STRAIN DIFFERENCES IN ACQUISITION AND EXTINCTION OF FEAR RESPONSES IN RATS

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Summary.—5 different strains of rats (Roman high and low avoiders, Maudsley reactive and non-reactive and random-bred animals) were subjected repeatedly to extinction trials following Pavlovian fear conditioning. The duration of the extinction trials was varied for different groups of animals. Fear was measured by latency of escape into the "safe" compartment in Exp. I and by step-down latency in Exp. II during a final fear-retention test. Results showed no differences between Roman high and low avoiders; for the Maudsley strains, however, results suggested that the higher the basal fear level the stronger is the acquired fear response and the more time is required for its extinction. Fearfulness in the animal and duration of extinction trials were jointly and severally causal in determining degree of extinction of the conditioned fear response.

Reports on the effectiveness of flooding techniques in treatments show highly contradictory results (Morganstern, 1973). When successful, flooding has the advantage of requiring fewer treatment sessions than other methods, and therefore proves economical. There is also evidence, however, that the presentation of fear cues without further administration of the UCS may cause sensitization rather than extinction of the fear response (Napalkov, 1963; Rachman, 1966).

Eysenck (1968) drew attention to this phenomenon, which he calls "incubation of fear," by theorizing that the fear response, evoked by fear cues without further accompaniment of the primary reinforcer, is an unpleasant experience in itself, and is capable of acting as its own reinforcer. Lader and Mathews (1968) also hypothesized a positive feedback mechanism that slowly increased the fear response from one trial to the next if components of the fear response were not allowed to habituate. Considering the course of the fear response during extinction trials, the duration of the exposure to fear cues is of paramount importance. Recordings of psychophysiological measures during flooding (Watson, Gaind, & Marks, 1972) show a sharp increase of the autonomic responses at the beginning of the session and a slow consecutive decrease resulting from habituation to the fear cues. Systematic variation of the duration of single extinction trials has yielded an enhancement of the fear response after short extinction trials, and a decrement after long extinction trials in humans (Miller & Levis, 1971) and rats alike (Rohrbaugh, et al., 1970, 1972, Silvestri, et al., 1970).

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If these results are caused by the differential habituation of autonomic system responses a differential baseline of responding to stressful stimulation, i.e., emotionality, can be expected to interact with time allowed for habituation. If on the other hand, the speed of extinction is related to the strength of formation of a classically conditioned response, i.e., conditionability, nonspecific arousal would be a crucial factor in determining the former. Lat (1967) found that animals of high levels of nonspecific arousal ("nonspecific excitatory level") surpassed those of low levels of arousal in the speed of acquisition of both an instrumentally conditioned response and a classically conditioned response. Strains bred for a differential baseline in either of the two dimensions thus provide a direct test of factors determining extinction. Two sets of strains and an appropriate control strain have been used in the present study. The control strain consists of randomly selected, outbred animals (RBS) comparable to the Wistar strain.

**EXPERIMENT I**

Bignami's Roman low and high avoider strains were chosen to assess the role of nonspecific arousal in determining speed of extinction of the Pavlovian fear response. These strains have been bred for high and low rates of active avoidance (Bignami, 1965) and have been shown not to differ in emotionality as measured in the open-field test (Broadhurst & Bignami, 1965). Differences between the two strains were attributed to differences in nonspecific arousal, since the latter finds expression in the level of general activity in animals (Lat, 1967). Lat (1967) established rearing frequency as the variable which best discriminated between low and high arousal strains. It was used here to verify the above assumption and to assess differences between the Roman strains and the controls, a strain of random-bred animals. Conditioning was carried out by means of inescapable shocks, and fear measured in a final retention test, using latency of escape into a "safe" area. Since administration of inescapable shocks can prevent the occurrence of escape responses in later trials (Seligman & Maier, 1967), a random sample of each tested strain was directly observed by E to record competing responses (freezing).

**Method**

**Animals.**—Ss were 280 naive rats, 140 of which were random-bred (RBS) hooded rats (Rattus norvegicus), 70 males (mean weight = 292.31 gm.) and 70 females (mean weight = 180.93 gm.). The remaining 140 animals were albinos of the Roman high and low avoider strains. The Roman high avoiders (RHA) totalled 70 of which 35 were male (mean weight = 261.06 gm.) and 35 female (mean weight = 178.43 gm.). Thirty-five of the 70 Roman low avoiders (RLA) were males (mean weight = 267.31 gm.) and 35 were females (mean weight = 172.79 gm.). The animals used for the present experiment...
were from the 25th and 26th generations of selective breeding. All animals were bred in the laboratory. They were housed in group cages, three rats per cage, with water and food available ad lib. Ss were 87 to 95 days old when testing started. All testing took place in the afternoon. A small sample of 25 animals underwent direct behavioural observation while being tested. They were 9 RBS animals (4 females and 5 males), 7 RHA (3 females and 4 males) and 9 RLA (4 females and 5 males).

**Apparatus.**—Inter-individual differences in faecal elimination rate were recorded in a standard open field test as described elsewhere (Broadhurst, 1960). The rearing cage consists of a clear Plexiglas cylindrical chamber (12 in. high X 11 in. in diameter). Changes in the ambient capacitance between the wire mesh floor of the chamber and the tinplate ceiling are detected by a proximity meter, the output of which is fed into a dc amplifier in order to trigger a relay which actuates an event recorder. The amplifier was calibrated such that rearing was recorded only when the animal stood on its hindlegs with its body fully stretched.

The main experiment was carried out with two shuttleboxes (AIM biosciences Ltd.), whose two compartments were lined with white and black cardboard respectively. One shuttlebox had a partition between the two compartments while the other allowed entry into the next-door compartment through an opening. All conditioning and extinction trials were administered in the shuttlebox with the partition, while the box with the opening was used for all exploratory trials and fear retention tests. Scrambled shocks could be administered with a shock generator (AIM bioscience) via the grid floor in the black compartment of the partitioned box. The floor of the other black compartment was connected to an ENM print-out timer, which was in operation whenever the rat was present. White noise of 64 dB at cage floor level was sufficient to mask all equipment noises. Testing was carried out in a darkened room. The only light source consisted of the house lights in the shuttleboxes.

An arrangement for direct behavioural observation consisted of a Rustrak event recorder with 8 channels. Each of these was connected to a button on a panel which was operated by E. During direct observation E sat in a distance of 2 metres from the testing chamber in darkness. Since E was also present in the room while testing animals which were not directly observed no change in experimental conditions was imposed by this additional procedure.

**Procedure.**—For the purpose of defining strain differences all animals were tested in the open field on two consecutive days and in the rearing cage for three sessions with an hour's rest between them during the week before the main experiment. Open-field testing lasted for 2 min. on each occasion. Total elimination was recorded. Ambulation was discarded since the validity of this measure proved to be doubtful (Whinbey & Denenberg, 1967). Rearing responses were recorded for 10 min. on each occasion.
Exploratory trial: The main experiment started with an exploratory trial lasting for 5 min. The animal was placed in the black compartment while having access to the white compartment. Latency of first crossing into the white compartment, total time in the black compartment and number of crossings were recorded. The total duration of the following behaviours was recorded: exploratory activity (running around and sniffing at floor level), rearing, preening, occupation with opening between compartments, and freezing. "Freezing" was defined as sitting motionless while exhibiting signs of alertness such as pricked ears.

Conditioning: Following exploration the animal was placed in the black compartment of the partitioned shuttlebox where 5 scrambled shocks were administered. The shocks lasted for 2 sec. and had an intensity of 0.4 ma. The first shock occurred within the first 30 sec. and the other four were randomly spread out over the next 4.5 min. After conditioning the rat was restricted to the white compartment for 5 min. The conditioning and resting phases were repeated once. In addition to the behaviours observed during the exploratory trials, various reactions to shock were recorded such as "dancing," jumping and vocalization ("Occupation with opening" was discarded since the partitioned box was used for conditioning). Behaviours were observed for the black and white compartments separately.

All animals underwent the exploratory trial and conditioning. They were then allocated to one of seven groups—10 random-bred animals and 5 Ss from each Roman strain per cell—on the basis of their crossing latency during exploration. This was done to achieve the same baseline for the pre-treatment measure in all groups.

Extinction: Trials of the same duration took place on Days 2, 4, and 7 after conditioning. For different groups they lasted for either 0, ½, 1, 3, 5, 15, or 30 min. each. During this time Ss were restricted to the black compartment as in the conditioning but without receiving any further shocks. The 0-extinction group stayed in the home cage until the fear retention test on the 9th day after conditioning. The observed animals from the three strains were balanced with respect to duration of extinction trials. The same behaviours as during conditioning were recorded with the exception of the shock contingent behaviour.

Fear-retention test: Ss were again placed in the black compartment for 5 min. while having access to the white compartment. Latency of first crossing, total time in the black compartment and number of crossings were measured as in the exploratory trial. Total duration of behaviours as listed for the exploratory trial were recorded. Faecal elimination was recorded throughout the experiment.

Results

All data, except for the ones collected during direct behavioural observation,
were subjected to analyses of variance (Multivariance, National Resources, Inc.), with a factorial design of 3 (strain) X 7 (duration of extinction trials) X 2 (sex). Orthogonal contrasts were used throughout and unequal cell frequencies taken into account (Clyde, et al., 1966).

Strain differences with respect to rearing and rate of emotional elimination. — The rearing scores for the 3 sessions were combined into a single score, since sessions did not interact with either sex or strain (Table 1). The strain effect was significant \( F = 73.57, df = 2/274, p < .0001 \); Roman high avoiders had a significantly higher rearing frequency than random-bred rats, and Roman low avoiders had a significantly lower rearing frequency than the latter, as tested with the Duncan t test \( (p < .01) \). Female animals had a significantly higher rearing frequency than males \( F = 34.517, df = 1/274, p < .0001 \).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roman High</td>
<td>116.400±36.848</td>
<td>146.257±27.083</td>
</tr>
<tr>
<td>Random-bred</td>
<td>105.500±26.435</td>
<td>122.286±33.866</td>
</tr>
<tr>
<td>Roman Low</td>
<td>61.114±23.468</td>
<td>81.971±32.537</td>
</tr>
</tbody>
</table>

The interaction between sex and strain was nonsignificant. The total sum of faecal elimination in the open field was transformed by \( \log (X + 1) \) in view of the J-shaped distribution of the data. The strain effect was again significant \( F = 20.60, df = 2/274, p < .001 \). Both Roman strains had a higher defecation rate than the random-bred rats \( (p < .01) \). The difference between the two Roman strains was nonsignificant. Females had a lower defecation rate than males \( F = 39.90, df = 1/274, p < .0001 \). The interaction of strain X sex was nonsignificant. As the random-bred rats were pigmented and the Roman strains albinos, open-field data from random-bred albinos\(^3\) (46 males, 26 females) were compared with the Roman strains to ensure that differences in faecal elimination were not merely due to differences in pigmentation (Fig. 1). The same results emerged. Duncan's multiple-range t test gave reliable differences in faecal elimination between random-bred albinos and both Roman strains within males as well as females \( (p < .01) \). Differences between random-bred albinos and random-bred pigmented animals were nonsignificant. The sex difference was significant in random bred albinos \( F = 4.67, df = 69, p < .005 \) as in all other strains.

Thus, the Roman strains have to be considered as a low and high arousal strain, which is obviously a reflection of the criterion for which they have been

\[^3\]We would like to thank Mr. David Sanders for providing us with data on random-bred albino rats.
selected. Animals characterized by high levels of arousal exhibit a higher degree of general activity, i.e., they rear more often and develop active avoidance responses more readily than animals characterized by low levels of arousal. Unexpectedly, both Roman strains were more fearful in the open field than the random-bred animals. This result can not be explained in terms of differences in pigmentation between strains but is evidently also a phenomenon brought about by the selection procedure to which the Roman strains were subject. Females showed a higher level of arousal as measured by the rearing test and less emotionality in the open field than males across all strains.

**Exploratory trial.**—The three measures recorded during the exploratory trials correlated highly. Product-moment correlations were for latency of first crossing and total time in black compartment, .838, for latency and number of crossings, −.782, and for total time and crossings, −.550 (df = 238, p < .01 for all three correlations). Although the correlations can be expected to be somewhat lower when calculated for Roman strains and random-bred strains separately, the reason for the correlations is obvious. The longer an animal
takes for the first crossing, the more time it will spend in the black compartment and the fewer crossings it will be able to make. The strain effect was significant for the three measures: first latency of crossing into the white compartment \( (F = 53.24, df = 2/238, p < .0001) \), and number of crossings \( (F = 61.88, df = 2/238, p < .0001) \). The effects of sex and duration of extinction trials were both nonsignificant and so were all interactions. Both Roman strains had a significantly longer crossing latency than random-bred animals \( (p < .01) \) and both of them spent more time in the black compartment than the latter \( (p < .01) \). The Roman strains did not differ from each other on these two measures. However, all three strains differed from each other with respect to number of crossings; random-bred animals crossed more often between the compartments than did Roman high avoiders \( (p < .01) \), which in turn crossed more often than Roman low avoiders \( (p < .01) \).

### Table 2

**Exploratory Trials: Means ± SDs of Latency of First Crossing, Total Time In Black Compartment and Number of Crossings For Random-bred Animals, Roman High Avoiders, and Roman Low Avoiders**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Latency</th>
<th>Total Time</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Random-bred Strain</td>
<td>115.07± 88.173</td>
<td>190.44±59.244</td>
<td>6.657±3.627</td>
</tr>
<tr>
<td>Roman High Avoiders</td>
<td>205.23± 94.600</td>
<td>238.03±61.971</td>
<td>2.229±2.991</td>
</tr>
<tr>
<td>Roman Low Avoiders</td>
<td>234.49± 90.896</td>
<td>260.47±55.546</td>
<td>1.600±2.226</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Random-bred Strain</td>
<td>102.74± 68.126</td>
<td>181.96±42.312</td>
<td>7.343±3.791</td>
</tr>
<tr>
<td>Roman High Avoiders</td>
<td>203.76±102.911</td>
<td>245.18±56.551</td>
<td>3.771±4.544</td>
</tr>
<tr>
<td>Roman Low Avoiders</td>
<td>231.45± 83.508</td>
<td>257.89±46.317</td>
<td>1.771±2.276</td>
</tr>
</tbody>
</table>

No differences were found among the observed animals between strains or sexes with respect to exploratory activity and occupation with the opening. However, strains differed in their rearing frequency as evaluated by the Kruskal-Wallis test \( (H = 9.92, df = 2, p < .01) \). Roman high avoiders had the highest rearing frequency during the exploratory trial, followed by random-bred animals. Roman low avoiders had the lowest rearing frequency. Only few animals showed preening activity and none of the observed animals in any of the three strains showed any freezing responses during the exploratory trial.

Results from the directly observed behaviour corroborated the ones obtained in the rearing cage; that is, Roman high avoiders tended to rear more often while exploring a novel environment than random-bred animals, and Roman low avoiders showed the least rearing activity. High avoiders were generally more active than low avoiders as can be seen from their higher incidence of crossing into the white compartment. However, the former crossed less often than random-bred animals and both Roman strains took longer to venture into the white
compartment but spent more time in the black compartment than the random-bred rats. Brightness is an essential factor in eliciting emotional responses in rats. Thus the more fearful Roman strains had a greater preference for the dark compartment.

Conditioning.—Total rate of faecal elimination during conditioning in the black compartment and during rest in the white compartment was transformed by log \((X + 1)\). The defecation rate was clearly higher during the stressful shocks in the black compartment than during rest in the white compartment. Strain had a significant effect on both measures \((F = 4.38, df = 2/238, p < .01\) for the black compartment, and \(F = 28.39, df = 2/238, p < .0001\) for the white compartment). Roman low avoiders had a higher defecation rate during conditioning than either random-bred animals \((p < .01)\) or high avoiders \((p < .05)\). The two latter do not differ from each other with respect to this measure during conditioning. They do differ, however, during the rest period in the white compartment. Both Roman strains have a higher defecation rate than the random-bred animals \((p < .01)\) and high avoiders have a higher rate than low avoiders \((p < .01)\). Sex had a significant effect only with respect to defecation in the white compartment \((F = 10.51, df = 1/238, p < .001)\); males had a higher rate of defecation than females during the rest period.

![FIG. 2. Mean rate of defecation of random-bred animals and Roman high (RHA) and low avoiders (RLA) during conditioning (black compartment) and rest (white compartment): Exp. 1](image)

The interaction of strain \(\times\) sex (Fig. 2) was significant for both measures \((F = 3.59, df = 2/238, p < .02\) for the black compartment, and \(F = 2.95, df = 2/238, p < .05\) for the white compartment). Duration of extinction trials was nonsignificant and so were all other interactions. The results obtained for defecation during rest in the white compartment substantiates the results from the exploratory trial, namely, that Roman strains are more fearful of the white compartment than the random-bred animals. The sex-strain-interaction
effect obtained for defecation in the white compartment revealed that males have higher defecation scores than females in both Roman strains whereas animals from both sexes in the random-bred strain showed virtually no defecation at all. Thus females are equally stress-resistant in the white compartment as they had been in the open-field test. A different picture emerges from the defecation data during conditioning. The interaction between strain and sex specified that the higher defecation rate of low avoiders was contributed by the females. This curious result might be easier to explain if we look at defecation in the context of other behaviours displayed during shock.

The measures recorded in the directly observed animals were analysed by means of nonparametric tests according to sex, strain, and differences between black and white compartments. The latter test was omitted for behaviour elicited by shocks which are "dancing," jumping, and vocalizing. Sex had no significant effect on any of the observed behaviours. Strain had a significant effect on freezing responses (Kruskal-Wallis $H = 13.58, df = 2, p < .01$) and vocalization ($H = 13.60, df = 2, p < .05$). Between the three strains, Roman low avoiders spent most time freezing and vocalized least, followed in the duration of both behaviours by Roman high avoiders. Random-bred animals spent the least time freezing and vocalized most. The duration of none of the other behaviours differed between strains. Differences between behaviours in black and white compartments were found in exploratory activity (Wilcoxon test for related samples $T = 72.0, N = 25, p < .01$) and preening ($T = 3, N = 24, p < .01$). Ss spent more time with exploratory activity and preening in the white compartment than in the black compartment.

We can thus summarize that Roman low avoiders tended to cling to the grid during administration of shocks while animals from the other strains involved in other activities which were most likely used to minimize contact with the grid. Roman low avoiders have therefore received shocks of longer duration than the other strains. Females are usually more stress-resistant than males. If stress is constituted by administration of footshocks, however, females can be expected to receive them at a higher intensity than males owing to the less insulatory skin tissue of their paws. This might explain, in post hoc fashion, why female Roman low avoiders should have the highest defecation rate during shocks. None of the observed behaviours recorded during extinction trials differed between strains or sex.

**Fear-retention test.**—The three fear measures correlated highly with each other; latency of first crossing had a correlation of .709 with total time and of -.529 with number of crossings. Total time had a correlation of -.384 with number of crossings. Strain had a significant effect on all three measures taken during fear retention: latency of first crossing into the white compartment ($F = 20.74, df = 2/238, p < .0001$), total time in black compartment ($F =$
9.27, df = 2/238, $p < .0002$), and number of crossings ($F = 37.40$, df = 2/238, $p < .0001$). Random-bred rats had a shorter mean latency of first crossing (31.140 sec.) into the white compartment than Roman high avoiders (96.203 sec., $p < .01$) and Roman low avoiders (92.809 sec., $p < .01$). Random-bred animals also spent less time in the black compartment (152.96 sec.) than Roman high avoiders (189.38 sec., $p < .01$) and Roman low avoiders (177.15 sec., $p < .01$), and cross more often (9.29) than both Roman strains (5.66, $p < .01$ for high avoiders, and 4.64, $p < .01$ for low avoiders).

The Roman strains only differed with respect to total time in black compartment: Roman high avoiders spent more time in the black compartment than Roman low avoiders ($p < .05$). Sex also had a main effect on the fear measures. Females had a shorter crossing latency than males ($F = 5.10$, df = 1/238, $p < .02$) and spent less time in the black compartment ($F = 9.72$, df = 1/238, $p < .002$). They also crossed back and forth more often than the latter ($F = 11.76$, df = 1/238, $p < .0008$). The effect of duration of extinction trials was only marginally significant for number of crossings ($F = 2.01$, df = 1/238, $p < .06$) and latency of crossing ($F = 1.97$, df = 1/238, $p < .07$). The interaction of duration $\times$ sex was significant for total time ($F = 2.72$, df = 1/238, $p < .01$). It is only among the longer extinction groups that females spent less time in the black compartment than males (Fig. 3). The interaction of duration and strain was marginally significant for total time in black compartment only ($F = 2.01$, df = 2/238, $p < .08$). All other interactions were non-significant. None of the observed behaviours differentiated between strains and sexes. Nor did they yield significant results when animals were split into groups with long extinction trials (15 and 30 min.) and short ones (0, 1, and 3 min.).

![Fig. 3. Mean total time in black compartment of male and female animals as a function of duration of extinction trials: Exp. 1](image-url)
Discussion

The two Roman strains proved to be a high and a low arousal strain as shown in the rearing test and by their rearing frequency during the exploratory trial. However, the failure to find a differential effect of arousal on the speed of extinction of the fear response, i.e., the interaction of duration of extinction trials × strain, could have been due to the confounding effect of emotionality. It was discovered here, as has been reported before (Broadhurst & Bignami, 1965), that the two Roman strains do not differ from each other in emotionality but show the same high level of emotionality as compared to random-bred animals. Indeed, the Roman strains behave on the same level of emotionality as Maudsley emotionally reactive animals. It is conceivable that the selection of animals rating either high or low in active avoidance favours animals of high levels of fearfulness. They tend to respond consistently over a great number of trials exhibiting either of the two adaptive responses to fear, flight or freezing. They thus achieve higher scores on either avoidance or non-avoidance than less fearful animals which show a less consistent pattern of responding.

Differences in fearfulness between Roman strains and random-bred animals also extended to the pre-treatment measures, such as the preference for the black compartment. This had confounding effects upon the conditioning procedure, as the Roman strains experienced the white compartment as the “safe” area during the rest phases, to a lesser degree than the random-bred animals as indicated by their higher rate of defecation (Fig. 2). Duration of extinction trials failed to have a systematic effect on the fear retention measures (Fig. 3) unlike in previous studies in adult rats (Rohrbough & Riccio, 1970, 1972) nor did the post-treatment measures show any consistent trends. None of the previous studies of adult rats used the escape response as a fear measure. Post hoc, it appears to be an inappropriate, i.e., non-linear, measure considering the high incidence of freezing in Roman low avoiders. Females showed higher arousal and lower emotionality than males. However, they reacted more strongly to the fear conditioning than males as is indicated by their higher scores in the final retention test. This could be due to sex-linked differences in the thickness of skin and therefore in resistance to electric current. Thus, females may have received higher intensity UCS than males.

Among the directly observed behaviours freezing was the best discriminating variable between pre- and post-conditioning, since it was totally absent in the non-fearful condition of the exploratory trial.

Experiment II

Maudsley emotionally reactive (MR) and non-reactive animals (MNR) were chosen to assess the effect of emotionality on extinction of the Pavlovian fear response as a function of duration of extinction trials. Both strains have been shown to differ reliably in their baseline of reactivity to stressful stimula-
tion (Broadhurst, 1969). It was therefore expected that they would also differ in the time needed for extinction of the fear response as compared to a control group. The experimental design of the present study was identical with that of Exp. I, except that the fear measure was changed. Latency of step-down from a "safe" platform on the grid was considered to be more appropriate than the escape response. The former measure takes the freezing response into account and also makes it possible to assess the growth of the fear response from the first conditioning trial to the second.

Method

Animals.—Ss were 150 naive male rats, 30 of which were random-bred hooded animals (Rattus norvegicus). Half of the remaining 120 rats were Maudsley emotionally reactive and the other half, Maudsley emotionally nonreactive (Broadhurst, 1960). Rats from the latter two strains were from the 49th and 50th generation of selective breeding. Emotionally actives (mean weight 258.08 gm.) are slightly smaller than random-bred rats (mean weight 264.66 gm.) and nonreactives are considerably smaller than both of the other strains (mean weight 210.66 gm.). All animals were 100 to 105 days old when testing started. They were housed in group cages, three rats per cage, with water and food available ad lib.

Apparatus.—The open-field test used is described elsewhere (Broadhurst, 1960). The rearing cage was the same as in Exp. I. The testing chamber for the main experiment was constructed in the laboratory work-shop. Its dimensions are .50 m (length) × .22 m (width) × .5 m (height). It was made from black perspex except for the lid and one wall which was made from transparent perspex to allow observation of the animal. One wall on the narrow side of the chamber could be exchanged with another one fitted with a platform measuring 3 in. (W) × 6.5 in. (L) × 4 in. (H). It too was made from black perspex with the top surface slightly roughened for better grip. The top of the platform was hinged so as to depress a micro-switch whenever the animal sat on it. The micro-switch was connected to an ENM print-out timer and the metal-bar floor of the testing chamber to an Aim BioScience shock generator. The arrangement for direct behavioural observation was identical to the one in Exp. I. White noise of 64 db at cage floor level masked all equipment noises during testing, and a red lamp above the testing chamber was the only light source in the otherwise darkened room.

Procedure.—The open-field and rearing test were carried out as reported in Exp. I. 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. **Conditioning:** Following this trial, the animal was put back into the testing chamber and the first foot-shock delivered within 30 sec.; administration of the following 4 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 to remain there for 5 min. It was immediately taken out upon stepping down on to the bars to avoid extinction. The animal was thus intended to discriminate between the "safe" platform and the "dangerous" bars in the absence of 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. During the phases of shock-administration, freezing behaviour was recorded for 30 sec. following each shock. So far, all animals received the same treatment. After conditioning, they were allocated to one of 6 different groups, 10 per cell, on the basis of their step-down latency during the first "rest" phase.

**Extinction.**—The animal was placed on the grid of the testing chamber in absence of the platform and remained there for either 0, 1, 3, 5, 10 or 15 min., three times, with a 48-hr. interval between trials. Animals of the random-bred strain were divided into 0-, 1-, and 5-min. groups. The 0-extinction group was left in the home cage for the whole time. During the last minute of each extinction trial E recorded duration of freezing behaviour so as to evaluate the progress of fear extinction from one trial to the next.

**Fear-retention test.**—Two days after the last extinction trial, animals were placed on the platform and left 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. Latency of first attempted step-down and number of attempted step-downs were recorded prior to the first actual step-down.

**Results**

All data were subjected to analysis of variance with a factorial design of 3 (strain) \( \times \) 6 (duration of extinction trials). Empty cells in the case of the random-bred strain and unequal frequencies were taken into account (Multivariate, National Resources, Inc.).

**Open-field and rearing test.**—Faecal elimination in the open-field of the two days was transformed into a sum and a difference score. Strains differed significantly with respect to both derived scores (sum: \( F = 212.57, df = 2/135, p < .0001 \); and difference: \( F = 6.80, df = 2/135, p < .001 \)). Reactives have a significantly higher defecation than random-bred animals (\( p < .01 \))
and nonreactives a significantly lower score than the latter \( (p < .01) \). Random-bred animals showed a considerable decrement in defecation on the second day of testing as compared with the two Maudsley strains \( (p < .01) \). In case of the nonreactives, the failure to habituate is due to their low defecation on Day 1. Reactives, on the other hand, must be assumed to have a low rate of habituation to stressful stimulation (Fig. 4).

The factor "duration of extinction trials" was also significant with respect to total elimination rate \( (F = 2.46, df = 5/135, p < .04) \). The 0-extinction group had a lower rate than the 1-min. group \( (p < .01) \) and so did the 5-min. group \( (p < .05) \). All other differences were nonsignificant. The interaction of strain and duration was also nonsignificant. No differences were found between the three strains with respect to the combined rearing frequency of the three sessions. Maudsley strains thus differ from random-bred rats in the basal level of emotionality which is higher in the case of reactives and lower in the case of the nonreactives. But the level of arousal or general activity is the same in all three strains. The differences in rate of defecation between extinction groups are an experimentally undesirable effect. They are worth noting in case they affect the outcome of the study.

**Exploratory trial.**—Maudsley strains and random-bred animals did not differ from each other with respect to preference for either the platform or the cage bars prior to conditioning. Neither of the three measures (latency
of step-down, total time on platform and number of step-downs) shows significant differences between strains or extinction groups.

**Conditioning.**—The freezing responses observed directly following the administration of each shock were transformed into orthogonal polynomials with a repeated measures design of 2 (session) × 5 (shocks). Only the total mean over the two sessions differentiated significantly between strains ($F = 10.65, df = 2/135, p < .0001$). Random-bred animals showed less freezing behaviour following shocks than reactives (Fig. 5) and the latter had lower scores than nonreactives ($p < .01$ in both cases). The three strains did not differ in their defecation rate during conditioning but they differed significantly in their step-down latency during rest phases (Fig. 6) ($F = 14.74, df = 2/135, p < .0001$, for rest I, and $F = 5.99, df = 2/135, p < .003$, for rest II). Reactives had a longer step-down latency after the first conditioning session than both random-bred animals ($p < .01$) and nonreactives ($p < .01$). Differences between the latter two were nonsignificant for both rest phases. The step-down latencies of the three strains approach each other after the second conditioning phase but reactives still took longer to step down on the grid than random-breds ($p < .01$) and nonreactives ($p < .05$). None of the attempted step-down responses yielded significant results.

![Fig. 5. Mean duration of freezing behaviour of random-bred animals, Maudsley emotionally reactives (MR) and nonreactives (MNR) during 30 sec. following termination of foot-shocks: Exp. II](image)

There two results, i.e., equal rate of defecation during conditioning but differences between strains in the step-down latency following conditioning, appear to be incompatible. It has to be concluded from them that the physical impact of shock evokes the same physiological responses in all three strains but that the formation of the conditioned fear response, i.e., the differentia-
tion between the "dangerous" grid and the "safe" platform, is subject to a different growth rate.

**Extinction trials.**—Strain had a main effect on rate of defecation during the three extinction trials ($F = 69.49$, $df = 2/135$, $p < .0001$ for Trial 1; $F = 44.93$, $df = 2/135$, $p < .0001$ for Trial 2 and $F = 16.48$, $df = 2/135$, $p < .0001$ for Trial 3). Nonreactives had a lower rate of defecation than random-bred animals in all three trials ($p < .01$). Reactives had a higher defecation rate than random-bred animals in the first two trials only ($p < .01$). The three strains tended to approach each other with increasing number of trials (Fig. 7). Duration of extinction trial also affected rate of defecation significantly for the simple reason that the longer animals were confronted with the CS the longer they had time to defecate.

Freezing measures were unaffected by the length of extinction trial, since they were recorded for the last minute of each trial only (Fig. 8). The 0-extinction groups were discarded and the freezing scores of the remaining groups transformed into orthogonal polynomials resulting in a mean score, a linear trend and a quadratic trend. Duration of extinction trial had a significant effect on the first two ($M: F = 3.32$, $df = 4/108$, $p < .01$; linear trend: $F = 2.69$, $df = 4/108$, $p < .03$).

The 15-min. group had a lower mean freezing score than the 5-min.
STRAIN AND CONDITIONED FEAR

FIG. 7. Mean rate of defecation of random-bred animals, Maudsley reactives (MR) and nonreactives (MNR) during the three extinction trials: Exp. II

FIG. 8. Mean duration of freezing behaviour as a function of duration of the three extinction trials. Freezing was measured during the last minute of each trial: Exp. II.

group ($p < .01$) and the 1- and 3-min. groups. All other differences between means are nonsignificant. Linear trends of the freezing duration in the three trials clearly appeared to be a function of the duration of the extinction trials. While duration of freezing responses remained the same over the three trials in the 1-min. group, it is decreased in the third trial in the 3-min. group and in the second and third trials in the 5- and 10-min. groups. The 15-min. group had the same short duration of freezing in all three trials. Strain only had a marginal effect on the linear trend but the interaction of duration $\times$ strain was significant ($F = 2.40, df = 5/108, p < .04$). Clearly, a reverse process was taking place for nonreactives as compared with reactives and random-bred animals. Both of the latter shared a distinct trend towards less freezing the longer the extinction trials lasted, whereas in the case of the nonreactives freezing increased with increasing duration.

We can summarize that extinction trials were the more beneficial to fear reduction the longer they lasted. Reactives reacted more strongly to the CS-only exposure than the other strains as shown in the defecation data. Given these two results, it was surprising to find an increase in freezing duration in nonreactives while animals from the other two strains returned to more relaxed activities. We shall attempt an explanation of this result later.

Fear-retention test.—Duration of extinction trials had a significant effect on the latency of step-down response ($F = 6.00, df = 5/135, p < .0001$), total time on platform ($F = 4.16, df = 5/135, p < .001$), and a marginal effect on number of step-down responses ($F = 2.25, df = 5/135, p < .052$).
The 0-extinction group did not differ from the 1-min. group on any of the indices but the former had a longer step-down latency \( (p < .01) \) and spent more time on the platform \( (p < .05) \) than the 3-min. and all remaining groups.

The 1-min. group differed from the 3-min. group in step-down latency \( (p < .05) \) and from all other groups with longer trial duration. Animals from the 1-min. group spent more time on the platform than animals from the 10-min. group \( (p < .05) \) and also more than the 5- and 15-min. groups. Strain had a main effect of only marginal significance on step-down latency \( (F = 2.91, df = 2/135, p < .058) \) and a significant effect on total time on the platform \( (F = 5.48, df = 2/135, p < .005) \). Nonreactives spent less time on the platform than random-bred or reactive rats \( (p < .01) \). The interaction of strain \( \times \) duration had a significant effect on step-down latency \( (F = 2.93, df = 7/135, p < .007; \text{Fig. 9}) \) and an effect of marginal significance on total time on platform \( (F = 1.79, df = 7/135, p < .09) \).

**TABLE 3**

<table>
<thead>
<tr>
<th>Duration of Extinction</th>
<th>Maudsley Animals</th>
<th>Random-bred</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reactive</td>
<td>Nonreactive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total Time on Platform</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>533.788±173.043</td>
<td>210.458±262.063</td>
<td>334.908±247.596</td>
</tr>
<tr>
<td>1</td>
<td>404.002±264.273</td>
<td>157.734±207.733</td>
<td>357.938±220.200</td>
</tr>
<tr>
<td>3</td>
<td>297.370±248.879</td>
<td>126.416±177.102</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>158.406±197.593</td>
<td>140.418±191.688</td>
<td>171.868±183.553</td>
</tr>
<tr>
<td>10</td>
<td>172.026±141.797</td>
<td>176.546±238.391</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>138.496±138.166</td>
<td>140.466±181.398</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number of Step-down Responses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.900± .994</td>
<td>1.400± .966</td>
<td>2.100± 1.449</td>
</tr>
<tr>
<td>1</td>
<td>1.400± .966</td>
<td>2.300± 2.359</td>
<td>4.100± 2.470</td>
</tr>
<tr>
<td>3</td>
<td>4.400± 3.950</td>
<td>2.200± 2.201</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.700± 4.923</td>
<td>3.600± 3.864</td>
<td>4.000± 3.162</td>
</tr>
<tr>
<td>10</td>
<td>5.800± 3.910</td>
<td>3.300± 3.592</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>4.600± 3.147</td>
<td>2.700± 2.111</td>
<td></td>
</tr>
</tbody>
</table>

Within the 0- and 1-min. extinction groups, reactives took longer to step down on the grid than either of the other two strains. Strain had a main effect on number of attempted step-downs \( (F = 5.48, df = 2/135, p < .005) \). As can be expected from above results, reactives made more attempted step-downs than nonreactives \( (p < .01) \), since the latter made their actual step-down response at an earlier stage than the former. None of the other data
relating to attempted step-down responses yielded positive results. The rate of defecation during the fear-retention test showed a similar pattern as the step-down data (Fig. 10). Strain had a significant effect on defecation \((F = 25.09, df = 2/135, p < .0001)\) and so did duration of extinction trials \((F = 3.16, df = 5/135, p < .01)\). The interaction between the two, however, was nonsignificant.

Nonreactives had a lower rate of defecation than random-bred animals \((p\)
The latter two groups failed to show significant differences with respect to faecal elimination. The difference between the 0-extinction group and the 5-min. group is significant \((p < .05)\) as is the difference between the former and the 10-min. group and 15-min. group; the 1-min. group and 15-min. group also differ significantly \((p < .05)\). The results on step-down responses suggest that reactives retained a higher fear level following extinction trials of short duration than either of the other two strains. Following long extinction trials, reactives assume the same low level of fear retention as nonreactives and random-bred animals. However, defecation data suggest that both reactives and random-bred animals were subjected to considerably more stress in the final fear test than nonreactives.

**Discussion**

Animals characterized by a high basal level of emotionality acquire stronger Pavlovian fear responses and need a longer time for their extinction than animals characterized by low levels of emotionality. Reactives showed higher fear levels, i.e., longer step-down latencies, following conditioning, than random-bred animals or nonreactives. This result conforms to former findings on Pavlovian fear conditioning (Singh, 1959; Singh & Eysenck, 1960) involving reactives and nonreactives. Their results showed a higher rate of conditioned suppression in reactives than in nonreactives, i.e., the bar-pressing response of reactives was disturbed to a greater extent than that of nonreactives. No extinction trials and 1-min. extinction trials had a differential effect on fear retention for the three strains: Maudsley reactives retained a higher fear level than the two other strains. Thus, the basal level of emotionality is a primary factor in the acquisition and extinction of Pavlovian fear responses, interacting powerfully with the duration of extinction trials.

The decision whether the high resistance to fear extinction in reactives was a function of their high basal level of emotionality or merely due to greater strength of their classically conditioned fear response can ultimately only be decided by adjusting the conditioning procedure such that all animals attain fear responses of the same strength. However, this question might be a superficial one as far as clinical implications are concerned. What matters for the clinical context is the finding of the extinction of high levels of fear following long extinction trials. None of the three strains showed an increase in fear as compared to the control, i.e., 0-extinction, group. This failure to find an incubation effect might be due to the long intervals between extinction trials. In the studies which report an incubation effect in adult rats (Rohrbough, et al., 1970, 1972), the intervals between conditioning, extinction and retention test never exceeded half an hour. Comparatively, intervals of two days were used in the present study. Eysenck (1968) suggested that concomitant with the increase of the fear response owing to CS-only exposure, a process of loss
of retention is taking place. It is conceivable that intervals of two days between extinction trials cancelled out the increase of the fear level and thus prevented it from becoming manifest. Results from the extinction trials actually show that two extinction trials of 5 min. each, with a two-day interval between them are more beneficial, i.e., result in fewer freezing responses at the end of the trial than a single extinction trial of 10 min. However, in the absence of a fear monitor during the time between extinction trials, any explanation of the failure to find an incubation effect must remain speculative.

A curious effect was obtained in the analysis of the freezing responses recorded directly by E. Although step-down measures and defecation rate indicated animals from the nonreactive strain to be the least fearful, they showed more freezing behaviour following shocks and in the extinction trials. This result seems incompatible with the common observation that freezing responses occur only in fearful animals and also with the observation made previously (Levine & Broadhurst, 1963) that nonreactives are superior to reactives in the acquisition of an active avoidance response. A highly speculative explanation would be to assume the existence of a hierarchy of fear responses ranging from freezing responses via flight to defecation. The choice of response would also be determined by the state of non-specific arousal the animal is in. As was shown in Exp I animals characterized by low levels of arousal favour freezing in response to stressful stimulation. The effect of the foot-shock appears to have been equally painful for all three strains as they did not differ in their rate of defecation during conditioning. Wilcock (1968) recorded the behaviour of reactives and nonreactives during administration of shocks together with the GSR, i.e., the voltage-drop across Ss. Nonreactives showed a higher resistance than reactives but both strains responded equally vigorously to shock. He concluded that the difference in skin resistance reflected a difference in autonomic functioning but ruled out a differential pain threshold in view of their similar behaviour. It is thus not susceptibility to shock, i.e., the unconditioned response, but the formation of the conditioned response in which the three strains differ.

**Résumé and Conclusions**

Both the experiments reported here give ample evidence for the genetic control of differences in the acquisition and extinction of fear responses, as evidenced in observed strain differences. In the first experiment, escape behaviour from fear stimuli conditioned to the black compartment of a shuttle box was used as a measure and Roman high and low avoiders were used as the experimental animals, together with a random-bred strain. The main findings were as follows: (1) The Roman strains are more fearful than random-bred animals, as shown by their higher rate of defecation in the open-field test. (2) Roman strain rats do not differ with respect to defecation but do differ with
respect to rearing in a special test used to measure arousal, Roman high avoiders showing greater arousal, as hypothesized. (3) On exploratory trials, random-bred animals crossed more frequently than did Roman high avoiders, and the high avoiders more than the low avoiders. This may be due to the greater fearfulness of the Roman strain animals making them prefer the security of the darker (black) compartment. (4) During conditioning, Roman low avoiders defecated at a higher rate than random-bred rats or Roman high avoiders but during rest in the white compartment, both Roman strains had higher defecation rates than random-bred animals, with Roman high avoiders defecating more than low avoiders. Roman strains are thus more fearful in the white compartment, which follows from points 1 and 3 above. (5) Behavioural observation shows that the low avoiders freeze most and vocalize least while random-bred animals freeze least and vocalize most. (6) On the fear-retention test, the random-bred animals showed shorter latencies, spent less time in the black compartment and crossed more often. There were few differences between the Roman strains. This is in good agreement with the general finding that fearfulness is the crucial variable in determining behaviour on this test. (7) The duration of the extinction trials failed to have a systematic effect on the fear-retention measures. This failure to replicate findings using other paradigms may be due to the inappropriate nature of escape responses as fear measures. The much more frequent freezing responses of the Roman strains, as compared with the random-bred animals, would clearly work against the hypothesis when escape from the black compartment is used as the index of fearfulness. The study in general demonstrates the hypothesized relative independence of fearfulness from arousal and indicates that it is strain differences in fearfulness which determine very largely the pattern of reactivity shown by animals in the experimental situation.

In the step-down test, Maudsley emotionally reactive and nonreactive animals were used as well as a random-bred strain (RBS). The main results were as follows. (1) Reactives showed a higher defecation rate than random-bred animals and these, higher defecation than nonreactives. Random-bred animals showed a significant decrement in defecation on the second day. (2) There were no differences in rearing between the strains, thus demonstrating that the animals differed with respect to emotionality (fearfulness) but not with respect to arousal. (3) Freezing behaviour following shock showed differences between nonreactive, reactive, and random-bred animals with nonreactives having the highest freezing duration; the reasons for this curious inversion are not known, but a hypothesis has been suggested in the text. (4) There were no strain differences in defecation during conditioning, suggesting that such differences do not appear, following the UCS, but are a consequence of conditioning. (5) Step-down latencies during rest were longest for the reactives, sup-
porting this view. (6) During extinction trials, defecation scores ordered the strains reactive > random-bred > nonreactive; rates of defecation became more similar with increasing number of trials. (7) On the fear-retention test, longer extinction trials produce shorter step-down latencies. (8) Reactives show longer latencies and more attempts to step down; non reactives show less defecation. Following long extinction trials, reactives assume the same low level of fearfulness as do nonreactives and random-bred animals.

The main conclusion of the experiments reported here is that the basal level of emotionality (fearfulness) is a primary factor in the acquisition and extinction of fear responses and that this basal level interacts powerfully with the duration of extinction trials, in the sense that emotional animals show little extinction when trials are short, but do extinguish to the same level as non-emotional animals when trials are long. Thus we have good evidence of the relevance to fear extinction of genetic factors determining the level of emotional responsiveness, of duration of extinction trials and of the interaction of the two. This may be relevant to the practice of behaviour therapy, particularly the technique of "flooding" or fear response evocation with response prevention. As reviews by Staub (1968) and Woods (1974) make clear, there is much evidence to suggest that duration of extinction trials with human patients seem crucially relevant to outcome; short periods may produce incubation of fear (paradoxical enhancement), while long periods may produce extinction. These findings are in good agreement with our experimental conclusions, as well as with Eysenck's (1968) theory regarding the nature of this phenomenon.

Evidence for interaction between level of emotionality and duration of extinction trials in humans is quite non-rigorous and anecdotal, but clinical practice has suggested that in cases of neurosis presenting with very strong emotional reactions, flooding may be the more successful technique of treatment, while with less strong emotional reactions, desensitization might be preferred. This impression, purely subjective as it is, gains some strength from our results on interaction and it may be suggested that clinical trials comparing desensitization and flooding in patients showing different degrees of emotional reactivity might, with advantage, be undertaken. It would, of course, be unethical to vary length of extinction trials, as the evidence is already fairly convincing that short periods would not help the patient in the flooding procedure and might very well make him worse.

One last comment may perhaps be made with respect to animal experimentation in this field generally. Usually the experimenter decides ab initio on the measure to be used for his main effect, e.g., step-down latency or latency of crossing in our case. Our results suggest, and general observation confirms, that continuous surveillance and measurement of the animal's activities throughout the experiment may cast doubt on the wisdom of the choice made, and may contribute
important evidence towards a proper interpretation of the results achieved. Emotional activation can be shown by other types of behaviour than that originally selected, e.g., freezing, vocalization, rearing, etc., and some of these behaviours may be incompatible with that chosen for measurement. Freezing is clearly incompatible with crossing from one compartment to the other in the shuttle box. There are obvious difficulties in combining alternative behaviour patterns into a single index of "fearfulness," but it may be better to make such an attempt than to use a single measure which is not monotonically related to the underlying variable which one is attempting to index. Altogether, animal work is perhaps too much characterized by reliance on statistical procedures and neglect of observational ones. Our results forcefully suggest a return to more naturalistic types of observation, without neglecting the usual experimental safeguards.

Our results also suggest that the different responses to fear-producing situations may arrange themselves in a hierarchy, such that a certain type of reaction, e.g., crossing, may be produced at one level of fear, another response, e.g., freezing, at another level. There is little evidence in the literature on such hierarchical arrangements of responses, or on the possibility that these might differ from strain to strain. Research into this question would seem urgently required if the problems raised by the lack of monotonicity in measurement of fearfulness is ever to be solved. Criticisms by ethologists of experimental psychologists along these lines have often been repeated. They would seem to require an experimental answer.

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