VULNERABILITY OF SKELETAL AND AUTONOMIC MANIFESTATIONS OF A CER TO THE AMNESIC EFFECTS OF ECS

by

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ABSTRACT

VULNERABILITY OF SKELETAL AND AUTONOMIC MANIFESTATIONS OF A CER TO THE AMNESIC EFFECTS OF ECS.

by Alan D. Springer

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A series of experiments was performed to probe the phenomenon of sparing of memory by electroconvulsive shock (ECS) when amnesia is assessed by autonomic indices. Differential vulnerability of memory as indexed by skeletal and autonomic responses was determined to be a function of a high threshold to disruption for autonomically indexed memories. Automomic responses spared by ECS were found to reflect underlying memory rather than artifact produced by nonassociative factors resulting from the interaction of CS, US and ECS. Furthermore, an analysis of the retrograde amnesia gradients seen with skeletal and autonomic responses demonstrated that memorial processes associated with the former response category required more time to achieve a stable nondisruptable format from which information could be retrieved than the latter. Inasmuch as retrograde amnesia can be induced for both skeletal and autonomic components of a learned response, it is concluded that the memorial processes underlying these diverse response components are similar in nature with respect to their interaction with ECS. A model is proposed that attempts to relate the rate of achieving memory stability to complexity of the conditioned response.

Acknowledgements

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VULNERABILITY OF SKELETAL AND AUTONOMIC MANIFESTATIONS OF A CER TO THE AMNESIC EFFECTS OF ECS

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Upon examining the effects of various disruptive agents on memory as indexed by autonomic or skeletal responses, a general consistency in results across disruptors emerges.

Neuroleptic drugs are exemplary of disruptive agents which differentially affect autonomic and skeletal responses. These drugs interfere with retention of prior training if they are administered prior to the retention test. However, after the effect of the drug on the subject has subsided, impairment in retrieval of information is no longer evident. In a study using rats, haloperidol, perphenazine, and chloroprothixene did not similarly affect a conditioned hyperthermia and an operant skeletal response that were acquired simultaneously (Delini-Stula, 1971). These drugs were effective in disrupting operant skeletal behavior, but had no effect on conditioned hyperthermia. Rats injected with another neuroleptic drug, chlorpromazine, prior to testing for memory of passive avoidance training displayed poor retention for the appropriate skeletal response (Iwahara, Iwasaki, & Hasegawa, 1968). Nevertheless, micturition, a common response in footshock (FS) motivated tasks, was apparent after injection of chlorpromazine.

Several studies using amnesic agents to probe memorial processes in animals have also reported that autonomic responses are not disrupted by these agents whereas skeletal responses are. Among the first investigators to suggest that electroconvulsive shock (ECS) might not induce

amnesia for autonomic responses were Chorover and Schiller (1966). On the test trial of a study using a one-trial passive avoidance task, they noticed that animals that were amnesic on the basis of a skeletal index defecated and urinated as much as animals that received FS without ECS. Defecation in rats trained using a one-trial passive avoidance task has been more thoroughly investigated by Yaginuma and Iwahara (1971). Subsequent to training and ECS delivery, memory was assessed by a latency measure, as well as, by counting boluses on the test trial. Latency measures implied that the rats were amnesic; however, profuse defecation argued that autonomic responses were resistant to the amnesic consequences of ECS.

Another autonomic response that has been examined is conditioned bradycardia. Using a Pavlovian conditioning paradigm, Mendoza and Adams (1969) presented rats with a single buzzer-FS pairing followed six seconds later by ECS or no ECS. When tested 24 hours after training, animals that had previously received ECS or no ECS following the CS-US pairing demonstrated equivalent bradycardia to the buzzer. Furthermore, the two groups did not differ with respect to defecation. In addition, bradycardia and respiration changes were spared by ECS in a classical conditioning paradigm using ewes (Naitoh, 1971). Classically conditioned bradycardia in goldfish also appears resistant to the amnesic effects of puromycin, an antimetabolite (Schoel & Agranoff, 1972).

Vulnerability of a bradycardic response to ECS has also been explored in a step-through, passive avoidance task (Hine & Paolino, 1970). Rats tested 24 hours following training with FS and ECS proved to be behaviorally amnesic as they readily walked into the punishment compartment. However, when other trained and convulsed animals were restricted

to the start chamber for purposes of recording EKG, they displayed as much bradycardia as animals that had received FS only and were similarly tested.

If in fact memories indexed by autonomic responses are not disrupted by ECS, it would seem reasonable to assume that the mechanisms and processes governing autonomically manifest memories may differ from those underlying skeletally manifest memories. (Miller and Springer, (1973) describe several studies in which a noncontingent FS was effective in reversing ECS-induced amnesia following training and ECS. Given that these deficits can be reversed, it is plausible to assume that ECS primarily impairs retrieval rather than consolidation processes.) The finding that autonomic responses are resistant to disruption by ECS and other amnesic agents generalizes to several different species and tasks. Nevertheless, at least two laboratories have obtained ECS-induced amnesia for an autonomic response, conditioned bradycardia.

In one study rats were exposed to a tone-FS pairing followed by ECS (Caul & Barrett, 1972). Subsequently, the animals were presented with the CS and heart rate was recorded. In addition, the CS was presented while the animals were drinking and the time to resume drinking was recorded. Both the autonomic (bradycardia) and skeletal (cessation of drinking) expressions of memory were disrupted by ECS. Devietti and Kallioinen (1972) have also obtained amnesia for bradycardia in rats using FS followed by ECS.

It is important to point out that different skeletal indices of amnesia also lead to different conclusions about retention of the learning experience. Carew (1970) has observed amnesia following ECS in both passive avoidance step-down and step-through tasks. Yet upon closer

examination of the short latency step-down or step-through responses that indicated basic amnesia, Carew found that subjects were avoiding the particular locus of previous FS delivery.

Recently, Adams and Calhoun (1972) punished drinking animals with FS followed by ECS. In a retention test 168 hours after training, latencies to begin drinking did not provide any evidence for spontaneous recovery of memory. However, when total number of licks made during the test session was recorded, an amnesic group tested at 168 hours after training made significantly fewer licks as compared to a pretraining session than did a comparable group of rats tested 24 hours after training. Again, it appears that different measures of skeletal responses following ECS are discrepant in that latencies to begin drinking did not provide any hint of spontaneous recovery of memory, while examination of the change in number of licks per session indicated the return of memory.

The discrepancies apparent in the comparison of different skeletal indices of memory are similar to the discrepancies observed between autonomic and skeletal indices of memory. If these various response measures are actually measuring a unitary memory it would be difficult to resolve the discrepancies observed with different measures. More plausibly, the memory of training events that is retrieved at the time of testing is comprised of discrete components (e.g., stimulus, response, and associative). Different measures of retention are probably tapping the degree to which retrieval of the disparate components of the memory has been disrupted. If so, reports of amnesia observed with one measure and not with another are not necessarily contradictory.

However, before a meaningful statement can be made concerning the effects of a disrupting stimulus on different response measures, it

would seem necessary to a) examine both responses within the same experiment, b) record both responses from the same animal, and c) obtain both response measures at the same time. Although these methodological prerequisites are best met in the Carew (1970) and Adams and Calhoun (1972) studies, they are lacking in the type of skeletal versus autonomic response comparisons cited above. Mendoza and Adams (1969) and Devietti and Kallioinen (1972) reported the consequences of ECS on an autonomic but not on a skeletal response. Hine and Paolino (1970) examined both types of responses within a single experiment but recorded them in different groups of animals under different testing conditions. Caul and Barrett (1972) used the same animals to record autonomic and skeletal responses, but did so at different times.

Moreover, the interaction of skeletal movement and heart rate changes has not received adequate attention in some of the earlier studies. A reduction in heart rate is characteristic of fearful rats. However, a decrease in activity level could produce bradycardia which might be mistaken for fear-induced bradycardia, or might alternatively magnify a memory-linked bradycardia (Howard & Obrist, 1971). This factor may be of particular importance in the Hine and Paolino (1970) study. In their study, heart rate was recorded while restricting the animals' movements to the start compartment thereby reducing activity level. Unfortunately, an alternative approach, recording heart rate while the amnesic rats are making a stepthrough response, would probably result in the masking of any bradycardia by an activity-induced tachycardia.

In order to circumvent this problem, a task should be used that does not necessitate large scale movement by the animal to display

either retention or unavailability of memory. A conditioned emotional response (CER) lick suppression paradigm seems to fulfill this requirement. Animals are presented with the CS of a previously received CS-US pairing while they are drinking water. Highly fearful rats will stop drinking. Thus the time to resume drinking is the quantitative skeletal measure of fear. While the animal is drinking its skeletal activity is minimal; therefore, CS onset only leads to a small reduction in activity as fearful rats stop drinking. This small reduction in activity is unlikely to affect heart rate. As amnesic rats fail to respond to the CS, amnesia is apparent when there is no significant change in activity level. Moreover, since all animals are standing still as the CS is presented, any increase in activity would attenuate fear produced heart rate decelerations. Consequently, the lick suppression paradigm lends itself to a more conservative evaluation of the effect of ECS on conditioned bradycardia than would a step-through task.

Another potential activity-linked confound might be activity level changes produced by the non-associative effects of FS and ECS which might in turn affect heart rate. Insofar as none of the studies cited above included a control group that received noncontingent FS and ECS, it is difficult to conclude that any bradycardia they observed in amnesic animals was truly memorial in nature.

Those studies which report amnesia for bradycardia assessed amnesia by making comparisons with a trained but nonconvulsed group. That is, Devietti and Kallioinen (1972), as well as, Caul and Barrett (1972) determined whether amnesia existed for the bradycardic response by ascertaining whether animals that had received CS US ECS displayed as much fear with respect to this measure as did a group receiving CS US

no ECS. On the other hand the two studies that failed to observe amnesia for bradycardia (Mendoza & Adams, 1969; Hine & Paolino, 1970) assessed amnesia or its absence by comparing animals that had received CS US ECS against rats that had no opportunity to learn the CS-US association. Therefore, it is possible that the differences in conclusions reached by these studies might be idiosyncratic to the particular method used in assessing amnesia. To avoid this potential pitfall it might prove beneficial to compare amnesic animals against a trained but nonconvulsed group, as well as, a group that is not exposed to the CS-US contingency. Parenthetically, the lack of consistency in the use of control groups in assessing amnesia is also related to the issue of whether the term "amnesia" is to be applied only to a complete lack of memory or to a measurable memory deficit. Very often when an amnesic group is compared to a trained but nonconvulsed group of subjects it differs significantly from the latter group thereby indicating amnesia. However, in comparing the "amnesic" group with a nonmemorial control it is not unusual that these groups will also significantly differ. This would then suggest that ECS may not yield total amnesia but does induce significant amnesia, i.e., attenuation of manifest memory. Ideally, the amnesic group will not differ from nonmemorial controls while it will differ from a trained group. However, amnesia is not ordinarily an all-or-none phenomenon within indices, and different indices used to assess amnesia often yield appreciably different results. Understanding these discrepancies that arise using different measures of amnesia would seem of special importance.

Among the other factors which may have influenced the results concerning amnesia for bradycardia are ECS intensity and duration. ECS intensity

appears to be a critical variable that will be discussed in detail later. However, the ECS durations used in the various experiments are highly similar and small differences in ECS duration do not appear to be at all correlated with observing or not observing amnesia for bradycardia. Another factor which might be related to the different findings could be the particular portion of the heart rate record that is sampled. For example, Hine and Paolino (1970) focused their attention on six alternate fifteen second epochs after CS onset while Caul and Barrett (1972) analyzed three consecutive six second intervals following CS onset. As with ECS duration, the particular portion of the heart rate record that is sampled does not appear to be correlated with any consistent findings.

The present series of studies attempt to delineate the circumstances that lead to sparing of autonomically indexed memories by ECS. These studies will attempt to resolve the disparate results obtained with respect to amnesia for autonomic and skeletal responses by incorporating the suggestions presented previously.

Experiment 1

An initial experiment was performed to establish the FS intensity necessary to produce substantial skeletal lick suppression with a single CS-US pairing. Preliminary studies found that long FS durations (greater than one second) resulted in maximal fear being associated with the grid floor delivering the shock rather than with the CS. Using a 500 msec. FS reversed this effect.

Method

Subjects. Thirty-eight naive, male albino, Sprague-Dawley descended rats weighing 145-170 gm. on Day 1 of the experiment were purchased from a commercial breeder (Carworth, New City, New York). The subjects were individually housed in continuous light, maintained on ad lib water and 10 gm./day of powdered Purina Rat Chow. Although the food deprivation schedule was not necessary in the present study, it was used to maintain consistency with later experiments which required the deprivation schedule.

All water bottles were removed from the cages twenty-four hours prior to Day 1 of the experiment, whereupon the rats were allowed ad lib access to Purina Rat Chow pellets. The rats were permitted access to water for one hour each day approximately 1.5 hours after completion of the daily experimental schedule.

Apparatus. The apparatus consisted of an operant chamber measuring 22 x 28 x 41 cm. The floor of the device was constructed of 18 parallel, evenly spaced, stainless steel rods 0.3 cm. in diameter. Two walls of the chamber were constructed of white Plexiglas while the door and the facing rear wall were constructed of transparent Plexiglas. A hole 3.5 cm. in diameter was made in the center of one white wall 3 cm. from the

floor. Recessed 2 cm. behind the hole was a water tube. The number of licks emitted was recorded by having the rats break a photobeam positioned across the mouth of the lick tube each time they licked (Martonyi & Valenstein, 1971). The entire operant chamber and watering assembly was placed inside a sound attenuating box such that the open roof of the operant chamber met the ceiling of the sound attenuating enclosure. A black, four-sided plywood insert which blocked access to the water spout was used to change the stimulus properties of the operant chamber. The insert measured 26.5 x 19.0 x 38.5 cm. and covered the ceiling and walls of the operant chamber excluding the hinged wall that was the door to the chamber.

A Scientific Prototype 4025J white noise generator provided the CS which was 15 db. above a background of 55 db. The white noise was presented through a speaker positioned beneath grid level and mounted behind the wall containing the water spout. FS was produced by a 5000V transformer with a 120K ohm dropping resistor in series with the animal. A Variac (0 - 130 vac) provided the input to the transformer. The output of the shock source was connected to two parallel daisy chains of NE2H neon bulbs which were in turn connected to the grids of the operant chamber. The sole illumination source for the apparatus was provided by a 2.8 watt bulb.

Procedure. Days 1-4 involved placing each rat in the operant chamber, allowing it to find the water tube, and to emit 200 licks. On Day 5 the black insert was placed inside the operant chamber. The purpose of the insert was to change the cues of the apparatus on the training day so as to minimize the number of fear associations with the test environment. Therefore, on the test day when the insert was removed, most of the

acquired fear was not demonstrated until the onset of the CS, rather than being exhibited to the apparatus cues which were encountered as soon as the animal was placed in the chamber.

After randomly assigning each animal to one of four groups on Day 5, every animal was habituated in the chamber for 2 minutes. Subsequently, the white noise was presented for 18 seconds of which the last 500 msec. included FS. Various groups received either 0 (n=8), 0.5 (n=12), 1.0 (n=13), or 4.0 (n=15) ma., rms at 60 Hz. of FS. The subjects were removed to their homecages within 6 seconds of FS offset.

On Day 6 the insert was removed and the rats were allowed to find the tube and lick. Immediately after the 100th lick the CS was presented and the time to complete 10 additional licks was recorded. Any rat that took longer than 300 seconds to complete the 10 licks was removed to its homecage and given a score of 300 seconds. This criterion was maintained in the remaining experiments.

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Insert Fig. 1 about here.

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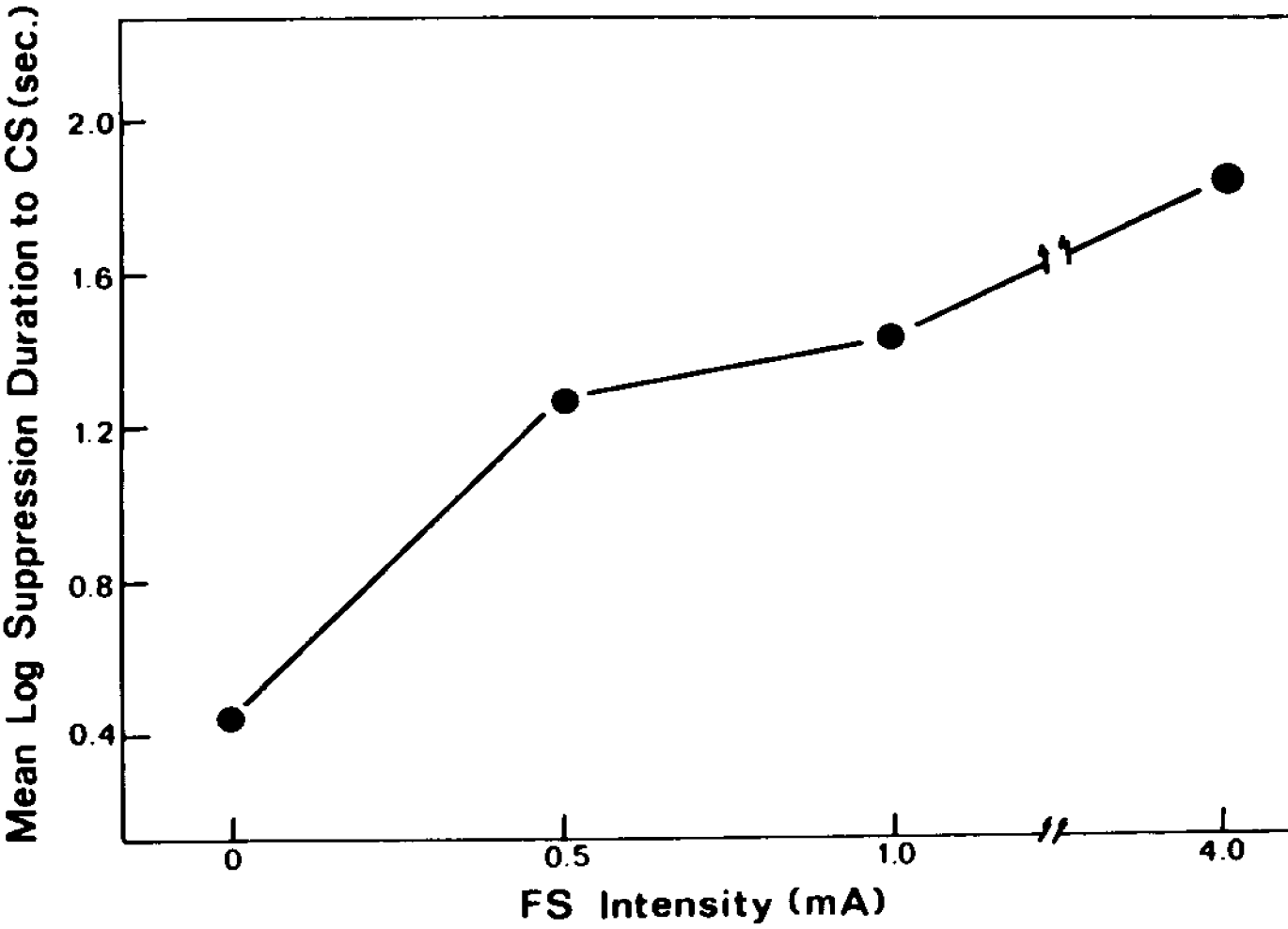
Results and Discussion

All latencies from lick 100-110 were converted to logs to permit parametric analysis. Use of this transformation was maintained throughout all five experiments.

Experiment 1 demonstrated that 4 ma. of FS paired with white noise produced substantial fear as measured by a skeletal index of lick suppression (Fig. 1). An analysis of test trial latencies from lick 100-110 yielded a significant treatments effect ($F=5.66$, $df=3/44$, $p<.01$).


Mean Log Suppression Duration to CS (sec.)
2.0
1.6
1.2
0.8
0.4
0
0.5
1.0
4.0
FS Intensity (mA)

A two-tailed *t* test between the groups receiving 0 and 4 ma. of FS ($p<.001$) demonstrated the efficacy of training with 4 ma. of FS.

Experiment 2

This experiment attempted to determine the minimum ECS current that would yield amnesia for the CS-US pairing using a skeletal index of amnesia.

Method

Subjects. Fifty-eight rats similar to those described in the preceding experiment were used. The food deprivation schedule prior to Day 1 of the experiment (described in Experiment 1) served to maintain the rats at a low weight in order to reduce the frequency of ECS-produced paraplegia. Maintenance schedules were identical to those described earlier.

Apparatus. In addition to the apparatus described in the preceding experiment a Lafayette 82403 constant current shock source was used to deliver ECS through modified alligator clips attached to the pinnae of the subjects. ECS delivery wires were suspended from an overhead pulley and counterweighted to minimize inhibiting effects of the clips and wires upon the movement of the animal. Twisting of the ECS delivery wires by rotational movement was minimized by a small lightweight commutator.

Procedure. On Days 1-4 each animal was placed in the chamber and allowed to emit 200 licks before being returned to its home cage. On Day 5 the insert was placed inside the chamber whereupon the animals were fitted with earclips and placed inside the apparatus. After 2 minutes of habituation, various groups received the CS, FS, and either 0 (n=12), 25 (n=10), 35 (n=12), 45 (n=12), or 55 (n=12) ma. ECS (rms, 60 Hz.). The subjects were randomly assigned to one of these five groups. ECS was delivered for 0.3 seconds immediately upon FS offset. On Day 6 the insert was removed in order to test the rats as in the preceding

experiment. The animals were tested without earclips.

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Insert Fig. 2 about here.

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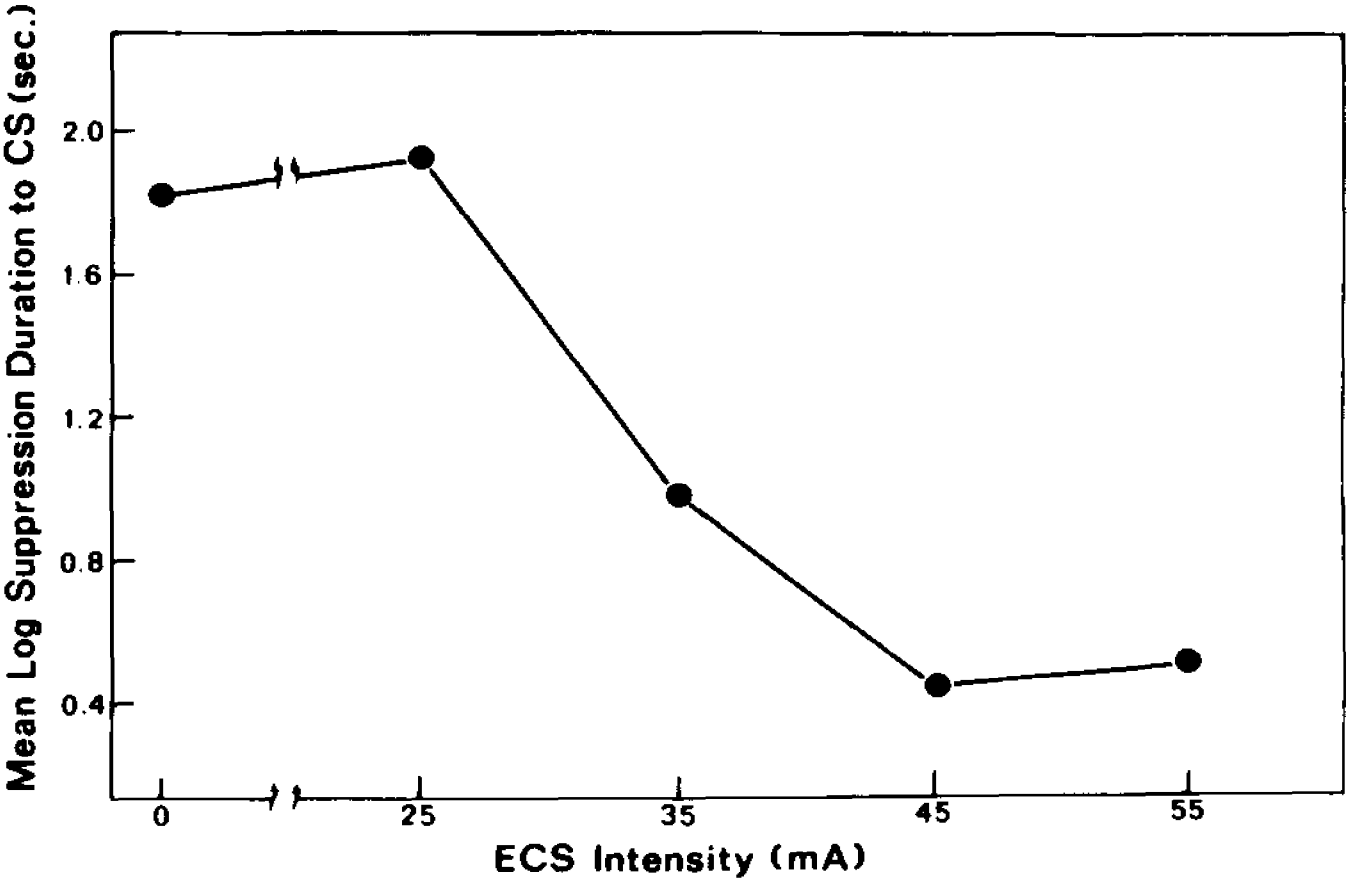
Results and Discussion

Both 45 and 55 ma. of ECS produce significant amnesia (Fig. 2). Thirty-five ma. of ECS yielded intermediate amnesia, while 25 ma. produced a convulsion without impairing memory. An ANOVA on latencies from lick 100-110 for the five ECS intensity groups proved significant (F=12.86, df=4/53, $p<.001$). A two-tailed t test between the 45 ma. and 0 ma. groups was highly significant ($p<.00001$).


Mean Log Suppression Duration to CS (sec.)
2.0
1.6
1.2
0.8
0.4
0
25
35
45
55
ECS Intensity (mA)

Experiment 3

If there are uniquely different processes underlying the consolidation and retrieval of skeletal versus autonomic responses, expression of the latter might not be impaired by any ECS intensity. Alternatively, if the processes underlying these responses are qualitatively similar, these responses might differ only with respect to their threshold of vulnerability to ECS. Consequently, it might be expected that the lowest ECS intensity that eliminates skeletal responses should leave autonomic responses intact. However, at high ECS intensities both skeletal and autonomic responses should be impaired.

Those studies that obtained amnesia for bradycardia (Caul & Barrett, 1972; Devietti & Kallioinen, 1972) used 100 and 92 ma. of ECS respectively, while the studies which claimed to have observed sparing of autonomic responses from disruption by ECS (Yaginuma & Iwahara, 1971; Mendoza & Adams, 1969; Hine & Paolino, 1970) used 28, 35, and 100 ma. of ECS respectively. It seems likely that skeletal and autonomic responses may differ in their their thresholds to disruption by ECS. That Hine and Paolino (1970) did not observe amnesia with their heart-rate measure despite the high ECS current used might be attributed to their recording technique which was discussed previously. The present study compares the effects upon amnesia of two ECS intensities (45 and 100 ma.) for both skeletal and autonomic responses.

A Pavlovian conditioning task is used in this experiment because of the previously cited advantages inherent in a lick suppression paradigm. Furthermore, a classical conditioning task was used in the two studies that found results anomalous to the trend of greater resistance to disruptability of autonomic responses. Certainly the

robustness of the sparing phenomenon would be better supported if the failures to observe this phenomenon within the classical conditioning paradigm could be explained.

The major differences between this study and earlier works is a) heart rate and skeletal responses are recorded simultaneously in the same animal, b) two autonomic responses are examined to determine whether they respond similarly to ECS, c) two ECS intensities are examined, and d) controls for nonassociative effects of ECS on skeletal and autonomic responses are incorporated in the design.

Method

Subjects and Apparatus. Ninety-five rats similar to those described earlier weighing 170-215 gm. were obtained. This experiment was performed in two replications. Twenty-four hours prior to Day 1 of the experiment, two No. 1 stainless steel safety pins were inserted subcutaneously on either side of each subject's thorax. All apparatus were identical to those already described. The sound attenuating chamber also served as a Faraday cage.

Heart rate recording and analysis. The EKG was amplified by a device that minimized movement and cable motion artifacts (Brakel, Babb, Manhnke, & Verzeano, 1971). Following amplification of the R-wave, the signal was filtered and then detected by a voltage comparator (Springer & Miller, 1973) (see Appendix). The output of the voltage comparator triggered a Beckman 9857B cardiotachometer. Connection between the subject and preamplifier was made with a Microdot Mini-Noise (# 202-3808) cable which terminated at one end with mini alligator clips. A Scientific Prototype MC4 mercury commutator prevented twisting of the recording cable. A harnesss modelled after the Lehigh Valley

rat saddle further reduced motion artifact by pressing the alligator clip-safety pin junction snugly against the thorax. The saddle restricted the animals' movement to some small degree.

Analysis of heart rate data consisted of sampling two interbeat intervals (IBIs) at the beginning of each of four successive 0.5 second epochs immediately prior to CS onset. The eight pre-CS IBIs were converted to bpm and averaged. Similarly, heart rate changes during the CS presentation were monitored by recording two successive IBIs at the end of each of 12 successive 0.5 second epochs immediately following CS onset. Each IBI was separately converted to bpm and the pairs were averaged. The pre-CS heart rate was then subtracted from each of 12 CS heart rate measures, 140.0 added to all numbers to convert negative values to positive ones, and the square root taken. A square root transformation served to increase the normalcy of the skewed heart rate data distributions.

Procedure. On Days 1-4 of the experiment each animal was placed in the operant chamber whereupon it was permitted to find the water tube and to emit 200 licks. On Days 2 and 4 each rat wore a harness in the chamber. In addition, on Day 4 each subject was fitted with a recording cable. On Day 3 the animals wore earclips while in the chamber. Thus Days 2 through 4 cumulatively allowed the animals to habituate to the harness, recording cable, and earclips. Training on Day 5 began by inserting the black, wooden insert into the chamber and subsequently placing the rats in the chamber for two minutes with earclips affixed. Following the two minutes of habituation, various treatments were administered. One set of animals (n=18) received the CS, US, and pseudo-ECS (CS US PECS) in order to demonstrate acquisition of fear. These

animals were earclips but did not receive ECS and were removed to the home cage within six seconds following FS offset. Two amnesic groups received the CS, FS and ECS at 45 ma. (CS US ECS (45 ma.)) (n=15) or 100 ma. (CS US ECS (100 ma.)) (n=17). A fourth group, which was to provide a stringent control in the assessment of startle responses, was not presented with the CS during the 18 second interval prior to the US but was given the US and ECS (45 ma.) (PCS US ECS (45 ma.)) (n=17). The last group of rats was intended to control for nonassociative changes in responses that might result from the interaction of white noise, FS and ECS. These animals were placed in the chamber for 90 seconds whereupon the CS was presented for 18 seconds. The subjects were then removed to the home cage and returned to the chamber one hour later. After an additional delay of 30 seconds within the chamber, they were given FS and ECS (45 ma.) (CS DELAY US ECS (45 ma.)) (n=15).

On Day 6 each animal was returned to the chamber (less insert) with harness and recording cable attached. Upon the subject's making lick 100, the CS was presented and time to reach lick 110 was recorded. The CS remained on from lick 100 until the time the animal was removed. Heart rate was recorded from the time the animal was placed inside the chamber, and the recording was continued for approximately 60 seconds following lick 110. Number of boluses deposited in the box by each subject was also noted. The data of 13 rats were not used due to excessive artifact in the EKG.

Results

When latencies to make lick 100-110 were examined the CS US PECS group differed significantly from all remaining groups indicating that significant memory was not evident in these groups and that amnesia as

indexed by the skeletal response had been achieved with as little as 45 ma. of ECS. The CS US ECS (45 ma.) group differed from both the PCS US ECS (45 ma.) and CS DELAY US ECS (45 ma.) groups. On the other hand, the CS US ECS (100 ma.) group did not differ from both the PCS US ECS (45 ma.) and CS DELAY US ECS (45 ma.) groups. Analysis of the heart rate data found that the CS US PECS and CS US ECS (45 ma.) groups did not differ (unlike the similar comparison for the skeletal measure) while the former group differed from the remaining three groups. As with the skeletal measure, the CS US ECS (45 ma.) group also differed from the PCS US ECS (45 ma.) and CS DELAY US ECS (45 ma.) groups. Similarly the CS US ECS (100 ma.) differed from the PCS US ECS (45 ma.) and CS DELAY US ECS (45 ma.) groups. Analysis of the defecation data found intergroup relationships that were identical to those found for the heart rate data except that the CS US ECS (100 ma.) group did not significantly differ from the PCS US ECS (45 ma.) and CS DELAY US ECS (45 ma.) groups.

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Insert Fig. 3 and Table 1 about here.

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An unequal n analysis of variance on latencies to resume drinking found the replication (F=0.05, df=1/72, p >.50) and treatment x replication factors (F=0.36, df=4/72, p >.50) to be nonsignificant. The treatment factor proved significant (F=17.34, df=4/72, p<.001) and two-tailed t tests were performed to determine the source of the effect (Table 1). Group means are graphically presented in Fig. 3.

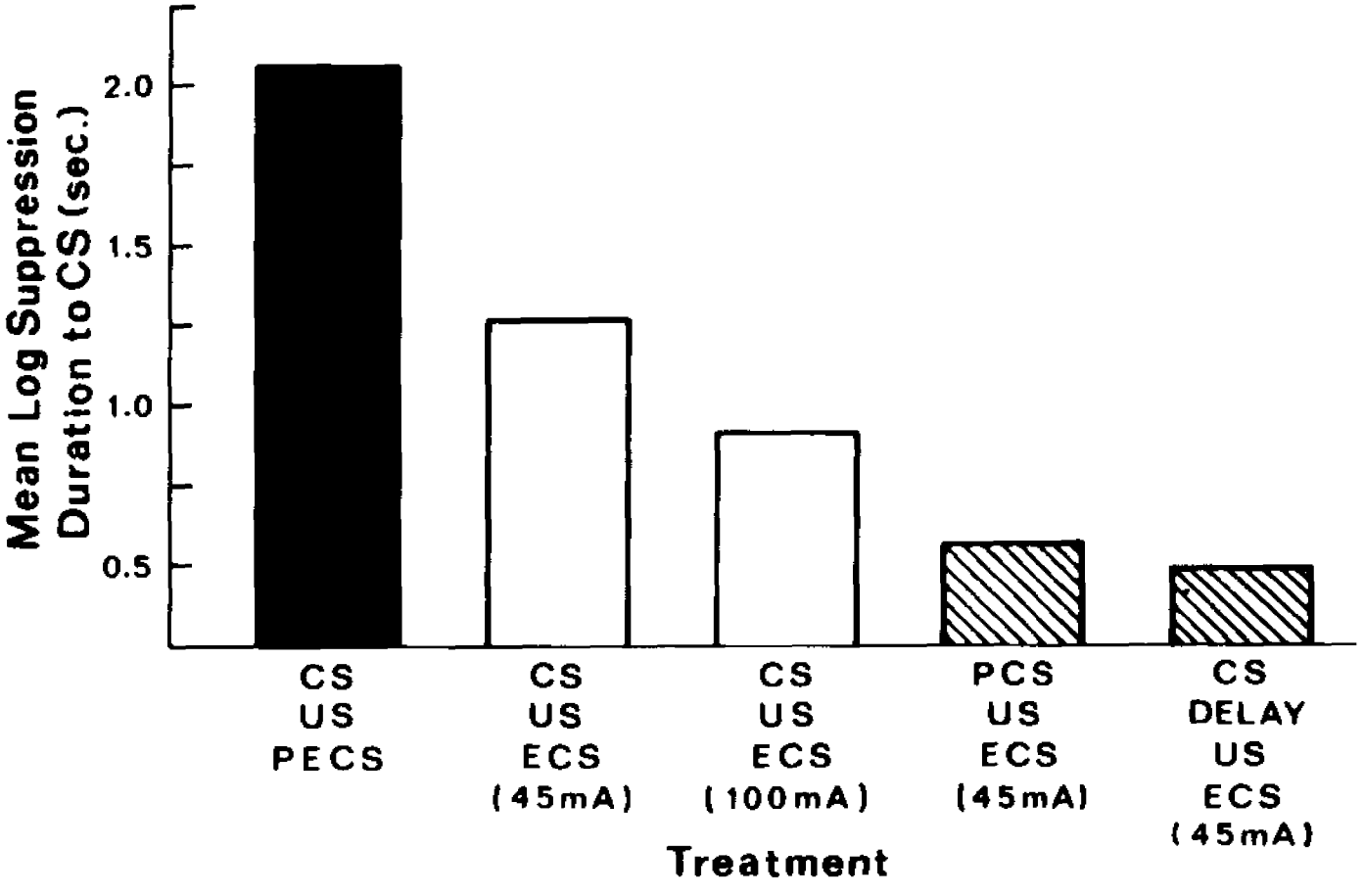
Mean Log Suppression Duration to CS (sec.)
2.0
1.5
1.0
0.5
CS US PECS
CS US ECS (45mA)
CS US ECS (100mA)
PCS US ECS (45mA)
CS DELAY US ECS (45mA)
Treatment

TABLE 1

TREATMENTS AND SIGNIFICANCES OF MEAN LOG SUPPRESSION DURATION TO CS FOR EXPERIMENT 3

TREATMENT	p VALUES OF DIFFERENCES BETWEEN GROUPS			
	CS US ECS (45 ma.)	CS US ECS (100 ma.)	PCS US ECS (45 ma.)	CS DELAY US ECS (45 ma.)
CS US PECS	<.01	<.000001	<.000001	<.000001
CS US ECS (45 ma.)		>.10	<.002	<.002
CS US ECS (100 ma.)	>.10		>.05	>.05
PCS US ECS (45 ma.)	<.002	>.05		>.50
CS DELAY US ECS (45 ma.)	<.002	>.05	>.50	

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Insert Fig. 4 and Table 2 about here.

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As Fig. 4 indicates, the startle response in PCS US ECS (45 ma.) has declined considerably by interval 6. Consequently, the analysis of the heart rate data was performed from intervals 6-12. It can be argued that the heart rate response over intervals 1-5 for the CS US ECS (45 ma.) and CS US ECS (100 ma.) groups is not a startle response and actually reflects memory. However, the actual effect of ECS on the CS-US pairing is not patently clear. ECS might be impairing the CS US bond, memory of both the CS and US, memory of the CS only, or memory of the US only. If, as is likely, ECS impairs memory of the CS presented during training, CS US ECS (45 and 100 ma.) subjects' reaction to the CS on the test trial is probably a function of a startle response to the effectively novel CS and not due to memory of the training trial. Therefore, in order to most conservatively analyze the heart rate data of the present, as well as, later experiments, the data of intervals 1-5 were not analyzed. The analysis was begun at interval 6 as the startle response in the PCS US ECS (45 ma.) group had approached baseline by this time. Furthermore, data after interval 12 were not analyzed because there is a tendency for the groups to begin converging with respect to the heart rate measure.

An unequal n, repeated measures ANOVA on the heart rate data found a significant treatments ($\underline{F}$=7.74, $\underline{df}$=4/72, $\underline{p}$<.0005) and intervals effect ($\underline{F}$=7.66, $\underline{df}$=6/432, $\underline{p}$<.0005). The replication factor ($\underline{F}$=0.26, $\underline{df}$=1/72, $\underline{p}$ > .50), replication x treatment ($\underline{F}$=0.81, $\underline{df}$=4/72, $\underline{p}$ > .50), treatment x interval ($\underline{F}$=0.99, $\underline{df}$=24/432, $\underline{p}$ >.25), and three-way interaction ($\underline{F}$=1.19, $\underline{df}$=24/432, $\underline{p}$ > .25) did not achieve significance. Two-tailed $\underline{t}$ tests

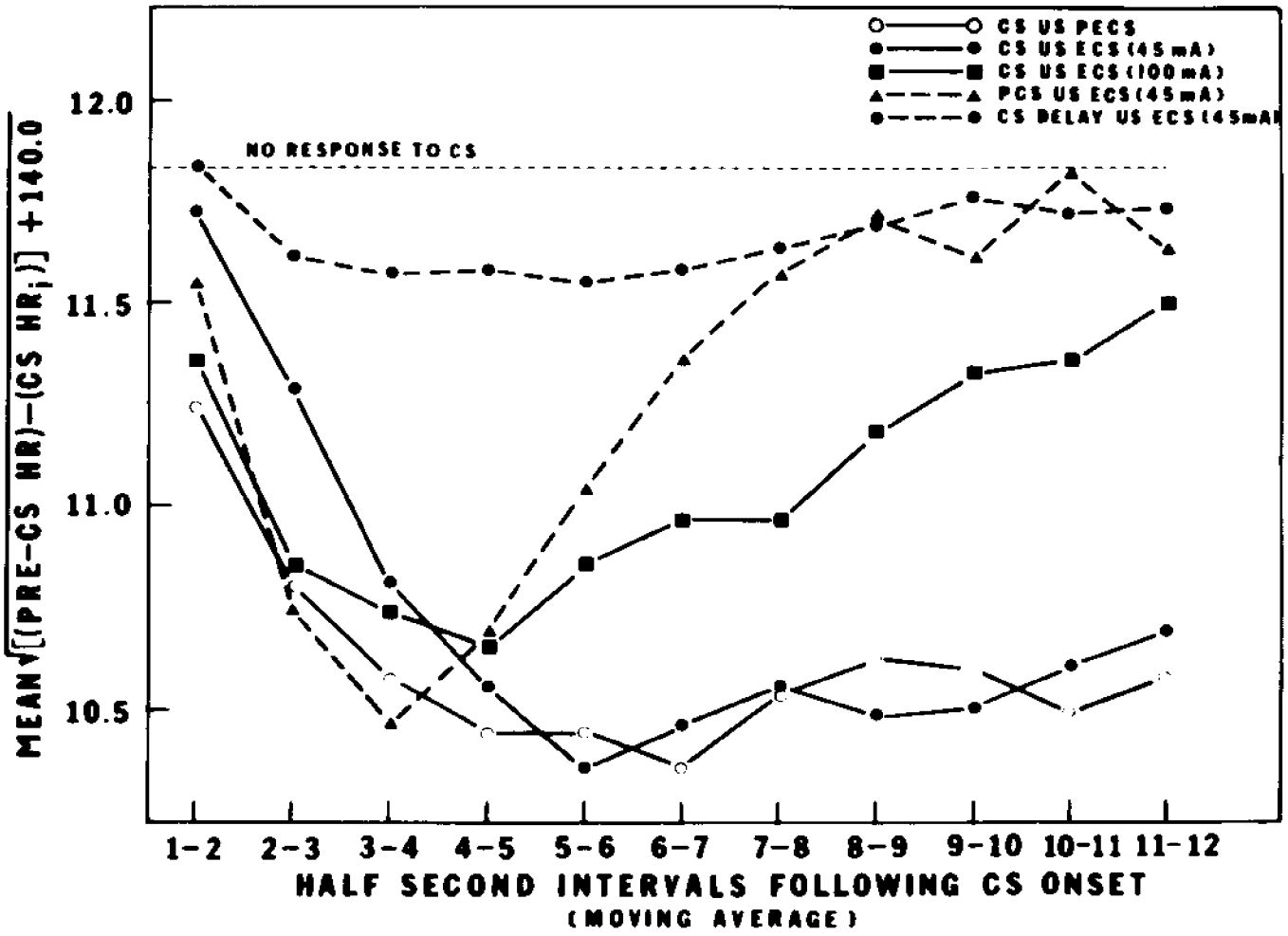
CS US PECS
CS US ECS (45 mA)
CS US ECS (100 mA)
PCS US ECS (45 mA)
CS DELAY US ECS (45mA)
NO RESPONSE TO CS
12.0
11.5
11.0
10.5
MEAN√[(PRE-CS HR)−(CS HR_i)] +140.0
1-2
2-3
3-4
4-5
5-6
6-7
7-8
8-9
9-10
10-11
11-12
HALF SECOND INTERVALS FOLLOWING CS ONSET
(MOVING AVERAGE)

TABLE 2

TREATMENTS, MEANS, AND SIGNIFICANCES OF HEART RATE CHANGES FOR EXPERIMENT 3

TREATMENTS	MEANS[a]	p VALUES OF DIFFERENCES BETWEEN GROUPS			
		CS US ECS (45 ma.)	CS US ECS (100 ma.)	PCS US ECS (45 ma.)	CS DELAY US ECS (45 ma.)
CS US PECS	10.54	>.50	<.02	<.0001	<.000001
CS US ECS (45 ma.)	10.58		>.05	<.001	<.005
CS US ECS (100 ma.)	11.23	>.05		<.02	<.02
PCS US ECS (45 ma.)	11.67	<.001	<.02		>.50
CS DELAY US ECS (45 ma.)	11.67	<.005	<.02	>.50	

[a]Obtained by averaging each of 7 means of intervals 6-12.

on mean heart rate across intervals 6-12 for various groups were performed (Table 2).

- -

Insert Fig. 5 and Table 3 about here.

- -

Defecation scores (Fig. 5) were analyzed by an ANOVA. The replication (F=0.98, df=1/72, $p > .50$) and treatment x replication factors (F=1.88, df=4/72, $p > .10$) were not significant while the treatment factor (F=6.81, df=4/72, $p < .001$) achieved significance. Comparisons were made between groups to determine the source of the treatment effect using two-tailed t tests (Table 3).

Discussion

The long latencies from lick 100-110 of the CS US PECS group as compared to all remaining groups indicates that as little as 45 ma. of ECS delivered following the CS-US pairing effectively induces experimental amnesia for skeletal responses. Amnesia in the CS US ECS (45 ma.) group is substantial when compared against the CS US PECS group. However, the CS US ECS (45 ma.) group differed from the PCS US ECS (45 ma.) and CS DELAY US ECS (45 ma.) groups, thus suggesting that the amnesia was not as intense as that of group CS US ECS (100 ma.). Group CS US ECS (100 ma.) differed from group CS US PECS and did not differ from the PCS US ECS(45 ma.) and CS DELAY US ECS (45 ma.) groups. Therefore, it is apparent that 45 ma. of ECS yields significant but not maximal amnesia using the skeletal index.

When defecation scores were analyzed, it appeared that 45 ma. of ECS did not induce amnesia for this autonomic response since groups CS US PECS and CS US ECS (45 ma.) did not differ from one another.

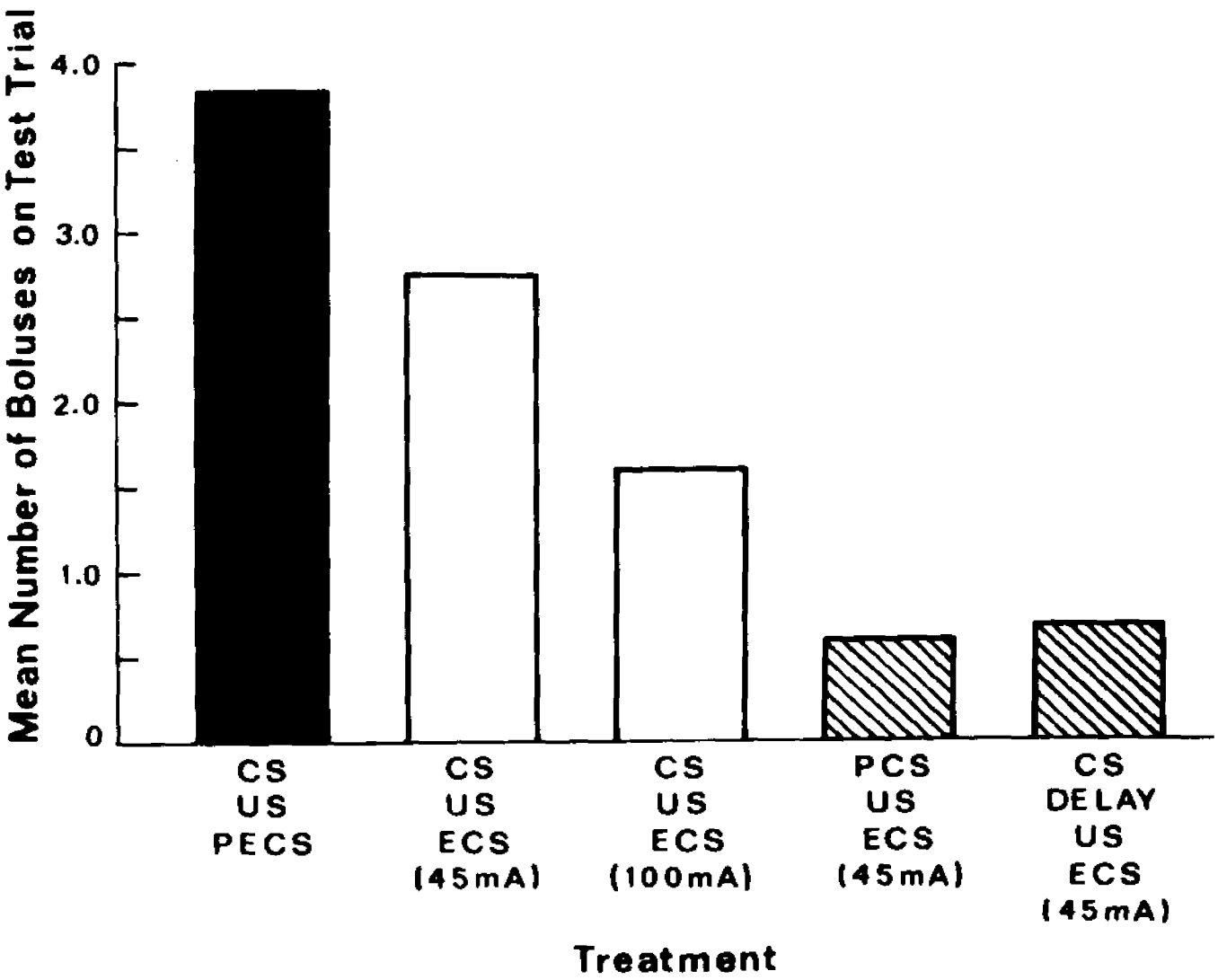
Mean Number of Boluses on Test Trial
4.0
3.0
2.0
1.0
0
CS US PECS
CS US ECS (45mA)
CS US ECS (100mA)
PCS US ECS (45mA)
CS DELAY US ECS (45mA)
Treatment

TABLE 3

TREATMENT AND SIGNIFICANCES OF MEAN BOLUSES ON TEST TRIAL FOR EXPERIMENT 3

TREATMENT	p VALUES OF DIFFERENCES BETWEEN GROUPS			
	CS US ECS (45 ma.)	CS US ECS (100 ma.)	PCS US ECS (45 ma.)	CS DELAY US ECS (45 ma.)
CS US PECS	>.10	<.005	<.0001	<.0001
CS US ECS (45 ma.)		>.10	<.005	<.01
CS US ECS (100 ma.)	>.10		>.10	>.20
PCS US ECS (45 ma.)	<.005	>.10		>.50
CS DELAY US ECS (45 ma.)	<.01	>.20	>.50	

However, 100 ma. of ECS appeared to have produced amnesia when the defecation index of memory was examined since groups CS US PECS and CS US ECS (100 ma.) differed substantially. Moreover, any memory observed in group CS US ECS (45 ma.) was unlikely to be a function of nonassociative effects caused by the interaction of the CS, US, and ECS as group CS US ECS (45 ma.) differed from both control groups PCS US ECS (45 ma.) and CS DELAY US ECS (45 ma.).

One problem in interpreting the defecation data is that on the average subjects from different treatments spent dissimilar amounts of time in the operant chamber on the test day. For example, subjects in group CS US PECS remained in the chamber for a greater interval than those of group CS DELAY US ECS (45 ma.) since the former subjects suppressed drinking for a considerably longer period than those of the latter group. Although this is a potential confound, observations of defecation over time suggest that maximal defecation ensues with CS onset in all groups and does not continue indefinitely until the animal is removed. Moreover, the CS US ECS (45 ma.) group spent as much time in the test chamber as did the nonmemorial control groups but defecated significantly more than these groups, further indicating that the time factor was not critical.

The heart rate data argue that 45 ma. of ECS does not significantly affect a bradycardiac response to the CS; however, 100 ma. of ECS proved a more effective amnesic agent. Memory observed in the CS US ECS (45ma.) group using the heart rate measure was not a function of CS US ECS interactions resulting in nonassociative responses as this group differed from both groups PCS US ECS (45 ma.) and CS DELAY US ECS (45 ma.). These results parallel those described for the defecation data. As might be predicted, the EKG and defecation data are more similar to one another

than they are to the skeletal data.

The present experiment confirms the findings of Mendoza and Adams (1969) who claimed that 35 ma. of ECS does not lead to amnesia for bradycardia and those of Yaginuma and Iwahara (1971) who demonstrated that 28 ma. of ECS does not induce amnesia when a defecation measure of memory is used. Both failures to observe the sparing of bradycardia in rats using approximately 100 ma. of ECS (Caul & Barrett, 1972; Devietti & Kallioinen, 1972) have also been replicated. However, in the present experiment the amnesia assessed by bradycardia following 100 ma. ECS is apparently less robust than the amnesia assessed by skeletal indices in the same animals. As stated previously, the CS US ECS (100 ma.) group differed from the nonassociative controls for the heart rate but not the skeletal index. Minimally the data obtained with 45 ma. of ECS argue that autonomic responses do in fact have a higher threshold to disruption by ECS than skeletal responses.

The issue of whether ECS affects autonomic responses as it does skeletal responses has important implications with respect to whether different classes of information are processed in similar or in distinctly different fashions. If ECS has no effect on autonomic responses while impairing skeletal responses, it would be reasonable to assume that the mechanisms that are necessary for the ultimate manifestation of the memory might differ for disparate types of information. However, the present finding of disruptability of autonomic responses, albeit with higher current levels, suggests that similar processes underlie the memories responsible for autonomic and skeletal conditioned responding.

Experiment 4

Typically, immediate postrial administration of ECS results in amnesia for training events. ECS delayed by seconds or minutes following training has little effect on memory with temporal parameters being a function of training task, species, mode of ECS delivery, and ECS intensity. This invulnerability is indicative of information achieving a nondisruptable, stable format within seconds of training, i.e., the memory is sufficiently consolidated and prepared for retrieval to be manifest on the test trial (Miller & Springer, 1973).

Although RA gradients have been traced using skeletal indices of memory, similar gradients have not been recorded for autonomic response indices. Moreover, a comparison of the RA gradients for autonomic and skeletal response indices might be helpful in explaining why autonomically indexed memories have a higher threshold to disruption by ECS than skeletally indexed memories. Perhaps memory components responsible for autonomic responses achieve a stable form more rapidly than skeletal responses. For example, the results of Experiment 3 would be explained if, for 45 ma. ECS, autonomically indexed memories were found to be prepared for retrieval in less than the 0.5 second duration of the FS while skeletally indexed memories took longer than 0.5 seconds. An examination of the RA gradients should reveal any such differences in stabilization times. Alternatively, skeletally and autonomically indexed memories may stabilize at the same rate with some other factor determining their differential vulnerability to ECS. The present experiment will compare the RA gradients for lick suppression, heart rate, and defecation indices of memory using an ECS current intensity that with a sufficiently short delay produces amnesia observeable with each index

of memory.

Method

Subjects. One hundred sixty two rats similar to those in Experiment 1 were obtained and maintained as previously described. This experiment was performed in three replications.

Apparatus and Procedure. Only one piece of apparatus was altered from Experiment 3. ECS was increased to 170 ma. by using two Lafayette shock sources connected in parallel. This was done to intensify the amnesia for all measures so as to magnify differences in memory resulting from small changes in ECS delays.

Days 1-4 were identical to those of Experiment 3. On Day 5 all rats were randomly assigned to one of six groups. One set of animals received a CS-US pairing followed by pseudo ECS (CS US PECS) (n=25). The remaining five groups received a CS-US pairing followed by various delays of ECS; 60 seconds (CS US 60" ECS) (n=26), 15 seconds (CS US 15" ECS) (n=26), 8 seconds (CS US 8" ECS) (n=22), 3.5 seconds (CS US 3.5" ECS) (n=25), 0 seconds (CS US 0" ECS) (n=26). Testing procedures on Day 6 were identical to those in the preceding experiment. The data of 12 subjects were not analyzed due to excessive artifact in the EKG.

Results

Analysis of latencies from lick 100-110 indicated that the 60 second delay between FS offset and ECS onset was sufficiently long to permit skeletally indexed memory to reach a nondisruptable state as the CS US PECS and CS US 60" ECS groups did not differ from one another. The CS US PECS group differed from the CS US 0" ECS group indicating that immediate ECS had induced significant amnesia. Lack of amnesia is also evident in the CS US 60" ECS group as it differs significantly from

the CS US 0" ECS group. Inasmuch as the CS US 15" ECS group did differ from the CS US PECS group it appears that memory, indexed skeletally, requires between 15 and 60 seconds to reach a state that cannot be disrupted by ECS. For the heart rate measure the CS US PECS differs only from the CS US 0" ECS group. Thus memory indexed by bradycardia appears to reach a stable state between 0 and 3.5 seconds after FS offset. When defecation scores are analyzed, the CS US PECS group differs from the CS US 3.5" ECS and CS US 0" ECS groups, while all groups differ from the CS US 0" ECS group. Therefore, memory indexed by defecation appears to reach an invulnerable condition between 3.5 and 8 seconds after FS.

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Insert Fig. 6 and Table 4 about here.

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An ANOVA of latencies from lick 100-110 (Fig. 6) found a significant treatments effect (F=6.97, df=5/132, p<.001). Both the replications effect (F=1.53, df=2/132, p >.10) and treatment x replication interaction were not significant (F=0.75, df=10/132, p >.50). Two-tailed t tests were performed (Table 4) to determine which group differences were the source of the treatment effect.

- -

Insert Fig. 7 and Table 5 about here.

- -

A repeated measures ANOVA on the heart rate data (Fig. 7) revealed reliable treatment (F=3.52, df=5/132, p<.01) and interval effects (F=17.13, df=6/786, p<.001). Significance was not achieved in the replication factor (F=0.32, df=2/132, p >.50), replication x treatment (F=0.79, df=10/132, p >.50), replication x intervals (F=0.95, df=12/786,

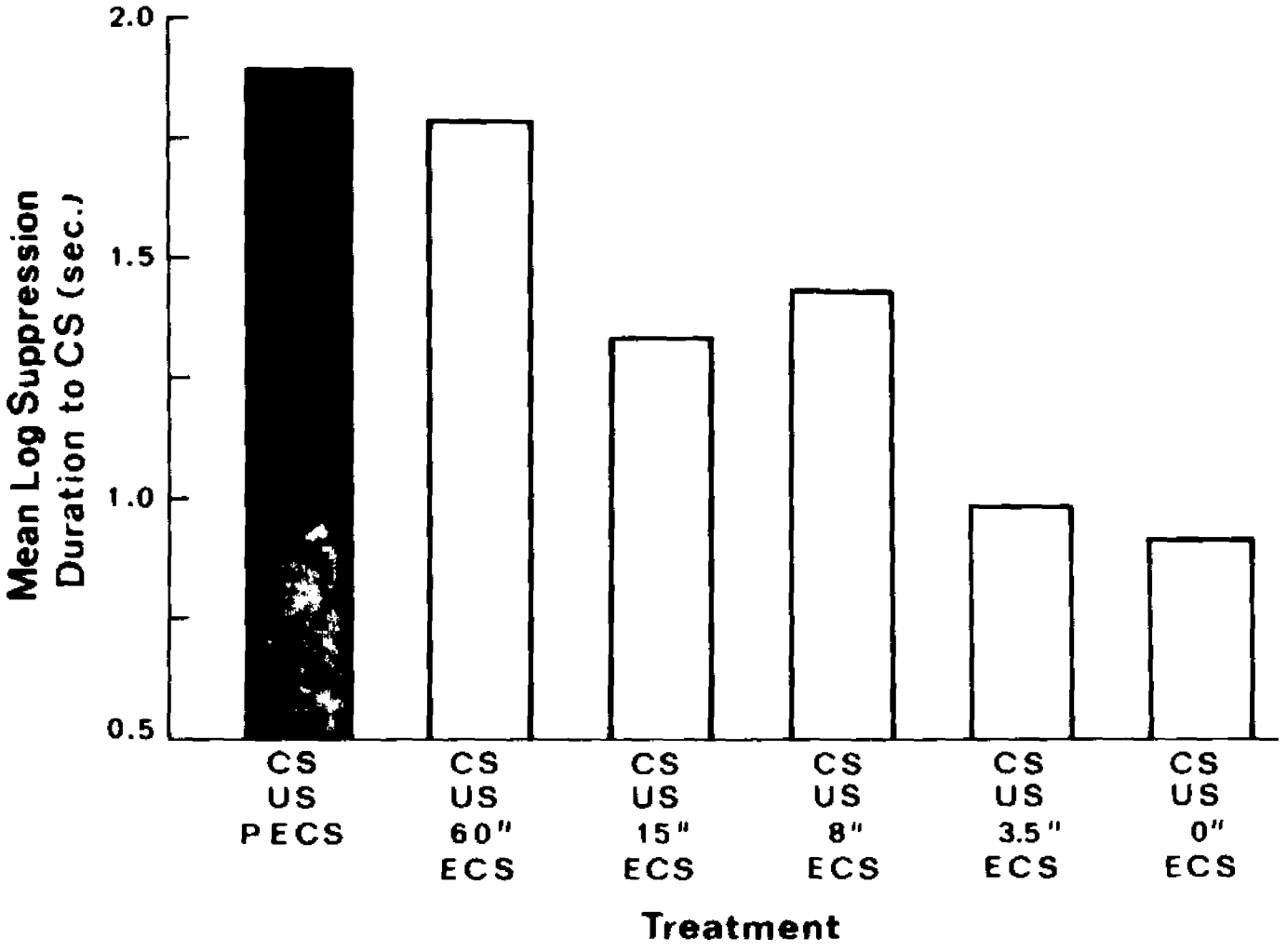
Mean Log Suppression
Duration to CS (sec.)
2.0
1.5
1.0
0.5
CS US PECS
CS US 60" ECS
CS US 15" ECS
CS US 8" ECS
CS US 3.5" ECS
CS US 0" ECS
Treatment

TABLE 4

TREATMENTS AND SIGNIFICANCES OF MEAN LOG SUPPRESSION DURATION TO CS FOR EXPERIMENT 4

TREATMENT	p VALUES OF DIFFERENCES BETWEEN GROUPS				
	CS US 60" ECS	CS US 15" ECS	CS US 8" ECS	CS US 3.5" ECS	CS US 0" ECS
CS US PECS	>.50	<.02	>.05	<.0001	<.0001
CS US 60" ECS		<.05	>.20	<.001	<.001
CS US 15" ECS	<.05		>.50	>.10	>.05
CS US 8" ECS	>.20	>.50		>.05	<.05
CS US 3.5" ECS	<.001	>.10	>.05		>.50
CS US 0" ECS	<.001	>.05	<.05	>.50	

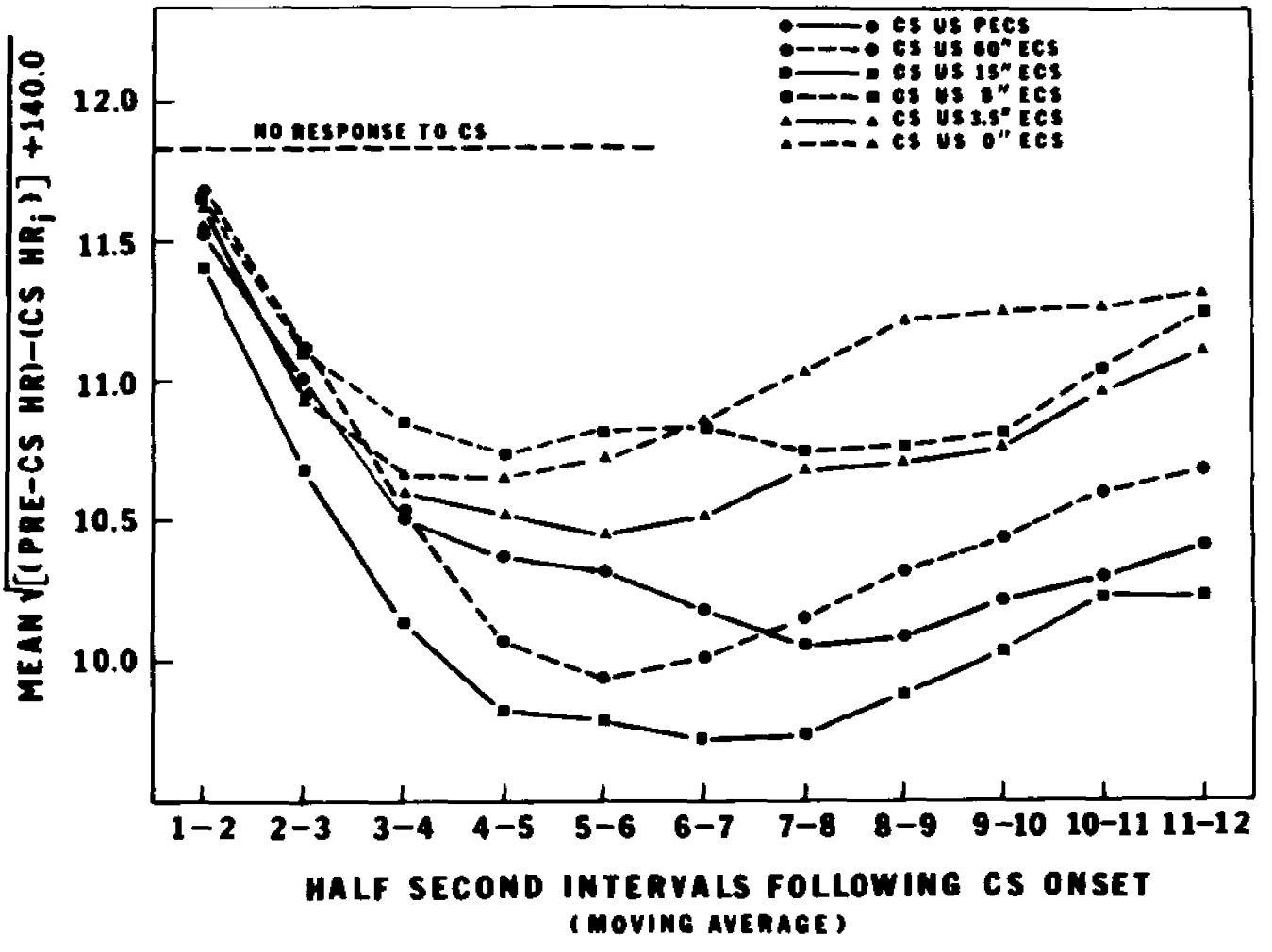
CS US PECS
CS US 60" ECS
CS US 15" ECS
CS US 8" ECS
CS US 3.5" ECS
CS US 0" ECS
NO RESPONSE TO CS
12.0
11.5
11.0
10.5
10.0
MEAN √[(PRE-CS HR)−(CS HRj)] +140.0
1–2
2–3
3–4
4–5
5–6
6–7
7–8
8–9
9–10
10–11
11–12
HALF SECOND INTERVALS FOLLOWING CS ONSET
(MOVING AVERAGE)

TABLE 5

TREATMENTS, MEANS, AND SIGNIFICANCES OF HEART RATE CHANGES FOR EXPERIMENT 4

TREATMENT	MEANS[a]	p VALUES OF DIFFERENCES BETWEEN GROUPS				
		CS US 60" ECS	CS US 15" ECS	CS US 8" ECS	CS US 3.5" ECS	CS US 0" ECS
CS US PECS	10.23	>.50	>.20	>.05	>.05	<.005
CS US 60" ECS	10.37		>.20	>.05	>.10	<.01
CS US 15" ECS	9.97	>.20		<.01	<.02	<.001
CS US 8" ECS	10.94	>.05	<.01		>.50	>.50
CS US 3.5" ECS	10.80	>.10	<.02	>.50		>.10
CS US 0" ECS	11.14	<.01	<.001	>.50	>.10	

[a]Obtained by averaging each of 7 means of intervals 6-12.

$p > .25$), treatment x interval ($F=1.01$, $df=30/786$, $p > .25$), nor three way interaction ($F=0.89$, $df=60/786$, $p > .50$). Two-tailed t tests were used to ascertain the source of the treatments effect (Table 5). The intervals effect was not of particular interest since it indicated a progressive diminution in bradycardia from interval 6 through 12 for all groups.

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Insert Fig. 8 and Table 6 about here.

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Analysis of the defecation data (Fig. 8) found significant treatment ($F=5.58$, $df=5/132$, $p<.001$) and replication factors ($F=8.24$, $df=2/132$, $p<.001$). However, since the replication x treatment factor was not significant ($F=0.95$, $df=10/132$, $p > .25$), the replication effect was assumed to stem from differences in animal shipments and was not perceived as a major concern. The source of the treatment effect was analyzed by two-tailed t tests (Table 6).

Discussion

Insofar as RA gradients assess the amount of time necessary for information to reach a stable nondisruptable format from which information may be readily retrieved, it appears that different components of a unitary memory achieve this state at different times.

The t tests on latencies from lick 100-110 indicate that between 15 and 60 seconds are necessary for the skeletal response to achieve a stable state that is not modifiable by ECS. On the other hand, a stable state is attained for the heart rate response between 0 and 3.5 seconds following FS offset and a similar state is achieved for the defecation response between 8 and 15 seconds.

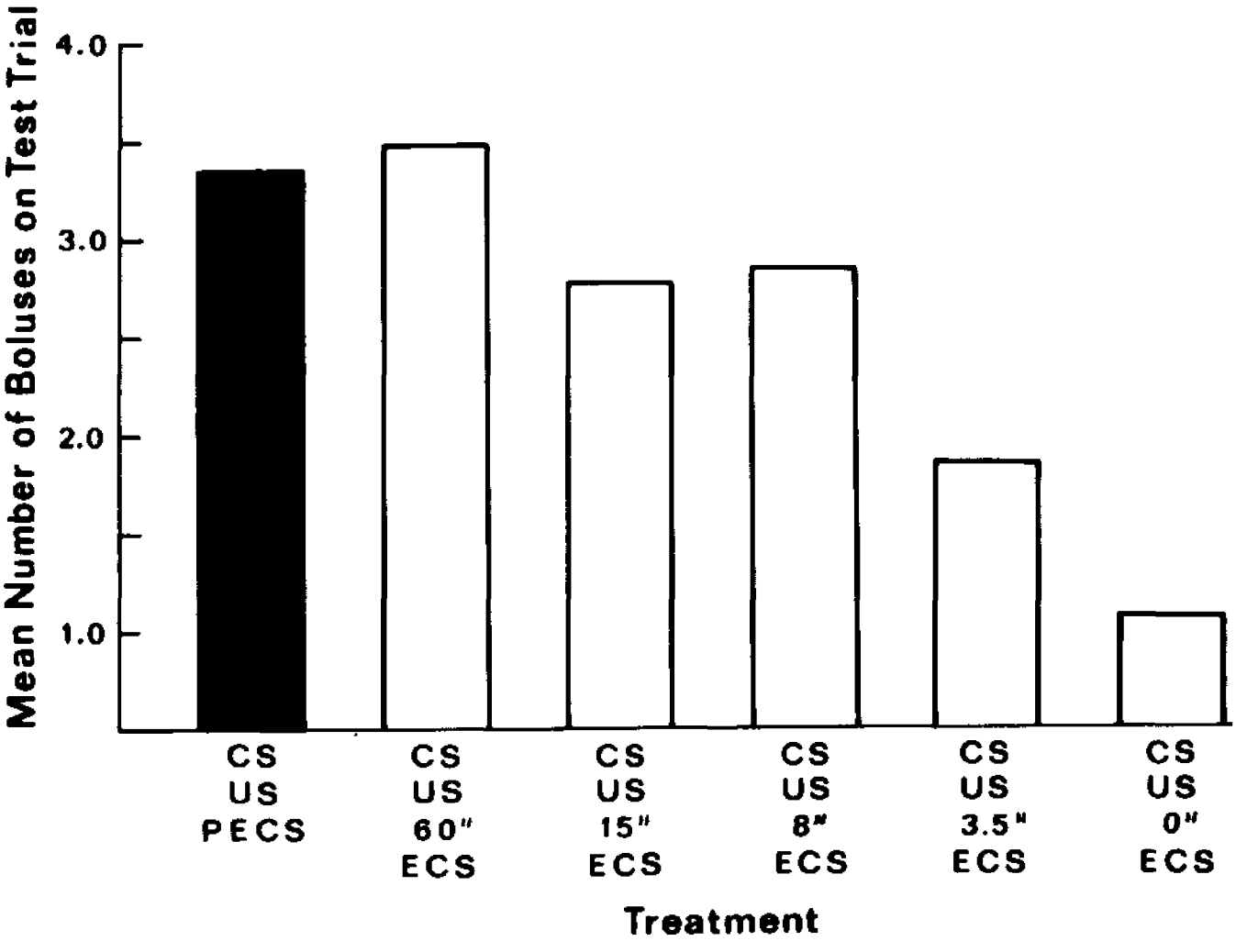
Mean Number of Boluses on Test Trial
4.0
3.0
2.0
1.0
CS US PECS
CS US 60" ECS
CS US 15" ECS
CS US 8" ECS
CS US 3.5" ECS
CS US 0" ECS
Treatment

TABLE 6

TREATMENTS AND SIGNIFICANCES OF MEAN BOLUSES ON TEST TRIAL FOR EXPERIMENT 4

TREATMENT	p VALUES OF DIFFERENCES BETWEEN GROUPS				
	CS US 60" ECS	CS US 15" ECS	CS US 8" ECS	CS US 3.5" ECS	CS US 0" ECS
CS US PECS	>.50	>.20	>.20	<.02	<.001
CS US 60" ECS		>.10	>.10	<.002	<.0001
CS US 15 " ECS	>.10		>.50	>.10	<.005
CS US 8" ECS	>.10	>.50		>.05	<.005
CS US 3.5 " ECS	<.002	>.10	>.05		>.10
CS US 0" ECS	<.0001	<.005	<.005	>.10	

Differences in stabilisation times could very well explain the difference in threshold of vulnerability to ECS for skeletal and autonomic responses. If the CS-autonomic CR component stabilizes sooner than the CS-skeletal CR component, the CS-autonomic association should be stronger than its skeletal counterpart at any given time following learning until both association strengths reach an asymptotic level. Thus, ECS induced disruption of CS-CR bonds should be achieved more readily for the CS-skeletal bond than for the CS-autonomic bond.

Experiment 5

Prior to further consideration of factors which may affect stabilization rates for memory, it is necessary to establish that any behavior seen with a delay in ECS is truly memorial in nature rather than a function of nonassociative factors.

Experiment 3 demonstrated that the problem of nonassociative factors does not arise when an ECS of 45 ma. is delivered immediately following the CS-US pairing. Nevertheless, 170 ma. is sufficiently different from 45 ma. of ECS to necessitate adding controls for nonassociative factors in Experiment 4. It must also be shown that ECS delivered 60 seconds after FS offset as opposed to immediately after FS offset does not create a nonassociative change in behavior which might be mistaken for memory. Furthermore, although 100 ma. of ECS yielded amnesia for bradycardia in Experiment 3, the magnitude of the amnesia was not maximal. Therefore, the present study also compares a group that receives 170 ma. of ECS immediately following FS offset with a group that does not have an opportunity to learn the CS-US contingency in order to assess the amount of amnesia produced.

Method

Subjects and Apparatus. Eighty eight rats similar in description to those used in preceding experiments were obtained. All apparatus were identical to those used in Experiment 3. The present study was performed in two replications.

Procedure. Days 1-4 were identical to those described in Experiment 3. On Day 5 the animals were randomly divided into five groups. One group received CS, FS and PECS (CS US PECS) (n=20); the second group received CS and FS followed by ECS 60 seconds after FS offset (CS US 60" ECS) (n=17); the third group received ECS immediately after the

CS-US pairing (CS US 0" ECS) (n=18); the fourth group received the CS followed one hour later by FS followed 60 seconds later by ECS (CS DELAY US 60" ECS) (n=14); and the fifth group received the CS followed one hour later by FS and immediate ECS (CS DELAY US 0" ECS) (n=15). All ECS values were 170 ma. The rats were tested on Day 6 as described in Experiment 3.

Replication two of the preceding experiment contained in addition to the previously described groups, a) CS US 15" ECS, b) CS US 8" ECS, c)CS US 3.5" ECS, d) CS US 0" ECS, e) CS US 60" ECS, and f) CS US PECS, animals that received treatments g) CS DELAY US 60" ECS and h) CS DELAY US 0" ECS. Groups run in the present experiment were identical to the last five groups in the replication described above. Therefore, the data from replication two of Experiment 3 served as one of the two replications of the current study. The data of eight rats were not used due to excessive artifact in the EKG.

Results

Memory observed in a group receiving a 60 second delay between FS offset and ECS was not a function of nonassociative factors as demonstrated by appropriate controls. One-hundred-seventy ma. ECS delivered immediately upon FS offset produces significant amnesia for lick suppression, heart rate and defecation as the amnesic groups did not differ from their nonmemorial controls (CS DELAY US 0" ECS). This was not the case in Experiment 3 where the group receiving CS US ECS (100 ma.) did significantly differ from a nonassociative control with respect to the heart rate measure. Thus, it appears that 170 ma. yields more amnesia for memory as indexed by heart rate than does 100 ma. of ECS, while amnesia according to defecation and skeletal indices has asymptoted with 100 ma. of ECS.

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Insert Fig. 9 and Table 7 about here.

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An ANOVA of latencies from lick 100-110 found a significant treatment (F=14.31, df, 4/74, p <.001) effect. The replication (F=3.51, df=1/74, p >.05) and the replication x treatment factors were not significant (F=1.50, df=4/74, p >.10). Two-tailed t tests were used to determine the source of the treatment effect (Table 7).

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Insert Fig. 10 and Table 8 about here.

- -

Heart rate scores (Fig. 10) were analyzed with a repeated measures ANOVA. Significance was observed for the treatment (F=5.27, df=4/74, p<.001) and interval factors (F=9.89, df=6/444, p<.001) as well as for the treatments x intervals interaction (F=1.78, df=24/444, p<.05). This interaction was probably a function of a fairly flat heart rate response of control groups CS DELAY US 60" ECS and CS DELAY US 0" ECS over intervals 6-12 and a progressive diminution in heart rate deceleration in the three experimental groups over the same intervals. Significance was not achieved by the replication factor (F=0.09, df=1/74, p >.50), the replication x intervals (F=1.82, df=6/444, p >.05), replication x treatment (F=0.14, df=4/74, p >.50) or three way interaction (F=0.90, df=24/444, p >.25). The source of the treatments effect was analyzed by using two-tailed t tests (Table 8).

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Insert Fig. 11 and Table 9 about here.

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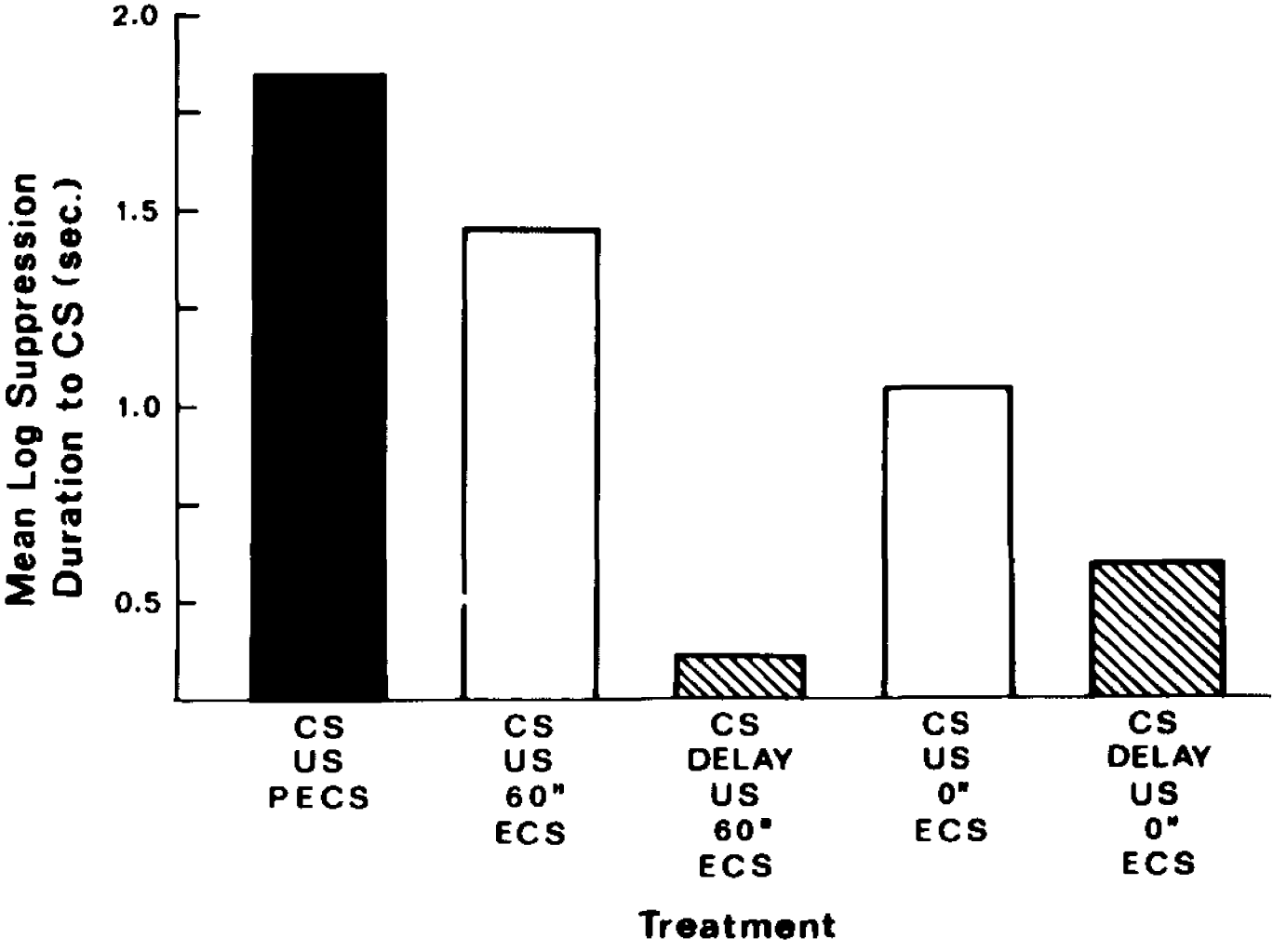

Mean Log Suppression Duration to CS (sec.)
2.0
1.5
1.0
0.5
CS US PECS
CS US 60" ECS
CS DELAY US 60" ECS
CS US 0" ECS
CS DELAY US 0" ECS
Treatment

TABLE 7

TREATMENTS AND SIGNIFICANCES OF MEAN LOG SUPPRESSION DURATION TO CS FOR EXPERIMENT 5

TREATMENT	p VALUES OF RELEVANT DIFFERENCES BETWEEN GROUPS			
	CS US 60" ECS	CS US 0" ECS	CS DELAY US 60" ECS	CS DELAY US 0" ECS
CS US PECS	>.05	<.001	<.000001	<.000001
CS US 60" ECS		>.05	<.0001	———
CS US 0" ECS	>.05		———	>.05
CS DELAY US 60" ECS	<.0001	———		>.20
CS DELAY US 0" ECS	———	>.05	>.20	

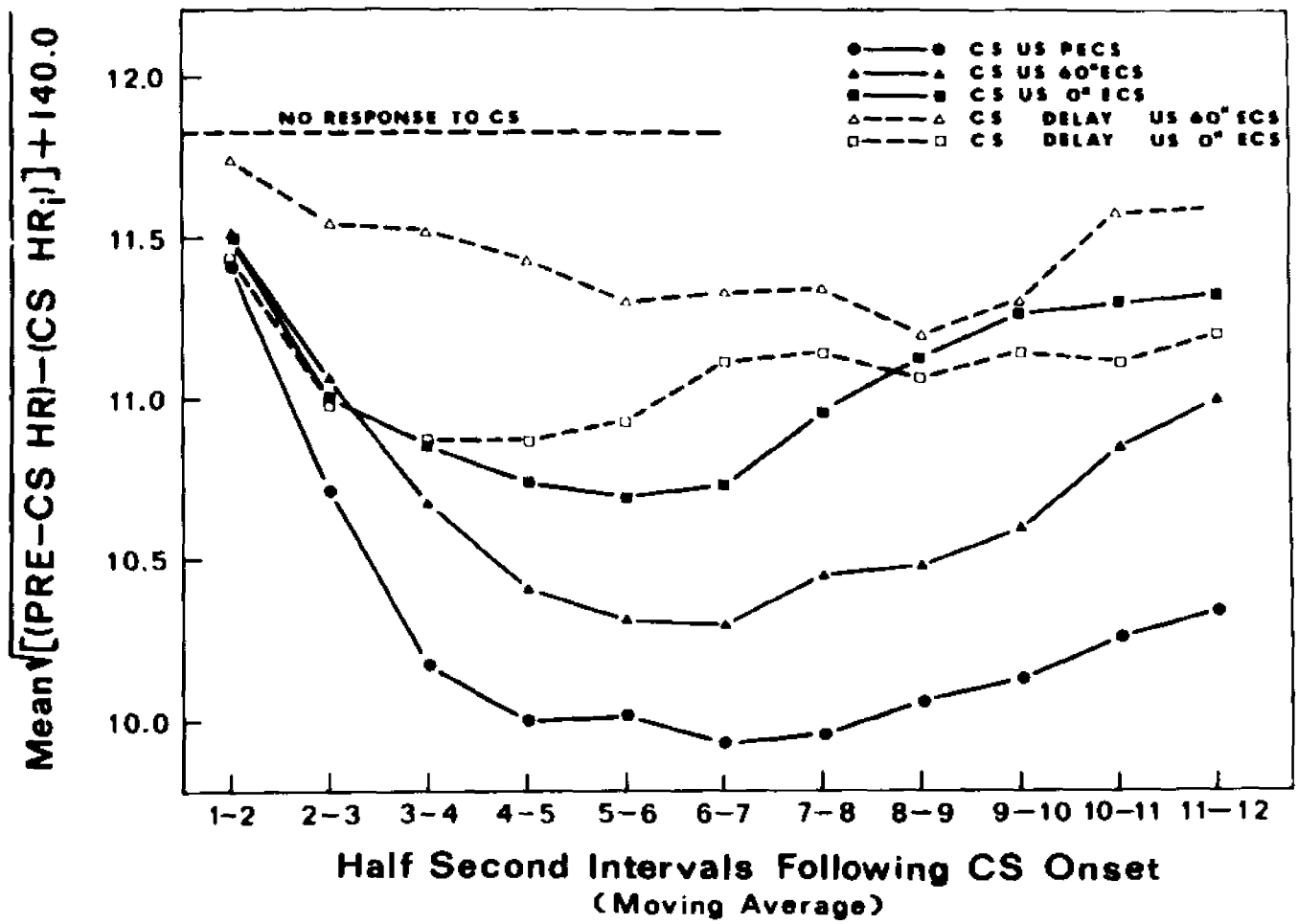

Mean√[(PRE−CS HR)−(CS HRi)]+140.0
12.0
11.5
11.0
10.5
10.0
NO RESPONSE TO CS
CS US PECS
CS US 60°ECS
CS US 0° ECS
CS DELAY US 60° ECS
CS DELAY US 0° ECS
1–2
2–3
3–4
4–5
5–6
6–7
7–8
8–9
9–10
10–11
11–12
Half Second Intervals Following CS Onset
(Moving Average)

TABLE 8

TREATMENTS, MEANS, AND SIGNIFICANCES OF HEART RATE CHANGES FOR EXPERIMENT 5

TREATMENT	MEANS[a]	p VALUES OF RELEVANT DIFFERENCES BETWEEN GROUPS			
		CS US 60" ECS	CS US 0" ECS	CS DELAY US 60" ECS	CS DELAY US 0" ECS
CS US PECS	10.12	>.05	<.005	<.001	<.005
CS US 60" ECS	10.64		<.05	<.01	———
CS US 0" ECS	11.11	<.05		———	>.50
CS DELAY US 60" ECS	11.40	<.01	———		>.20
CS DELAY US 0" ECS	11.13	———	>.50	>.20	>.20

[a]Obtained by averaging each of 7 means of intervals 6-12.

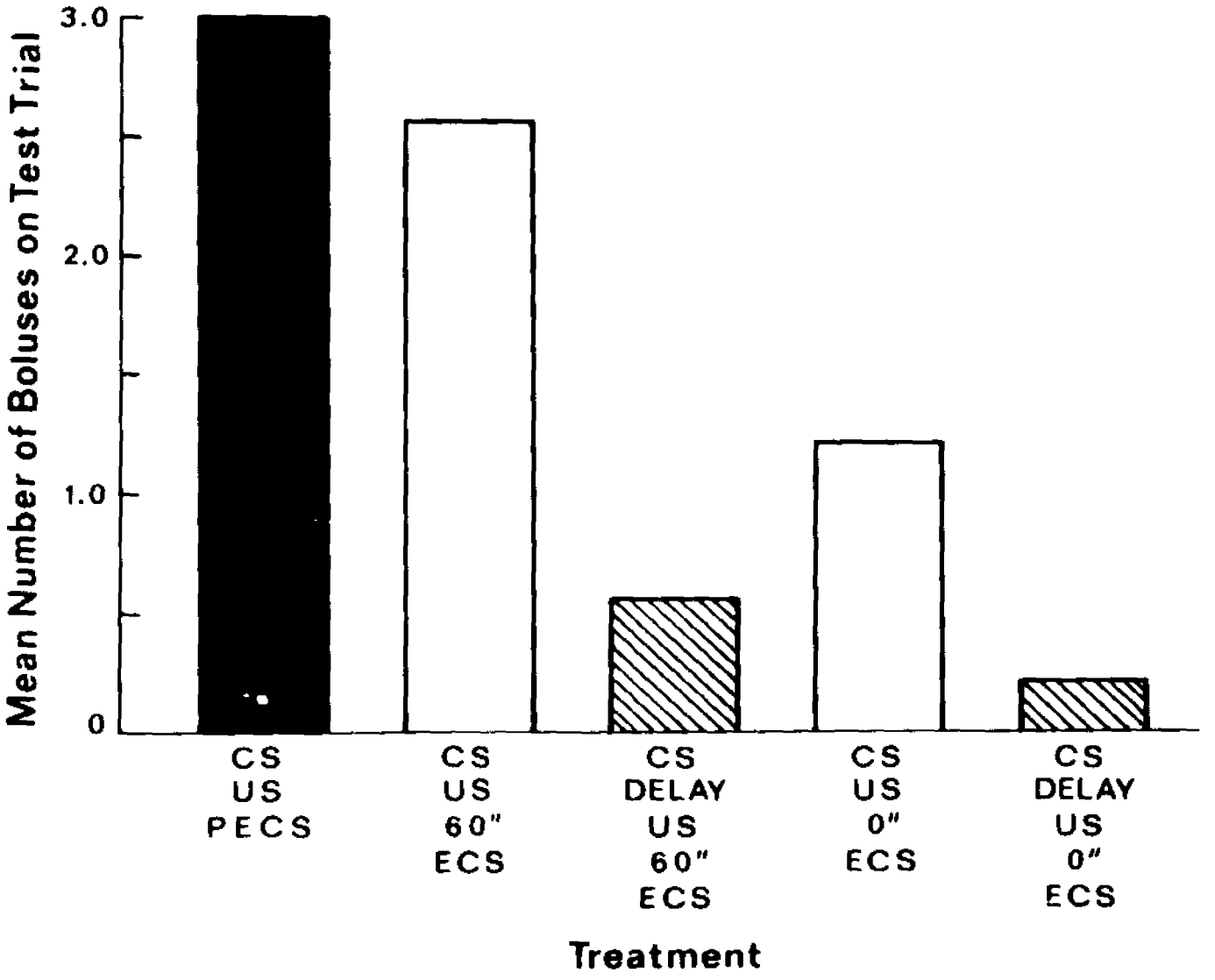

Mean Number of Boluses on Test Trial
3.0
2.0
1.0
0
CS US PECS
CS US 60" ECS
CS DELAY US 60" ECS
CS US 0" ECS
CS DELAY US 0" ECS
Treatment

TABLE 9

TREATMENTS AND SIGNIFICANCES OF MEAN BOLUSES ON TEST TRIAL FOR EXPERIMENT 5

TREATMENT	p VALUES OF RELEVANT DIFFERENCES BETWEEN GROUPS			
	CS US 60" ECS	CS US 0" ECS	CS DELAY US 60" ECS	CS DELAY US 0" ECS
CS US PECS	>.50	<.005	<.001	<.0001
CS US 60" ECS		<.02	<.002	———
CS US 0" ECS	<.02		———	>.05
CS DELAY US 60" ECS	<.002	———		>.20
CS DELAY US 0" ECS	———	>.05	>.20	

An ANOVA of the defecation data (Fig. 11) found a significant treatment (F=8.46, df=4/74, p<.001) and replications effect (F=10.87, df=1/74, p<.005). However, the treatments x replication interaction was not significant (F=1.09, df=4/74, p > .25) and therefore the significant replication factor was not of major concern. Two-tailed t tests (Table 9) were used to determine the source of the treatment effect.

Discussion

Inasmuch as groups CS US 60" ECS and CS DELAY US 60" ECS differed on each of the three memory indices it is apparent that the behavior of the CS US 60" ECS group reflects underlying memory rather than being a function of nonassociative factors. The CS US 0" ECS group was consistent in its performance across all indices as it differed from the CS US PECS group but not from the CS DELAY US 0" ECS group at each index. Consequently, it appears that 170 ma. of ECS produces more amnesia than 100 ma. of ECS. Taken together, these data indicate that the RA gradients obtained in Experiment 4 do in fact represent gradients of memory rather than non-associative factors.

General Discussion

The present studies have resolved the discrepancies in the literature concerning the differential susceptibility of autonomic and skeletal responses to ECS by demonstrating that autonomic responses have a higher threshold to disruption than skeletal responses. Thus, it seems reasonable that the processes underlying the establishment of a retrievable autonomic or skeletal CR appear to be qualitatively similar in nature to the extent that these processes interact with ECS. However, the question of why these responses differ in their thresholds to disruption remains to be answered.

Experiment 4 argues that the various components of the overall response require different amounts of time to reach a stable, non-disruptable state of preparedness for retrieval. The skeletal component required the longest time to reach a stable state (between 15-60 seconds), followed by the defecation (between 3.5-8 seconds) and heart rate components (between 0-3.5 seconds). The unique stabilization times that are required by these various expressions of memory are consistent with the existence of a gradient of ECS current intensities necessary to yield asymptotic levels of amnesia for different response types. Across Experiments 3-5, it appears that 45 ma. yields asymptotic amnesia for the skeletal response while 100 and 170 ma. of ECS are required to obtain asymptotic amnesia for the defecation and heart rate responses respectively.

One possible explanation of this gradient would be to assume that each of these CRs differs along a continuum of neural complexity. Complexity may simply be defined as the number of synapses or neurons that mediate the behavior. As the complexity of the CR increases, a greater number

of synapses would be involved in the association between the CS and CR at the time of learning. If the number of synapses involved in forming the CS-US bond increases with complexity of the CR, then it is likely that the amount of time required to form a nondisruptable CS-US bond is also a function of the complexity of the CR. This is not to say that at the time of acquisition the subject is acquiring a particular CR which is identical in form for each task used to measure the CR. Instead, at the time of acquisition the subject is probably learning to make a particular CR in the presence of a relevant CS, but the ultimately observed CRs are dependent upon an interaction of the acquired associations and the testing environment.

The skeletal response examined in the present experiment (lick suppression) probably requires a constellation of discrete muscular movements. The overall integration of these skeletal movements is likely to require the participation of many neurons in the cortex. On the other hand, the heart rate response is fairly simple in that the heart has a very limited repertoire of responses available. Heart rate can basically go up, go down, or remain the same, and deceleration is primarily controlled by the vagus. The defecation CR is probably midway in complexity between the skeletal and heart rate CRs in that it requires an integration of autonomic and skeletal components as the external anal sphincter is skeletally innervated (Mountcastle, 1968).

One prediction based on these assumptions might be that ECS, as well as other disruptive agents differentially affect disparate points on a hierarchy of responses which vary along a continuum of complexity. The most complex responses require the longest times to reach a stable format and are, therefore, more susceptible to disruption than responses lower in the hierarchy which attain a stable state rapidly.

This model can also be adapted to incorporate instrumental responses. Skeletal operant responses would probably be considered more complex than most autonomic CRs, in that they are likely to entail more integration. Consequently, they might be seen as potentially more disruptable by amnesic agents. Thus, in a passive avoidance task ECS would disrupt the associations of the skeletal response that led to FS punishment. However, the less complex, classically conditioned fear associated with apparatus cues is likely to be less affected by ECS. This would tend to explain the often noted, although rarely reported, observation of defecation in rats that are tested following passive avoidance training with FS followed by ECS.

Within the hierarchy of complex responses, different types of skeletal operants could also be distinguished. Carew (1970), as previously noted, found that a latency index of amnesia for passive avoidance training did in fact indicate that the animals were amnesic. However, the subjects avoided the locus of previous FS thus implying a lack of amnesia. Carew concluded that the latter measure was more sensitive than the latency measure in assessing amnesia. An alternative analysis of Carew's data involves examining the delay between the subject's last movement within the punishment compartment preceding FS and FS onset as compared to the delay between the subject's last attending to contextual stimuli in the safe compartment and FS onset. The delay in reinforcement that is experienced in learning the locus of FS is appreciably smaller than the delay in reinforcement that is experienced in acquiring a passive avoidance response that distinguishes the safe from punishment chamber. Consequently, at this level of analysis, the go-no go response may be considered to be the product of a more complex association than the

avoidance of FS location. Therefore, in Carew's study, the two measures of retention may not be tapping the same memory with differential sensitivity, but may be reflecting the degree to which two types of responses, differing in complexity, are disrupted by ECS. As such, these data are viewed as being consistent with the present model.

An alternative to the complexity explanation of differential vulnerability of skeletally and autonomically indexed memories might assume that as a result of the CS-US pairing fear becomes associated with presentation of the CS. In an animal that does not receive ECS each of the indices (skeletal, heart rate, and defecation) indicates that the subject is extremely fearful. However, ECS delivered following the CS-US pairing reduces the fear associated with the CS and the diverse indices are differentially sensitive in their ability to reflect residual fear. Thus, fear conditioned in the present experiments has been sufficiently attenuated by ECS that the skeletal measure is fairly non-responsive to it. On the other hand, the defecation and heart rate indices are more sensitive in their responsiveness to fear and indicate considerable fear in the subject despite the overall level of fear being reduced by ECS. This model is more parsimonious than the complexity model; however, it does not account for the differential sensitivity of the various indices in indicating the presence of fear. The complexity model encompasses a more physiological interpretation than the latter model.

APPENDIX

Method for Recording Mean Heart-Rate

Experiments examining heart-rate in rats frequently employ a polygraph to provide a written record of the EKG. One method of computing mean heart-rate over a defined interval from this record is to count the number of R-waves occurring within the given interval. This measure is time consuming to obtain and contains an average error of $\pm \frac{1}{2}$ beat. An on-line method for computing heart-rate involving a polygraph pen activating a microswitch on each R-wave has been described (Fitzgerald, Vardaris, & Teyeler, 1968). This method requires extensive peripheral equipment in addition to the cost of the polygraph and does not eliminate the inherent error of $\pm \frac{1}{2}$ beat. Alternatively, with a cardiotachometer, a voltage proportional to each interbeat interval may be recorded; however, converting this measure to mean heart-rate is a laborious procedure. The circuit described below does not necessitate a polygraph or any other expensive electronics. It requires minimal data analysis to obtain mean heart-rate and permits easy elimination of the above mentioned error of $\pm \frac{1}{2}$ beat.

Although any preamplifier can be used in the present system, the one that is currently being used in our laboratory was originally designed for recording multiple unit activity (Brakel, Babb, Mahnke, & Verzeano, 1971) but is more than adequate for amplifying the EKG in moving rats. The output of the preamplifier is filtered by a low-pass filter (LPF) set to pass frequencies below 300 Hz. (see Fig. 12). Aside from removing high frequency artifacts from the EKG, it also yields an additional gain of 10 over the gain of 4000 provided by the preamplifier. The next stage is a voltage comparator (VC) whose output

goes from -14 to +14 volts each time the amplitude of the R-wave exceeds a variable tiggering threshold. Subsequently, the output of the VC initiates a one-shot (OS) which in turn activates the coil of a low current-drawing relay. Total cost of the LPF, VC, OS, and relay is under $40 and the LPF, VC, and OS may be purchased in preassembled form (Geonics Ltd., Toronto, Canada).

Low Pass Filter

The 3 dB points may be varied by changing C_1 and C_2: Frequency (in Hz.)=22/C_1 (in microfarads) = 1/C_2. The assembled unit is available as Geonics #1341-300.

Voltage Comparator

Since the LPF inverts the R-wave, it was necessary to activate the VC with negative signals. Positive signals may be used to trigger the VC by connecting point "a" to the positive instead of negative power supply terminal. Triggering threshold may be determined by the following formula: Threshold voltage = 15Y/(5600+R_x). The assembled VC may be purchased as Geonics #1257.

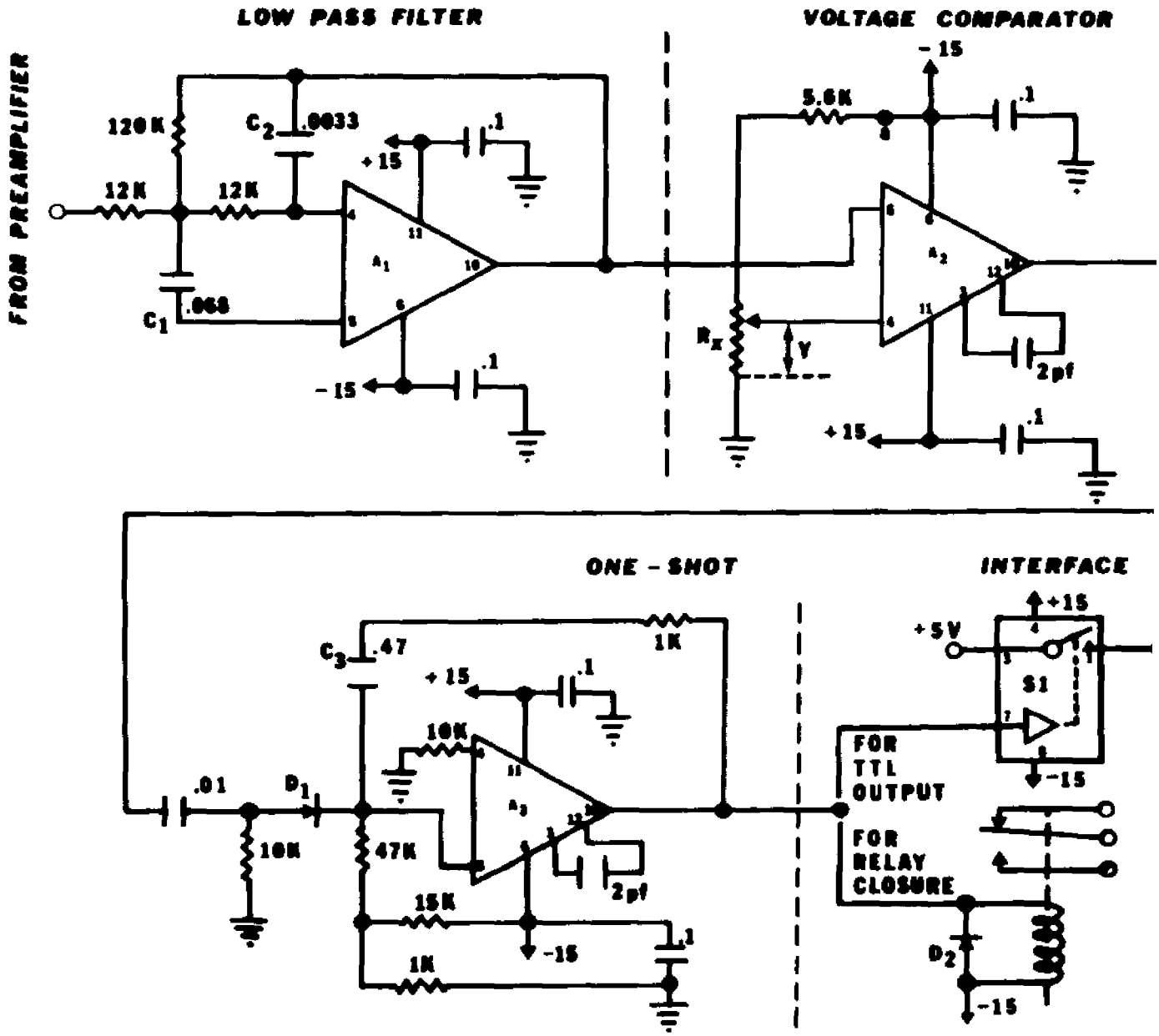
One-Shot

While the LPF attenuates high frequency signals, to further prevent tiggering on artifacts between R-waves, the trace of the OS was made 75 msec. long. A heart-rate of 600 bpm slightly exceeds the maximum heart-rate observed in rats and is equivalent to an interbeat interval of 100 msec. Subtracting relay opening time of 15 msec. leaves an 85 sec. interval of which the extended OS pulse duration deactivates the circuit for 75 msec. The trace of the OS may be altered by changing C_3. The trace (in msec.) = 160 x C_3 (in microfarads). The assembled unit is available as Geonics #1261-B.

Relay or Switch

Output of the OS is limited to a maximum of +15 volts at 10 ma., therefore any relay that is rated for 24-30 volts at less than 10 ma. may be used. Our circuit used an Elec. Spec. #71GB4R (American Design Components, New York, N.Y.). A solid state switch allows the output of the OS to be converted to TTL levels.

Using this circuit, R-waves may be recorded on counters that are enabled through a stepping switch controlled by an interval timer. This method is fairly simple but prone to inaccuracy. One beat may be missed or an extra beat counted as a function of when between beats the interval is initiated and terminated. This factor will produce an average error of $\pm\frac{1}{2}$ beat and a maximum error of ± 1 beat. For a heart-rate of 360 bpm recorded over a 15 sec. interval, the error may be as high as 1%. Decreasing the recording interval to 5 sec., the error may be as high as 3%. Clearly, this procedure becomes unacceptable as the recording interval is further reduced. A more accurate procedure, particularly useful for recording heart-rate over a brief interval, is currently being used in our laboratory. It consists of allowing the first R-wave during the desired recording interval to initiate a msec. timer and having the first R-wave after the desired interval stop the timer. By recording the number of beats and actual interval in msec. between the initiating and terminating beats, an extremely accurate mean heart-rate may be readily computed. For recording heart-rate at successive intervals a buffered high speed parallel print out counter can be used to record both the time in msec. and the number of R-waves within each interval.


LOW PASS FILTER
VOLTAGE COMPARATOR
FROM PREAMPLIFIER
120K
12K
12K
C_2 .0033
C_1 .068
+15
-15
.1
A_1
5.6K
A_2
R_X
Y
2pf
ONE - SHOT
INTERFACE
C_3 .47
1K
10K
.01
D_1
10K
47K
15K
1K
A_3
2pf
+5V
S1
FOR TTL OUTPUT
FOR RELAY CLOSURE
D_2

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