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Rats' Reactions to a Predator: Modification by Chlordiazepoxide

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Rats were exposed to a natural predator (a ferret) in a runway. Flight and freezing defensive reactions were measured by recording location and time-sampled codes of behaviour categories. Chlordiazepoxide attenuated reactions to the predator as measured by the location variable, while the behaviour-observation data showed that the drug enhanced predator-induced immobility, but attenuated frequency of locomotion. This interaction between drug and type of species-specific defensive reaction may resolve contradictory findings on effects of Chlordiazepoxide on escape and avoidance of electric shock.

Blanchard and Blanchard (1971) studied the behaviour of rats exposed to a natural predator (a cat) rather than to a conventional unconditioned stimulus (US), electric shock. Impetus for this research has come from Bolles' trenchant criticisms of conventional avoidance experiments for ignoring ecological and biological variables, especially the role of species-specific defensive reactions (SSDRs). SSDRs are "innate defensive reactions which occur when animals encounter any new or sudden stimulus." (Bolles, 1970, p. 33). They are commonly flight, freezing or threat behaviours.

The natural predator technique has been used to study the ontogeny of defensive reactions in the rat (Bronstein & Hirsch, 1976), the effects of limbic lesions (Blanchard & Blanchard, 1972a, b; Kim, Kim, Kim, Kim, Chang, Kim, & Lee, 1971), and of the effects of the anticholinergic drug scopolamine (Plotnick, Mollenauer & Snyder, 1974; Mollenauer, Plotnick & Southwick, 1976) on rat defensive behaviours. The present experiment tested the effects of the benzodiazepine, Chlordiazepoxide, on rats reactions when exposed to a predator (a ferret). Chlordiazepoxide (Librium) is widely prescribed as an anti-anxiety agent (Clarke & del Guidice, 1970). It has been shown to attenuate fear as measured by conditioned suppression (Geller, Kulak & Seifter, 1962; Vogel, Beer & Clody, 1971) and avoidance of a negative conditioned stimulus (CS) (Kumar, 1971), but has been reported both to enhance (Sachs, Weingerten & Klein, 1966) and interfere with (Cicalia & Hartley, 1965) conditioning of shock escape and avoidance. Drug-induced changes in the SSDR repertoire may account for this discrepancy, by making the repertoire more or less compatible with the experimental task (Mollenauer et al., 1976).

Research using a natural predator as the aversive stimulus poses methodological difficulties which have led some researchers (Mollenauer, et al., 1976; Mollenauer, White, Plotnic & Tiffany, 1979) back to using a mechanical device in place of the predator animal. Blanchard used a feral cat, which remained immobile throughout the experiment, and his apparatus therefore did not need to provide for physical separation of rat and predator. Ferrets, in contrast, are generally active, and the rat requires physical

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protection from predation. In the control condition, (i.e. no predator) Blanchard used an empty apparatus. This poses the difficulty that the rats' behaviour in the predator condition may be due to the novelty of the predator as a stimulus. Finding a stimulus in the control condition which matches the predator along dimensions of novelty, complexity and activity, but not in the predator dimension is difficult, if not impossible. Here we used an unfamiliar conspecific.

Method

Subjects

The subjects were 48 New Zealand Hooded and 48 Wistar/Sprague Dawley rats, approximately 100 days old, equally divided between males and females (mean weights 384 and 234 gm respectively). They were housed three or four to a group, with ad lib food and water, on a reversed day-night cycle, and were experimentally and predator naive.

The predator was a young female ferret *(Mustela putorius),* laboratory reared in quarters entirely separate from the rats. In control conditions, the place of the predator was taken by a non-experimental rat of the same strain, age and sex as the particular experimental subject.

Apparatus

Two identical wooden enclosures, each 60 x 60 x 30 cm, with a perspex roof, were used as arenas for the predator and the predator-control rat respectively.

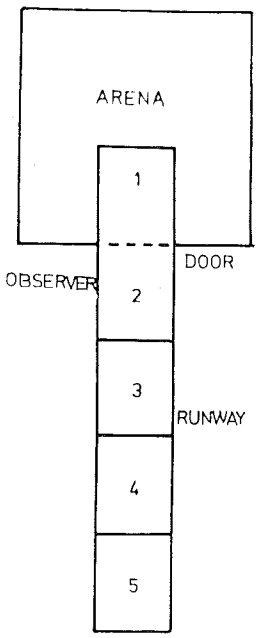

Figure 1. The arena and runway.

Table 1
The Categories of General Behaviour

Name	Abbr.	Definition
Grooming	(G)	Rat licks, scratches, or cleans any part of its body.
Locomotion	(L)	Rat moves on all four limbs — includes walking or running but not moving solely on rear legs.
Rearing	(R)	Rat raises itself so that it is supported by its hind legs only.
Sniffing	(S)	Rapid movement of the whiskers, usually accompanied by nose twitching, neck stretching, and the sound of sniffing.
Immobility	(I)	Complete cessation of all movement except for the whiskers.
Freezing	(F)	Complete cessation of all movement except for movements associated with breathing.
Approach-Avoidance	(A-A)	Rat stands over a hurdle making moves to enter another cell and then withdrawing. This 'hovering' is usually done with the front portion of the body. Recorded if animal is withdrawing immediately after attempting to enter a new cell.

Attached to one wall of the arena there was a perspex enclosure 20 x 25 x 18 cm, slotted at intervals over its front wall. This enclosure (cell 1) was separated by a guillotine door from a metal runway, 20 x 100 x 30 cm. The floor of the runway was divided into four equal areas (called cells 2 to 5) by 1 cm high wooden hurdles. A floor plan of the arena and runway is shown in Figure 1 . The arena and the runway were each illuminated by a 22 watt fluorescent lamp, suspended approximately 1 m above the floor, and white noise (approximately 80 dB) masked extraneous sounds. The experimenter sat on a stool at the junction of arena and runway to make observations, which were cued by a brief auditory signal emitted every four seconds by a timer.

Procedure

Each group of cage-mates was randomly assigned to an experimental condition, and the experimental conditions were run in random order. Testing each group took two days . Day 1 was used for habituation to the apparatus by an8-min confinement to the runway, with the arena empty. On Day 2, the ferret or the control rat was placed in the arena at least 30 min before the testing began. The subjects were weighed, then injected i.p. with either Chlordiazepoxide (4mg/kg), or an equivalent volume of distilled water. This dose of Chlordiazepoxide was selected following previous work in this laboratory (Hughes, 1972) which found ataxia in some rats at 5 mg/kg, and consequent interference with mobility. After 30 min in the home cage, the subject was placed in the perspex enclosure, and confined for 30 sec. The guillotine door was then raised, the latency to emerge into the runway was recorded, and for the next 8 min the animal's location (cell 1 to 5) and behaviour category (Table 1) was recorded every 4 sec. General activity was measured by counting each hurdle crossing on a hand counter, and recording the total every 40 sec.

Results

At the end of each observation period, 14 scores were calculated for each subject: latency to leave Cell 1 ; and frequencies of observation in five runway locations, and seven behaviour categories. (A table of within-cells means and standard deviations is available from the second author.) All variables were analyzed by a multi-variate analysis of variance (MANOVA). Setting criterion at $p < 0.01$, the main effects of Strain, Predator, Drug, and the interactions Predator x Drug, and Strain x Gender x Drug were significant. The Gender main effect was not significant, and in the data presented below, with one exception, data were collapsed over the sex variable. Neither latency, nor lines crossed contributed significantly to the multivariate discrimination between groups, and these variables are not presented further. Three behaviours, freezing, grooming,and approach-avoidance occured very infrequently, and the measures were collapsed under Other behaviour (Table 2).

Table 2 (section A) shows the differences which contributed to the significant multivariate Strain main effect. Compared with hooded rats, Wistars were more often observed to be either Immobile, or Locomoting, and less likely to be Rearing or Sniffing. This pattern of strain differences persisted over Drug and Predator conditions.

Reactions to the predator

Figure 2 shows the frequency with which subjects were observed to be in different locations in the runway (Cell 1 was the closest to the arena), as a function of the Predator variable. The standardized discriminant function equation for the Strain main effect showed very low weightings on the location variates, so in Figure 2, data were collapsed across Strain as well as Gender.

When there was no predator in the arena, Cell 1 was occupied most frequently, Cell 2 less frequently, and Cells 3 to 5 infrequently. When the predator was present, rats were much more likely to be in Cell 2 or Cell 5 than in Cell 1.

Section B of Table 2 shows the frequency of observation of the behaviour categories, with data collapsed over Gender and Strain. The frequencies of Locomotion and Immobility increased when the predator was present, while Rearing was suppressed. Sniffing was largely unaffected.

Table 2

Mean frequencies of observation of Immobility (I), Locomotion (L), Rearing (R), Sniffing (S), and Other Behaviour (O).

	I	L	R	S	O
A					
Wistar	23	38	24	30	5
Hooded	7	32	32	42	7
B					
No drug/ No predator	6	31	40	37	6
Predator Alone	15	40	18	40	7
Drug Alone	9	33	38	35	5
Drug/Predator	28	37	18	31	6

Effects of Chlordiazepoxide

The effect of Chlordiazepoxide on location is shown in Figure 2. The drug had a negligible effect when the predator was absent. When the predator was present, however, drugged rats were more likely to be in Cell 1, and less likely to be in Cell 5 than non-drugged rats. They still, however, preferred Cell 2, as did the non-drugged rats.

As Table 2 Section B shows, drugged rats not exposed to the predator had a higher frequency of Immobility than in the control condition (no drug, no predator). Otherwise, the drug alone had little effect. Drugged rats exposed to the predator showed the highest frequency of Immobility, while the frequency of Locomotion was intermediate between control and predator-alone conditions, i.e., there was a slight attenuation of the predator-induced increase in locomotion. Rearing was not affected by the drug alone, and the drug did not alter the suppression of Rearing when the predator was present. Sniffing was suppressed by the drug, and the drug plus predator.

The Strain x Predator x Drug interaction appears to be due to greater reactivity of Wistar subjects, especially Wistar males, to the drug, on a number of measures, of which Locomotion was significant by univariate F test. Wistar rats locomoted more frequently than Hooded rats, (Table 2A) and Chlordiazepoxide had little effect on Locomotion in females of either strain, (it increased the mean score by 1 in both

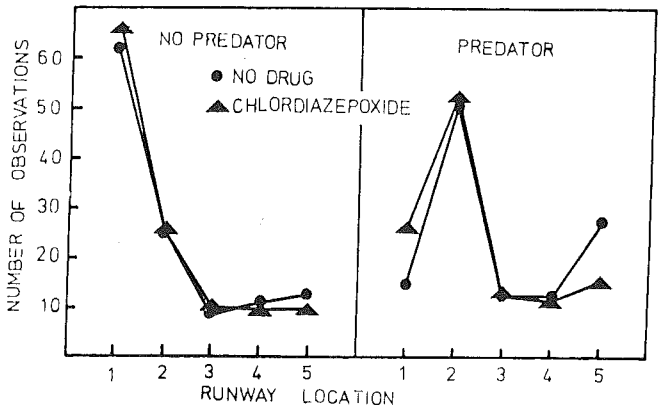

Figure 2. Location in the runway in drug and no-drug conditions when the predator was present and when another rat was present in the arena.

groups) but the drug increased Locomotion in Hooded males (from a mean of 31 to 39), but suppressed it in Wistar males, reducing their mean score from 44 to 33.

Temporal trends. Temporal trends over the 8-min observation period were examined by dividing each score (except latency) into three scores, representing successive 160 sec observation periods. Locomotion and Lines Crossed decreased with time, but the second MANOVA showed no significant interactions of time with other variables: the data reported above would have changed little if a shorter observation period had been used.

Discussion

When placed in close proximity to a predator, rats' behaviour was different from the behaviour of rats in the control condition. Chlordiazepoxide modified the subjects' reactions to the predator. Can these changes in behavior be related to SSDRs? SSDR theory would predict freezing and/or flight to be the most probable responses when the predator was nearby (Bolles, 1970). There is little consistency in the definition of freezing in the literature, some authors adopting a very stringent criterion for the cessation of activity (Curti, 1935), while others infer freezing from locomotion scores (Blanchard & Blanchard, 1971). We differentiated freezing from immobility, and found that freezing occurred with negligible frequency. The frequency of freezing is known to be reduced by the availability of an escape route, the subjects' familiarity with the apparatus, and a highly discriminable threat stimulus, (Blanchard & Blanchard, 1969; Blanchard, Fukunaga & Blanchard, 1976) and these conditions were met in our study. The related behaviour, Immobility, while never occurring with more than moderate frequency, did contribute significantly to the measured differences between Strain, Predator and Drug conditions. Immobility was increased by both the drug and the predator, and this was additive, so that maximum Immobility was observed in the Predator plus Drug condition.

Of the potential measures of the flight SSDR, latency to leave Cell 1 decreased when the predator was present, but not significantly. Lines crossed, a measure of general activity, correlated positively with Locomotion ($r = 0.35$), and females had higher scores than males, agreeing with previous findings (Hughes, 1972) but these effects were not significant. Locomotion increased significantly when the predator was present, but the clearest effect of the predator was seen in the location data, the rats moving either to Cell 2 or to Cell 5. There was a significant correlation ($r = 0.37$) between location in Cell 2 and Immobility, suggesting that the Immobility response occurred after movement away from the predator. Chlordiazepoxide clearly attenuated these reactions to the predator.

Can these findings be related to the interpretation of Chlordiazepoxide's effect on instrumental performance in escape or avoidance situations? Immobility was increased by both the predator and the drug, and reached maximum frequency when both

variables were present. If this behaviour alone had been used to make inferences about fear, it could have been concluded that Chlordiazepoxide increased fear. In experimental situations in which freezing and immobility are very probable reactions, Chlordiazepoxide is likely to interfere with active escape and avoidance performance, because of its effect of increasing freezing and imobility. This was the case in the Cicalia and Hartley (1965) study, where intense, unavoidable shocks were used, and subjects had to run down a runway to escape. In contrast, in experiments in which flight reactions are prepotent, the locomotion data of the present study suggest that Chlordiazepoxide should not impair performance.

In the present study, Chlordiazepoxide had effects on location scores which were congruent with fear reduction, and did so at a lower dose than that typically reported as effective in studies using electric shock as the fear-eliciting stimulus. It provides a demonstration of the utility of the natural predator technique in the analysis of drug effects on avoidance, and also the value of using multiple measures of behaviour.

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