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Effects of Chlordiazepoxide on Tail Pinch-Induced Eating in Rats

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ROBBINS, T. W., A. G. PHILLIPS AND B. J. SAHAKIAN. *Effects of chlordiazepoxide on tail pinch-induced eating in rats.* PHARMAC. BIOCHEM. BEHAV. 6(3) 297–302, 1977. – The effects of 5, 10, and 20 mg/kg chlordiazepoxide on tail pinch (TP)-induced behavior were investigated. Five mg/kg enhanced TP-induced eating in terms of both latency and duration. Twenty mg/kg had decremental effects. All doses of the drug reduced the incidence of clip-directed behavior, but increased locomotor activity during the TP trials in a dose-dependent manner. On control trials, the drug increased locomotor activity at the low dose and eating at the high dose. The results are examined in terms of the various behavioral actions of the minor tranquilizers. The implications for the behavioral and neuropharmacological mechanisms underlying TP-induced and other forms of stimulus-bound behavior are discussed.

Tail pinch-induced eating Stress Chlordiazepoxide

TAIL-PINCH (TP)-induced eating in sated rats is a reliable phenomenon, which resembles natural feeding, and which has been compared to eating produced by electrical stimulation of the brain (ESB) [1]. TP-induced eating probably arises from a heightened responsiveness to environmental stimuli caused by a mild, nonspecific stressor [2]. The response does not appear to depend on the activation of pain mechanisms since it is best obtained by applying a pinch of minimal intensity [1,2].

It remains uncertain to what extent ESB-induced eating arises from the stimulation of neural circuitry specifically involved in the regulation of food intake [20,30] as opposed to the activation of a nonspecific mechanism, sensitive to environmental contingencies, and similar to that advanced to explain TP-induced behavior above [27,28]. The relative role of these factors in the regulation of the normal feeding pattern also remains to be determined.

Minor tranquilizing drugs of the benzodiazepine class, such as diazepam, have characteristic effects upon both natural feeding [19] and ESB-induced eating [25]. For example, diazepam increases eating in sated rats, an effect attributable to a specific effect of the drug on food intake mechanisms [31]. Diazepam also enhances ESB-induced eating, both by lowering the threshold in eaters and by producing stimulus-bound eating in noneaters [25]. These effects have been ascribed to an anti-anxiety action of the drug which is hypothesised to attenuate emotional responses which may accompany ESB and be incompatible with eating [30].

This study sought to extend the parallels existing between ESB-induced and TP-induced behavior [1, 12, 27], by studying the effects on TP-induced responses of another drug of the benzodiazepine class, chlordiazepoxide. It has recently been suggested that TP-induced and ESB-induced behaviors might be related to amphetamine-induced stereotypy [2,27], since, in addition to various behavioral similarities, all of these behaviors are mediated in part by the nigro-striatal dopamine (DA) system [2, 6, 18]. TP-induced behavior and stereotyped behavior are, however, affected by modulatory influences of other neurotransmitter systems [2, 8, 23]. Therefore it was hypothesised that chlordiazepoxide, which affects both the catecholaminergic [26] and serotonergic [29] neurotransmitter systems, and which facilitates amphetamine-induced stereotypy [3], as well as ESB-induced eating [25], would also have a modulatory influence on TP-induced eating.

METHOD

Animals

Thirty six male hooded rats (Animal Suppliers), 50 days of age at the beginning of the experiment, were used. These were housed in cages of four and supplied with food and water ad lib. The rats had been accustomed to the laboratory for a two week period and were well-handled for several days prior to the experiment.

Apparatus and Procedure

TP testing was carried out in a dimly illuminated open field (78 × 78 × 43 cm), with a grid of 25 squares painted

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on the floor, which was littered with food pellets. Each rat was given two TP trials separated by 0.5 hr on each of two successive days. Each TP trial was immediately preceded by a one minute control trial, on which no TP stimulus was applied. The TP stimulus was a paper clip (7.3 × 1.8 cm), padded with electrical masking tape and adjusted to exert a constant mild pressure. It was applied approximately 3.8 cm from the tip of the tail. This form of stimulation permitted freedom of movement in the open field.

Behavior was monitored by two observers with a six pen event recorder, which registered the latency and duration of eating as well as other oral behaviors, such as gnawing and licking and clip-directed behavior. Gnawing and licking of the clip were included in the latter category. Locomotor activity was measured by the frequency of whole-body entries into the different squares of the open field. To avoid error and ambiguous cases, latency was measured of the first bout of eating $\geq$4 sec in duration. The TP trial continued for 60 sec, after the initial latency. In this way, measurements of latency and duration of eating remained unconfounded.

The drug used was chlordiazepoxide hydrochloride (Librium, Roche), dissolved in 0.9% saline. Injections were made intraperitoneally 30 min prior to the 1st test trial, on each of the two test days. Twelve rats received 0.9% saline control injections, 12 rats received 5 mg/kg, 6 rats received 10 mg/kg and 6 rats 20 mg/kg chlordiazepoxide. All testing was conducted on a blind basis and occurred during the light portion of the animals' light-dark cycle (light 8.00–20.00 hr.).

RESULTS

Since the data generally did not meet the requirements for parametric tests, the nonparametric Kruskal-Wallis one way analysis of variance [24] was employed in preliminary tests, on median data obtained on the 4 trials for individual animals. This was followed by planned, orthogonal Mann-Whitney U tests [24] between the various drug doses of chlordiazepoxide and the control group.

Control Trial

Spontaneous eating. No spontaneous eating by rats treated with saline was observed. Low doses of chlordiazepoxide induced sporadic eating in some rats (see Table 1). However, there was a pronounced increase in spontaneous eating seen after 20 mg/kg (Fig. 1). The duration of eating was significantly enhanced (Kruskal-Wallis, χ^2 = 13.76, $p<0.01$, 0 vs 20 mg/kg, U = 6, $p<0.02$). This was despite an evident ataxia in the rats treated with 20 mg/kg. The animals generally would show a degree of unco-ordinated locomotor activity, followed by a still, hunched posture often in the centre of the field. The eating observed was not a vigorous response, but was manifested by the slow nibbling of small fragments of food pellets. Licking of urine and wet faeces also occurred.

TABLE 1

INCIDENCE OF SPONTANEOUS EATING, AND TP-INDUCED BEHAVIORS

Trial	Behavior	Dose MG/KG Chlordiazepoxide	Frequency of Animals Exhibiting Behavior (Number of Times Behavior Shown/4 Trials)					
			0/4	1/4	2/4	3/4	4/4	TOTAL
Control	Spontaneous Eating	0	12	0	0	0	0	12
		5	8	4	0	0	0	12
		10	4	1	1	0	0	6
		20	0	1	1	2	2	6
TP	TP-Induced Eating							
		0	1	0	3	2	6	12
		5	0	1	0	2	9	12
		10	0	1	2	2	1	6
		20	2	2	1	1	0	6
TP	Clip-Directed Behavior							
		0	0	0	0	4	8	12
		5	0	2	2	6	2	12
		10	0	1	1	2	2	6
		20	0	1	2	3	0	6
TP	TP-Induced Licking							
		0	5	5	2	0	0	12
		5	11	1	0	0	0	12
		10	5	1	0	0	0	6
		20	3	3	0	0	0	6

Notes: Incidence rates based on following minimal durations: (1) TP-induced and spontaneous eating $\geq$4 sec. (2) Clip-directed behavior $\geq$1 sec. (3) TP-induced licking $\geq$1 sec.

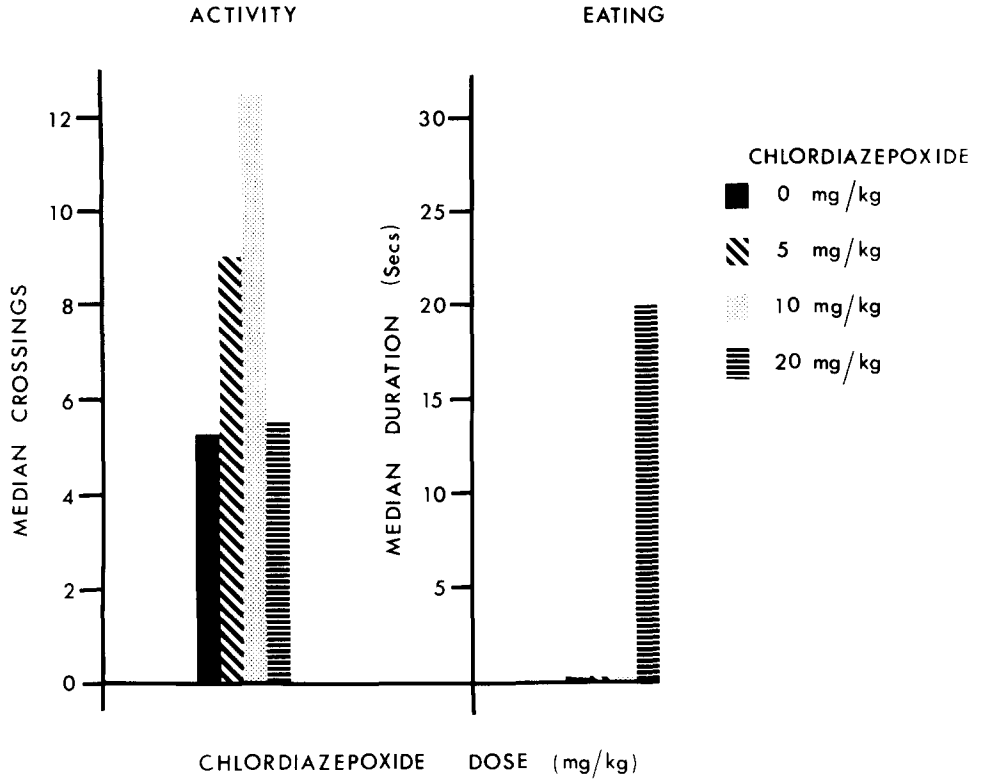

FIG. 1. Effects of chlordiazepoxide on locomotor activity and eating on control trials. Data are expressed as medians of median scores collected over four trials for individual animals.

Locomotor activity. There was a small stimulatory effect on locomotor activity by low doses of chlordiazepoxide (χ^2 = 8.18, $p<0.05$) (see Fig. 1). The increase produced by 5 mg/kg was significant (U = 29, $p<0.02$), but mainly present after Trial 1 (see Table 2). It was interesting to observe that 20 mg/kg only produced a marked decrement in activity on the first two control trials, despite the ataxia it produced in all of the rats.

TP Trials

TP-eating: latency. Chlordiazepoxide had a biphasic effect on eating latency (χ^2 = 13.07, $p<0.01$) (Fig. 2). Five mg/kg produced a significant shortening of the latency (U = 33.5, $p<0.05$), whereas 20 mg/kg produced a significant delay in the initiation of the response (U = 9, $p<0.02$). The effects of 10 mg/kg of the drug were not significant (U = 29), presumably as a reflection of the opposing actions of the 5 and 20 mg/kg doses. For this dose level, it is interesting that, whereas on the first day (Trials 1–2) it had effects similar to those of 20 mg/kg, on the second day they were similar to those at 5 mg/kg. As in previous work [12,22], a distinct reduction of latency was evident over successive trials, (Table 2).

TP-eating: duration. The effects of chlordiazepoxide on eating duration paralleled those on eating latency (χ^2 = 14.81, $p<0.01$) (see Fig. 2). Five mg/kg significantly increased the duration of eating (U = 23.5, $p<0.02$), whereas 20 mg/kg significantly reduced it (U = 13, $p<0.05$), and 10 mg/kg had no effect (U = 29, $p>0.05$). Again increases in duration of the response were evident over successive trials for the 0, 5 and 10 mg/kg groups, with the exception of Trial 2 for the 0 mg/kg group and Trial 4 for the 10 mg/kg group (Table 2).

Clip-directed behavior. There were usually several attempts made by the animals to displace the clip from their tails especially on initial trials (Table 2). The median durations, in seconds, of clip-directed behavior are given in Fig. 2. There was a marked reduction of clip-directed behavior produced by all doses of chlordiazepoxide, (χ^2 = 10.75, $p<0.02$), 5 mg/kg and 20 mg/kg of the drug significantly reduced the duration of the behavior (U = 21.5, $p<0.002$ and U = 12, $p<0.05$, respectively), but the effects at 10 mg/kg just failed to attain significance (U = 14.5, $p>0.05$).

TP-induced locomotor activity. Chlordiazepoxide affected locomotor activity on TP trials in ways markedly different to its effects on control trials (Fig. 2). There was a monotonic increase in locomotor activity of increasing doses of the drug (χ^2 = 14.87, $p<0.01$). Ten and 20 mg/kg significantly increased activity (U = 5, $p<0.02$, and U = 4.5, $p<0.02$, respectively). Locomotor activity was generally greater during control trials, except at the 20 mg/kg dose level (Table 2).

General Behavioral Observations

TP elicited a number of other behavioral patterns apart from eating, although this was by far the most prevalent response. Licking, of urine, or faeces, was observed (Table 1) and tail-preening was noted on five occasions. Bouts of vocalisation occurred on 6 trials (5 rats), at 0 mg/kg, and

TABLE 2

MEDIAN SCORES FOR BEHAVIORS IN CONTROL + TP TRIALS OVER TRIALS 1–4

Behavior	Dose MG/KG Chlordiazepoxide	Trial 1	2	3	4
Control Trials					
Eating	0	0.0	0.0	0.0	0.0
Duration	5	0.0	0.0	0.0	0.0
(secs)	10	0.0	0.0	0.0	0.0
	20	0.0	19.2	19.8	25.8
Locomotor	0	32.0	6.0	3.5	1.5
Activity	5	34.0	11.0	7.0	2.0
(Crossings)	10	32.5	12.0	12.0	3.0
	20	12.0	2.5	9.0	0.0
TP Trials					
Eating	0	17.4	7.5	23.4	37.2
Duration	5	45.0	44.4	52.8	48.6
(secs)	10	0.0	27.6	38.4	16.8
	20	0.0	3.0	0.0	0.0
Eating	0	47.5	40.5	24.0	22.0
Latency	5	39.5	20.0	11.5	4.0
(secs)	10	60.0	40.5	24.5	10.0
	20	60.0	58.0	60.0	58.0
Locomotor	0	3.0	4.5	4.0	4.5
Activity	5	9.5	3.5	2.0	3.5
(Crossings)	10	34.5	1.5	3.0	1.0
	20	27.0	11.5	27.5	11.0
Clip-Directed	0	16.2	15.6	9.0	9.6
Behavior	5	7.8	7.2	1.8	0.0
(Duration;	10	3.0	19.2	10.2	3.6
secs)	20	0.0	0.0	4.2	7.2

also on 6 trials (4 rats), at 5 mg/kg, but there was only one case at each of the 10 or 20 mg/kg doses.

Locomotion was inhibited during TP trials after Trial 1 at 0 and 5 mg/kg. The stimulation of locomotor activity seen at the 10 and 20 mg/kg doses was remarkable since the animals had clearly lost the coordination of forwards progression. The drugged rats were splayed in posture bilaterally, as they lurched forwards in uncoordinated but continual movement. Backwards locomotion was seen once and apparently spontaneous jumping on three occasions.

The eating response itself appeared to be similar to natural feeding; the animals generally held pieces of food between their forepaws and nibbled and ingested small fragments. Shredding or gnawing of the food pellet, and coprophagia were occasionally observed.

Eating at the 0 mg/kg dose level tended to be divided into a few bouts (median 3.5). The 5 mg/kg dose level produced a small reduction in this fractionation of response (median = 2.5 bouts; U = 33, $p<0.05$). On the far fewer occasions when TP-induced eating was elicited at higher dose levels, the behavior also tended to be fractionated (10 mg/kg; median = 3.0 bouts; 20 mg/kg; median = 3.75 bouts). However, whereas the fractionation of eating at the lower dose levels was generally produced by tail-directed behaviors or licking, that at the higher doses was the result of hyperactivity.

Finally, whatever the form of the response induced by TP, it was generally highly idiosyncratic for each animal, and was frequently reproduced on successive trials. In this sense it resembled the rigid behaviors resulting from ESB-induced eating that are described by Wise [30].

DISCUSSION

Chlordiazepoxide had several clear effects on TP-induced behavior, depending on dose. The low dose (5 mg/kg) enhanced TP-induced eating, as measured either by latency or duration of response. The high dose (20 mg/kg) had converse effects, reducing TP-induced eating, whereas 10 mg/kg had effects intermediate between these.

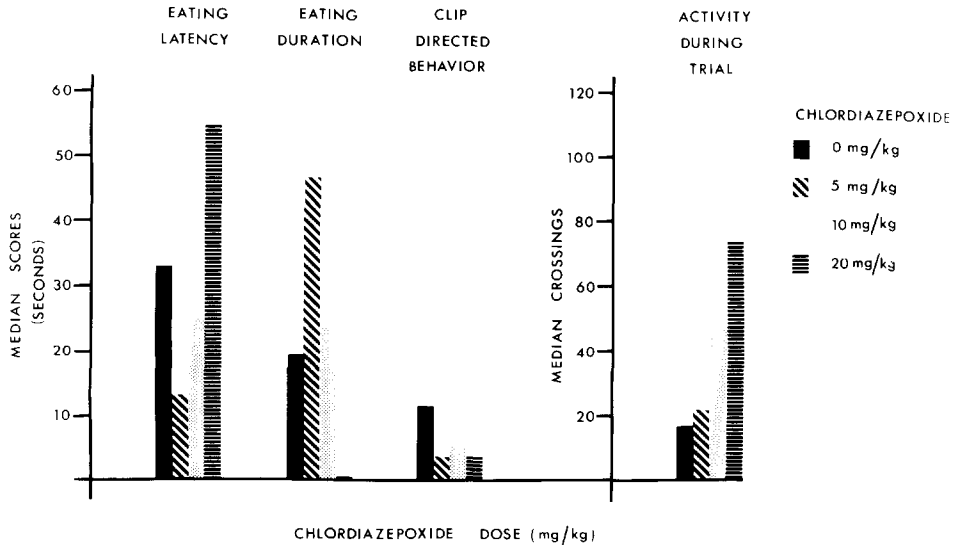

FIG. 2. Effects of chlordiazepoxide on behavior elicited by TP. Data are expressed as medians of median data collected over four trials for individual animals.

These effects of chlordiazepoxide could result from several of the controversial and diverse behavioral actions of the drug. Chlordiazepoxide has been reported to produce sedation and ataxia [15], stimulate appetite [19], increase low rates of responding perhaps by response disinhibition, [15], and alleviate anxiety, both clinically [11] and in animal experiments involving conflict situations [15]. There is also evidence that the benzodiazepine class of drugs can antagonize the physiological manifestations of "stress" [4, 5, 7, 9, 10, 13, 14], although it is unclear how this effect is related to the psychological actions of the drug.

The facilitatory effect of the low (5 mg/kg) dose of chlordiazepoxide on TP-induced eating is analogous to the effects of diazepam on TP-induced eating of novel food [21]. The present effect is unlikely to have resulted from sedative, ataxic or appetite stimulatory properties of chlordiazepoxide as none of these effects were observed on control trials. Two alternative hypotheses are advanced to account for the facilitatory effect.

The first hypothesis suggests that chlordiazepoxide attenuated aversive correlates of TP stimulation. Although TP stimulation elicited reliable feeding behavior, it also had mildly aversive correlates in this study, since clip-directed behaviors and fractionation of the eating pattern were often observed. By eliminating emotional responses incompatible with eating, a facilitatory effect on ingestive behavior was produced. This explanation is similar to that advanced to explain the facilitatory effects of diazepam on ESB-induced eating [25] and chlordiazepoxide on brain stimulation reward [16,17].

However, it appears that emotional behavior is not always incompatible with TP-induced behavior, as treatment with FLA-63 causes an increase in vocalization and other signs of distress while at the same time enhancing TP-induced eating [2]. A second hypothesis would attribute the facilitatory actions of chlordiazepoxide to a non-specific facilitatory effect on responding, unrelated to an attenuation of aversive effects [15].

An important aspect of the present results is the decremental effect of the high dose (20 mg/kg) of chlordiazepoxide on TP-induced feeding, particularly as it was accompanied by a remarkable stimulation of locomotor activity during the TP trial. It is unclear whether this switch in responding represents an increase or decrease in TP-effects. It is improbable that the inhibitory effects on eating resulted from sedation or ataxia, since there was a strong stimulation of eating observed during the control trial at this dose level, in agreement with earlier results [19]. The suppressant effect on TP-eating would also thus appear to be dissociated from the appetite stimulatory action of the drug. Valenstein [28] has suggested that both ESB and TP-induced behaviors may be representative of a class of stress induced coping responses. The reduction in TP-induced feeding could then be hypothesized to result from an attenuation of stress by the high dose of chlordiazepoxide. This interpretation is supported by findings that stress induced either by electroshock or by immobilisation, causes an increase in cortical NA turnover [5,26], an effect also observed following TP [2]. Chlordiazepoxide, in doses of 10 mg/kg and greater, has been found to antagonise stress-induced elevations of noradrenaline turnover [5,26]. Such an effect would also be consistent with the reductions in clip-directed behavior and vocalisation observed at this dose level, although it would not, by itself, explain the locomotor stimulatory action.

Further elucidation of these actions may require neuropharmacological analyses. TP-induced oral behavior depends on the integrity of the nigro-striatal (DA) system [2]. However, since TP itself does not increase DA turnover, it has been suggested that the behavior is also controlled by changes in other neurotransmitter systems which modulate the nigro-striatal DA projection [2]. This view is supported by the finding that FLA-63, an inhibitor of noradrenaline (NA) synthesis, facilitates TP-induced eating [2]. The benzodiazepine drugs may exert their effects on TP-induced eating by similar influences. These drugs, including chlordiazepoxide, reduce the turnover of brain DA and NA [26] as well as of serotonin [29]. Moreover, benzodiazepines can facilitate amphetamine-induced stereotyped behavior [3], as well as ESB-induced eating [25], both behaviors, like TP-induced eating, being dependent on the intact functioning of the nigro-striatal (DA) system [3,18]. The similar effects of benzodiazepines on amphetamine-induced stereotyped behavior and these two forms of stimulus-bound behavior support the notion that these activities may be mediated in part by common mechanisms, resulting from analogous behavioral antecedents [1, 2, 12, 27].

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