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The Effect of Diazepam on a Conditioned Emotional Response in the Rat[1]

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MÉTHOT, C. AND R. DEUTSCH. *The effect of diazepam on a conditioned emotional response in the rat.* PHARMACOL BIOCHEM BEHAV **20**(4)495–499, 1984.—Benzodiazepines have been shown to stimulate appetite and to affect behaviour in experimental situations that are viewed as providing a model of anxiety. However, the "anti-anxiety" effect is normally demonstrated in situations that use food to motivate behaviour. In the present research, the effect of diazepam was tested on the suppression of drinking brought about by a tone that had been previously paired with shock. Diazepam produced a marked decrease in the amount of time it took the animal to resume drinking, thus providing support for the anti-anxiety effect. Both acute and chronic modes of administration were effective, but the effect was even more clear-cut with chronic administration.

Diazepam Benzodiazepines CER Fear Anxiety

BENZODIAZEPINES, including diazepam (DZ) and chlordiazepoxide (CDP), have been found to act as anticonvulsants and muscle relaxants, as well as having a variety of behavioural effects, including appetite-stimulation, and reduction of conflict behaviour in mice, rats, cats, and monkeys [2, 26, 31, 37]. These early findings gave rise to at least two major bodies of research: that pertaining to the appetite-stimulating effect and to the so-called anxiolytic or "anti-anxiety" effect. The present research is concerned with the possible confounding between these two actions. It will be argued on the basis of the research surveyed that benzodiazepines appear to have a direct or "primary" effect [7] on feeding but not on drinking. Experimental results will then be presented supporting an anxiolytic action of benzodiazepines using a procedure unconfounded by the appetite-stimulating action.

Primary or direct effects of benzodiazepines on feeding have been defined as involving, for example, a change in appetite, taste preference and satiety "as opposed to secondary or indirect effects on feeding which derive from the drugs' capacity to attenuate fear, anxiety or avoidance response" ([7], p. 14). Benzodiazepines induce feeding in both satiated [4, 13, 21, 23, 24, 36] and food-deprived animals [18, 23, 29, 35] in both familiar [9, 23, 24, 29, 36] and unfamiliar [10, 12, 29, 36] testing situations, during both day and night time [36], with familiar [7, 9, 10, 16, 24, 36] and novel [9, 16, 19, 25] foods of solid [9, 10, 12, 13, 16, 18, 27, 29, 36] and liquid [19, 21, 25, 34] form. Finally, benzodiazepine-induced feeding bears a noticeable, although incomplete, resemblance to deprivation-induced feeding. Cooper [8] and others [10, 12, 29, 35] have shown that, in both cases, the latency to eat is reduced, the total feeding duration is extended, and there is a selective increase in the time devoted to eating familiar food. Wise and Dawson [36] found that satiated rats, having learned to lever press for food under conditions of normal food deprivation, will do so under DZ in a dose-related manner. They also found that, like deprivation-induced feeding, feeding induced by DZ responds to post-ingestional factors signalling satiety, in that it is inhibited by stomach loads of food but not by sham or water loads. On the other hand, it appears that not all the effects of food deprivation are mimicked by DZ: rats given DZ do not show an increase in general activity, do not readily learn to lever press for food, and the learning that does take place does not transfer well to a food-deprived condition [36].

Although the effect of benzodiazepines on drinking has not been studied as exhaustively, evidence suggests that benzodiazepines do not have a direct effect on this behaviour. Specifically, these drugs have not been found to induce drinking, as they do feeding, in non-deprived animals [8,13]. Thus, non-deprived rats, under DZ, will lever press for food but not for water, although the same rats will lever press for water when water-deprived [36]. Soubrie, Angelis, Simon, and Boissier [30] did report an increase in time spent drinking in water deprived rats, but there was no significant effect on drinking latency (water intake data were not provided). The finding that benzodiazepines shorten feeding latency, but have no effect on, or actually increase, drinking latency, has been confirmed by others [11]. This set of findings suggests that, in marked contrast to the effect of benzodiazepines on feeding, their effect on drinking is not direct and is quite different from "natural" drinking.

Numerous approaches have been used to show that benzodiazepines affect the behaviour of animals in experimental situations that can be viewed as models or analogues of human anxiety. One popular technique is the punishment discrimination procedure developed by Geller and Seifter

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[17]. This procedure consists of stabilizing lever pressing responses on a VI schedule, and then introducing a CRF schedule, signalled by a tone, where each response is both rewarded with food and punished with shock. Rats treated with benzodiazepines will tolerate more shocks than controls; these results have been interpreted as being due to disinhibition of a previously-suppressed behaviour. Benzodiazepines have also been found to reduce the time to return to an interrupted behaviour in a conditioned emotional response (CER) paradigm [32], increase resistance to extinction on a CRF schedule [14], accelerate the extinction of a conditioned taste aversion [5], reduce food [19,25] or container [33] neophobia, reduce fear present in an open field [3], reinstate the level of lever pressing decreased by electric shock [1] or by shifting rats from a concentrated to a dilute sucrose reward [34], and increase tail-pinch-induced eating [27]. These results were seen as consistent with an interpretation of decreased anxiety or fear present in the situation.

Although food was used in all of these studies, the effect of benzodiazepines on food intake was specifically tested in only two of them. Johnson [19] found that while "naive" (first exposure to milk) rats injected with DZ drank significantly more sweet condensed milk than saline-injected controls, DZ had no effect on intakes of "trained" (familiarized) rats. However, the trained rats were selected on the basis of their substantial milk intake (11.5 ml), so the failure of DZ to increase intake may be attributed to a ceiling effect. Britton and Britton [3] observed that rats treated with DZ consumed more food than controls in an open-field test, but did not differ from controls when tested in the home cage. A ceiling effect is likely to have occurred in this case as well, since home cage controls ate approximately 3.4 grams, and the highest intake for any group under any condition was 4.2 grams. Drug-treated rats would have had to eat very large amounts in order for the difference to be statistically significant. Although it is possible that, as the authors argue, the effect of DZ on food intake in the open field indeed represents an anxiolytic effect, the fact that DZ has a clear effect on appetite in a wide variety of situations makes this argument less than completely convincing.

The general conclusion that emerges from a review of these studies is that interpretations based on anxiolytic effects remain problematic because of the effects of benzodiazepines on food intake. The present research was concerned with testing the anxiolytic action of DZ in a procedure that does not use food. This is in marked contrast to studies such as those of Tenen [32], which used a drinking response, but the fluid was a sucrose solution, and can thus be classified as "liquid food." In addition, acute administration of DZ was compared to chronic administration. This allows comparison of the depressant (i.e., sedative) and disinhibitory (i.e., anti-anxiety) effects of DZ on behaviour, since it has been shown [22,36] that the sedative effect develops tolerance with repeated administration. Thus, the sedative effect may interfere with demonstration of the anti-anxiety effect when the drug is administered acutely, but should no longer do so when chronic administration is used.

METHOD

Animals

Thirty-six male, experimentally naive, Wistar albino rats served as subjects. They were housed individually in a colony room on a 12:12 hour light-dark cycle with light on at 7:00 a.m., and were on ad lib food and water, unless indicated otherwise. The amount of water consumed was measured each day.

Apparatus

Two plastic Skinner boxes, fitted inside standard Grason-Stadler rat chambers, were placed in a room adjoining that containing the programming and monitoring equipment, hence isolated from external stimuli. The box used for conditioning was connected to a Grason-Stadler shock generator set at an intensity of 1.6 mA and a duration of 0.5 seconds. The other box, used for testing, differed from the first in that a water bottle could be attached at the front of the plastic door, 3.5 cm above the grid floor. A running time meter indicated the time the rat spent touching the spout. These contacts were registered on a pen-recorder where the onset and offset of a tone stimulus, produced by a Foringer click generator controlled by a clock, and the occurrence of each 0.5 second, were also marked. It was therefore possible to measure accurately the time elapsing between an animal stopping and resuming its drinking behaviour (recovery time).

Procedure

The procedure used in this investigation was Tenen's [32] CER paradigm, in which recovery time, defined as the time it takes the animal to resume an interrupted task, is used as the measure of CER strength. The shock level (1.6 mA instead of 1.5 mA) and the intertrial period (6 minutes instead of 45 seconds) were the only modifications to the procedure. The intertrial period had to be lengthened to accomodate the control procedure where tone and shock were explicitly unpaired (EU Control procedure). Results from a pilot study, using these 2 new parameters, replicated those obtained by Tenen, in that a CER group had significantly shorter recovery times than Backward Conditioning, Shock Only, or EU control groups, $F(3,15)=4.26$; $p<0.02$.

Rats were assigned to six groups: three were CER trained and three were in the EU Control procedure. The three groups in each condition were distinguished by the injections they were to receive: Chronic DZ, Acute DZ, or Saline.

The experiment was conducted in three phases:

1. Injection only. For 22 days, rats were injected with DZ suspended in propylene glycol (5 mg/ml) (Chronic groups, 2.5 mg/kg IP) or physiological saline (Acute and Saline groups, equal volume of 0.85% NaCl). The DZ dose, the same as that used by Wise and Dawson [36], is a rather low one, thus making it more likely that the "characteristic features" of the drug's effect will be shown [37]. On the last day, the animals were put on a water deprivation schedule, with a 30 min/day access to water, until the end of the experiment.

2. CER training and injection. On Day 23, each rat was placed in the testing chamber until it had accumulated 20 seconds of drinking time. It was then transferred to the shock chamber where the CER and EU subjects received differential treatments. After one minute had elapsed, the CER animals were presented with a tone (CS) for 10 seconds. During the last 0.5 seconds, an electric shock (US) of 1.6 mA was administered. The animals were then left undisturbed for 5 minutes and 50 seconds, for a total trial duration of 6 minutes. Each animal was subjected to three trials. For

the EU animals, the times at which the CS or the US was to be presented were randomly selected, with the constraint that the stimuli would not be presented within 2 minutes of each other. After the CER/EU trials, each rat was injected (Chronic groups with DZ, Acute and NaCl groups with NaCl), returned to the home cage, and allowed 30 minutes access to water.

On Day 24, all rats participated in two sessions of 20 seconds' drinking time, followed by the appropriate injection and access to water.

3. Injection and test. The next 12 days were designated test days. On these days, each rat received an injection (DZ for the Chronic and—for the first time—the Acute groups, NaCl for the Saline groups) and allowed to accumulate 20 seconds of drinking time. The CS was then turned on, and the time to resume drinking after the onset of the tone (recovery time) was measured. The US was not presented on any of the test days.

Data Analysis

The analyses of variance reported are 2×3 factorial univariate analyses with one repeated measure, where the F-ratio is calculated using Wilk's lambda criterion. The two between factors were: Conditioning, with 2 levels (CER and EU), and Drug, with 3 levels (Acute, Chronic and Saline). The repeated measures factor was days of testing. Differences between groups were assessed using orthogonal comparisons.

RESULTS

Water Intake

Additional support for DZ not having a direct effect on drinking was provided by the finding of no significant differences in water intake between groups on any of the Injection Only days ($p>0.1$).

Conditioning and Drug Effects

Both the Drug, $F(2,30)=46.54$, $p<0.001$, and the Conditioning, $F(1,30)=67.45$, $p<0.001$, main effects were highly significant. The interaction was also highly significant, $F(2,30)=24.54$, $p<0.001$; this interaction may be interpreted by looking at the between-group comparisons in detail (see Fig. 1).

The conditioning procedure was effective in producing a suppression of drinking, as shown by the longer recovery times apparent in the Saline-CER group compared to those from the Saline-EU group over the 12 days of testing, $F(1,30)=108.46$; $p<0.001$. DZ significantly reduced the strength of the CER: rats from both Chronic-CER and Acute-CER groups displayed shorter recovery times than those in the Saline-CER group, $F(1,30)=137.31$; $p<0.001$. Chronic administration of DZ proved to be more effective in reducing the consequences of the tone-shock contingency; this is shown by the fact that the Acute-CER group differed significantly from the Acute-EU control, $F(1,30)=6.47$; $p<0.01$, whereas the Chronic-CER group did not differ from the Chronic-EU group, $F(1,30)=1.61$; n.s.

Finally, the significant effect of the repeated measures factor, $F(5,30)=34.68$; $p<0.001$, indicates a general decrease in recovery time with repeated testing, as expected.

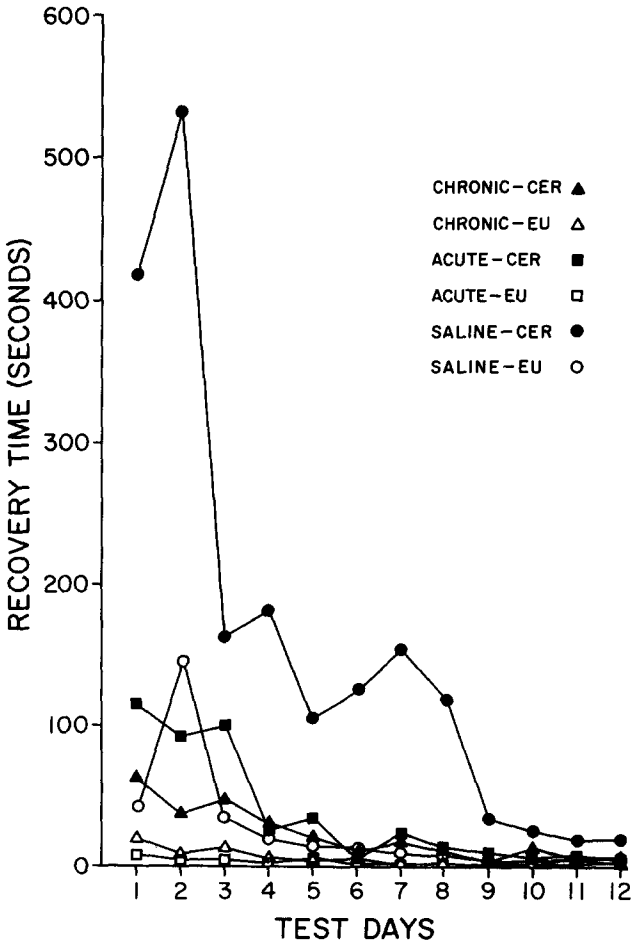

FIG. 1. Recovery Time. Mean group performance for each test day.

DISCUSSION

It has been suggested that the response-releasing properties of benzodiazepines should be tested in operant situations using rewards other than food [36], in order to eliminate the possible confounding with the appetite-stimulating effect of benzodiazepines. The dependent variable in the research reported here was recovery time: the length of time the subject interrupted its drinking upon presentation of a fear stimulus. Whereas benzodiazepines have a direct effect on feeding, there is no comparable effect on drinking. Recovery time of drinking has been shown to be a sensitive measure of conditioned emotional response or fear [32]. This was confirmed in the present study, as evidenced by the longer recovery times of the Saline-CER animals compared to those in the Saline-EU group.

The hypothesis that DZ has anti-anxiety action [21,22] was strongly supported by the present results. Thus, DZ greatly attenuated the conditioned fear response, as shown by the fact that rats from both Drug-CER groups had significantly shorter recovery times than those in the Saline-CER group.

An alternative to the hypothesis that benzodiazepines have an anti-anxiety effect is the view that they produce

response perseveration, which can be traced back to an interference of the drug with the mechanisms controlling the processing of the response-associated cues but not with those controlling the processing of stimuli scheduled independently of the subject's behaviour. For example, Dantzer [15] argues that, in a punishment situation, the dominant cues (lever, food cup) are associated with food and that their presence is response-independent. Shock, on the other hand, is response-dependent, and benzodiazepines are viewed as interfering with the processing of response-dependent cues.

Although this hypothesis can account for behaviour in the punishment situation, and for the fact that benzodiazepines are more effective on behaviour suppressed by contingent shock than noncontingent shock [20], it fails to account for results where benzodiazepines appear to interfere with the processing of response-independent cues [10, 13, 36]. Thus, in the present research, the tone-shock pairings occurred in a situation different from the one in which drinking was trained, and shock was delivered independently of the animal's behaviour. If DZ affects only the processing of response-dependent cues, it should not have affected the fear-eliciting property of the CS. Contrary to this prediction, recovery times were much shorter in the drug-injected groups than in the Saline-CER group.

Chronic administration of DZ was compared to acute administration to evaluate the possible role of tolerance. Since the sedative effect of benzodiazepines has been shown to undergo tolerance, whereas other effects, such as disinhibition or appetite-stimulation, do not [23,36], chronic administration of DZ provides a way of "unmasking" effects that are resistant to tolerance. In the present research, both chronic and acute administration reduced the affect of the tone-shock contingency, but chronic administration was more effective than acute, in that recovery times in the Chronic-CER group were not significantly different from those in the Chronic-EU control, whereas the Acute-CER group had significantly longer recovery times than the Acute-EU control. Thus, chronic administration of DZ demonstrates the anti-anxiety effect more clearly, but the fact that the difference between the Chronic-CER and Acute-CER groups was abolished by the fourth test day suggests that tolerance to the sedative effect in this situation develops quite rapidly.

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