were due to conditioning by prior treatments, since the tumors of three previously untreated dogs showed extensive necrosis 12 hours after the PACC + CA regimen. Nor can the tumoricidal response be ascribed to release of protein A from the immunoadsorbent column, since there were no significant changes in radioactivity in the column before and after extracorporeal perfusion.

Augmentation of the tumoricidal response after the PACC + CA regimen may be related to observations in which CA, in the presence of TAA, exerted a synergistic inhibitory effect on tumor cell replication in tissue culture (*5*, *6*), perhaps due to antibody-enhanced phosphorylation and subsequent increased incorporation of the drug into the DNA of cell nuclei. The TAA, measured by radioimmunoassay, are now known to be activated in plasma after perfusion over PACC in vivo and in vitro (*3*, *4*). Indeed, canine IgG and C'3 were found deposited in tumor cell membranes 12 hours after treatment with PACC and PACC + CA. The distribution and concentration of these deposits were comparable after the two treatments, but light microscopy showed that necrosis was far greater after the PACC + CA treatment.

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Stress-Induced Eating Is Mediated Through Endogenous Opiates

Abstract. *The interaction of endogenous opiates and stress-induced eating in rats was evaluated by pharmacological manipulation. Eating induced by the tail-pinch method was inhibited by the opiate antagonist naloxone; after being repeatedly stressed over a 10-day period and then given naloxone, the rats behaved in a manner indistinguishable from the "wet-dog" shakes of opiate withdrawal. Thus endogenous opiates may have a role in the control of stress-related eating, a finding that may have therapeutic implications for humans.*

Mildly pinching the tails of rats reliably induces a syndrome of eating, gnawing, and licking in the presence of food (*1*). This response has interesting parallels with stress-induced eating in humans (*2*). The endorphins may be involved in the modulation of feeding drives in food-deprived rats (*3*) and in the development of obesity in genetically obese mice (*4*). In addition, there are striking pharmacological parallels between the behavioral response to tail pinching and schizophrenia (*5*), and a number of studies have suggested a role of endogenous opiates in the genesis of some types of schizophrenia (*6*). We report here that the opiate antagonist naloxone suppresses ingestive behavior without affecting gnawing or licking behavior in rats stressed by the tail-pinch method.

We modified the tail-pinch method by using a plastic hemostat (MacBick Co.), which gives better control of the range of pressures that can be exerted than a surgical hemostat. Testing was carried out in a 22 by 17 cm plastic box containing two pellets of Purina rat chow (6 to 9 g).

There are several fine points to the tail-pinch method. For example, gentle side-to-side oscillations of the hemostat may facilitate the onset of the oral behaviors. (In the majority of animals, the behaviors are induced before the onset of pain, as indicated by squeaking.) In addition, certain methodological precautions are necessary: (i) the hemostat and the hand of the investigator must be kept out of sight of the rats, (ii) the rat's tail must not become twisted, and (iii) the rat must be adapted to the experimental environment and should not be engaged in exploratory behavior at the time tail pinching begins. Provided these caveats are observed, oral behaviors can be reliably induced in all rats tested. However, only 72 percent of all the rats we have tested ate; 20 percent gnawed without ingestion and 8 percent merely licked. In general, once a rat has ingested food in one trial, it will eat during subsequent trials spaced 15 minutes apart.

In the experiment described here we used adult male Sprague-Dawley rats (175 to 225 g) that had not been deprived of food; all had been shown to ingest food during a 2-minute period of mild tail pinching. Naloxone (4 or 2 mg/kg, parenterally) decreased food intake, and this decrease was more marked 30 minutes than 15 minutes after the injection (Fig. 1). Naloxone did not suppress gnawing, which was present in all the naloxone-treated animals, including those that failed to ingest any food. A number of these rats demolished one or both of the pellets without ingesting any of them. Naloxone at 1 mg/kg did not significantly suppress eating, although two of the eight rats given this dose failed to ingest any food. At the highest dose of naloxone (4 mg/kg), a number of the rats squeaked at tail-pinch pressures below those necessary to induce eating, gnawing, or licking during the control trial. For this reason, we pinched these rats again (35 minutes after naloxone was given), using a pressure slightly less than that which elicited squeaking. This pressure was insufficient to induce the oral behaviors in these animals.

In contrast to the effects of naloxone, parenteral injections of saline tended to increase the amount of food ingested during the second and third trials (Table 1). To exclude the possibility that naloxone exerted its effect by decreasing the general arousal of the animal, we used diazepam (0.5 mg/kg) as a sedative control. Not only did diazepam fail to sup-

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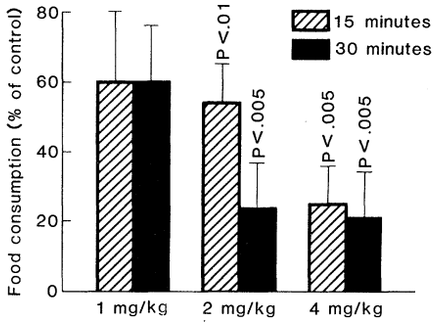

Fig. 1. Effect of parenteral administration of naloxone on tail-pinch-induced eating in rats. Tails were pinched mildly for 2 minutes after the onset of gnawing, and the amount of food consumed was determined by weighing the food pellets before and after this period. The amount consumed during the control period (2 minutes) was 0.64 ± 0.03 g (mean ± standard error). The rats then received a subcutaneous injection of saline or the drug. Tails were pinched mildly for 2 minutes at 15 and 30 minutes after the injection. The paired Student's *t*-test (two-tailed) was used to compare data for significance. There were eight animals in each group.

press food intake, in the first trial it actually increased food intake significantly. This is in keeping with previous reports that diazepam may act as an appetite stimulant (*7*).

To check our methodology, we administered the carboxyl terminal octapeptide of cholecystokinin (CCK) (5 μg/kg), which has been shown to suppress tail-pinch-induced eating in rats (*8*). Our results confirm the suppressive effect of CCK octapeptide on tail-pinch-induced ingestion, but, again, we could not discern any effect of CCK on gnawing. Antelman and Szechtman (*9*) demonstrated that tail-pinch-induced behavior is critically dependent on the integrity of the nigrostriatal dopamine system: a high dose of the dopamine antagonist haloperidol (2.5 mg/kg) totally suppressed both gnawing and eating. This suggests that dopamine antagonists may block eating behavior by suppressing gnawing, which is a prerequisite for ingestive behavior in the rat, whereas naloxone and CCK octapeptide appear to produce their effects by inducing satiety without inhibiting gnawing and licking.

Since intraventricular administration of β-endorphin induces eating (*10*), and since we found that naloxone inhibits tail-pinch-induced eating, it appears that stress-induced eating is primarily mediated through an increase in the levels of endogenous opiates. Because β-endorphin levels have been shown to be elevated in the pituitary of genetically obese mice (*4*), and since hypophysectomy alters the endorphin-mediated responses to acute and chronic pain (*11*), we examined the effects of tail pinching on six animals that had been hypophysectomized. Licking, gnawing, and eating were induced in all six animals (0.53 ± 0.13 g of food was ingested in 2 minutes). This excludes the pituitary from a role in the ingestive behavior that accompanies tail pinching.

Further evidence that the effects of stress-induced eating are mediated through the endogenous opiate system was obtained in an experiment in which we administered mild tail pinching several times a day for 10 days (*12*). Two hours after the last session we observed the behavior of the rats for 15 minutes and then administered naloxone (1 mg/kg, subcutaneously) and again observed behavior for 15 minutes. The behavior of these animals was compared with that of six animals whose tails had not been pinched. The animals that had been stressed all demonstrated behavior classically associated with opiate withdrawal. During the period after saline administration there was a marked increase in head shaking in the tail-pinched animals (12.3 ± 1.8 versus 1.5 ± 0.6 shakes; $P < .001$). After receiving naloxone, the tail-pinched animals demonstrated classical "wet-dog" withdrawal behavior, including body shakes (8 ± 2 shakes versus 0 in the controls), forepaw tremor, muscle twitching, and diarrhea. We suggest that repeated tail pinching activated the endogenous opioid system and that naloxone precipitated acute withdrawal from the animals' "self-addiction."

Although most authorities accept the relative specificity of naloxone (*13*), a few have suggested that at very high doses, the drug may have some nonspecific effects (*14*). The relatively high doses of naloxone used in this study were within the levels still considered by most to be specific for opiate receptor blockade (*15*). The use of higher doses than are necessary to reverse the effects of opiate administration is justified because the opiate alkaloids that produce major effects on the central nervous system require 40 to 60 times more naloxone for reversal than does morphine. Also, the endorphins require significantly more naloxone for reversal than equipotent doses of opiate alkaloids (*16*). Experiments in our laboratory have shown that intraventricular administration of the long-acting endogenous opiate analog D-Ala-Met-enkephalin ($10^{-7}M$) can partially reverse the suppressive effect of intraventricular thyrotropin-releasing hormone ($10^{-6}M$) on tail-pinch-induced ingestive behavior (65 ± 13 versus 13 ± 11 percent, $P < .001$). This strengthens the hypothesis that opiates are involved in stress-induced eating.

Table 1. Effect of parenteral administration of saline, CCK, haloperidol, and diazepam on tail-pinch-induced eating in rats. The experimental procedure is as described in the legend to Fig. 1. There were eight animals in each group.

Compound	Food consumption (percentage of control, g)	
	Fifteen minutes after injection	Thirty minutes after injection
Saline	170 ± 51	116 ± 13
CCK octapeptide (5 μg/kg)	45 ± 13*	32 ± 10†
Diazepam (0.5 mg/kg)	192 ± 37‡	164 ± 36
Haloperidol (2.5 mg/kg)	0†	0†

*$P < .01$. †$P < .005$. ‡$P < .025$.

In the tail-pinched rat, endogenous opiates may be released (due to the discomfort of the pinch) for their analgesic effect, with increased food ingestion being an ancillary effect. This would be compatible with the theory that stress-related hyperphagia is a food-directed manifestation of a more general hyperresponsivity to environmental stimuli (*17*). A less likely possibility is that the rat may eat because of some intrinsic analgesic action of the ingested food. Support for this possibility is provided by Zioudrou *et al.* (*18*), who found, in casein and gluten, "exorphins" that bind specifically to opiate receptors.

In conclusion, stress-induced eating in sated rats is mediated through endogenous opiates, whereas other stress-induced behaviors, such as gnawing, appear to be independent of the endogenous opioid peptide network. We suggest that these findings have potentially important implications for the etiology and treatment of stress-induced obesity in humans.

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The Spinal Frog Takes into Account the Scheme of Its Body During the Wiping Reflex

Abstract. *The hindlimb of the spinal frog produces a wiping reflex evoked by electrically or chemically stimulating distal skin of the forelimb. The reflex was released in frogs supported on a flat surface or suspended. It was found to have two stages. During the first, the frog fixed the hindlimb in an intermediate posture irrespective of forelimb position. In the second, the movement depended on forelimb position, which determined the final posture of the hindlimb. In this final posture, all joints except the hip joint were fully extended; the hip angle was correlated with forelimb position and varied on repeated wipings. When the stimulus was applied to the skin of the back, the pattern of final postures was the same, but the intermediate postures differed. The organization of the wiping reflex is discussed in light of the hypothesis that movement is evoked according to changes in the equilibrium (postural state) of the system.*

Since the last century, it has been known that the spinal frog can remove a stimulus from the body skin by coordinated movements of the limbs (*1*-*3*). Pflüger (*3*), astonished at the perfect nature of this reaction, the "wiping reflex" (WR), supposed the existence of a sort of "spinal soul." Even a completely deafferentated limb can perform an effective WR (*4*).

The WR has been used to test motor behavior in ontogenesis (*5*), to study interrelations between skin and neurons (*6*-*9*), to analyze the influence of descending systems on the motor functions of the spinal cord (*10*), and to reveal the possibility of learning in the spinal frog (*11*).

The spinal frog can also remove the stimulus from the skin of the contralateral hindlimb or the ipsilateral forelimb (*4*, *9*, *10*). Since the stimulated limb may take various positions, the coordinates of the same stimulated spot will differ in space. We decided to analyze the cinematics of the respective movements.

The spinal cords of 15 frogs anesthetized under ether were transected at the level of the calamus scriptorius. The frog was placed in the refrigerator for 2 to 4 hours or until the next day, when the experiments were performed. The transection was checked by the absence of the head skin reflexes. After the experiments, the frogs were fixed in Formalin; then we verified the transection visually through a craniotomy. The experiments were carried out on frogs that were either suspended or supported on a flat surface. In the latter case, the head of the frog was pinned down, and the forelimbs and one of the hindlimbs were stretched out by threads to limit their movements.

The WR was evoked by local electrical stimuli or by chemical stimuli. The electrical stimulation was bipolar (interelectrode distance, 0.2 mm; duration of pulse, 0.5 msec; frequency, 30 pulses per second; current, 0.05 to 0.5 mA). The chemical irritation was evoked by applying a filter paper soaked in a 2 to 5 percent solution of H_2SO_4 (*4*). The movie camera above the frog's back registered its movements against the background of the measuring grid.

Figure 1 shows the movement of the most distal point of the hindlimb in the suspended frog in response to electrical stimulation of the forelimb. Trajectories are shown for two positions of the forelimb. In both cases, the same spot on the skin was stimulated, but its position in space changed by about 2.5 cm. In both cases, also, the movements were successful and the stimuli were removed. The movements started with the flexion of the whole limb, bringing the tips of the toes to the base of the forelimb (frames 8 to 10 for one movement and 5 and 6 for the other). The frog preserved this intermediate posture for 100 to 200 msec. During this stage, the immobility of the toes as well as of the whole limb was maintained. The intermediate posture was independent of the position of the forelimb, but further decisive ballistic movement depended on forelimb position. The movement by the toes to remove the stimulus led to a new posture (frames 14 and 15 for one movement and 11 and 12 for the other). During this final posture, all joints of the hindlimb were almost fully extended except the hip; its angle depended on the position of the stimulus in space. At the end of the WR, the paw returned to its initial position.

With electrical stimulation, no repeated movements were observed, whereas with chemical stimulation they did take place. As a rule, the first movement was the most precise one, and the number of

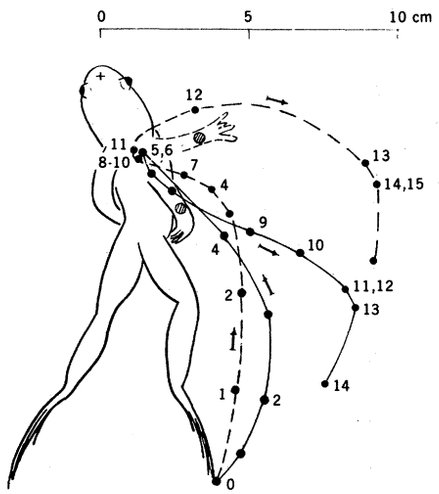

Fig. 1. Movements of the hindlimb in a suspended spinal frog during the WR evoked by electrical stimulation of the forelimb (hatched circle). The forelimb is shown in two different positions. Dashed and solid lines represent trajectories of the hindlimb when the forelimb was set into the upper or lower position, respectively; frames (16 per second) are numbered from the beginning of movement. The cross marks the point from which the frog was suspended.

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