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Stress-Induced Hyperphagia and Obesity in Rats: A Possible Model for Understanding Human Obesity

Abstract. *Mild tail pinch administered to rats several times daily in the presence of sweetened milk induced immediate hyperphagia and led to considerable gain in body weight. Parallels are drawn with stress-induced hyperhagia and altered affective states in obese humans.*

Hyperphagia, and the obesity which typically accompanies it, is one of the major problems of modern society. Although the causal factors underlying hyperphagia are numerous, in many cases they appear to be related to stress (*1*). For example, many obese patients tend to eat when they are emotionally tense or during other unpleasant states, such as depression and boredom. Reports suggest that eating diminishes or prevents these states (*2*). Usually there is a well-defined pattern to the hyperphagia, and, in most cases, a definite finickiness exists. While bland foods are not eaten to excess, palatable foods stimulate hyperphagia (*1, 3*). When food is not readily available, such as when obese individuals are forced to adhere to strict dieting conditions, there is an enhanced reactivity to other goal objects; for example, there is a higher incidence of other oral activities such as smoking, and there is an increase in sexual activity (*1, 4*). Stress-related hyperphagia may therefore be a food-directed manifestation of a more general hyperresponsivity to environmental stimuli (*3*).

Recent research in this laboratory has shown that a mild nonspecific stress, tail pinch, reliably induces eating in sated rats (*5*). This behavior occurs with short latency, appears quite normal, and proceeds without obvious pain. Such animals are finicky, displaying an increased preference for highly palatable fluids and familiar foods (*6*).

The behavior observed during tail pinch is not limited to eating, but appears to be appropriate to the particular goal object at hand. For instance, if rat pups are present during tail pinch, maternal behavior occurs (*7*). A similar stimulus, tail shock, induces copulatory behavior in naive male rats in the presence of a receptive female, and aggression in the presence of another male (*8*). When all goal objects have been removed from the testing arena, the incidence of grooming (washing of the face and flanks) and nail pulling of the paws increases markedly. There also appears to be an increased tendency to vocalize and to attempt to escape, which suggests that the performance of a goal-directed behavior may mask the perception of aspects of the pinch related to stress.

There are some interesting, although indirect, parallels between eating in rats induced by tail pinch and hyperphagia in humans. Both may reflect a stress-related increase in responsiveness to environmental stimuli. This study provides evidence for a more direct parallel: in rats with access to a highly palatable fluid food, chronic stress induced by repeated tail pinch leads to dramatic hyperphagia, weight gain, and visible obesity.

Twenty-four adult female rats (Sprague-Dawley) were determined to readily ingest milk from a hand-held drinking burette during mild tail pinch. They were randomly assigned to one of three experimental conditions: (i) surgically intact ($N = 6$), (ii) ovariectomized ($N = 10$), or (iii) ovariectomized and injected daily with hormone replacement (1 μg of estradiol benzoate) ($N = 8$). Three animals from each of these conditions were assigned to the pinched group, and the rest served as weight-matched controls. Both groups were individually housed and allowed free access to sweetened milk (*9*) and tap water. In addition, the experimental animals received six daily pinch sessions (10 to 15 minutes each, spaced at equal intervals throughout the 24-hour cycle) in the presence of a hand-held burette containing milk (*10*) for up to five consecutive days. The tail was pinched between 1 and 2 inches from the tip with a hand-held, 25-cm hemostat, padded at the tips with foam rubber. Testing was conducted in a wire mesh cage 15 inches (38 cm) square.

Only slight pressure was required to induce ingestion of milk (it rarely approached the first notch of the hemostat) and infrequently resulted in any indication (for example, squealing) that the animals were attending to the pinch. Immediately after the beginning of the pinch, animals would explore the testing arena for a few seconds and then begin licking the milk tube. Ingestion usually proceeded continuously for several minutes at a time and

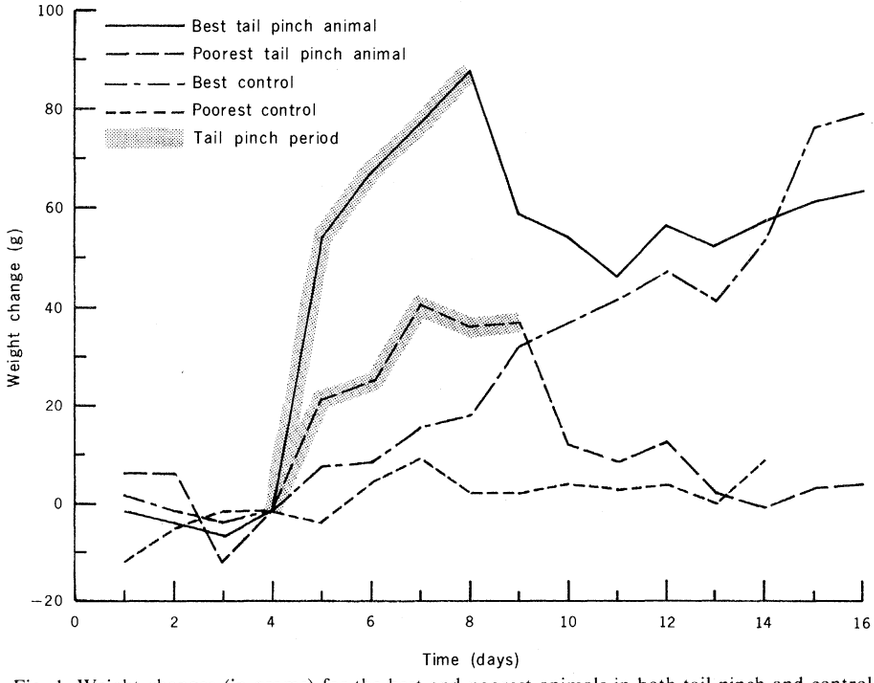

Fig. 1. Weight changes (in grams) for the best and poorest animals in both tail pinch and control groups. Data reflect comparisons before, during, and after the period of testing.

was punctuated by bouts of grooming. Frequency and duration of grooming tended to increase during a session as total intake increased. Sessions were terminated after an extended bout of grooming, or active rejection of the milk tube, or, by the experimenter, when a pause occurred after the animal had ingested 15 ml of milk.

All pinched animals became hyperphagic and obese. Although there were no differences in caloric intake between experimental and control groups before the procedure was begun (Table 1), during the experimental period all pinched groups combined showed a mean intake of 181 kcal in 24 hours in contrast to 71 kcal in 24 hours for the control groups.

As a consequence of this extreme hyperphagia, weight increased markedly and rapidly (Fig. 1). Mean daily weight gain during the experimental period for all experimental animals combined was 14.0 g (range, 7.8 to 22.5 g). This amounted to a mean gain of 71 g for the entire pinch period (range, 39 to 90 g) (*11*). These data stand in stark contrast to the 17 g (mean) gained by control animals during the same period (range, 4 to 34 g).

Toward the latter part of the experimental period, when obesity was clearly evident, pinched animals tended to become satiated earlier in a given session and were inclined to become agitated and to spill milk when the pinching persisted. Ingestion of milk in the home cage and body weight were monitored after cessation of the experimental treatment. The home cage intake, which had fallen to zero very early in the pinch phase, recovered within 2 to 3 days, and weight gradually fell to control levels. These results are in complete accord with those reported on obesity induced by electrical stimulation of the lateral hypothalamus (LH) (*12*) and suggest that tail pinch, like LH stimulation, does not abolish the ability to regulate weight but only temporarily overrides it.

Our results demonstrate for the first time that a chronic nonspecific stress is capable of inducing and sustaining clear-cut hyperphagia and weight gain in the rat. Moreover, the daily weight gain observed following the stress is at least comparable to that typically reported with either LH stimulation (*12*) or lesions in the region of the ventromedial nucleus of the hypothalamus (VMH) (*13*). The VMH syndrome has, to date, been considered the classical animal model of experimentally induced hyperphagia and obesity (*13*). However, since this syndrome results from deliberate brain damage (*1*) its usefulness as a model for human hyperphagia and obesity seems rather limited. Moreover, there is no evidence that hyperphagia which accompanies VMH lesions is stress-related, and

Table 1. Daily intake and daily and total changes in body weight for experimental (pinched) and control (unpinched) animals in periods before and during the tail-pinch experiment. Means, ranges (in parentheses), and percentage of change are shown. Abbreviations: Ovex, ovariectomized animals; Ovex-replaced, ovariectomized animals that received daily injections of 1 μg of estradiol benzoate; *N*, number of animals.

Surgical condition	*N*	Number of pinch sessions	Daily intake (kcal)			Daily weight change (g)			Total body weight (g)		
			Before experiment	During experiment	Change (%)	Before experiment	During experiment	Change (%)	Initial	Final	Change (%)
Intact		28 (26–32)									
Pinched	3		75 (64–80)	172 (155–190)	+129	+1.4 (−2.0–+6.7)	+10.8 (7.8–12.8)	+670	349 (305–392)	412 (344–475)	+18.0
Unpinched	3		62 (45–80)			+1.2 (−3.7–+6.7)	+2.9 (1.0–4.8)	+142	335 (304–362)	345 (308–386)	
Ovex		27 (22–30)									
Pinched	3		70 (43–88)	175 (160–200)	+150	+5.9 (+0.2–+11.5)	+14.8 (11.0–22.5)	+151	344 (280–404)	411 (335–494)	+19.5
Unpinched	7		71 (57–84)	76 (69–87)	+7	+4.2 (+1.0–+7.3)	+5.0 (2.4–6.8)	+19	307 (284–336)	330 (296–370)	+7.5
Ovex-replaced		30 (30–30)									
Pinched	3		64 (57–68)	195 (180–215)	+202	+0.2 (−0.3–+0.6)	+16.3 (14.4–17.2)	+805	295 (287–304)	377 (367–390)	+28.0
Unpinched	5		67 (46–80)	69 (48–85)	+3	+1.1 (−0.7–+2.3)	+2.5 (−0.2–+4.4)	+127	286 (276–303)	299 (288–312)	+4.5

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metabolic interpretations are currently favored (*13*). Considering the striking parallels which appear to exist between stressed rats and obese humans, we feel that stress-induced overeating might be a more realistic model system for the study of some kinds of nongenetic obesity in humans.

In conclusion, it should be mentioned that the nigrostriatal dopamine system appears essential for the manifestation of behavior stimulated by tail pinch (*5*). We have recently proposed that this system is indirectly activated during tail pinch, and that this activation results in an enhanced awareness of, and responsiveness to, environmental stimuli, with consequent induction of appropriate goal-directed behaviors (*5*). Chronic activation of this system could underlie stress-related hyperphagia in humans. To the extent that this formulation is correct, it may have important implications for the understanding and possible treatment of human eating disorders.

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10. In contrast to the readiness with which rats will consume milk under stress, drinking of water and licking at an empty spout have low probabilities of occurrence. Therefore, no water tube was used in this study.
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sen to de-emphasize differences between experimental groups. Our intention in using different stressed groups was to demonstrate the ubiquity of our phenomenon (which we have succeeded in doing) and its independence of the effects of ovarian hormones which are known to modulate food intake and body weight [G. N. Wade, *J. Comp. Physiol. Psychol.* **88**, 183 (1975)].
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Tracing Axons and Axon Collaterals of Spinal Neurons Using Intracellular Injection of Horseradish Peroxidase

Abstract. *Intracellular injection and subsequent histochemical localization of horseradish peroxidase have been used to stain the soma, dendrites, axons, and axon collaterals of spinocervical tract neurons and unidentified dorsal horn neurons in the cat. This technique may be used in combination with the intracellular injection of Procion yellow to demonstrate by light microscopy connections between physiologically typed vertebrate neurons.*

Techniques for staining neurons by the intracellular injection of dye through a microelectrode have made it possible to study morphological connections between physiologically identified neurons (*1*). Among the many substances that have been injected into nerve cells, cobaltous chloride and Procion yellow have provided the most complete picture of physiologically identified neurons. But, neither Procion yellow nor cobaltous chloride is ideal for studying the connections between vertebrate neurons. Their major disadvantage is that their rate of movement along axons appears to be positively related to the diameter of the processes (*2*). Although Procion yellow stains somata and dendrites, it has severe limitations for staining vertebrate axons and particularly axon collaterals (*3*, *4*).

To overcome the above difficulties we developed a technique in which we use orthograde axonal transport (*5*) followed by histochemical localization of intracellularly injected horseradish peroxidase (HRP). An advantage of using axonal transport is that there is evidence to suggest that substances are transported along axons at rates independent of the axonal diameter (*6*).

Experiments were performed on chloralose anesthetized cats (70 mg per kilogram of body weight) that were immobilized with gallamine triethiodide and given artificial respiration. Spinocervical tract neurons, or other dorsal horn neurons which received cutaneous inputs (*7*), were impaled with broken glass microelectrodes (*4*) that had been filled under pressure with 4 percent HRP (type VI, Sigma) in 0.05*M* tris-HCl buffer which contained 0.2 mole of KCl per liter (*p*H = 8.6). Successful penetrations were made with electrodes having a resistance of 5 to 20 megohm, which proved very stable for the passage of both depolarizing and hyperpolarizing currents. Horseradish peroxidase was passed from the microelectrodes by applying a depolarizing current of 20 to 40 na for 450 msec, every 600 msec. Neurons that received from 170 to 750 na/min were successfully filled with HRP. After the injection the animals survived for 4 to 9 hours.

Generally the soma-dendritic spike of impaled neurons decayed within the first minute after penetration. The presence of postsynaptic potentials from natural stimulation or electrical stimulation of cutaneous nerves or of the dorsal columns was used as an indication that the electrode was inside a cell. Postsynaptic potentials were usually negative during depolarizing pulses but positive during hyperpolarizing pulses. Furthermore, during hyperpolarizing pulses, spinocervical tract neurons showed an axon spike upon stimulation of the ipsilateral dorsolateral funiculus at the third cervical segment (*7*).

After several neurons were injected, the cats were perfused first with saline and then by 10 percent buffered formalin (*p*H 7.6) (*8*). The spinal cord was removed and stored for 3 days in cold (2°C) buffered formalin, washed in 0.1*M* phosphate buffer

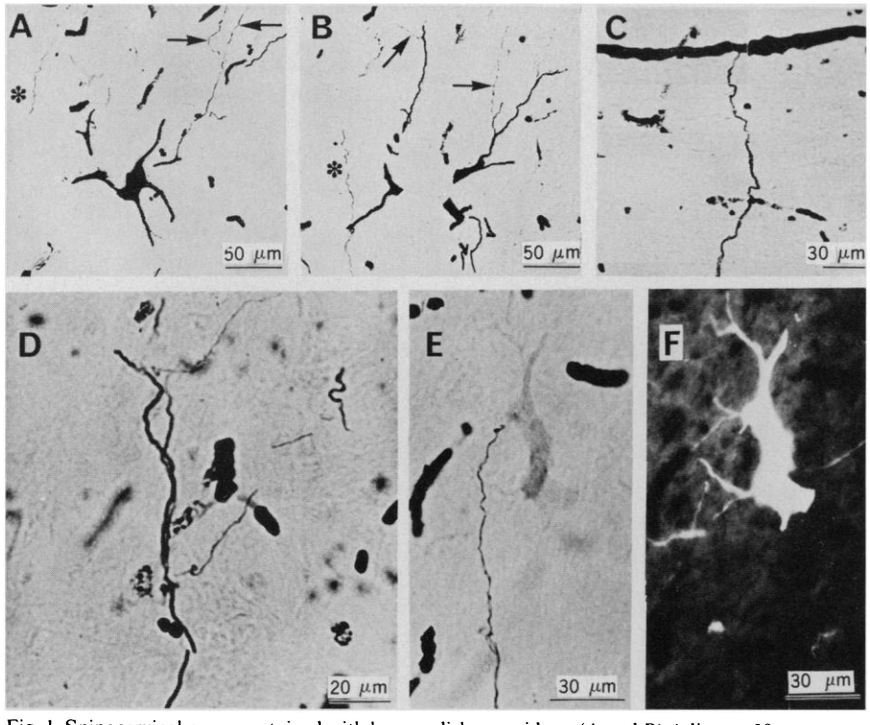

Fig. 1. Spinocervical neurons stained with horseradish peroxidase. (A and B) Adjacent 30-μm transverse sections through a spinocervical tract neuron (375 na/min). Before penetration, this neuron was excited by all hair types and by skin pressure on the lateral surface of the foot. Staining of fine dendrites is indicated by arrows, and the presence of axon collaterals is indicated by asterisks within the dendritic field of the neuron. (C) Axon of a spinocervical tract neuron running through the dorsolateral funiculus and dropping off a collateral 3.1 mm from the cell body. This plate is a montage built from ten adjacent, 30-μm sagittal sections. (D) Several fine dorsally directed collateral branches which could be traced back to the collateral shown in (C). These endings were situated in the gray matter 1.3 mm ventral to the parent axon. (E) A collateral from a spinocervical tract neuron ending on, or very close to, dorsal horn neuron injected with Procion yellow, as photographed with ordinary illumination. (F) The same neurons as in (E) but as photographed with fluorescent illumination. Dark bodies not associated with nervous elements are blood corpuscles stained by the incubation medium.