DARK AVOIDANCE LEARNING AND THE EFFECTS OF CARBON DIOXIDE ON MEMORY DISRUPTION IN THE COCKROACH PERIPLANETA AMERICANA

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
KATHRYN LEE LOVELL
1973

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ABSTRACT

DARK AVOIDANCE LEARNING AND THE EFFECTS OF CARBON DIOXIDE ON MEMORY DISRUPTION IN THE COCKROACH *PERIPLANETA AMERICANA*

By

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The time course of acquisition and retention of dark avoidance learning in cockroaches was investigated. Carbon dioxide was administered after training to determine if the degree of disruptibility of the memory depends on the interval between training and CO_2 administration.

Cockroaches were trained to avoid the dark side of a box using electric shock as negative reinforcement. The majority of the acquisition of learning, as measured by the number of shocks initiated, occurred within the first minute of training. Retention was measured in two ways: 1) change in the amount of time spent on the dark side (dark preference) during 3 minute periods before and after training; 2) retraining to avoid the dark. There was no decrease in retention up to 2 hours after 1 hour of training according to both of the retention measures.

Carbon dioxide was administered to roaches immediately or 1 hour after training and retention was measured 2 hours after training. When CO_2 was given immediately after training, no retention was observed 2 hours later in either retention measure. When CO_2 was given 1 hour after training some retention was observed during testing, but there was a difference in the amounts of retention seen in the dark preference and retraining measures. These results indicate that memory phases which differ in their susceptibility to disruption by CO_2 can be observed in cockroaches.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Biophysics

1973

Dedicated to H. P. L.

ACKNOWLEDGEMENTS

The technical assistance of N. St. Pierre has been very helpful. Dr. E. M. Eisenstein, my advisor and chairman of this thesis committee, has greatly aided my scientific development in the course of this research. The love and understanding of my husband Bob has been deeply appreciated throughout the duration of this work.

The author has been supported by an NSF predoctoral fellowship and is currently a trainee on NIH training grant No. T01-GM01422-08. The research was carried out under NSF grant GB 23371 to Dr. E. M. Eisenstein.

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INTRODUCTION

The time courses of acquisition and retention of a learned task have been investigated in many animals. In addition, the effects on learned behavior of various physical and chemical agents have been studied in an attempt to understand the processes underlying learning and memory. While most of this work has been performed on vertebrates, an increasing amount is being done on insects, particularly cockroaches. It is of interest to compare characteristics of learning and memory phenomena across the animal kingdom. In this way it may be possible to separate those processes common to all animals with a nervous system and those processes which vary among animals and thus may depend on the specific circuitry of the animal's nervous system. In many respects, the results of learning experiments on insects are similar to those on higher animals. For example, the interference theory of Jenkins and Dallenbach (1924) which postulates that forgetting is mainly due to interpolated activity has been confirmed on cockroaches. Minami and Dallenbach (1946) compared the effects of forced activity and of inactivity on relearning. Forced activity after learning decreased retention of a dark avoidance response in cockroaches while inactivity greatly increased retention compared to controls given corresponding intervals of normal rest. Kamin (1957) observed that retention of dark avoidance in rats decreased to a minimum at 1 hour and subsequently increased over a period of 19 days. A similar pattern of retention has been seen by Eisenstein (1970) in an investigation of shock avoidance learning in the headless cockroach. A "Kamin effect" with a retention minimum at 2 days was observed by Alloway (1969) in the grain beetle, using a number of spaced training trials.

Many agents, such as electroconvulsive shock (ECS) (Jarvik 1972), carbon dioxide (CO_2) (Taber & Banuazizi 1966; Freckleton & Wahlsten 1968), inhibitors of protein, RNA and DNA synthesis (Glassman 1969), cholinesterases and anti-cholinergics (Deutsch 1971), have been found to disrupt memory or interfere with learning. The results of experiments in which such agents were administered to vertebrates after a learning task have suggested to many the existence of at least two stages of memory, commonly termed "short term" and "long term" stages. Generally in such experiments, retrograde amnesia is observed if the agent is administered immediately after training. Retrograde amnesia is not observed if the agent is administered some period of time after training (the period may vary from seconds to hours depending on the agent and the conditions of the experiment). Results have been interpreted as suggesting "consolidation" of the memory trace from a "short term" stage to a "long term" stage such that disruption may occur during the short term stage, but not during the long term stage. However, the short term and long term descriptions of memory stages must be considered relative to the specific experimental variables. For example, Paolino, Quartermain and Miller (1966) showed that ECS and CO_2 produce retrograde amnesia with different time courses in rats and probably act via different mechanisms. There may be many phases or steps in the physiological formation of memory, but in any case the different susceptibilities of the memory to disruption at different times after training indicate the changing nature of the memory trace.

Carbon dioxide has not been as widely used in memory disruption experiments as ECS and drugs, but it has been shown to produce retrograde amnesia in both insects (Freckleton & Wahlsten 1968) and mammals (Taber & Banuazizi 1966; Paolino, Quartermain & Miller 1966) when given immediately after training. Taber and Banuazizi (1966) administered CO_2 to mice at several intervals up to 2 hours after one-trial passive avoidance training. They observed a temporal gradient of retention impairment with statistically significant disruption up to 30 minutes after acquisition. However, Paolino, Quartermain and Miller (1966), using similar although not identical experimental procedures with mice, reported a maximum effective interval to produce retrograde amnesia of less than 5 minutes. The disruption mechanism would appear to be sensitive to variables which have not been identified.

The experiments reported here investigate the time course of acquisition and retention of a dark avoidance task in cockroaches. Cockroaches, which normally prefer dark, were trained to avoid the dark side of a box using electric shock as negative reinforcement. In addition, CO_2 was administered either immediately or 1 hour after training to determine if the degree of disruptibility of the memory depends on the interval between training and CO_2 administration. In insects, CO_2 produces an anesthetic effect of rapid onset and short duration. Convulsions occur initially, followed by quiescence. Locomotor behavior is normal after recovery from the anesthesia.

METHODS

General Procedures

Adult male cockroaches, _Periplaneta_ _americana_, were kept together in a large bin until the day of the experiment. Each experimental animal was removed and exposed to CO_2, an anesthetizing agent, so that a piece of gauze could be waxed to its back to serve as a handle. Each roach was then kept in an individual container with access to water for the duration of the experiment. Figure 1 illustrates the chamber in which each roach was trained individually. One side (the

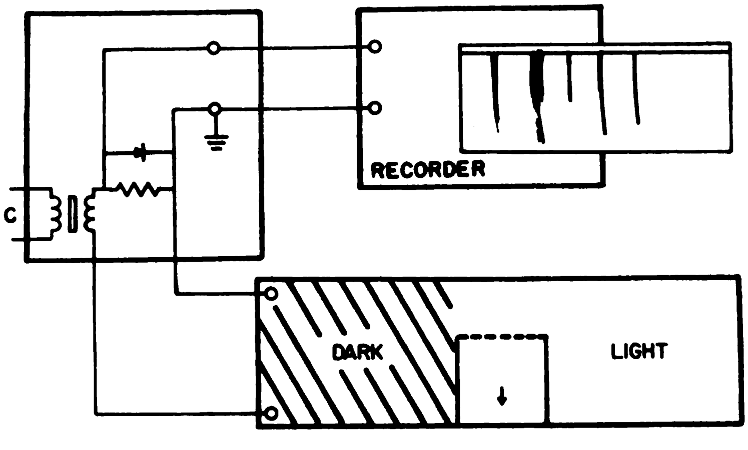

Figure 1. Top view of the experimental chamber and wiring diagram for shock delivery and recording of shock bouts. The chamber is a 8 x 25 x 11 cm high plexiglas box. The source of the shock is 60 cycle AC current (C) reduced to approximately 3v with a variable transformer. The sample polygraph recording shows five typical shock bouts. The deflections from the base line indicate when the animal is on the dark side receiving shock. When the roach leaves the dark side, the pen returns to the baseline. The magnitude of deflection is of no consequence. See text for explanation of training procedure.

dark side) was covered externally with dark paper. Room light served as the source of illumination for the light side. The floor of the light side was covered with plexiglas. Shock grids on the floor and three of the interior walls of the dark side served to deliver an electric shock whenever the animal entered. The shock grids were formed by photoetching copper film on a glass epoxy backing to form 2 mm wide strips separated by 0.8 mm. The grids were covered with solder to prevent oxidation. A polygraph recorded the shocks taken by the animal. A tracing of a typical recording is shown in Figure 1. From this record the number of times the animal entered the dark side to initiate a shock (number of bouts) and the length of each shock bout could be measured. For the analysis of learning the following three measures were calculated for each consecutive 5 minute period: the number of bouts, the length of time shocked, and the time shocked per bout (length of time shocked divided by the number of bouts).

Acquisition and Retention Studies

The avoidance training and testing consisted of the following procedure.

a) The animal was placed in the area indicated by the arrow facing in the direction of the arrow (Figure 1) with the shock turned off.

b) After the animal turned around and entered one side, the sliding partition indicated by the dashed line was replaced. The side entered was recorded.

c) Beginning at the time the animal entered one side, the amount of time he spent on the dark side during a 3 minute period was measured and the number of times he left the dark side was noted. This was a pre-training dark preference test with no shock given.

d) The shock was then turned on and the animal was trained for 1 hour; i.e. the animal was allowed to wander within the chamber with no outside interference. The pattern of shocks initiated was recorded as described above.

e) The animal was placed in the home cage and kept in the dark for varying time periods, after which retention was tested. Each animal was given only one retention test.

f) The testing procedure for retention was similar to the training procedure, i.e. a 3 minute dark preference test was given, followed by retraining with shock for 20 minutes.

With this procedure, retention could be measured in two ways. Comparison of the 3 minute dark preference tests before and after training gave an indication of retention of dark avoidance behavior independent of shock at the time of measurement. The records of shocks received during training and retraining, in addition to giving a second measure of retention, were used to distinguish between avoidance and escape modes of behavior. The change in the number of bouts can be considered as a change in the animal's avoidance of the dark. The change in time shocked per bout can be considered as a change in escape behavior, since this is a measure of the time an animal takes to escape from the dark side after entering. The measure of total time shocked represents a combination of avoidance and escape behaviors.

Carbon Dioxide Studies

The training procedure in the CO_2 experiments was the same as described above except that the animals were given 15 minutes of training and 5 minutes of retraining, since the results from the acquisition and retention studies showed that acquisition rapidly approached its asymptote within 5 to 15 minutes. The following groups were used to test the effects of CO_2 on retention.

1) CO_2 was given immediately after training and the animal was tested 2 hours after the end of training (CO_2 - 0 hour group).

2) CO_2 was given 1 hour after training and the animal was tested 2 hours after training (CO_2 - 1 hour group).

3) The animal was tested 2 hours after training; no CO_2 was given (no CO_2 group).

4) After the 3 minute dark preference test the animal was exposed to the training apparatus for 15 minutes with no shock given (pseudo-training); 1 hour later CO_2 was administered; the animal was tested by the regular testing procedure 2 hours after the pseudo-training (CO_2 control group).

In all cases where CO_2 was given, the animals were removed from their home cages and placed in plastic containers in which one side was a mesh screen. This container was put, screen side down, into a Coors porcelain Buchner funnel through which CO_2 was delivered. The animals were exposed to CO_2 gas from a tank for 45 seconds. Usually the animals had convulsions about 10 to 15 seconds after initial exposure and the convulsions lasted 5 to 15 seconds. The animals were quiescent for the remainder of the 45 second exposure. Following exposure the animals were replaced in their home cages until time for testing.

RESULTS

Acquisition

Avoidance measure. The number of shock bouts initiated by roaches during a 1 hour dark avoidance training period is shown in Figure 2. This measure indicates how often an animal entered the dark side, initiating a shock. A change in the number of shock bouts

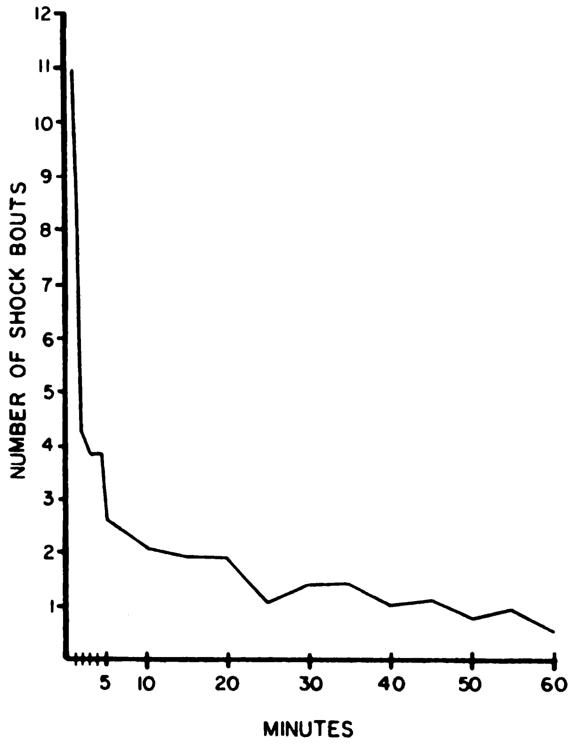

Figure 2. Mean number of shock bouts initiated by roaches for each consecutive 5 minute period. This is a measure of avoidance behavior. Values for each consecutive minute during the first 5 minutes are shown on the same scale. The training curve gives average values for a group of 40 roaches trained for 1 hour.

initiated can be considered a measure of avoidance behavior modification. More than half the total decrease in the number of shock bouts occurred by the second minute, indicating that the majority of learning occurs in the first minute.

Escape measure. Figure 3 illustrates the time shocked per bout during training, that is, the average time taken by an animal to escape from the dark side for each entrance into the dark. This can be considered a measure of escape behavior. There does not appear to be any significant modification of escape behavior during the training period. The learning curve obtained from the measure of total time

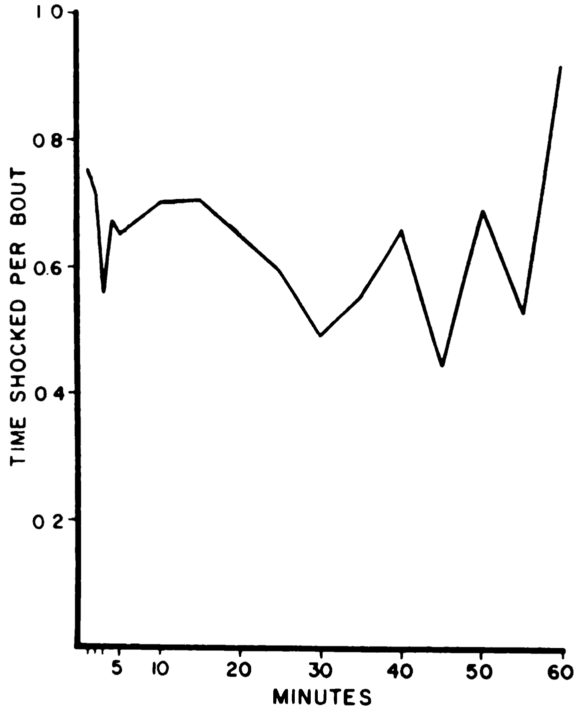

Figure 3. Mean time shocked per bout for each consecutive 5 minute period. This was calculated by dividing the total time shock was received by the number of bouts. Values for each consecutive minute during the first 5 minutes are indicated on the same scale. Experimental conditions as in Figure 2. The time shocked per bout is a measure of escape behavior. No evidence of escape learning can be seen.

shocked is very similar to that obtained from the measure of number of shock bouts. This is expected since the measure of total time shocked is a combination of avoidance and escape behaviors and no escape learning is observed. Thus, learning is determined by the avoidance component of behavior with no observable contribution by the escape component.

Retention

Retention of dark avoidance learning was measured by giving a dark preference test and by retraining animals after various time periods following training. One measure of retention, the percentage decrease in the number of shock bouts between training and retraining, was calculated using the number of shock bouts during both the first 1 minute and first 5 minute periods. Both groups of percentage values were in the same range. The initial 5 minute periods of training and retraining were used in analyzing retention. Figure 4 shows the amount of retention as indicated by retraining. There is good retention up to 2 hours. There is no significant difference among the retention points measured at different intervals. Retention as measured by the 3 minute dark preference test is shown in Figure 5. There is no decrease in retention during the first hour and subsequently retention appears to drop, but the amount of retention is still significant after 3 hours ($P < 0.025$, 1-tailed Wilcoxson). The amounts of retention for both retention measures (dark preference and retraining) are in the same range. Comparison of these results with the retention results of animals trained for 15 minutes (the no CO_2 group in the CO_2 experiments) shows that the amount of retention seen after 2 hours is

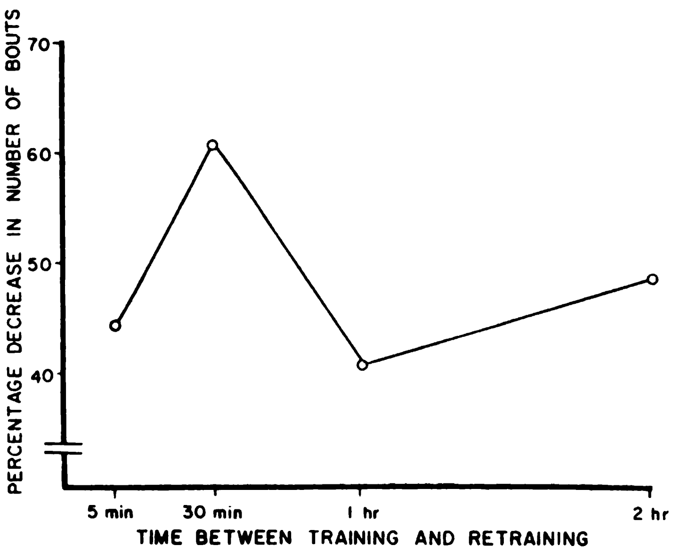

Figure 4. Percentage decrease in number of bouts between the first 5 minute periods of training and retraining. The percentage decrease was calculated as: (number of bouts during training minus number of bouts during retraining) / (number of bouts during training). A higher percentage value indicates greater retention. Each retention point represents an average value for 10 roaches. The amount of retention is significant out to 2 hours ($P < .005$, 1-tailed Wilcoxson).

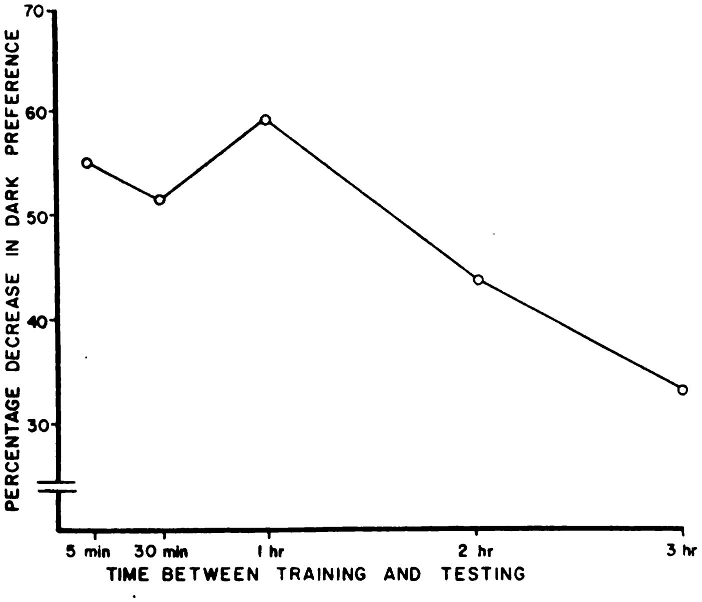

Figure 5. Percentage decrease in dark preference before training and before retraining. The percentage decrease was calculated as (initial minus final)/initial. A higher percentage value indicates greater retention of dark avoidance. The amount of retention at 3 hours is significant ($P < .025$, 1-tailed Wilcoxson). Each retention point represents an average value for 10 roaches.

in the same range in animals trained for 15 minutes or 1 hour.

A number of controls indicated that the change in dark preference can be directly attributed to learning by the animal to avoid the dark side and not to other variables. The amount of time spent by the roaches on the dark side did not change over a period of 3 hours if no shock training was given. Avoidance of the dark side cannot be the result of an odor or secretion left by previous roaches which were shocked, since dark preference did not depend on whether other roaches had been shocked in the apparatus immediately before measurement of the dark preference. In addition, the apparatus was cleaned well with alcohol between training sessions. The modification of dark preference behavior also cannot be attributed to a difference in activity as a result of shock received, since the number of times the animals crossed between the light and dark sides during the dark preference test was not significantly different before and after training in any of the retention groups.

Observations of animals during the 3 minute dark preference tests clearly revealed differences in behavior before and after training. When initially placed in the apparatus, the cockroaches had a choice of entering the light or dark side. Animals demonstrated a tendency to initially enter the dark side less often after training than before training in all retention groups (Table 1). The change in the number of roaches which initially enter the dark side can be considered as an additional measure of retention. Generally, the roaches observed before shock training tended to spend more time in the dark at the beginning of the 3 minute period and gradually enter the light for longer periods to explore. In contrast, roaches observed in the 3

Table 1. Number of animals initially entering dark side in dark preference test

Retention group (time between training and testing)	Before training	After training
5 minutes	8	6
30 minutes	6	4
60 minutes	7	5
120 minutes	9	5

minute period after training tended toward the opposite behavior -- they spent more time in the light initially and gradually started to spend longer periods in the dark. This latter behavior would be expected since some extinction of the dark avoidance behavior would occur when the animal enters the dark without receiving shock.

Effects of Carbon Dioxide on Retention

The effects on retention of carbon dioxide given immediately or 1 hour after a 15 minute training period were investigated using the four groups described in the Methods section. The change in dark preference between the 3 minute test periods before and after training is shown in Figure 6. CO_2 itself, in the absence of training, does not alter the dark preference of animals during a 2 hour period (CO_2 control group). There is significant retention ($P < 0.005$, 1-tailed Wilcoxson) of dark avoidance learning after 2 hours (no CO_2 group). If CO_2 is administered immediately after training there is no evidence of retention at 2 hours. However, if carbon dioxide is given 1 hour after training there is good evidence of some retention at 2 hours, since the difference between the CO_2 - 0 hour and CO_2 - 1 hour groups is significant at the 0.05 level (1-tailed Mann-Whitney U test). Also, the difference between the no CO_2 and CO_2 - 1 hour groups is not significant ($P > 0.05$, 1-tailed Mann-Whitney U test). However, the amount of retention for the CO_2 - 1 hour group is only significant at the level of $0.05 < P < 0.10$, (1-tailed Wilcoxson test), indicating that some disruption has occurred. (In the analysis of the data in Figures 6 and 7, previous data on the time course of memory disruption justifies the use of a 1-tailed test.) Thus the memory appears to be completely disruptible by CO_2 immediately after training and less disruptible 1 hour after training.

The effects of CO_2 on retention as measured by retraining are shown in Figure 7. Again there is a significant degree of retention when no CO_2 is given ($P < 0.005$, 1-tailed Wilcoxson). The group given CO_2 immediately shows no evidence of retention. There is no significant difference between the retraining values for the CO_2 - 0 hour and CO_2 - 1 hour groups. However, the direction of the changes for the two groups is the same as for the dark preference measure of retention.

These results lead to the conclusion that administration of CO_2 definitely interferes with retention of dark avoidance learning when given immediately after training. There appears to be a decrease in the

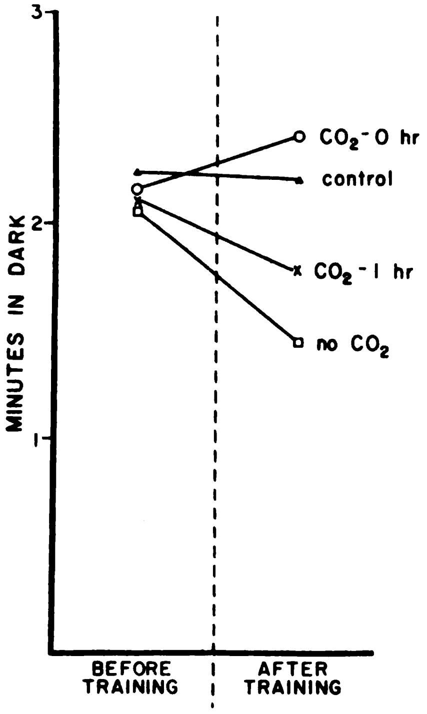

Figure 6

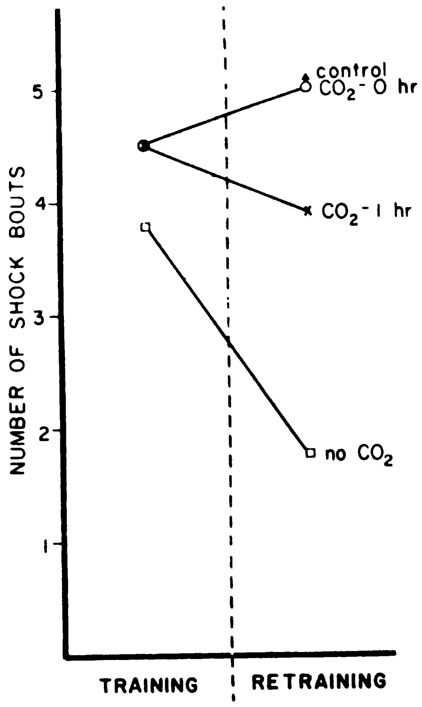

Figure 7

Figure 6. Mean number of minutes spent on the dark side during the 3 minute dark preference tests before training and 2 hours after training. The different groups assess the effects of CO_2 administration on retention. The amount of retention for the no CO_2 group is significant ($P < 0.005$, 1-tailed Wilcoxson). The amount of retention for the CO_2 - 1 hour group is not significant at the 0.05 level ($0.05 < P < 0.10$, 1-tailed Wilcoxson). The changes in dark preference for the CO_2 - 0 hour and CO_2 - 1 hour are different at the 0.05 level (1-tailed Mann-Whitney U). The changes in dark preference for the CO_2 - 1 hour and no CO_2 groups are not significantly different ($P > 0.05$, 1-tailed Mann-Whitney U). There were 14 animals in the no CO_2 group and 10 animals in each of the other groups.

Figure 7. Mean number of shock bouts initiated during training and during retraining 2 hours later. The different groups assess the effects of CO_2 administration on retention. The amount of retention of the no CO_2 group is significant ($P < 0.005$, 1-tailed Wilcoxson). The training-retraining differences for the no CO_2 and CO_2 - 1 hour groups are different at the 0.05 level (1-tailed Mann-Whitney U). The training-retraining differences for the CO_2 - 0 hour and CO_2 - 1 hour groups are not significantly different ($P > 0.05$, 1-tailed Mann-Whitney U).

susceptibility of the memory to CO_2 disruption 1 hour after training. The time course of this effect needs to be more thoroughly investigated.

Addendum

Replication of some of these experiments was attempted as a preliminary to further experiments on CO_2 effects. The cockroaches used for the replication experiments were from a different source (Insect Control and Research, Baltimore, Md.) than the source of roaches (Dept. of Agriculture) for the original experiments. Although both batches of animals looked healthy, they differed markedly in initial preference for the dark side, measured in the 3 minute dark preference test before training. For the purpose of analyzing such differences, three groups of roaches were considered: Group I - 50 roaches used in the experiments on acquisition and retention (Dept. of Agriculture stock); Group II - 47 roaches used in the experiments on CO_2 effects (Dept. of Agriculture stock); Group III - 25 roaches used in the replication experiments (Insect Control and Research stock). The average initial dark preference of Group I was 127.4 seconds; that of Group II was 131.3 seconds; that of Group III was 155.4 seconds. There was no significant difference ($P > .6$, 2-tailed Mann-Whitney U) between Groups I and II, but the initial dark preference of Groups II and III were significantly different at the .002 level (2-tailed Mann-Whitney U).

Dark avoidance learning occurred among all three groups, but less retention was seen for the animals in Group III. The amount of retention measured at ½ hour for Group III was significant at the .05 level (1-tailed Wilcoxson), but the Group III animals showed only a

12.4% decrease in dark preference and a 16.9% decrease in number of shock bouts initiated -- much less than any of the groups in the original experiments. It would be expected that animals with a stronger dark preference would find it more difficult to retain a behavior which opposed that preference. There are probably a variety of factors involved in regulating dark-related behavior of cockroaches. It is possible that differences in rearing conditions, current environmental influences, or genetic factors are responsible for the differences in behavior seen among the groups of cockroaches. Factors such as these should be considered if problems arise in replicating this type of experiment.

DISCUSSION

The design of the training task in these experiments allowed the separation of the avoidance and escape components of learning. It can be concluded that in this situation, avoidance learning is the dominant mode occurring. Escape learning made no observable contribution to reducing the number of shock bouts initiated. Disterhoft (1972) arrived at the same conclusion when cockroaches were trained to lift a leg to avoid shock. During dark avoidance training in the present work, over half the decrease in the number of shock bouts occurred by the second minute. Considering the differences in the types of training, this time course agrees well with the results of Eisenstein (1968) and Disterhoft (1972), who reported that asymptotic avoidance learning in cockroaches was found by 1.5 minutes of leg lift training.

One of the major topics of investigation in the field of learning and memory is the time course and nature of memory consolidation. The theory that two stages of memory - "short term" and "long term", characterized by different degrees of disruptibility and probably by different physiological stages - exist has been widely proposed on the basis of memory disruption experiments with vertebrates. Little is known about the transition between memory states and the factors which influence the time course of the transition. The transition time course refers to the changes in memory occurring during the time in which one stage of memory declines and the subsequent stage grows. This does not necessarily imply a causal connection between the two stages and they may have either sequential or parallel developments. The transition time courses between different phases of memory may be dependent on many factors, including the modality of learning (e.g. escape or avoidance learning), the nature of the reinforcer (e.g. positive or negative), the strength of the memory, the conditions of training, genetic factors, and environmental conditions (e.g. temperature or activity).

One interpretation of the minimum point of the "Kamin effect" is that it represents a point in the transition time course where "shorter term" memory has decreased to a low level and "longer term" memory has not yet been established to a large extent, with the result that very little retention is observed. The observation of a Kamin effect in rats (Kamin 1957) and cockroaches (Eisenstein 1970) suggests the possibility of multiple phase memory in both vertebrates and invertebrates. Another manifestation of the characteristics of more than one memory stage could be a difference in the susceptibility of the memory to disruption at various times after training. The present experiment showed that cockroaches were susceptible to memory disruption by CO_2 and the degree of disruption depended on whether CO_2 was administered immediately or 1 hour after training. These results are consistent with the existence of more than one memory stage in cockroaches. This possibility has also been suggested for insects by Alloway (1972).

Memory disruption experiments on a wide variety of animals should be given careful consideration as they suggest similarities in memory processes in vertebrates such as mammals and fish and invertebrates such as insects. They may also indicate some of the differences in these processes, either in transition time courses or mechanism of disruption. In goldfish given puromycin immediately after learning, a retention loss was not observed until at least 6 hours after training (Davis & Agranoff 1966) and there was an apparent growth of amnesia from 1 to 6 hours when ECS was administered 20 seconds after passive avoidance training in rats (Geller & Jarvik 1968). These results suggested the possibility that in vertebrates, a major mechanism of retrograde amnesia is not the disruption of the short term memory trace, but the inhibition of a transition step to a long term memory stage. In contrast, in cockroaches given CO_2 immediately after learning there is no evidence of retention 2 hours later. The roach data suggest two possible interpretations. 1) The time course of decay of a short term component is more rapid in roaches than in higher animals. This would imply that some retention should be detectable immediately after CO_2 administration. 2) The administration of CO_2 to roaches obliterates the memory trace. This would imply that no retention would be detected after CO_2 is given. Resolution of these alternatives could have important implications for characterizing the nature of the transition between more and less disruptible phases of memory.

During the period between training and testing the roaches were kept in their home cages in the dark. A question might be raised concerning whether keeping animals in the dark following training to avoid a dark place serves as an extinction paradigm. In early experiments on dark avoidance training of cockroaches, Turner (1912) concluded that "the change in behavior of these insects is not due to a physiological reversal of the phototropic responses of the roaches, but a case of learning, by experience, to avoid a specific dark place." Such a result implies that extinction would not occur in a dark home cage because dark, per se, is not the stimulus cue. This is consistent with the retention results presented here, showing that there is no significant difference in retention for roaches kept in the dark for varying periods after training.

One of the difficulties in all studies of memory disruption is the inability to determine the precise physiological mechanism by which the disruption is produced. There have been several investigations describing the effects of CO_2 on various physiological processes (Ward 1971; Brooks 1957) and behavioral activities (Ribbands 1958) of insects, but the mechanism of action of CO_2 on insects has never been clearly shown. Hypotheses implicating pH, anoxia, and changes in permeability are the explanations most often suggested. In addition to these possibilities, Wasterlain (1970) reported that rats repeatedly exposed to an atmosphere of CO_2 have a lower rate of brain RNA synthesis than controls submitted to a similar degree of anoxia (by exposure to nitrogen gas) but not exposed to CO_2. Whether such a change in RNA synthesis affects memory and whether it also occurs in insects is not known.

There are several effects of CO_2 anesthesia which might be responsible for the observed retention impairment, including convulsions, anoxia, and any of the chemical effects of CO_2 in the animal. Other anesthetic gases (which produce anoxia and may or may not cause convulsions) can be used to investigate the necessity of these effects for producing retrograde amnesia. In contrast to CO_2 anesthesia, nitrogen anesthesia produced no amnesic or impairing effects on retention in mice (Taber & Banuazizi 1966), thus eliminating anoxia as a major factor in the amnesic effect of CO_2. Since nitrogen produced clonic convulsions before unconsciousness in mice, while CO_2 did not, convulsions (at least in mice) are not directly involved in memory disruption. However, these results cannot be applied to insects without further investigation, since the gases may have different effects in different animals. Thus, the use of various anesthetic gases can determine if anoxia and convulsions are important elements in memory disruption in insects. In addition, variations in the amount of CO_2 administered could determine if the degree of disruption is dependent on dose, for example through concentration of a reaction product in the animal. These aspects, as well as a more detailed study of the time course of disruption, need to be more fully investigated.

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