Locomotor activation induced by infusion of endorphins into the ventral tegmental area: Evidence for opiate–dopamine interactions

(naloxone/mesocorticolimbic dopamine)

LUIS STINUS, GEORGE F. KOOB, NICHOLAS LING, FLOYD E. BLOOM, AND MICHEL LE MOAL

The Salk Institute, P.O. Box 85800, San Diego, California 92138

ABSTRACT   β-Endorphin in nanomole quantities produced a stimulation of locomotor activity when infused into the region of the dopamine cell bodies of the ventral tegmental area (VTA) in rats. α-, γ-, and des-Tyr-γ-endorphin produced similar effects, but the D-alanine analogues of α- and γ-endorphin produced a larger and longer-lasting activation, presumably reflecting their resistance to degradation. This locomotor activation was reversible by pretreatment with naloxone and by destruction of the terminal projections of the mesocorticolimbic dopamine system originating in the VTA. These results demonstrate that locally infused endorphin can interact with the opioid receptors in the VTA, and they suggest a means by which endorphins activate limbic excitability.

The identification and localization within the central nervous system of endogenous peptides with opiate-like actions suggested that these substances may act as central neurotransmitters (1, 2). Both [Met]enkephalin [β-lipotropin-(61–65)] and β-endorphin (β-END) [β-lipotropin-(61–91)] exhibit opiate-like activity when injected into the ventricles (3, 4). These effects have included analgesia, catatonia, loss of corneal and tail pinch reflexes, and wet-dog shakes (3), and β-END appeared to be more potent than [Met]enkephalin (5–7). In addition, most of these effects are reversible by naloxone. These preliminary results suggested to some that the endorphins could be an etiological factor in mental illness (3, 8), but the nature and specification of this action remains to be determined.

Opiate receptors and enkephalin-like peptides have been found in the region of the ventral tegmental area (VTA) (9), and in at least some accounts receptors appear to be localized on mesolimbic dopamine neurons (9, 10). These mesolimbic dopamine neurons are thought to be involved in the locomotor stimulation produced by psychomotor stimulants (11, 12) but also may have a role in general activation and the actual organization of behavior (13, 14). In fact, some authors have suggested that the activation of the mesolimbic dopamine system may be an important model of schizophrenia (15). This intriguing similarity of the putative function for the mesolimbic dopamine system and the endogenous opiate systems requires further analysis of their interaction at the behavioral level, and our initial studies have concentrated on the activation of the mesolimbic dopamine system as measured by simple locomotor activity.

Recent work has demonstrated that morphine and [D-Ala^2, Met]enkephalin when injected into the VTA can produce significant increases in locomotor activity that may reflect an activation of these mesolimbic dopamine neurons (16, 17). The purpose of the present study was to determine whether β-END also interacts with the opiate receptors in the VTA to produce

locomotor activity and, if so, to pursue the pharmacological specificity of this effect with other endorphins, endogenous catecholamines (11), and naloxone.

METHODS

Animals and Surgery. Male Sprague–Dawley (350-g) rats were stereotaxically implanted with bilateral stainless steel guide cannulae (23 gauge) aimed at the VTA (coordinates: 2.3 mm anterior to interaural zero, 0.5 mm lateral and 5.6 mm ventral from the skull surface with the incisor bar 5.0 mm above interaural line). All animals were housed individually and were allowed at least a 1-week recovery period before the start of behavioral testing. In the lesion experiment, in addition to the above procedures, rats were injected bilaterally with 6-hydroxydopamine (8 μg of base in 2 μl over 14 min) in the nucleus accumbens and were allowed 2 weeks of recovery. Control rats were injected with the vehicle solution (0.9% saline containing ascorbic acid at 0.2 mg/ml). Thirty minutes before injection of the 6-hydroxydopamine, the rats were pretreated with pargyline (50 mg/kg intraperitoneally).

Microinfusion and Materials. β-END [β-lipotropin-(61–91)], α-END [β-lipotropin-(61–76)], γ-END [β-lipotropin-(61–77)], and des-Tyr^1-γ-END [β-lipotropin-(62–77)], as well as [D-Ala^2]-α-END and [D-Ala^2]-γ-END, were synthesized at The Salk Institute. They were stored at 20°C in 200-μg freeze-dried samples. The appropriate amounts of opioids were prepared by addition of sterile 0.9% saline (151 mM). During the infusion procedure, cannulae (30 gauge) were lowered into the VTA (8.9 mm from the skull), and bilateral infusion of 1 μl of solution was performed over 105 sec by using a microdrive pump. Doses are expressed in nmol and reflect the *total amount* injected on *both* sides of the VTA. The cannulae were left in place for 1 min after injection to allow for diffusion. Before testing, all rats were given a preliminary saline infusion in order to habituate them to the procedure and also to minimize mechanical effects during experimental infusions. Naloxone dissolved in saline was injected subcutaneously (0.5 ml of solution).

Behavioral Measures. Quantitative analysis of horizontal locomotor activity was recorded in two types of photocell cages. Activity was measured in circular corridors, 12 cm wide and 170 cm long, in which four beams of infrared lights traversed the corridor 3 cm above the floor. Interruption of these infrared beams registered counts on an automatic recorder outside the testing room. In the β-END/naloxone study, locomotor activity was measured in wire mesh cages, 25 × 36 × 20 cm, equipped with two infrared photocells, whose interruption registered counts on counters outside the testing room. Before any activity measures, rats were habituated to the testing box for 24 hr. On
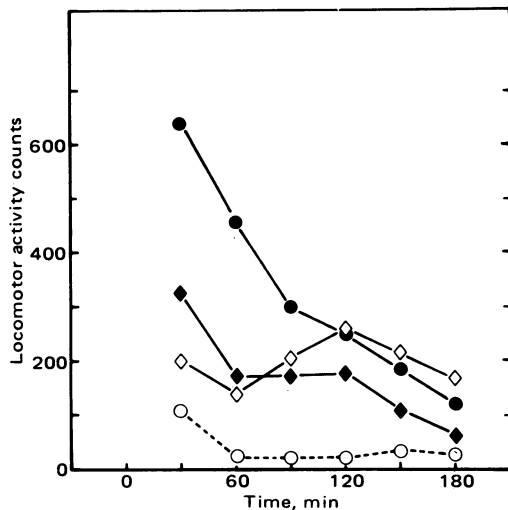
Abbreviations: END, endorphin; VTA ventral tegmental area.

FIG. 1. Stimulation of locomotor activity induced by simultaneous bilateral VTA infusion of β-END. O, Vehicle, n = 6; ●, 2 μg, n = 6; ◆, 5.0 μg, n = 2; ◇ 10 μg, n = 8. These treatments correspond to total infusions of 0, 0.6, 1.5, and 2.9 nmol of β-END.

the test day they were habituated for 1 hr before infusion, infused with peptide, and returned to the apparatus for 1–3 hr depending on the experiment.

Statistical Analysis. Locomotor activity counts were subjected to parametric statistical analysis using the repeated measured analysis of variance with the least squares computation for unequal numbers (18). For these analyses, each separate injection was considered an independent observation and the repeated observations over time during that injection were considered the repeated measure. Individual comparisons were made by using Student's t test.

RESULTS

Locomotor Activation Produced by Endorphins. Bilateral infusion of 1-μl solutions into VTA containing a total of 2.0, 5.0 or 10.0 μg of β-END (0.6, 1.5, and 2.9 nmol, respectively) induced a long-lasting increase in locomotor activity (Fig. 1), which persisted for 120–180 min (group effect, F = 3.65, df = 3, 18; P < 0.05; group × time interaction, F = 2.73, df = 5, 15; P < 0.01). At the high doses of β-END (2.9 nmol) the initial behavioral activation was associated with an initial period of head-down sniffing (and immobility) which lasted 60 min and was followed by a second phase, in which increased locomotor activity predominated. This initial increase in stereotyped behavior yields the inverse relationship between dose and locomotor activation seen in Fig. 1, particularly because the peak
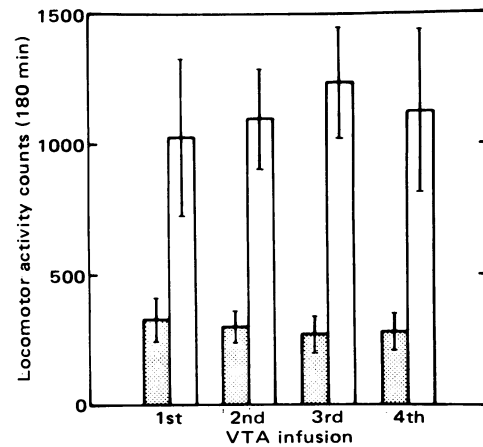

FIG. 2. Locomotor response to repeated bilateral VTA infusion of 1 μl of saline (stippled bars; n = 4) or 2 μg (6 nmol) of β-END (open bars; n = 4). As in all following figures, error bars indicate SEM. One infusion was given every 2 days.

activation appears to be produced by a dose between 0.30 and 1.50 nmol (see Fig. 4). In addition, repeated infusions of β-END (0.6 nmol) into the VTA at intervals of 48 hr or more showed that β-END-treated rats have a stable response from test to test (Fig. 2), with no obvious development of tolerance.

Although all the opiate peptides induced some activation, they were not equally potent in inducing locomotor stimulation (Fig. 3, Table 1). The largest increase was the β-END at the lower dose. γ-END and des-Tyr^1-γ-END did increase locomotion, but these effects were minimal and mainly localized to the first 30 min (Fig. 3). [D-Ala^2]-α-END and [D-Ala^2]-γ-END, slowly metabolized analogues of α- and γ-END, produced significant increases in locomotor activity during the first 30 min, and the behavioral activation was as long-lasting (120 min) as with β-END (Table 1).

Naloxone Antagonism of Endorphin Effects. The locomotor activation induced by infusion of 0.06 nmol of β-END into the VTA was significantly blocked by subcutaneous pretreatment with naloxone at 1 mg/kg (F = 12.57, df = 1, 14; P < 0.01) (see Fig. 4). Naloxone only partially antagonized the effects of 0.30 nmol of β-END (F = 4.58, df = 1, 14; P < 0.05) and had no effect on the locomotor stimulation produced by 1.5 nmol (F < 1.0). This shift in the dose–response relationship with naloxone pretreatment was also evident after infusion of [D-Ala^2]α-END. The behavioral activation induced by infusion of 0.6 nmol of [D-Ala^2]-α-END into the VTA was completely blocked by simultaneous subcutaneous injection of naloxone at 1 mg/kg (F = 5.27, df = 1, 9; P < 0.05) (see Fig. 5). Naloxone at a dose of 1 mg/kg also partially antagonized the effects of

Table 1.   Dose-dependent locomotor stimulation induced by bilateral infusion of endorphins and related peptides into the VTA

| Amount of opiate infused per rat, nmol | Locomotor activity counts (120 min) | | | | | |
	β-END	α-END	[D-Ala]-α-END	γ-END	Des-Tyr^1-γ-END	[D-Ala]-γ-END
0.6	1030 ± 200** (8)		739 ± 169* (7)			810 ± 205* (6)
1.5		484 ± 126 (5)		323 ± 32 (8)		
3.0	784 ± 253 (8)		1296 ± 152** (5)		484 ± 57** (7)	1098 ± 270** (5)
6.0		527 ± 148 (8)		403 ± 44* (7)	444 ± 45** (8)	

In parentheses are the numbers of rats in each group. Locomotor activity was recorded in a circular corridor for 120 min. Control group score was 232 ± 39 (mean ± SEM) for six rats. The total amount of the peptide infused is indicated in the left column. The molarity of the solution itself varied with dose; 0.6 nmol of peptide produced a 0.3 nM solution. An overall analysis of variance revealed a significant group effect (F = 3.95, df = 12, 75; P < 0.01).
* Significantly different from control, P < 0.05 by Student's t test.
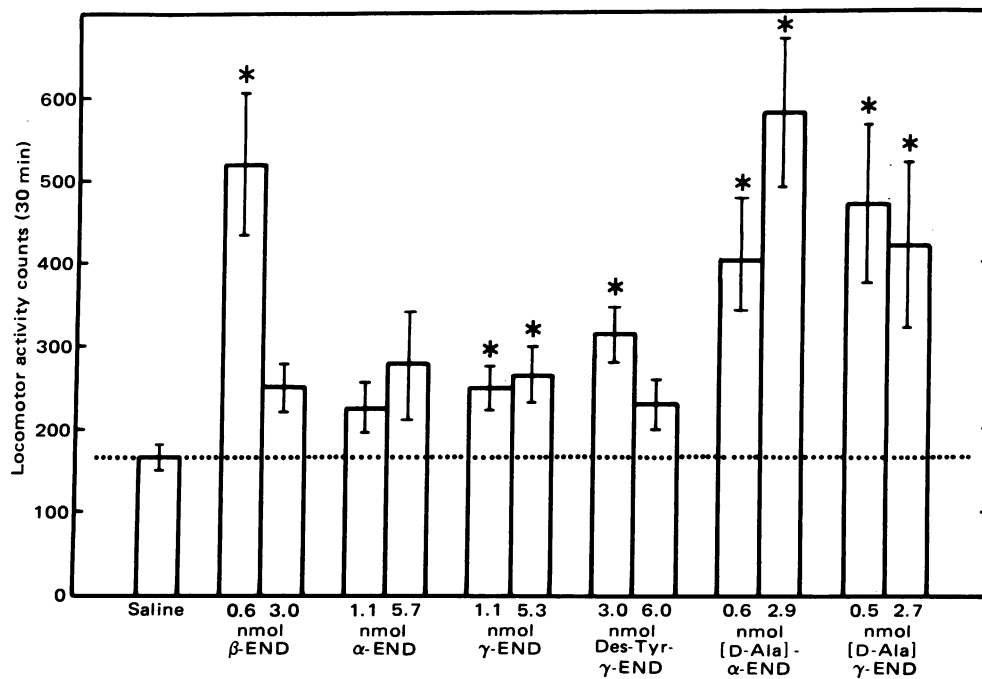** Significantly different from control, P < 0.01 by Student's t test.

FIG. 3. Locomotor response induced by bilateral VTA infusion of endorphins and related peptides during the first 30 min after injection. An overall analysis of variance revealed a significant group effect ($F = 4.63$, $df = 12, 75$; $P < 0.01$). $n \geq 5$ for each group. * Significantly different from saline, $P < 0.05$ by Student's t test.

3.0 nmol of [D-Ala^2]-α-END ($F = 17.76$, $df = 1, 7$; $P < 0.01$) but actually increased the locomotor stimulation produced by 15.0 nmol of [D-Ala^2]-α-END. To block the effect of 15.0 nmol
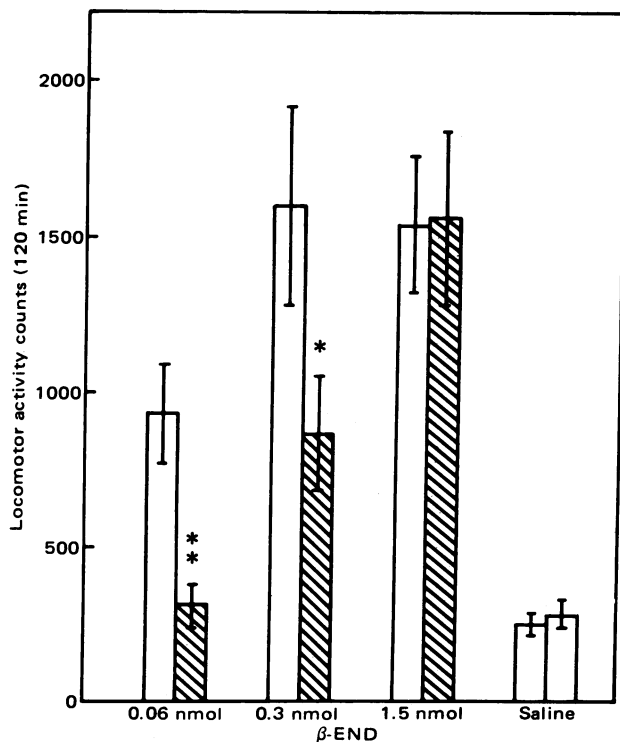

FIG. 4. Locomotor response after simultaneous bilateral VTA infusions (open bars) of a total of 0.2, 1.0, and 5.0 μg of β-END, corresponding to 0.06, 0.30, and 1.5 nmol, respectively, and previous subcutaneous injection of naloxone (1 mg/kg) (hatched bars). $n \geq 6$ for each group.
* Significantly different from endorphin without naloxone, $P < 0.05$, group effect, two-way analysis of variance with repeated measures on one factor.
** $P < 0.01$.

of [D-Ala^2]-α-END required a dose of naloxone of 5 mg/kg ($F = 13.13$, $df = 1, 10$; $P < 0.01$).

Role of Mesocorticolimbic Dopamine Neurons. The destruction of the dopamine terminals by injection of 6-hydroxydopamine into the nucleus accumbens completely blocked the locomotor stimulation induced by VTA infusion of 0.6 nmol of β-END (Table 2) ($F = 13.9$, $df = 1, 13$; $P < 0.01$), whereas the locomotor response to VTA saline was identical for sham-operated rats and rats with nucleus accumbens lesions. In this experiment the lesion of dopamine neurons was verified by psychopharmacological tests (11). As previously demonstrated,
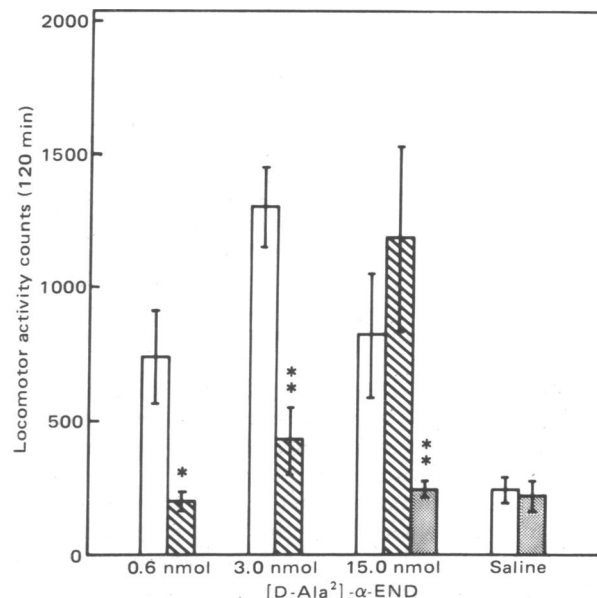

FIG. 5. Locomotor response after simultaneous bilateral VTA infusion of a total of 1.0, 5.0, and 25.0 μg of [D-Ala^2]-α-END (0.6, 3.0, and 15.0 nmol, respectively) and subcutaneous injection of naloxone at 0, 1 (hatched bars), or 5 (stippled bars) mg/kg. $n \geq 4$ for each group. Significant differences are indicated as in Fig. 4.

Table 2.   Endorphin locomotor stimulation blocked by destruction of the terminals of the mesocorticolimbic dopamine system

| | Time of test, min | Locomotor activity counts | |
Treatment		Sham ($n = 9$)	Nucleus accumbens, 6-hydroxydopamine ($n = 6$)
Saline VTA	120	202 ± 29	198 ± 30
β-END (0.6 nmol) VTA	120	1349 ± 247	198 ± 19**
d-Amphetamine (1.5 mg/kg) intraperitoneal	180	2752 ± 358	90 ± 19**
Apomorphine (0.1 mg/kg) subcutaneous	60	98 ± 17	2010 ± 464**

Destruction of the innervation of the mesocorticolimbic dopamine system by injection of 6-hydroxydopamine into the area of the nucleus accumbens produced a blockade of the locomotor stimulation normally induced by bilateral VTA infusion of 2.0 μg (0.6 nmol) of β-END.
** $P < 0.01$, compared to sham-operated rats, by Student's t test.

the lesion blocked the locomotor response to d-amphetamine ($F = 32.8$, $df = 1, 13$; $P < 0.01$) and induced a supersensitive response to apomorphine ($F = 125.5$, $df = 1, 13$; $P < 0.01$).

DISCUSSION

The present study demonstrates that β-END and endorphin analogues produce a dose-dependent increase in locomotor activity when infused into the VTA. These increases in activity can be blocked by naloxone and thus appear to be a direct result of stimulating opiate receptors located there. The relationship with dose for β-END probably reflects the development of behavioral patterns that are incompatible with horizontal locomotion. Support for this hypothesis comes from the experiment with [D-Ala²]-α-END, in which a low dose of naloxone actually increased activity to a high dose of the endorphin. This shift to the right of the dose–response functions of β-END and [D-Ala²]-α-END (Figs. 4 and 5) by naloxone suggests a dynamic competition between agonist and antagonist at the level of the receptor, and it appears that a high dose of naloxone can overcome this competition and produce a complete block of the locomotor activity.

The injection of the α and γ analogues of β-END produced similar effects during the first 30 min, but the D-Ala analogues of α and γ endorphin caused a much longer-lasting activation, presumably reflecting their resistance to metabolic destruction. The fact that [D-Ala²]-α-END and [D-Ala²]-γ-END produced similar effects on locomotor activity with central injections appears to be in contradiction to some of the results reported in other studies on extinction with peripheral injections. Here, α-END characteristically inhibits extinction of both active and passive avoidance and γ-END and des-Tyr¹-γ-END facilitate such extinction (19–21). Whether this difference in the relationship of α- and γ-END reflects a difference in behavior, in dose, or in some unknown intermediate process remains to be determined.

What is clear, however, is that the activation induced by these endorphin compounds depends upon an intact mesolimbic dopamine system, because destruction of the terminal projections of this system located in the area of the nucleus accumbens—and at some distance from the site of the peptide infusions—completely blocks these effects. How this interaction occurs at the neuronal level remains to be explored, but several hypotheses may be considered. Activation of the dopamine neurons may be mediated via presynaptic inhibition of an as-yet-undetermined inhibitory system (22, 23). An alternative explanation may be an inhibition of dendritic release of dopamine onto some afferent input such as serotonin or γ-aminobutyric acid.

The actual mechanism in the normal brain for this endorphin–dopamine interaction remains unclear because no endorphin fibers have been localized to the VTA with current methods (24). Thus, endogenously released endorphin would have to act via the cerebrospinal fluid. Injection of β-END intraventricularly does produce an augmentation of locomotor activity (25), but the doses necessary are considerably higher than those reported here. In fact, the present study demonstrates a probable site of action for the behavioral activation produced by intraventricular β-END.

Regardless of the ultimate cellular mechanism of this opiate–dopamine interaction, the importance of this interaction at the functional level cannot be discounted. The mesocorticolimbic dopamine neurons have been implicated in the psychopathology of schizophrenia (15, 26–28), and recent results have shown that the opiate peptides may also have a role. This opiate–dopamine interaction at the level of the cell bodies of the mesocorticolimbic dopamine system may offer important clues for our understanding of the complex pathology of these and similar mental diseases.

1. Hughes, T., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A. & Morris, H. R. (1975) *Nature (London)* **258**, 577–579.
2. Simantov, R. & Snyder, S. H. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2515–2519.
3. Bloom, F., Segal, D., Ling, N. & Guillemin, R. (1976) *Science* **194**, 630–632.
4. Jacquet, Y. F. & Marks, N. (1976) *Science* **194**, 633–634.
5. Belluzzi, J. D., Grant, N., Garsky, V., Saratakis, D., Wise, C. D. & Stein, L. (1976) *Nature (London)* **260**, 625–626.
6. Buscher, H. H., Hill, R. C., Romer, A., Cardinaux, F., Closse, A., Hauser, D. & Pess, J. (1976) *Nature (London)* **261**, 423–425.
7. Chang, J., Fong, B. J. W. & Pert, A. (1976) *Life Sci.* **18**, 1473–1481.
8. Terenius, L., Wahlstrom, A., Lindstrom, L. & Widerlou, E. (1976) *Neurosci. Lett.* **3**, 157–162.
9. Pollard, H., Llorens, C., Bonnet, J. J., Constentin, J. & Schwartz, J. C. (1977) *Neurosci. Lett.* **7**, 295–299.
10. Versteeg, D. H. G., DeKloet, E. R. & De Wied, D. (1979) *Brain Res.* **179**, 85–92.
11. Kelly, P. H., Seviour, P. W. & Iversen, S. D. (1975) *Brain Res.* **94**, 507–522.
12. Kelly, P. H. & Iversen, S. D. (1976) *Eur. J. Pharmacol.* **40**, 45–56.
13. Le Moal, M., Stinus, L. & Galey, D. (1976) *Exp. Neurol.* **50**, 521–535.

14. Le Moal, M., Stinus, L., Simon, H., Tassin, J. P., Thierry, A. M., Blanc, G., Glowinski, T. & Cardo, B. (1977) in *Advances in Biochemical Psychopharmacology*, eds. Costa, E. & Gessa, G. L. (Raven, New York), Vol. 16, pp. 237–245.
15. Stevens, S. & Livermore, A. (1978) *Neurology* **28**, 36–46.
16. Broekkamp, C. L. E., Phillips, A. G. & Cools, A. R. (1979) *Nature (London)* **278**, 560–562.
17. Joyce, E. M. & Iversen, S. D. (1979) *Neurosci. Lett.* **14**, 207–212.
18. Winer, B. J. (1971) *Statistical Principles in Experimental Design* (McGraw-Hill, New York).
19. De Wied, D., Bohus, B., Van Ree, J. M. & Urban, I. (1978) *J. Pharmacol. Exp. Ther.* **204**, 570–580.
20. De Wied, D., Kovacs, G. L., Bohus, B., Van Ree, J. M. & Gr, H. M. (1978) *Eur. J. Pharmacol.* **49**, 427–436.

21. Le Moal, M., Koob, G. F. & Bloom, F. E. (1979) *Life Sci.* **24**, 1631–1636.
22. Costa, E., Fratta, W., Honig, J. S., Morini, F. & Tang, H. Y. T. (1978) in *The Endorphins*, eds. Costa, E. & Trabucchi, M. (Raven, New York), pp. 217–226.
23. Schwartz, J. T., Pollard, H., Llorens, C., Malfroy, B., Gros, C., Pradelles, P. L. & Dray, F. (1978) in *The Endorphins*, eds. Costa, E. & Trabucchi, M. (Raven, New York), pp. 245–264.
24. Bloom, F. E., Battenberg, E. L., Rossier, J., Ling, N. & Guillemin, R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1591–1595.
25. Segal, D. S., Browne, R. G., Arnsten, A., Derrington, D. C., Bloom, F. E., Guillemin, R. & Ling, N. (1979) in *Endorphins and Mental Health Research*, eds. Usdin, E., Bunney, W. E., Jr. & Kline, N. S. (Macmillan, London), pp. 307–324.
26. Carlsson, A. (1978) *Am. J. Psychiatry* **135**, 164–173.
27. Hornykiewicz, O. (1978) *Neuroscience* **3**, 773–783.