Block 1, the C57BL/6J mice that received EA showed a decrease in errors on Block 2 and an increase in errors on Block 3. Specific comparisons between EA and CA groups of Strain C57BL/6J (Wimer, 1962, p. 344) indicated that the former group made significantly more errors than the latter group on Block 1 ($F = 6.39$, $df = 1/132$, $p < .05$) and Block 3 ($F = 14.71$, $df = 1/132$, $p < .01$), but the difference at Block 2 was not significant ($F = 2.52$, $df = 1/132$, $p > .05$). In contrast, EA mice of Strain DBA/2J made significantly more errors than CA mice of Strain DBA/2J on the second block of trials only ($F = 4.30$, $df = 1/132$, $p < .05$). For Experiment 2, where ether (EB) or control handling (CB)

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Fig. 1. Mean errors for blocks of 10 trials each for all experimental conditions.

was presented before each trial, only the observed decrease in errors from block to block was significant ($F = 37.59$, $df = 2/88$, $p < .01$).

Figure 2 illustrates the time scores for both experiments. For Experiments 1 and 2 the DBA/2J strain swam significantly faster than the C57BL/6J strain when times were averaged over blocks and treatments ($F = 77.32$, $df = 1/44$, $p < .01$; $F = 30.55$, $df = 1/44$, $p < .01$). For Experiment 1 there was a significant Strain by Treatments by Blocks interaction ($F = 3.14$, $df = 2/88$, $p < .05$). It may be seen in Fig. 2 that this interaction reflected the fact that C57BL/6J-EA, C57BL/6J-CA, and DBA/2J-EA Ss showed a decrement in swimming times from Block 1 to Block 3, while the DBA/2J-CA Ss showed an improvement. For Experiment 2 there was a significant Treatment by Blocks interaction ($F = 5.73$, $df = 2/88$, $p < .01$). Regardless of the strain, Ss that received EB swam significantly faster on the first block of trials than the Ss that received CB ($F = 11.18$, $df = 1/132$, $p < .01$); however, the largest time difference was seen for the C57BL/6J strain. For this strain, the EB Ss were clearly faster on Block 1 than the CB Ss.

DISCUSSION

Wimer (1968) and Wimer et al (1968) reported shorter passive avoidance and shock escape latencies for Strain C57BL/6J than for Strain DBA/2J when trials were presented at least 24 h apart. In the present experiment there were no differences in errors between the two strains, and swimming times were generally shorter

for the DBA/2J strain. Wimer (1968) and Wimer et al (1968) reported a facilitation in learning for DBA/2J mice that were etherized immediately after each trial and no learning differences between etherized and nonetherized C57BL/6J mice. In contrast, Experiment 1 of the present study indicated an overall increase in errors for both strains with posttrial etherization, although the C57BL/6J mice were most adversely affected. It appears, then, that ether effects on learning may be expected to vary as a function of task demands.

The fact that an Ether by Strain

interaction was obtained in the Wimer (1968), Wimer et al (1968), and the present experiments suggests that ether effects on learning vary among diverse genetic stocks and strains. For this reason it is not surprising to find conflicting results with regard to ether effects on learning in experiments that have utilized different strains or stocks. For example, Abt et al (1961) and Essman & Jarvik (1961) have reported a decrement in shock avoidance learning in Swiss-Webster mice, rather than the learning facilitation for DBA/2J mice reported by Wimer's group.

Animals in all groups showed a normal gain of weight over the experimental period, there was no mortality in either the ether group or the control group, and Ss that received ether prior to a trial showed even faster swimming speeds than did the control Ss. It appears, then, that cumulative effects of etherization produced by hypoxia or anoxia cannot explain the decrement in performance for the Ss etherized after a trial. Among others, Pearlman et al (1961) have suggested that ether-produced decrease in amplitude and frequency of electrical activity of the nervous system interferes with the process of memory trace formation. The present data are not inconsistent with this hypothesis. There are, however, two alternative hypotheses worthy of consideration: (1) adverse side effects accompanying ether induction negatively reinforces activity which precedes its administration or (2) ether-induced activity, e.g., jumping and struggling, interferes with the retention of stimulus-response

Fig. 2. Mean swimming time for blocks of 10 trials each for all experimental conditions.

sequences which precede ether administration. These hypotheses receive support from two studies. Jacobs & Sorenson (1969) found that passive avoidance learning in Swiss-Webster mice was retarded by immersion in hot or cold water for 0-2 sec after a trial. Elias & Simmerman (1970) found that a blast of air after daily discrimination trials resulted in an increase in swimming times and an increase in errors for Strain C57BL/6J under exactly the same conditions as the present experiment, with the exception of the ether treatment. The fact that aversive stimulation has a deleterious effect on learning suggests that decrements in learning following posttrial etherization cannot be solely attributed to the CNS-depressing effects of ether.

There was no difference in errors for Ss that received ether as opposed to control handling before each trial. However, Ss of both strains showed significantly faster swimming times on Block 1 as a function of pretrial etherization. This suggests that the stimulating effects of light ether anesthesia, described by Cherkin (1968b), enhanced drive or arousal level but did not affect learning or memory per se. The difference in swimming times between ether and control Ss was much less for the DBA/2J strain. In fact, the DBA/2J mice that received pretrial etherization actually showed an increase in swimming time from Block 1 to Block 3. The fact that the DBA/2J strain was much less affected by pretrial etherization than the C57BL/6J strain may be related to a naturally higher level of arousal or a greater responsiveness to water escape incentive in the former strain which was not increased appreciably by pretrial etherization. This hypothesis receives support from the observation that swimming times were faster for the DBA/2J controls than for the C57BL/6J controls for Experiments 1 and 2. These data suggest that pretrial etherization may provide a useful tool for studying strain differences in drive or arousal mechanisms.

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Tricarboxylic acid cycle levels following avoidance acquisition by rats*

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To gain further information on the relationship between intermediary metabolism and acquisition, rats were trained in a two-trial avoidance task and sacrificed for chemical analysis. Rough quantitative measures of five acids of the tricarboxylic acid cycle were obtained. After the second trial, Ss were assigned to groups by their behavior: passive avoidance, approach, or active avoidance. Kruskal-Wallis analyses of variance indicated that differences in citrate and in succinate levels were reliable. It cannot be concluded that the results were associated with learning.

Because numerous drugs affecting intermediary metabolism (e.g., amphetamines, phenothiazines) have been shown to have significant behavioral effects, measurement of some intermediary metabolites during a behavioral situation might be informative in elucidation of the physiological mechanisms underlying such behavior. Since the tricarboxylic acid cycle (TCAC) is so closely related to numerous metabolic processes, measurement of some of the substrates of this cycle would seem to be appropriate to tracing metabolic changes occurring during a behavioral task. Even if the relevant metabolic changes do not directly involve the

TCAC, the importance of this cycle to almost all synthetic tasks might allow the enumeration of the relevant reactions by the use of metabolic inhibitors.

METHOD

If transient metabolic changes are to be studied, a rapidly acquired task would be most appropriate. For this reason, a one-trial avoidance task was used. This situation allows exact comparisons to be made because all Ss receive the same external conditions (CS, US, ITI, and second CS) regardless of their behavior. Because these conditions are constant for all Ss, the probability that observed metabolic changes were associated with external conditions was low.

SUBJECTS

The Ss were 15 male Sprague-Dawley rats about 55 days old and weighing between 180 and 200 g upon arrival. All Ss were group housed and had free access to food and water until 24 h before testing. Ss were handled daily and individually placed

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