C. J. Sanders, *Nature (London)* **288**, 475 (1980)].

6. The narrow plume dispenser contained 1.0×10^{-5} μg of synthetic (±)-periplanone B adsorbed onto 1 cm^2 of Whatman No. 1 filter paper; the wide plume dispenser contained 5.0×10^{-5} μg adsorbed onto a 15.0 by 0.5 cm filter paper strip. Males oriented in a similar manner to narrow plume sources of 10^{-6} to 10^{-3} μg of (±)-periplanone B.
7. *Periplaneta americana* males orient downwind on a servosphere in an air current of 24 cm/sec without pheromone [W. J. Bell and E. Kramer, *J. Insect Physiol.* **25**, 631 (1979)]. When sex pheromone is present in the air current, a distinct behavioral change is stimulated, and the males orient upwind.
8. Mean plume widths ± standard error at 0.5, 1.0, 1.5, and 2.0 m from the source for the narrow plume ($N = 5$) were 6.18 ± 0.5, 8.16 ± 0.9, 8.25 ± 1.0, and 13.37 ± 5.9 cm, respectively, and for the wide plume ($N = 5$) were 20.85 ± 1.0, 27.99 ± 0.5, 37.75 ± 0.8, and 44.03 ± 1.5 cm, respectively.
9. J. P. Miller and W. L. Roelofs, *J. Chem. Ecol.* **4**, 187 (1978); R. T. Carde and T. E. Hagaman, *Environ. Entomol.* **8**, 475 (1979).
10. A possible explanation of these turns is that the insect encounters nonuniformities of concentration in the plume. This may occur near the more irregular boundaries, as suggested by R. H. Wright [*Can. Entomol.* **30**, 81 (1958)], but is not expected to be significant at the central portion of the plume where many of the turns are observed. It is not known if a filament could actually be detected by a cockroach or whether it perceives only a spatially averaged response from the 5.0-cm antennae. It is equally unknown if a cockroach can temporally perceive a concentration profile across the width of the plume.
11. D. Marsh, J. S. Kennedy, A. R. Ludlow, *Physiol. Entomol.* **3**, 221 (1978).
12. I thank C. Still for the generous supply of synthetic sex pheromone. Supported by NSF psychobiology program (BNS-06284) and a grant from the University of Kansas biomedical support fund (to W. J. Bell).

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Endogenous Opiates and Stress-Induced Eating

In discussing their results on an animal model developed in our laboratory (*1*), Morley and Levine state that the opiate antagonist naloxone attenuates stress-induced eating in rats (*2*). This model has a number of similarities to emotionally related overeating in humans (*3*), and the reported involvement of endogenous opiates suggests new treatments for this disorder.

We have been unable to repeat Morley and Levine's observations in a series of eight experiments with both Sprague-Dawley and Wistar rats given two doses of naloxone (4 and 10 mg/kg) and test-fed with Laboratory Chow and palatable chocolate chip cookies (*4*). In no instance did we observe an attenuation after naloxone in the amount eaten or the duration of food-directed oral behavior during tail-pinch stress (TP).

A possible reason for these contradictory results may be found by considering gnawing and eating as distinct behaviors. The more time that is spent in gnawing (without ingestion), the less that can be spent in biting and chewing (with swallowing). Morley and Levine found that, while only 20 percent of their animals gnawed prior to naloxone administration, this increased to 100 percent after the drug was given (*2*). Thus, their conclusion that "naloxone suppresses ingestive behavior without affecting gnawing" is misleading since they actually appear to have observed an increase in gnawing behavior at the expense of eating.

We think there is a logical reason that increased gnawing might have been observed. Animals that normally eat quietly during mild TP may be induced to shred or demolish food pellets by increasing the pressure to painful levels (*1*). We thus interpret Morley and Levine's statements that "a number of these rats demolished one or both of the pellets without ingesting" and "rats squeaked at tail-pinch pressures below those necessary to induce eating, gnawing, or licking during the control trial" as evidence for pain. Naloxone may, for example, have lowered a nociceptive threshold such that pressures that ordinarily are compatible with eating during TP may now become painful and produce gnawing or shredding of the food. Alternatively, naloxone may have potentiated the release of striatal dopamine (DA) during TP (*5*), in analogy to its enhancement of DA release by amphetamine and increased stereotypy (*6*). We have discussed the similarities between the neural effects of amphetamine and TP (*1, 7*), and in either case an excessive release of DA may be conducive to gnawing instead of eating.

In addition to facilitating the effect of the indirect DA agonist amphetamine, naloxone also potentiates the actions of the DA antagonist chlorpromazine (*8*). We found that low doses of the DA antagonist haloperidol attenuate TP-induced oral behaviors (*1*) and we examined whether naloxone would potentiate that effect. Our results support the concept that, while naloxone alone again had no effect upon TP behavior, a combination of naloxone plus haloperidol compared to haloperidol alone significantly suppressed oral behaviors (*9*). Our data suggest that any effects of naloxone on TP behaviors may be mediated indirectly through DA. There is also abundant biochemical and anatomical evidence for interactions of enkephalin and DA (*10*). In addition, the pharmacological specificity of naloxone has been questioned (*11*), and we thus believe that Morley and Levine's unequivocal conclusion that "stress-induced eating is mediated through endogenous opiates" is highly speculative.

Morley and Levine say that their hypothesis was supported by the observations that chronic TP produced "self-addiction," which was manifested by naloxone-precipitated withdrawal symptoms (*2*). We have been unable to reproduce this effect in experiments with rats given chronic TP in the presence and absence of food. This detail was not specified in Morley and Levine's report and is important because rats pinched in the absence of food show considerable agitation and escape attempts (*1*). Such attempts result in tail damage, and continued TP is clearly very painful to the animals. Rats pinched in the presence of food do not exhibit such marked pain responses after long-term TP. Our protocol was similar to that of Morley and Levine (*2, 12*). Neither TP group in our experiment exhibited marked withdrawal behaviors when tested after day 10 of TP (mean 0.53 per 15 minutes after saline and 0.68 per 15 minutes after naloxone). In contrast, the morphine-dependent rats exhibited 15.0 vigorous withdrawal behaviors in the 15-minute period after naloxone. We thus find no evidence of opiate dependence in rats given long-term TP, even when this involved pain.

In addition to these troublesome failures to confirm Morley and Levine's conclusions in our laboratory, we feel that it is necessary to comment on some other inadequacies in their presentation. The most important of these are inaccurate or misleading attributions to others (*13*), and the failure to distinguish their own data from those of others (*14*). Their acknowledgement to one of us [reference 19 in (*2*)] was without our knowledge or endorsement of the results or interpretations.

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4. Groups consisted of four to eight adult male rats that had been screened for reliable TP-induced eating. This screening procedure, typically performed on the day preceding the experiment, apparently is the only major difference between our method and that of Morley and Levine (*2*). We have also performed an experiment similar to that described by Lowy *et al.* [M. T. Lowy,

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R. P. Maickel, G. K. W. Yim, *Life Sci.* **26**, 2113 (1980)] and failed to observe their reported decrease of TP eating after naloxone. We have also used greater numbers of TP trials with the same negative results [N. L. Ostrowski, N. Rowland, T. L. Foley, J. L. Nelson, L. D. Reid, *Pharmacol. Biochem. Behav.* **14**, 549 (1981)]. In each case we measured the amount consumed, amount spilled, and duration of food-directed oral behavior.
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9. Haloperidol (0.2 mg/kg) was injected intraperitoneally 1 hour before the start of four 2-minute TP trials, and naloxone (10 mg/kg, subcutaneously) was given 15 minutes before the test ($N = 8$ to 9 per group). A positive trial was defined as more than 10 seconds of eating or gnawing. As before, rats injected with naloxone alone showed TP behavior on 97 percent of the trials. Rats given haloperidol showed oral behaviors on 84 percent of the trials, while those receiving both haloperidol and naloxone ate on only 55 percent of the trials ($P < .05$ compared to haloperidol; $P < .005$ compared to naloxone). The amounts consumed closely paralleled the trials data.
10. J. C. Schwartz *et al.*, *Adv. Biochem. Psychopharmacol.* **18**, 245 (1978).
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12. We used less total time of TP than Morley and Levine: five 2-minute trials per day for 10 days, including subgroups with and without food present during TP. Additional rats were made dependent on morphine (two daily injections of 10 to 30 mg/kg for 1 week). On the test day, rats received saline, and were observed for 15 minutes, then were given naloxone subcutaneously (1 mg/kg), and observed for another 15 minutes. Not one truly vigorous withdrawal response (as opposed to normal "twitching") was recorded in any of the TP rats in either period.
13. Morley and Levine cite Antelman *et al.* [reference 5 in (*2*)] as evidence for a parallel between TP and schizophrenia, yet we later specifically pointed out the differences [Antelman and Caggiula in (*1*)].
14. Morley and Levine report that diazepam did not decrease TP eating, yet do not refer to any one of three previous descriptions of facilitation of TP eating after anxiolytics [S. M. Antelman, N. E. Rowland, A. E. Fisher, *Physiol. Behav.* **17**, 743 (1976); T. W. Robbins, A. G. Phillips, B. J. Sahakian, *Pharmacol. Biochem. Behav.* **6**, 297 (1977); M. B. Wallach, M. Dawber, C. McMahon, C. Rogers, *ibid.*, p. 529]. The data from their experiments with diazepam, cholecystokinin, and haloperidol (none of which are new) are given in their table 1, yet this table is cited only once in their text in relation to a saline-injected group. Their text also reads as if we (*1*) used the high dose of 2.5 mg/kg haloperidol; this is at least six times the highest dose that we typically use.
15. Supported by NIH grants MH32306 and RSDA00238 (S.M.A.) and AM26231 (N.R.). We thank Donna Kocan for help with the experiments.

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We were somewhat surprised at the inability of Antelman and Rowland to reduplicate our results in view of independent confirmation that naloxone reduces tail-pinch–induced eating (*1*). Furthermore, we have shown that (i) naloxone's suppressive effect on tail-pinch–induced eating could not be reversed by central administration of known pharmacological agents that enhance appetite and (ii) naloxone inhibits tail-pinch–induced eating in mice (*2, 2a*). The discrepancy between the experiments of Antelman and Rowland and ours may result from their use of trained animals, whereas we used naïve animals. Panksepp *et al.* (*3*) have provided evidence that endogenous opioids play a role in social behavior and learning situations. The introduction of a learning paradigm may have confounded Antelman and Rowland's results.

Apart from the direct evidence that endogenous opioids are involved in tail-pinch–induced eating, there is a large amount of indirect evidence. Neck scruff pinch (which also induces eating) produces a cataleptic state that is reversed by prior treatment with naloxone (*4*). We have observed cataplexy in some animals during tail pinch. This tonic immobility observed during tail pinch closely resembles morphine cataplexy (*5*). The tonic immobility induced by body pinch is widely reported to be mediated by opiates and serotonin (*6*). Ornstein (*7*) has demonstrated that tail pinch will suppress wet-dog shakes in rats in a manner similar to morphine. The suppressive effect of a number of peptides on stress-induced eating is partially reversed by the concomitant administration of a long-acting enkephalin analog (*2, 8, 8a*). We have found that a 10-minute tail-pinch period in the presence of wood chips produces a significant reduction in immunoreactive dynorphin [a leucine enkephalin containing endogenous opiates that induce feeding (*9*)] levels in rat brain.

If endogenous opioids were involved in tail-pinch behaviors, one would expect tail pinch to produce analgesia. Antelman *et al.* noted that tail pinch induced apparent indifference to pin prick (*10*). Tail pinch also produces naloxone-reversible analgesia when the hot-plate test is used (*11*). Pain (nociception) is a well-recognized activator of endogenous opiates (*12*). Antelman and colleagues (*10*) have consistently argued that tail pressure when applied correctly is not painful. However, Rowland and Marques (*13*) point out that, at the very least, tail pinch represents an annoying stimulus and that the "demarcation between pain and annoyance (or stress) is a fuzzy line at best." Other evidence that nociception plays an integral role in tail-pinch behaviors includes the following. (i) Tail-pinch behaviors are blocked by a local anesthetic ring block of the tail; (ii) painful stimuli applied to other parts of the body, such as foot and neck, induce feeding; and (iii) diabetic animals with increased tail-flick latencies have a prolonged latency for induction of tail-pinch behaviors compared to their littermate controls (*2a, 14*). We recognize that the demonstration that tail-pinch–involved nociception is necessary but not sufficient evidence to infer opiate mechanisms as nonopiate analgesia is well recognized.

Antelman has stressed the similarities between the neural effects of amphetamine and tail pinch. Amphetamine-tolerant guinea pigs exhibit supersensitivity to naloxone with respect to feeding behavior (*15*). Long-term amphetamine exposure results in higher levels of β-endorphin in the hypothalamus in guinea pigs and acute infusion of dextroamphetamine induces increases in plasma β-endorphin in humans (*16*). These findings suggest that endogenous opiates have a role in amphetamine-induced behaviors and thus by implication in tail-pinch–induced behaviors.

Antelman and Rowland observed fewer withdrawal behaviors in that their total tail-pinch time amounted to less than 20 percent of ours. However, they previously reported the induction of tolerance to food ingestion in rats subjected to tail pinch over a long period and commented that the rats became "agitated" (*17*); both of these observations provide further evidence for a possible autoaddiction to endogenous opiates resulting from long-term tail pinch. As mentioned (*7*), the ability of tail pinch to block wet-dog shakes is particularly relevant in this regard.

Thus, there appears to be adequate evidence supporting the view that endogenous opiates are involved in tail-pinch–induced food ingestion. Although we (*18*) originally also took the view that tail pinch represents a model of stress-induced eating, our ongoing studies have led us to believe that the predominant tail-pinch behavior is gnawing (chewing), with any associated eating representing an epiphenomenon. Support for this concept comes from the observation that tail-pinch activation is mediated by dopaminergic mechanisms (*10*), since dopaminergic agonists are recognized as producing oral stereotypy rather than ingestion (*19*). In addition, Rowland and Marques (*13*) have stated that "because many rats shred the food without eating we believe that ingestion may be incidental to the predominant motor act of biting." Should this be the case, the argument presented above by Antelman and Rowland that the decreased eating in our rats was due to increased gnawing would not apply. Our response to the final paragraph of Antelman and Rowland is included in (*20*).

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20. We regret that Antelman did not consider his personal communication concerning methodological detail as advice. Our table 1 (*18*) was meant to demonstrate that tail-pinch behavior in our hands was similar to that reported by others and as such implicitly acknowledged the existence of prior studies. We did not mean to suggest that our dose of haloperidol was the same as that used by Antelman and his colleagues.

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Limitations in Identifying Neurotransmitters Within Neurons by Fluorescent Histochemistry Techniques

Grace and Bunney (*1*) purport positively to identify neurons as dopaminergic by intracellularly injecting L-dopa and subsequently inducing fluorescence in those cells by treating the tissue with glyoxylic acid. This method is based on the assumption that "only the dopamine reacts with formaldehyde vapor or glyoxylic acid to form fluorescent compounds" (*1*). On the contrary, our studies (*2*) show that L-dopa itself fluoresces when treated with the SPG (sucrose, phosphate buffer, and glyoxylic acid) method of de la Torre and Surgeon (*3*), and that the emission maximum of L-dopa is exactly the same as that of dopamine (Fig. 1). Furthermore, the products of the same biosynthetic pathway, epinephrine and norepinephrine, also fluoresce at the same λ_{max} when treated with glyoxylic acid. Additionally, L-dopa fluoresces if the tissue is treated by the paraformaldehyde (FGS) method (*4*). In this case the spectral emission of the L-dopa fluorophore is also very similar to that of the dopamine fluorophore. Therefore, it appears that neither the SPG nor the FGS methods of fluorescent histochemistry allow one unequivocally to distinguish the L-dopa fluorophore from the dopamine fluorophore.

The implication of Grace and Bunney that lack of fluorescence in the nondopaminergic cells of the zona reticulata is evidence that L-dopa is not the glyoxylic acid reactant is open to question on at least two counts. The 10 to 30 minutes allowed to elapse before the animals were killed would certainly be sufficient time for degradation of the L-dopa by either the monamine oxidase or catechol-*O*-methyltransaminase pathways (*5*). There is no assurance in the report (*1*) that the amount of L-dopa injected into zona reticulata cells was comparable to that iontophoresed into the zona compacta of the substantia nigra. In view of the fact that L-dopa does fluoresce, careful and exact controls must be used. One would have to examine considerably more data than were given on the controls before one could reach any conclusions concerning the ability to identify dopaminergic neurons after intracellular injection of L-dopa.

The identification of putative transmitters on the basis of their fluorescence has been a problem for some time (*6*). When treated by any number of methods, monoamines, as well as many amino acids, fluoresce. Even tyrosine, the precursor of monoamines, has a fluorescent spectogram that would be difficult to

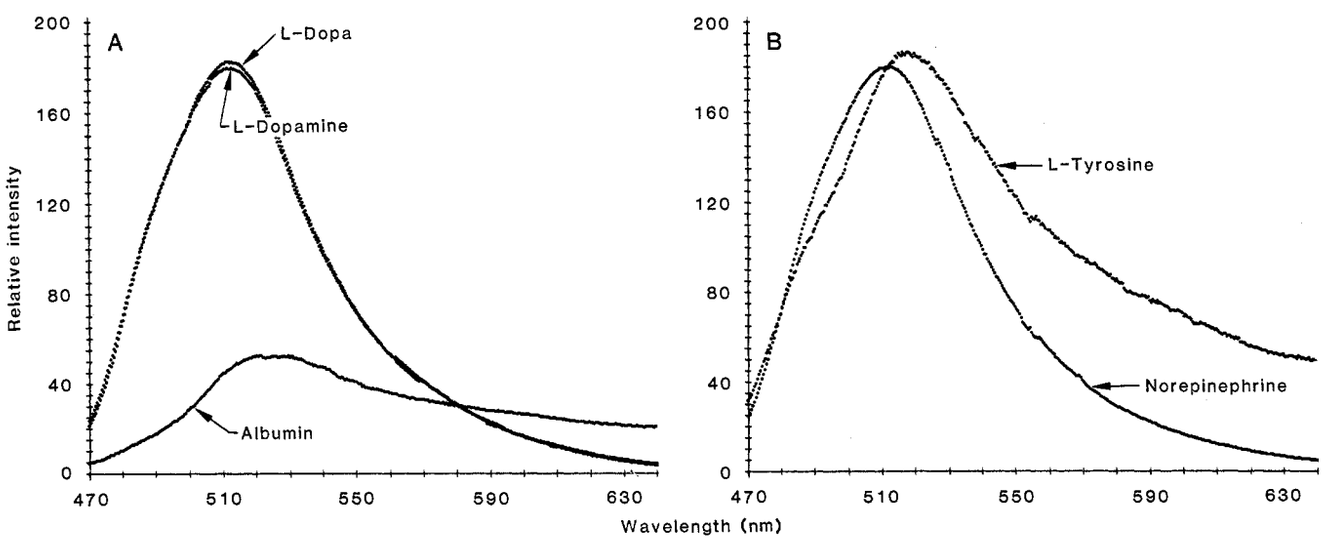

Fig. 1. Fluorescence emission spectra of L-dopa, dopamine, tyrosine, and norepinephrine in dried albumin droplets on glass microscope slides. Epifluorescent illumination was monitored and recorded as described (*2*). These are uncorrected records that are representative of at least three similar samples. The albumin was the only record for which we had to set the microspectrophotometer calibration at high gain. Hence, the relative intensities of the samples and the albumin are not accurately represented. The fluorescence of the monoamines is at least 1000 times that of albumin. Readings for each sample were taken every 0.44 nm and the point plotted is an average of ten readings taken at each 0.44-nm interval.

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