Retrograde Amnesia: Production of Skeletal but Not Cardiac Response Gradient by Electroconvulsive Shock

Abstract. *Rats given a single electroconvulsive shock immediately after but not 60 seconds after an aversive conditioning trial exhibited behavioral retention deficits 24 hours later in a one-trial passive avoidance task. In contrast to these differential performance deficits, similar heart-rate changes, indicative of fear retention, were seen in punished animals irrespective of the time of delivery of the shock. These data suggest retention of a generalized fear to the training experience that was not revealed by the behavioral measure. The potential usefulness of concomitant behavioral and physiological response assessment in consolidation research is discussed.*

Rats given an electroconvulsive shock (ECS) shortly after training in single-trial learning tasks exhibit behavioral performance deficits when tested for retention 24 hours later. The gradient generated by the inverse relation between the magnitude of these deficits and the training-ECS interval has generally been attributed to a retrograde amnesia resulting from disruption of memory consolidation (*1*). Some investigators, however, have applied this interpretation only to situations where training-ECS intervals on the order of seconds are used (*2*), while others discount effects of ECS on memory consolidation entirely (*3*).

Assessment of autonomic changes in ECS studies where punishment is applied should facilitate evaluation of behavioral data, but such measures have seldom been used in single-trial tasks. In one such study where heart rate was measured, it was reported that ECS, when presented within 6 seconds of a single buzzer-shock pairing, did not modify the bradycardia produced to the conditioned stimulus during a retention test 24 hours later (*4*). Although behavior was not reported for this task, the authors suggested that the cardiac data argued against consolidation disruption as an explanation of ECS effects. We think, however, that any interpretation of the data of Mendoza and Adams (*4*) with respect to the memory consolidation hypothesis is unwarranted since this study employed (i) familiarization to the training environment before training; (ii) no independent behavioral measure of memory; and (iii) only one training-ECS interval. The importance of measuring behavior and using more than one training-ECS interval in amnesia experiments is emphasized by the report that familiarization of rats to the training apparatus may decrease the period of vulnerability of memory fixation to ECS disruption to less than 0.5 second after training (*5*).

We report here the effects of ECS on one-trial aversive learning with both behavioral and heart rate responses as indices of retention under conditions that have, in our previous experience, repeatedly and consistently produced reliable, short behavioral gradients of retrograde amnesia (*6*). Two training-ECS intervals were investigated to ascertain the time dependency of these effects—one which consistently produces behavioral retention test deficits, the other which produces no such deficits. Cardiac responses, suggestive of fear retention, were seen under conditions where behavioral evidence of amnesia was confirmed.

We used 108 male rats (Holtzman strain; 90 to 120 days old). The animals were caged together until surgery and then were placed in individual wire-mesh cages with free access to food and water, which was available throughout the experiment. Two days before the first habituation session, animals were anesthetized with ether. Stainless steel surgical wire (Ethicon stranded) was inserted through the skin behind each pinna and tied flush to the skin. A third wire lead was implanted subcutaneously just above the sternum and exited on the dorsal surface of the neck between the two pinna leads where it was sewn into the skin. "Wire form" subminiature female pin contacts (Amphenol Co.) were soldered to 5-mm extensions of these leads to make connection with male contact pins soldered to ECS delivery and heart rate recording wires. These wires were enclosed in a shielded cable suspended from a counter-balanced, overhead boom which allowed free movement of the animal throughout the apparatus. The electrocardiogram (EKG), monitored between one of the pinna leads and the sternum lead, was recorded on an E & M physiograph. The ECS was delivered by way of the pinna leads from a Hans model 2-C seizure apparatus.

The one-trial passive avoidance apparatus (*6*) consisted of a small opaque plexiglass compartment (SC) connected to a larger compartment (open field with grid floor) by a guillotine door. An opaque guillotine door was used during habituation and training sessions, and a clear plexiglass door was used during retention testing.

Animals were habituated to the SC in four separate 3-minute sessions (*7*). Two sessions were given per day for two consecutive days, with sessions within a day separated by approximately 5 hours. During habituation, the opaque guillotine door was in place to prevent entrance into the open field. The animal, with recording leads attached, was placed in the SC, and the EKG was recorded for 3 minutes. The animal was then returned to its home cage.

A single training trial was given the day after the last habituation session, followed 24 hours later by a 3-minute retention test. During training, each animal, with its leads attached, was placed in the SC. After the guillotine door was opened, latency for placing all four paws into the open field was recorded to the nearest 0.1 second. The door was then closed, and one of five treatments was applied. For the three experimental groups, entry into the open field initiated the presentation of a 1.5-ma, 1.5-second footshock (FS) through the grid floor. In the FS learning group ($N = 18$), animals were removed 3 minutes after receiving FS and were returned to home cages. In the other two groups ($N = 18$ each) a single ECS (100 ma for 0.5 second) was administered either immediately (FS-ECS IMMED) or 60 seconds (FS-ECS 60S) after FS termination. Two control groups were trained. One group of animals (NFS-NECS) received no shocks. Each animal was returned to its home cage 3 minutes after entering the open field. A second group (ECS) received only ECS after entering the open field. In all groups that received ECS, recovery from the resultant tonic flexion or extension convulsions occurred in the home cage. Animals that failed to enter the open field within 180 seconds were discarded.

For the retention test, recording leads were attached and the animal was placed in the SC. With the exception of the FS-ECS 60S group, heart rate and latency data were collected separately for each training group in order to obtain cardiac data which were free from confounding effects of performing the behavioral response. Accordingly, each training group was divided into subgroups of equal sizes. Animals

in each subgroup were either confined to the SC for heart rate recording (clear plexiglass guillotine door in place) or given access to the open field area (guillotine door opened). The EKG was recorded for 3 minutes. Neither FS nor ECS was administered.

Median latencies to enter the open field during training and retention sessions for the five nonconfined groups are presented in Fig. 1. Every animal in both FS and FS-ECS 60S groups remained in the SC for 180 seconds (*8*), indicating maximum retention for the FS animals and failure of ECS to disrupt retention when delayed 60 seconds after FS administration. While not significantly different from either the NFS-NECS or ECS control groups, animals convulsed immediately after FS did differ significantly from both the FS learning group and FS-ECS 60S group [Kruskal-Wallis analysis of variance on retention latencies: $H = 29.2$, d.f. $= 4$, $P < .001$; significant Mann-Whitney comparisons: FS-ECS IMMED versus FS and FS-ECS 60S ($U = 4.5$), NFS-NECS and ECS versus FS and FS-ECS 60S ($U = 0.0$), all $P < .001$]. No significant differences were found between latency scores of the groups during training. These results confirm previous findings of a short behavioral gradient for this task (*6*).

Heart rate data were tabulated as number of beats per minute in six alternate 15-second epochs of the 3-minute test sessions. A trend analysis of variance performed on the intrasession retention data for the confined FS and NFS-NECS groups revealed significant heart rate changes ($F = 9.90$, d.f. $= 5/80$, $P < .005$). When compared with the nontreated control group, these changes, manifested as a decrease in rate for the FS group, were limited to the first two epochs ($t = 2.38$, d.f. $= 16$, $P < .025$) when habituation-retention group differences were analyzed. This conditioned bradycardia in shocked animals should be contrasted with the significant increase in heart rate in response to the FS in the training session. The training tachycardia was seen in all FS animals and lasted for most of the 3-minute recording period. The increase in rate during the first epoch after FS, when compared with a similar period during the last habituation session, was significant ($t = 2.86$, d.f. $= 8$, $P < .05$). During this post-FS period locomotor activity within the open field decreased markedly from preshock levels and was, in most animals, entirely absent. These

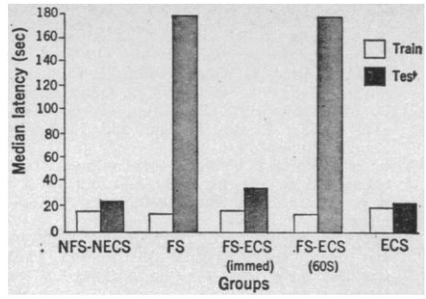

Fig. 1. Median latencies to enter the open field during training and retention test sessions. Timing of latency to enter the open field during both sessions was terminated at 180 seconds.

results suggest that mechanisms responsible for the production of bradycardia to conditioned and tachycardia to unconditioned stimuli during classical aversive conditioning and conditioned suppression training in rats (*8*) as well as conditioned suppression training in monkeys (*10*) may also be operating in a passive avoidance task, where the training environment acquires aversive cue properties (*11*).

The following discussion of heart rate data refer to those rates produced during the first 15-second epoch of a session. Since between-group averages for the last habituation session were not statistically different, these scores were equated. Retention scores were expressed as average rate changes from habituation scores for each group (Fig. 2). Analysis of variance of difference scores ($F = 115.8$, d.f. $= 5/45$, $P < .005$) and multiple comparison tests revealed that significant retention deficits occurred between groups that received FS during training and those for which FS was absent, irrespective of the presence or absence of ECS.

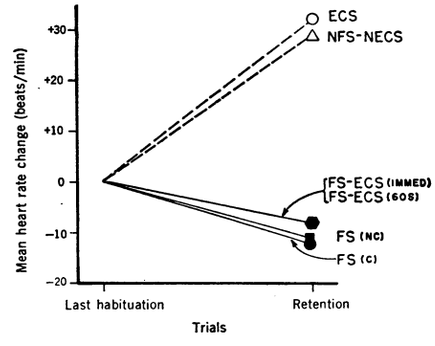

Fig. 2. Mean change in heart rate from the last habituation session to the retention test session. All groups, with the exception of the nonconfined (NC) FS group, were confined (C) to the small compartment during both sessions. Responses between the groups during the last habituation trial were not significantly different and were therefore equated.

That is to say, bradycardia with respect to cardiac responses of non-FS controls was produced for groups receiving ECS immediately as well as 60 seconds after FS, thus demonstrating the absence of a performance gradient with this measure. The fact that heart rate changes for the ECS group were similar in magnitude and direction to those of nontreated controls provides further support for the lack of aversiveness of ECS in this task. Lack of significant differences between FS confined and nonconfined groups suggests minimum influence of stimulus change, with respect to the presence or absence of the plexiglass door, during retention testing.

The major finding of this study, then, is the presence of an amnesic gradient when response latency is measured, but the lack of a gradient when heart rate is monitored. Assuming that both heart rate and response latency are valid measures of retention, cardiac responses may be more sensitive to training effects. While the underlying basis for the differences between the two measures is not known, the following speculation is offered for heuristic purposes. The bradycardia observed in animals that received ECS immediately after FS may actually reflect retention of a generalized fear. A possible mechanism that might account for this "sparing" effect is that fear conditioning to general environmental cues proceeds at an extremely rapid rate and that a longer time is required for the formation of associations with specific cues within the environment. Thus, placing animals back into the apparatus on the retention trial would reactivate a generalized fear response as measured by heart rate. However, the minimum association between pain produced by foot-shock and specific cues of the open field would not be sufficient to inhibit entrance into the open field (*12*). Indirect evidence for such "sparing" of memory in a task where a short training-ECS interval was used is provided in a study reported by Dawson and McGaugh (*13*). In their study of ECS effects on conditioned suppression of licking behavior in rats, these authors reported that animals which exhibited ECS-produced inhibition of suppression to the CS displayed significantly increased latencies to make a fixed number of licks during a pre-ECS period.

The questions raised by this study are important for several reasons. The use of a single dependent variable in consolidation research, while providing apparently unambiguous data, may ac-

tually mask the observation of important relationships. For example, the presence or absence of retention deficits may be inferred from our data, depending on the variable one chose to examine. The potential usefulness of both autonomic and behavioral variables in consolidation research might be applied to the problem of determining the nature of the process or processes that are disrupted by ECS, and whether "task" or "emotional" components, or both, of memory processes are being modified by ECS in aversive tasks.

Some of the current controversy about the concept of consolidation appears to be directed more to describing the range of conditions under which behavioral disruption will be produced by traumatic agents than to the assessment of the validity of the concept itself. Perhaps, even the question of validity will disappear as more specific postulates about memory mechanisms are developed. Both the judicious use of behavioral techniques and concurrent study of physiological mechanisms seem necessary if this goal is to be successfully approached.

BROMFIELD HINE
RONALD M. PAOLINO
Department of Pharmacology and Toxicology, Purdue University, Lafayette, Indiana 47907

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7. It is important to note that animals were habituated to the SC only and not to the large compartment where training to FS took place.
8. Since the variability of these groups' latencies was zero, nonparametric (distribution free) statistics were used for the analysis of the latency data.
9. R. D. Fitzgerald and T. J. Teyler, *J. Comp. Physiol. Psychol.* **70**, 242 (1970); L. de Toledo and A. H. Black, *Science* **152**, 1404 (1966).
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11. Additional evidence for single-trial cardiac conditioning was provided by the data from a shock-sensitization control group of nine rats. These animals were habituated and tested for retention in exactly the same manner as the other confined groups. On the training day, each animal was removed from the apparatus 1 minute after entering the open field from the SC. and was then returned to its home cage. One hour later, a 1.5-ma, 1.5-second alternating current shock was delivered to the tail of the restrained animal. The shock was administered outside of the training apparatus, and the rat was returned immediately to its home cage. The retention test was given 24 hours after tail shock. During retention testing, these animals exhibited an average increase of 17 beats per minute with respect to rates produced during the last habituation session (the group mean of 413 beats per minute for the habituation session was not significantly different from other habituation group means). This increase was similar in direction to that displayed by nonshocked and ECS controls. The retention heart rate of these sensitization controls was significantly greater than that of the FS learning group ($t = 1.61$; d.f. $= 16$; $P < .05$, one-tailed).
12. While this report was in press, D. Quartermain, B. S. McEwen, and E. C. Azmitia, Jr. [*Science* **169**, 683 (1970)], independently proposed a similar mechanism to account for the finding that a reminder shock would produce recovery of an ECS-disrupted passive avoidance response. According to these authors, however, the basis for the retained fear is an incomplete weakening, by ECS, of the association between specific training cues and fear, rather than the inability of ECS to disrupt a rapidly conditioned generalized fear response, as we suggest.
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14. Supported by Purdue Research Foundation predoctoral fellowship to B.H. and NIMH grant MH-15352.

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Emotionally Induced Increases in Effective Osmotic Pressure and Subsequent Thirst

Abstract. *Following a brief period of handling or enclosed rotation, rats increased the frequency of drinking relative to eating. Handling also delayed or eliminated eating behavior in hypoosmotic rats. Osmometric analysis revealed a rapid increase in serum osmolality during stress which may account for the emergence of thirst and disruption of eating.*

The incremental effect of stress, anxiety, and emotional excitement on the consummatory response of drinking has been discussed with respect to the induction of primary thirst (*1*) and to an increase in general drive, as hypothesized by Hull (*2*). Siegel and Siegel (*1*), who found an increase in water intake following faradic stimulation, explained their results by assuming that the supposed hemoconcentration resulting from emotional stress had produced primary thirst through a loss of water from the body cells. Similar results reported by Amsel and Maltzman (*3*) were attributed to a strengthened drive which augmented the learned response of drinking. Later, Siegel and Brantley (*4*) presented evidence that, in a situation somewhat more contrived than the Siegels' original conditions, food consumption could also be increased by emotional excitement. The original suggestion that emotional stress induces primary thirst remained, however, and recent research in support of an osmotic theory of thirst and hunger renews its relevance.

Briefly, we have reported that the initiation of drinking which follows food consumption in food-restricted rats is accompanied by a significant increase in serum osmolality (*5*), and the evidence suggests that primary thirst emerges during food ingestion following an increase in body-fluid osmolality. Similarly, the initiation of eating which follows water consumption in water-restricted rats is accompanied by a significant decrease in serum osmolality, and the suggestion was made that primary hunger results from a decrease in body-fluid osmolality (*6*). It follows, then, that if emotional stress were to change the effective body-fluid osmolality of an animal, the consummatory response which followed the stress would indicate the direction of that change. Also, the change should be measurable in the serum osmolality of the animal. A series of experiments reveal both of these expectations to be so.

The purpose of the first experiment was to determine if rarely handled rats would differentially increase one or the other consummatory response following a period of presumably stress-producing activity. If increased body-fluid osmolality elicits drinking and if stress increases body-fluid osmolality, stress should lead to increased drinking. Twenty-two male Holtzman albino rats (75 to 105 days of age and maintained with free access to food and water) were observed in their individual cages for three 2-hour periods, each period separated from the last by 7 days. In the first session, the rats were observed for the first hour, removed from their cages, individually handled (that is, held in the crook of the arm with no attempt to aggravate or hurt the animal) for 1.5 minutes, returned to their cages, and observed for 1 hour. The second session was a replication of the first, except that instead of being handled each animal was placed in a weighing container from an O'Haus animal scale and rotated slowly about several axes for 1.5 minutes before being returned to its cage. In the third session, no activity intervened between