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Tail Pinch Induces Eating in Sated Rats Which Appears to Depend on Nigrostriatal Dopamine

Abstract. *Mild tail pinch reliably and rapidly induced eating, gnawing, or licking behavior in all animals tested. Eating was by far the predominant response. Pharmacological analysis of the involvement of the brain catecholamines in tail-pinch behavior suggests that it is critically dependent on the nigrostriatal dopamine system.*

Studies in recent years have demonstrated that peripherally applied stressful stimulation, for example, electric shock to various parts of a rat's body, can reliably induce aggression or copulatory behavior, or both, depending on stimulus conditions (*1*). We report here that a similar stimulus, such as tail pinch, induces eating in sated rats when food pellets are present. Gnawing and licking are also observed, but these behaviors occur much less frequently than does eating.

Eating induced by mild tail pinch typically appears to be identical to normal eating. Immediately after pressure is applied to the tail, the animal begins to sniff and explore its environment for a few seconds. A food pellet is then picked up and held between the forepaws, and the animal begins to bite the pellet and chew. During a sustained pinch, animals pause and swallow quite normally between bites, and eating behavior is almost invariably maintained for the duration of the pinch. Relatively little spillage of food is seen in the tail pinch situation as the animals typically show unhurried consumption of a single pellet. In some animals, however, the pinch appears to represent a more stressful stimulus (as indicated by vocalization), and these animals often move from pellet to pellet and may spill or shred some of the food.

Tail pinch–induced consummatory behavior is an exceptionally reliable phenomenon, having been rapidly and repeatedly demonstrated in every animal in this study. This behavior does not appear to be causally dependent on the activation of pain mechanisms, as it can be reliably induced by applying a pinch of minimal intensity which does not produce vocalization. Since the brain catecholamines, norepinephrine (NE), and dopamine (DA) have frequently been linked to the control of eating (*2*), we examined their respective roles relative to tail pinch–induced eating. Our findings indicate that eating, as well as the other consummatory behaviors that we observed during tail pinch, is critically dependent on brain DA.

Male albino rats (weighing 250 to 350 g) were purchased from a number of suppliers (Marland Farms, Blue Spruce, Holtzman, and Zivic-Miller) to ensure that the tail-pinch phenomenon was not restricted to specific breeding conditions or populations. Rats were housed in pairs and maintained on a natural day-night cycle with food and water freely available. Testing was done during the daytime in shallow bowls 34.3 to 44.5 cm in diameter; each bowl contained six to ten pellets of Purina rat chow. A surgical hemostat, 25 cm long and insulated at the tips with foam rubber, was used for tail pinch. Testing consisted of five 20-second, predrug screening trials, each separated by 5 to 8 minutes, and, after an appropriate interval, five trials after the drug (or vehicle) treatment. All animals demonstrated eating, gnawing, or licking (hereafter referred to as tail-pinch behavior) within 20 seconds on 98 to 100 percent of both predrug and vehicle trials. All statistical comparisons are between drugs and vehicles.

Our initial experiment sought to determine the effects on tail-pinch behavior of pharmacological blockade of both brain NE and brain DA receptors. Haloperidol was chosen for this condition as it is known to antagonize both NE and DA receptors at moderate doses (*3, 4*). Doses of 0.2 and 0.4 mg/kg significantly blocked tail-pinch behavior on 44 and 52 percent of the total trials, respectively (Table 1); a dose of 0.1 mg/kg was ineffective in preventing this behavior. The blocking effect could not be attributed to nonspecific debilitation since the animals vocalized and moved about the testing chambers in an alert manner. During those trials in which tail-pinch behavior was initiated, it was typically maintained until the hemostat was removed. Nevertheless, animals treated with drugs at all doses showed significantly longer latencies to begin tail-pinch behavior than those receiving the vehicle (vehicle median, 2 seconds; 0.1 mg/kg median, 4 seconds; 0.2 mg/kg median, 10 seconds; 0.4 mg/kg median, 7.5 seconds; $P < .0005$ in all cases, U-test).

Since the results with haloperidol suggested catecholamine involvement in tail-pinch behavior, we attempted to parcel out effects that may have been due primarily to the action of NE or DA. We proceeded first by measuring the effect of the specific DA-receptor blocking agents, spiroperidol and pimozide (*3*), on tail-pinch behavior. Each of these agents significantly blocked the display of this behavior (spiroperidol: $F = 14.35$; d.f. = 3,19; $P < .01$; pimozide: $F = 4.09$; d.f. = 3,17; $P < .05$). As shown in Table 1, spiroperidol significantly blocked tail pinch–induced consummatory behavior on 50 percent of all trials at a dose of 0.125 mg/kg and virtually eliminated tail-pinch behavior at a dose of 0.25 mg/kg. The 0.062 mg/kg dose was without effect.

Pimozide, at 1 and 2 mg/kg, significantly reduced the display of tail-pinch behavior to 60 percent of the trials, whereas a dose of 0.5 mg/kg had no significant blocking action. During those trials in which tail-pinch behavior did occur, latencies were significantly extended after 0.125 mg of spiroperidol per kilogram (vehicle median, 3 seconds; 0.125 mg/kg median, 17 seconds; $P < .0005$, U-test) and all doses of pimozide (vehicle median, 2 seconds; 0.5 mg/kg median, 5 seconds; 1 mg/kg median, 12 seconds; 2 mg/kg median, 8 seconds; $P < .0005$ in all cases, U-test).

Spiroperidol and pimozide produced a moderate degree of ptosis in most, but not all, animals tested. There was no correlation, however, between the appearance of ptosis and blockade of tail-pinch behavior. Discontinuous lurching movements, observed with each drug, were more pronounced after spiroperidol and may be related to the extrapyramidal side effects ob-

served when this drug is given to schizophrenics (*5*). Each drug also produced a marked increase in the intensity of vocalization, but only during trials in which other oral behaviors were blocked. Since vocalization is one index of arousal, blockade of tail-pinch behavior is probably not related to a deficit in arousal, since, if anything, the animals appeared more aroused than normal. A similar dissociation between arousal or activation and the performance of consummatory behaviors has recently been reported (*6*).

These data imply that brain DA receptors may be critically involved in expression of the syndrome of eating, gnawing, and licking that occurs after tail pinch. Thus the wide safety margin between doses of spiroperidol effective in blocking tail-pinch behavior and those required to block NE receptors (*3*) argues against the possibility that spiroperidol antagonism of NE receptors can account for the results obtained. Moreover, the finding that pimozide at a dose of 2 mg/kg is no more effective in blocking tail-pinch behavior than a dose of 1 mg/kg also argues against a critical role for NE in tail-pinch behavior, since at doses above 1 mg/kg, pimozide is thought to antagonize both brain NE and brain DA receptors (*3*).

The possible role of brain NE receptors in tail-pinch behavior was directly examined by using α- and β-NE-receptor antagonists. Phentolamine, the α-blocking agent selected, is a potent competitive antagonist of brain NE, with no apparent action on DA receptors (*7*). Sotalol, the β-antagonist employed, is a potent blocker with considerably less local anesthetic properties than other such agents (*8*).

Blockade of brain NE receptors, with either α- or β-antagonists, had no effect whatever on either the induction of tail-pinch behavior or on latencies to induction (Table 1). The drug dosages employed in the present experiment were intentionally high and should therefore constitute a decisive test of any major involvement of NE in tail-pinch behavior. Despite their failure to interfere with tail-pinch behavior, the higher doses of both phentolamine and sotalol induced catalepsy, piloerection, hunched back, and ptosis between trials. These symptoms were especially marked with phentolamine, since ptosis continued and was even accentuated during tail pinch. Taken together with the findings reported above, these results suggest rather strongly that tail-pinch behavior is critically dependent on DA and not on NE. They do not, however, provide more than a hint that the particular DA neurons involved might be those of the nigrostriatal bundle (NSB).

In order to study the role of the NSB in tail-pinch behavior directly, we lesioned this bundle by intracerebral injections of 6-hydroxydopamine (6-OHDA) (*9, 10*) and tested the animals at different stages after the lesion (*10*). Injections of 6-OHDA significantly reduced the display of tail-pinch behavior ($F = 9.52$; d.f. = 3,22; $P < .01$). The group receiving its first tail pinch test after drug treatment at 27 hours after the lesion failed to initiate tail-pinch behavior on 33 percent of the trials (Table 1). This relatively modest effect on tail-pinch behavior was anticipated since 27 hours corresponds to the time of degeneration-induced release of DA (*10*). However, if tail-pinch behavior were critically dependent on the integrity of the NSB, it should have been severely impaired in animals tested at 48 hours after 6-OHDA. By that time all DA is thought to be gone from the striatum and there is a corresponding disappearance of DA storage granules (*10*). Nevertheless, the initiation of tail-pinch behavior was blocked on only 44 percent of the total trials when animals received their first trial after drug treatment at 48 hours (Table 1). Although the extent of blockade is statistically significant (Table 1) and comparable to that obtained with most of our pharmacological manipulations, our biochemical finding of a near total depletion of caudate DA (93 ± 3 percent) (*11*) would have led us to predict a greater behavioral deficit. These data suggest two possibilities: (i) DA fibers other than those of the nigrostriatal bundle may be critical for the initiation of tail-pinch behavior, or (ii) a few remaining DA fibers interacting with supersensitive DA receptors in the caudate may have been sufficient to allow the initiation of tail-pinch behavior on a substantial percentage of trials. The plausibility of the latter alternative is suggested by results obtained in a third group of animals tested initially at 48 hours after 6-OHDA with a pretreatment of 0.1 mg of haloperidol per kilogram. This dose of haloperidol, although it exerted no blocking effect on tail-pinch behavior in neurologically intact rats (Table 1), should be more effective in antagonizing supersensitive caudate receptors in denervated animals. In fact, although none of the animals tested at 48 hours after receiving 6-OHDA showed a complete blockade of tail-pinch behavior, three of five animals receiving the additional pretreatment with haloperidol showed a complete abolition of this behavior (Table 1). A similar effect was obtained in two of three animals in the 27-hour group when they were retested at

Table 1. The effects of pharmacological interference with brain DA or NE function, or both, on initiation and maintenance of tail-pinch behavior (TPB). Maintenance refers to TPB that is sustained until removal of hemostat from the animal's tail. *N* is the number of animals; the number of trials is $N \times 5$; S.E.M., standard error of the mean.

Treatment	Dose (mg/kg)	*N*	Trials on which TPB was initiated (mean % ± S.E.M.)	Trials on which TPB was maintained (No. positive/ No. tested)
Haloperidol	Vehicle	5	100 ± 0	25/25
	0.1	18	98 ± 2	87/88
	0.2	5	56 ± 15*†	14/14
	0.4	8	48 ± 10‡	12/19
Spiroperidol	Vehicle	5	100 ± 0	23/25
	0.062	5	88 ± 12	21/22
	0.125	8	50 ± 11‡	14/20
	0.250	5	12 ± 5‡	2/3
Pimozide	Vehicle	5	100 ± 0	25/25
	0.5	4	85 ± 15	16/17
	1.0	7	60 ± 12§	19/21
	2.0	5	60 ± 13§	9/15
Phentolamine	5.0	4	100 ± 0	20/20
	10.0	4	100 ± 0	19/20
	20.0	3	100 ± 0	14/15
Sotalol	20.0	4	95 ± 5	16/19
	40.0	4	100 ± 0	18/20
	80.0	4	100 ± 0	18/20
6-OHDA	Vehicle	13	98 ± 2	62/64
+ 27 hours	8 μg/4 μl	3	67 ± 10§	5/10
+ 48 hours	8 μg/4 μl	5	56 ± 13†	6/14
+ 48 hours and 0.1 mg/kg of haloperidol	8 μg/4 μl	5	40 ± 21‡	4/10
+ 27 hours and 48 hours	8 μg/4 μl	3	27 ± 27	1/4

*T-comparison with vehicle controls following one-way analysis of variance. For the analysis the percentages were subjected to an arc-sine square root transformation. †$P < .01$. ‡$P < .001$. §$P < .05$.

48 hours after 6-OHDA. These results suggest that blockade of supersensitive DA receptors, or exhaustion of the small amount of functional DA remaining in the striatum after the lesion, do indeed severely impair tail-pinch behavior in NSB lesioned animals. Furthermore, although NSB lesions did not always have a severe effect on the initiation of tail-pinch behavior (for example, in the 48-hour group) they did have a marked effect on the maintenance of this behavior (see Table 1). That is to say, in contrast to either vehicle-treated controls or animals receiving DA-receptor antagonists, animals with NSB lesions very often failed to maintain tail-pinch behavior until removal of the hemostat. Our results can be summarized as follows: (i) Mild tail pinch rapidly induced eating, gnawing, or licking in all animals tested, with eating the predominant response. (ii) Tail-pinch behavior appears to be critically dependent on the nigrostriatal DA system. This conclusion is further supported by our findings that both lesioning of terminal areas of the mesolimbic DA system and the administration of clozapine (a DA-receptor antagonist largely devoid of action on striatal receptors) failed to affect tail-pinch behavior (*12*).

In addition to eating, gnawing, and licking our laboratory has also demonstrated that tail pinch can reliably induce both drinking (of palatable fluids) and maternal behavior (*12*). The particular response pattern observed with tail pinch appears to be determined by the stimulus objects available in the environment and is always appropriate to those objects. Changing of available stimuli, for example, from food pellets to a drinking tube, produces an immediate "switch" in response, much as occurs during stimulus-bound behavior (*13*).

Overall, the tail pinch paradigm provides a new, unusually powerful, and especially simple tool for investigating the neural organization of behavior. The diversity of behaviors that can be induced by tail pinch suggests that the nigrostriatal DA system may be importantly involved in regulating the organisms' responsiveness to a wide variety of environmental stimuli.

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11. Animals were killed 24 hours following completion of the 48-hour tail pinch test. Caudate, hypothalamus, and neocortex were dissected from the brain of each animal and stored on Dry Ice for biochemical determination of catecholamines. Dopamine was measured in the caudate and NE in the hypothalamus and neocortex of six experimental (6-OHDA–treated) animals (animals pretreated with haloperidol were excluded from the analysis and two of the remaining eight animals died), and 17 controls (13 treated with vehicle, that is, 0.9 percent NaCl and 0.1 percent ascorbic acid, plus four uninjected controls), according to a modification of the fluorometric method of M. K. Shellenberger and J. H. Gordon [*Anal. Biochem.* **39**, 356 (1971)]. Assay values (in micrograms per gram) were as follows: hypothalamic NE: control, 1.66 ± 0.13; 6-OHDA, 0.94 ± 0.32; cortical NE: control, 0.26 ± 0.05; 6-OHDA, 0.20 ± 0.05; caudate DA: control, 7.73 ± 0.41; 6-OHDA, 0.57 ± 0.24. Values are uncorrected for recovery (DA, 84 percent; NE, 77 percent).
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