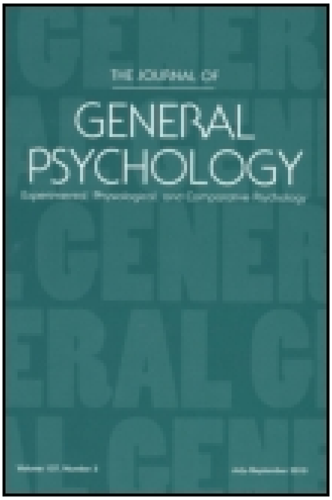

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Fear Conditioning and Extinction in Rats at Different Times of Day

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FEAR CONDITIONING AND EXTINCTION IN RATS AT DIFFERENT TIMES OF DAY*[1]

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SUMMARY

Random bred male animals (N = 48 rats) underwent classical fear conditioning, an extinction trial, and a subsequent fear retention test either in the morning or in the afternoon. Half of the animals in both groups had been deprived of food for 18 hours prior to testing and were offered some food in the final fear retention test, whereas the others carried on feeding ad lib. The four groups were further split up into groups of three animals with a duration of the extinction trial of either 0, 15, 30, or 60 sec. Food deprivation had no significant effect on the fear retention data. But animals which had been tested in the afternoon showed greater fear reduction following a short duration of extinction than after a long duration. Animals tested in the morning, on the other hand, profited more from longer duration of extinction.

A. INTRODUCTION

Few studies have been carried out on the role of daily rhythms in acquisition and extinction of learned responses despite the growing literature on the effect of circadian rhythms on most physiological parameters (4). When researchers are aware of the relevance of biological rhythms, *S*s are usually kept on a rigid lighting cycle and testing is administered at the same time within the cycle. Fear responses have been clearly shown to become associated with internal physiological states and their rhythms (8). Stroebel's study showed that the conditioned emotional response is acquired and extinguished more rapidly when trials are administered at the same time of day as compared to different times of day. Avoidance responses failed to exhibit this pattern. A similar

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study by Davies *et al.* (3) demonstrated a clear variation in passive avoidance responding as a function of the dark/light cycle with the avoidance peak during the middle of the light phase. However, animals had not undergone initial avoidance training in this study but had been given inescapable shocks during the acquisition phase. When rats were given passive avoidance training (5), the retention of this response also showed a cyclic pattern with its peak at multiples of 24 hours after acquisition. The association of the fear responses with the prevailing physiological state and the increased response rate at the cyclic recurrence of the state seems thus well established. In above studies fear conditioning was administered at all times of day, and it was the cyclic nature of the response which was of interest rather than differences between times of day. But there is also evidence that affective distress sustained varies according to diurnal rhythms of adrenaline level and this has been an acknowledged factor in human psychopathology for a long time (6).

The present study was planned to establish whether retention of a classically conditioned fear response tested shortly after its acquisition is superior in the morning or afternoon; and, furthermore, whether extinction trials of varying lengths produce different degrees of extinction in morning and afternoon. The fear response was acquired to a criterion, and its retention tested either immediately afterwards or else after 15, 30, or 60 sec of exposure to the CS only. The lighting cycle was held constant over the whole time. The periods of darkness in the laboratory extended from 21.00 to 4.00 hrs.

B. Method

1. *Subjects*

Forty-eight random-bred male rats took part in the experiment; the majority of the animals were brown hooded (17) and Agouti (19), but there were also eight Albinos among them, two black hooded animals, and two black animals. *S*s were housed in group cages, three per cage; they were 100 days old when testing started.

2. *Apparatus*

The open-field test and rearing cages were the same as used in previous experiments, and they are described elsewhere (1, 7). The conditioning chamber is also described under the apparatus section of previous reports. It was essentially a rectangular chamber of which one wall was removable so as to be exchanged with a wall carrying a platform. The top of the platform was hinged and depressed a microswitch whenever the animal sat on it. The microswitch was connected to a print-out timer, and the metal bar floor of the testing chamber to a shock generator.

3. *Design*

The three factors under investigation were duration of extinction trial, time of testing, and food deprivation. Half the animals were deprived of food for 18 hours prior to testing and were offered some food in the final fear retention test, whereas the others carried on feeding ad lib. Half of the animals within each of these two groups were tested in the morning between 10.00 or 12.00 hrs, the other half in the afternoon between 14.00 and 16.00 hrs. The four groups were further split up into groups of three animals with a duration of extinction of either 0, 15, 30, or 60 sec.

4. *Procedure*

All animals were tested in the open field and in the rearing cages during the week preceding conditioning. The main experiment started with an exploratory trial to familiarize the animal with the testing chamber.

a. Exploratory trial. The animal was placed on the platform of the testing chamber and left to explore the novel environment for 5 min. Latency of first step-down response, total time on the platform, and number of times on the platform were recorded. Afterwards the rat was taken out of the testing chamber and remained in a plastic bucket, while the wall carrying the platform was exchanged with the one without platform.

b. Conditioning. The animal was put back into the testing chamber and the first foot-shock (intensity .4mA, duration 2 sec) delivered within 30 secs; administration of the following four shocks was unevenly spaced within the next 4.5 min. The animal was then placed in the bucket again and the wall, fitted with the platform, inserted. The animal was seated on the platform and immediately taken out upon stepping down on to the bars to avoid extinction. In the absence of a step-down response the animal was left on the platform for 5 min. The animal was thus intended to discriminate between the "safe" platform and the "dangerous" bars without undergoing avoidance training. Shock-administration and "rest" on the platform were repeated once. Latency of step-down was recorded for both resting phases. In addition, latency of first attempted step-down and number of attempted step-down responses were recorded by *E*. "Attempted step-down" was defined as the animal stretching down at least one front paw towards the bars. Following the conditioning and rest phases animals were put back into the home cage and remained there for half an hour after which time the extinction trial took place.

c. Extinction. All *S*s, apart from the animals allocated to the zero-extinction group, were returned to the grid of the testing chamber in the absence of the platform and remained there for either 15, 30, or 60 sec. Afterwards they were taken out again and stayed in the home-cage for another half hour.

d. Fear retention test. Afterwards, animals were placed on the platform and remained in the testing chamber for 10 min. Latency of first step-down, total time on the platform, and number of times on the platform were recorded as in the exploratory trial. Animals which were subjected to food deprivation prior to the experiment were given food pellets on the cage floor opposite the platform. Defecation was recorded during conditioning and fear retention. Animals were tested individually at all times.

C. Results

Data were subjected to an analysis of variance with a factorial design of Two × Two × Four, the three factors being food deprivation *vs.* feeding, testing mornings *vs.* afternoons, and duration of extinction trial (i.e., 0, 15, 30, and 60 sec).

Coat color was included in the analysis after coding it according to intensity of pigmentation and amount of area covered by pigmentation (i.e., 1 = Albino, 2 = brown hooded, 3 = black hooded, 4 = brown, and 5 = black). Defecation data were transformed by log (X + 1) so as to normalize their distribution.

Total rearing scores and open-field defecation scores were evenly distributed among groups and so were animals' coat color: i.e., groups had a similar mean derived from color codes. Rearing correlated positively with number of step-downs during the fear retention test ($r = .331, p < .05$), indicating that high rearers returned to the platform more often than low rearing animals. However, in the absence of a similar result for number of step-downs during the exploratory trial not much importance can be attached to this correlation. Animals which defecated highly in the open-field tended to have less pigmentation in their coat than low defecators ($r = -.371, p < .05$) and took longer to step down from the platform after the first conditioning trial ($r = -.346, p < .05$).

Groups were well matched with respect to the measurements taken during the exploratory trial (mean step-down latency: 15.5 sec) and step-down latency after the first conditioning trial. All animals stayed on the platform for 5 min after the second conditioning trial. Animals subjected to food-deprivation showed less defecation during conditioning than those fed ad lib, for obvious reasons. The same result applies to defecation during the fear retention test. None of the main effects produced significant results with respect to the measures taken during the fear retention test. However, the first-order interaction of time of testing × duration of extinction trial had a significant effect on step-down latency ($F = 3.79$, $df = 3$ and 32, $p < .02$). Duncan's multiple range t test revealed significant differences between the 0 and 60 sec extinction groups tested in the morning ($p < .05$) as between the two 60 sec extinction groups tested in the morning and afternoon, respectively. Animals tested in the morning took longer to step down after short extinction trials than did animals tested in the afternoon. After long extinction trials this relationship was reversed and animals tested in the morning stepped down more quickly from the platform than did animals tested in the afternoon. See Figure 1.

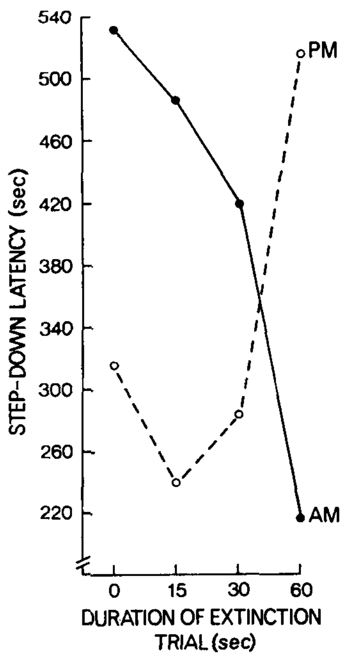

FIGURE 1
MEAN STEP-DOWN LATENCIES OF ANIMALS TESTED IN THE MORNING *vs.* AFTERNOON FOLLOWING VARIOUS DURATIONS OF EXTINCTION TRIALS

D. Discussion

The extent of sustained affective distress has previously been shown to coincide with the diurnal rhythm of adrenal cortical activity (2). The present study failed to confirm this finding; number of trials necessary to achieve the conditioning criterion—i.e. a step-down latency of 5 min—did not differ from morning and afternoon. Similarly, the higher retention of the conditioned fear response in the morning, as tested immediately afterwards, failed to reach significance level. It must be assumed that the experimental procedure—that is, the intensity of the US—was sufficient to evoke the hormonal mechanisms associated with fear despite different baseline levels in the morning and afternoon. The two times of day did, however, affect retention of the conditioned response following exposure of varying duration to the fear eliciting stimulus. The results indicate that retention of the fear response improves with time in the afternoon, whereas it deteriorates in the morning within the one-min period following acquisition.

The failure to obtain results for conditions food *vs.* no food indicates that animals were too frightened to approach the food, despite prior food deprivation. The incompatibility of feeding and fear reactions has been well established since Wolpe's experiments with cats (9). It can therefore be concluded that animals stayed on the platform because they were too afraid to step down and not because of other reasons, such as sleepiness.

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