Pharmacology Biochemistry & Behavior, Vol. 25, pp. 1279–1284, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Radio-Frequency Analysis of the Effect of Haloperidol and Cyclo(Leucyl-Glycyl) on Apomorphine-Induced Stereotypy

JEREMY Z. FIELDS,* LARRY P. GONZALEZ,† LAURENCE R. MEYERSON,‡
PAUL LIEBER,* JOHN M. LEE,†§ KATHY A. STEECE,†
F. A. DeLEON-JONES§ AND RONALD F. RITZMANN†§

**Research Service (151), Hines V.A. Hospital, Hines, IL 60141*
†Department of Physiology and Biophysics, University of Illinois at Chicago
P.O. Box 6998, Chicago, IL 60680
‡American Cyanamid Co., Medical Research Division, Ramapo College G-119
505 Ramapo Valley Road, Mahwah, NJ 07430
§Neurobiology Laboratory, Research Service (151), West-Side V.A. Hospital, Chicago, IL 60680

Received 23 April 1986

FIELDS, J. Z., L. P. GONZALEZ, L. R. MEYERSON, P. LIEBER, J. M. LEE, K. A. STEECE, F. A. DeLEON-JONES AND R. F. RITZMANN. *Radio-frequency analysis of the effect of haloperidol and cyclo(leucyl-glycyl) on apomorphine-induced stereotypy*. PHARMACOL BIOCHEM BEHAV **25**(6) 1279–1284, 1986.—Our previous studies indicated that the peptide cyclo(leucyl-glycyl) (cLG) prevents the development of supersensitivity to dopamine in several animal models at both biochemical and behavioral levels. We therefore tested cLG in a paradigm more commonly used to model tardive dyskinesia, namely chronic haloperidol administration to rats. We found that cLG administered subcutaneously at a dose of 8 mg/kg, blocked about 50% of the supersensitizing effects of haloperidol on apomorphine-induced stereotypic behaviors. Further, we used a novel method, radio-frequency analysis, that quantifies sniffing and other stereotypic movements. Unlike methods that rely on visual observation of stereotypy and utilize an ordinal scale, these measurements are rated by an automatic motility monitor and utilize a ratio scale. Unlike other automated motility monitors, this device can distinguish between various forms of stereotypic behaviors. Since parametric statistics can be used, there is a significant improvement in the efficiency of the task of rating and comparing stereotypy scores.

Dopamine Dopamine receptors Apomorphine Haloperidol Cyclo(leucyl-glycyl) Antipsychotics
Neuroleptics Stereotypy Animal model Tardive dyskinesia Supersensitivity

A number of pathological states have been associated with malfunctioning dopaminergic systems. One such syndrome, tardive dyskinesia, is thought to be due to hyperactivity in the nigrostriatal dopamine tract and has been suggested to be a result of protracted pharmacological blockade of striatal D-2 dopamine receptors by neuroleptics [30]. A commonly used animal model of tardive dyskinesia consists of chronic administration of haloperidol or other neuroleptics to rats. These rats become behaviorally supersensitive to dopamine agonists such as apomorphine (APO) [29]. That is, there is a leftward shift in the dose-response curve for APO-induced stereotypic behaviors such as sniffing, licking, and gnawing. Biochemical data indicate that parallel and consistent changes occur in the density of D-2 dopamine receptors in corpus striatum as measured either by tritiated haloperidol or spiroperidol binding [15,28]. The number but not the affinity for dopamine antagonists, at D-2 but not D-1 sites [19], is increased by chronic neuroleptic administration. These changes in receptor recognition sites at striatal dopaminergic synapses are thought to be the basis of the behavioral supersensitivity to dopamine.

While these dyskinesias occur spontaneously in humans, in rodents they do not. In order to unmask the latent biochemical supersensitivity to DA agonists that is a consequence of long-term haloperidol treatment in rodents, animals are generally injected with various doses of APO and subjectively rated for the occurrence of stereotypic behaviors by one or more observers using some form of rating scale. A common procedure has been to assign arbitrary numbers for their rating scale (e.g., 1=normal activity, 2=stereotypic locomotor activity, 3=stereotypic sniffing, 4=stereotypic gnawing) and then to sum the data to yield a total stereotypy score [10, 11, 28].

One drawback to this method is that the different stereotypies elicited in rats are probably mediated by different dopamine tracts within the central nervous system [6, 7, 14, 18]. Another drawback is that the data are actually on an ordinal scale. A third drawback of this method is that nonparametric statistics are required and these are generally less powerful than parametric methods [32]. A brief survey of the literature indicates that quite a few investigators inappropriately have used parametric statistics anyway, as if the data

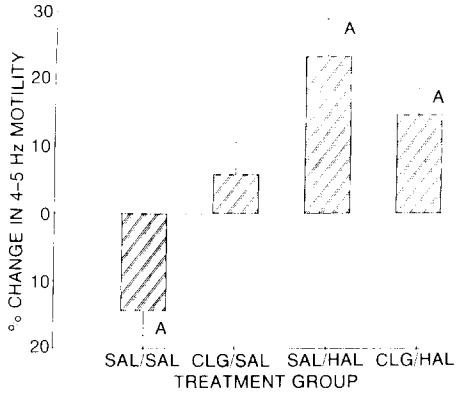

FIG. 1. Mean percent change in 4–5 Hz motility during the 20 minute period following apomorphine administration (0.5 mg/kg, IP), relative to predrug motility. A=$p<0.001$ compared to predrug controls. *Each group represented in this figure is significantly different from every other group at the $p<0.05$ level.*

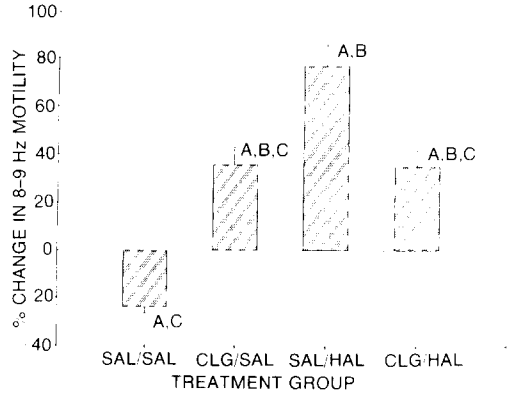

FIG. 2. Mean percent change in 8–9 Hz motility during the 20 minute period following apomorphine administration (0.5 mg/kg, IP), relative to predrug motility. A=$p<0.001$ compared to predrug controls; B=$p<0.01$ compared to group SAL/SAL; C=$p<0.01$ compared to SAL/HAL.

were on a ratio scale (where the distance between 1 and 2 is the same as the distance between 3 and 4 and where 4 is truly twice as big as 2).

Attempts to improve such measurements have led to the development of automatic activity monitors that can evaluate behaviors on an interval or ratio scale. Although a variety of automatic activity monitor systems are commercially available for the quantification of motor activity, these devices will not distinguish between different kinds of stereotypic behaviors such as head-bobbing, sniffing, licking and gnawing. Recently however, the use of a radio-frequency (RF) capacitance field device has been reported to have the ability to quantify and distinguish between a number of these specific stereotypic behaviors [8–9, 12, 13].

We have recently shown that the peptide cyclo(leucyl-glycyl) (cLG) can prevent the development of behavioral supersensitivity to dopamine agonists induced by either morphine in mice [26] or rats [16, 17, 23–25], 6-hydroxydopamine in mice [22], or haloperidol in mice [2]. In each case cLG inhibits the usual shift to the left of the dose-response curve for APO-induced behaviors. Attempting to discover the molecular mechanism for this neuromodulatory effect of cLG after chronic morphine administration to rats, we observed [16] that cLG can down-regulate binding of DA *agonists* to high affinity sites or conformations of the D-2 DA receptor. Moreover, this particular alteration of the D-2 receptor-effector complex correlated more strongly with the behavioral changes than did changes in parameters for *antagonist* ([^{3}H]spiroperidol) binding such as receptor density (Bmax) or receptor affinity, i.e., 1/Kd, for [^{3}H]spiroperidol.

In the present series of experiments we tested the ability of haloperidol and cLG to alter the sensitivity to APO using the RF capacitance device in order to test which types of stereotypic behaviors were modified by these treatments.

METHOD

Animals

Male, Sprague-Dawley rats, weighing 200 to 225 g, were purchased from Harlan (Indianapolis, IN). Animals were kept in groups, four per cage on a 12/12 dark/light cycle with free access to food and water.

Reagents and Drugs

Haloperidol was administered as McNeil's brand HALDOL. Apomorphine and dopamine were obtained from Sigma, cLG from Bachem, [^{3}H]spiroperidol from New England Nuclear and d-(+)-butaclamol from Research Biochemicals, Inc.

Drug Administration

After one week of acclimation, animals were divided into two experimental groups. Group I received a daily SC injection of saline (0.1 ml/100 g) for 21 days while group II received a daily SC injection of haloperidol (0.5 mg/kg) for the same duration. Injection volume was 0.1 ml/100 g body weight. Before the experiment started, each of these two groups was further subdivided into two subgroups (A and B). Subgroups IA and IIA received saline while subgroups IB and IIB received cLG as an SC injection (8 mg/kg). The cLG was administered once every three days for 21 days to coincide with every third haloperidol injection. This dose schedule was chosen because the plasma half-life of cLG is about 24 hr (unpublished data) and more frequent dosing may lead to an accumulation of cLG. We wished to avoid this accumulation because it is known that higher doses of cLG and similar peptides are as ineffective behaviorally as very low doses [3,4]. After 21 days, all drug administration was stopped.

Apomorphine-Induced Stereotypy

After an additional three days of withdrawal, all animals were challenged with an IP injection of apomorphine (0.5 mg/kg), a dose known to elicit both licking and sniffing behaviors in animals made supersensitive by chronic haloperidol but not in controls, between 9 a.m. and 12 noon. Animals were then evaluated for stereotypy by two methods. Method 1 involved visual observation of the animals. An animal was considered showing stereotypy if he exhibited continuous stereotypic sniffing. Each animal was observed for a 60 sec period at 5, 10, 15 and 20 minutes following the administration of APO. An animal was rated as positive for stereotypy if the predominant behavior for any of the observation periods was continuous stereotypic sniffing. Since licking

TABLE 1
STEREOTYPY FROM VISUAL OBSERVATIONS

Treatment Group	Stereotypy No. Positive/ No. Total	%
Saline-Saline	1/15	7
Peptide-Saline	4/15	27
Saline-Haloperidol	10/15	67*
Peptide-Haloperidol	7/15	50

Rats were treated as described in the text. Those animals exhibiting continuous sniffing, licking or gnawing were rated as positive for stereotypy. *$p<0.05$, Chi Square Test.

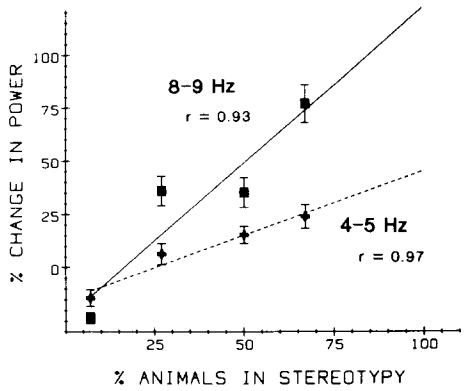

FIG. 3. Correlation between motility measurements and visual ratings of stereotypy. The figure represents the mean percent change in 4–5 Hz and 8–9 Hz power (±SEM) for each group versus the percent of animals rated by an observer as showing stereotypy. The broken and solid lines represent the linear regression curves, with r=correlation coefficient, for stereotypy ratings versus 4–5 Hz and 8–9 Hz power, respectively.

and gnawing occurs at higher doses of APO than does sniffing (unpublished observations), any animal whose predominant behavior was continuous stereotypic licking and/or gnawing was also rated as positive for stereotypy. All other animals were rated as negative for stereotypy. Statistical analysis of the data using this visual observation method was done using a Chi Square test [30].

Method 2, which was used simultaneously, involved the use of an electronic motility monitor. This apparatus consisted of a Stoelting activity monitor modified to permit objective measurement of repetitive behaviors. The use of this device has been described in detail elsewhere [8, 12, 13]. By motility, we refer to the output of the motility monitor which is an analog signal with a frequency of oscillation equal to the frequency of occurrence of movements within the field of the monitor. In this report, we use the terms "motility" and "movement" interchangeably. Quantification of movements is accomplished by spectral analysis of the frequency components of the analog output of the motility monitor. The resulting amplitude-frequency distribution has been shown [8,9] to accurately depict the occurrence of specific repetitive movements (sniffing, licking, head bobbing, etc.). The power spectrum of the transduced signal provides a statistically significant dose-response curve for several stimulant drugs [13] and thus permits the quantification of stimulant-induced stereotypy. This RF method permits the use of parametric rather than non-parametric analysis which therefore increases the power of the statistical inference and decreases the probability of a type II error.

For the measurement of motility, animals were placed in Plexiglas chambers, 19.0×13.0×8.0 cm, which were then placed in fixed positions within the movement sensor of the motility monitor. Analog-to-digital conversion of the transduced signal and subsequent analyses were performed on an Apple II+ microcomputer.

After 21 days of chronic treatment as described above, one animal from each of the four experimental groups was placed in one of the four Plexiglas chambers of the motility monitor. After a 15 min period for adaptation to the apparatus, motility was monitored for a period of 40 seconds. Following this initial (predrug) measurement, animals received the apomorphine challenge. Forty second epochs of motility were obtained immediately after injection and again at five, ten, fifteen, and twenty minutes post-injection. At the same time points stereotypy was scored through the use of behavioral observation (Method 1, above).

For the analysis of RF data, a Fast Fourier Transform was used to obtain power spectra for each one-second segment of the motility data, and the spectra were averaged across the 40 seconds of each sampling period. Following a log transformaton of the mean power spectra, multivariate analysis of variance (MANOVA) and univariate analysis of variance (ANOVA) with repeated measures were used to determine the significance of group differences at the various sampling periods.

RESULTS

Motility measurements of the four treatment groups did not differ significantly ($p>0.05$) prior to apomorphine administration. That is, neither haloperidol nor cLG altered any spontaneous behaviors that were detectable by the apparatus between frequencies of 1 and 30 Hz. However, multivariate analysis of variance indicated that the four groups exhibited significantly different ($p<0.05$) responses to an acute injection of apomorphine (0.5 mg/kg, IP). These group differences were due primarily to differences in 4–5 Hz motility ($p<0.05$) and in 8–9 Hz motility ($p<0.001$). Motility in these frequency bands has previously been reported [5] to reflect the occurrences of stereotyped licking (4–5 Hz) and sniffing (8–9 Hz) behavior. The effects of acute apomorphine on the motility in these frequency bands are illustrated in Figs. 1 and 2 for each of the four groups of animals.

Administration of apomorphine to animals treated chronically with saline alone (group IA) resulted in a significant decrease in 4–5 Hz motility ($p<0.001$) and in 8–9 Hz motility ($p<0.001$) compared to predrug measurements. Concurrent chronic administration of cLG (group IB) blocked the decrease observed in 4–5 Hz motility after apomorphine. The decrease in motility at 8–9 Hz in group IB was not only blocked but was actually increased ($p<0.001$) by apomorphine, in contrast to animals receiving only chronic saline (IA).

Animals chronically treated with haloperidol (IIA) exhibited significantly more motility in both the 4–5 Hz and 8–9 Hz frequency bands as compared with predrug motility levels ($p<0.001$) and showed significantly more 4–5 Hz ($p<0.05$) and 8–9 Hz ($p<0.01$) motility than any of the other treatment groups. Concurrent chronic administration of peptide along with haloperidol resulted in significantly less apomorphine-induced motility than observed in subjects receiving only chronic haloperidol (IIA) at both the 4–5 Hz band ($p<0.05$) and at the 8–9 Hz band ($p<0.01$). Peptide/haloperidol animals (IIB), however, showed significantly more ($p<0.05$) 4–5 Hz motility than did animals treated only with saline (IA) or with peptide (IB), and more 8–9 Hz motility ($p<0.01$) than those subjects treated chronically with saline alone (IA). Peptide/haloperidol animals (IIB) did not differ significantly ($p>0.05$) in their acute response to apomorphine in 8–9 Hz motility as compared to animals receiving peptide alone (IB).

When data from the visual observation method were analyzed (Table 1), a similar pattern of peptide and haloperidol effects was obtained. Although the haloperidol group (IIA) was statistically different than the saline group (IA), the "N" was not large enough to reach statistical significance for the comparison between the haloperidol (IIA) and peptide/haloperidol (IIB) groups.

The RF method of evaluating stereotypy, on the other hand, enabled us to make these distinctions, i.e., reach statistical significance, that were not possible, with the limited sample size, by the visual observation method. It is noteworthy that there is an identical rank order and a good correlation ($p<0.01$) between the two methods (Fig. 3).

DISCUSSION

The main finding of this study is that the peptide cLG can, when co-administered chronically with haloperidol, inhibit the neuroleptic-induced development of behavioral supersensitivity to apomorphine. Similar inhibitory effects of cLG, and the related peptide PLG (prolyl-leucyl-glycinamide, also known as MIF and MIF-1) have been independently observed by others at the biochemical level [1, 2, 4, 5, 20] where changes in [^{3}H]spiroperidol binding to sites on striatal membranes was measured. One study [2] also showed a similar effect of cLG on apomorphine-induced locomotion which is thought to be mediated mostly by mesolimbic dopamine neurons in rats [6, 7, 14, 18].

The current finding is also consistent with our own previous observations that cLG inhibits the development of supersensitivity to apomorphine in several animal models including mice treated with 6-hydroxydopamine to bilaterally lesion the nigrostriatal DA tract [22], mice treated chronically with haloperidol [2], and mice [26] and rats [16, 23–25] treated chronically with morphine.

At the one dose of cLG we studied, 8 mg/kg, the peptide inhibited about 50% of the development of behavioral supersensitivity to dopamine mediated by the rat nigrostriatal system. Since cLG and related peptides are known to have biphasic dose-response curves [3, 16, 20], such that high peptide doses have *less* effect than intermediate doses, it is possible that there exists for rats a more effective dose of cLG that can inhibit closer to 100% of the sensitizing effect of haloperidol. In mice cLG did inhibit about 100% of the haloperidol effect [2].

Failure to obtain complete inhibition could also be due to the fact that the peptide cLG, by itself (group IB), stimulates a partial up-regulation of the striatal dopamine system [17], that is, a partial supersensitivity to dopamine. In fact, the ability of PLG to potentiate L-DOPA effects in mice [17] led to clinical trials of this tripeptide and it was shown to have some anti-Parkinsonian actions [20]. This up-regulation may in fact be responsible for the biphasic dose-response curve for cLG-induced down-regulation. This pattern is reminiscent of the effects of direct-acting and indirect-acting dopamine agonists that by themselves induce an up-regulation of supersensitivity of the nigrostriatal DA system in rats but, when co-administered with neuroleptics, down-regulate the system [27].

This pattern, in which the supersensitive animals were down-regulated while the normo-sensitive animals were up-regulated, is also similar to the pattern of cLG effects we observed in rats treated chronically with morphine [16, 23–25]. In the morphine model, we were able to show that there is a change in striatal D-2 dopamine receptor binding that very strongly correlates with the behavioral changes. The D-2 binding parameter that correlated best with behavioral changes in all four treatment groups in that study was the affinity of dopamine agonists for the high-affinity conformation of the D-2 binding site ($r=0.98$). The fraction of receptors in the high-affinity state or conformation, the affinity and number in the low affinity state or conformation, and the affinity (Kd) and number (Bmax) of antagonist ([^{3}H]spiroperidol) binding sites correlated less well [16].

These biochemical findings in the morphine model led us to suggest that cLG is capable of down-regulating the D-2 dopamine receptor or at least of preventing its up-regulation to a fully supersensitive state. We are currently determining whether there are similar biochemical correlates for the behavioral data on stereotypy presented in the current study involving chronic haloperidol.

In contrast to the above two studies in rats, the inhibitory effects of cLG in mice appears to be more complete. cLG completely prevented the development of behavioral supersensitivity to dopamine agonists that develops following treatment of mice with 6-hydroxydopamine [22]. cLG also completely prevented the development of behavioral supersensitivity to dopamine agonists that developed following chronic treatment of mice with haloperidol [2]. Since only one dose of cLG was evaluated in each study, this species difference in extent of inhibition by cLG may be related to non-overlapping maxima in the biphasic dose-response curve for cLG in each species.

The RF method is also of interest because is overcomes an inherent problem in visually rating stereotypic behaviors. In any visual method, it is necessary to establish an arbitrary cutoff point and rate each animal or each group as positive or negative for stereotypy.

Other systems rely on a total stereotypy score summing scores for different stereotypies. An inherent problem with that approach is that different brain regions may mediate each stereotypy. Moreover, correlations of behavioral changes with neurochemical changes will be more difficult to make. For instance, stimulation of striatal DA receptors appears to be most important for stereotypic sniffing and gnawing whereas mesolimbic areas such as nucleus accumbens and olfactory tubercle appear to be more important for stereotypic locomotor activity with the striatum playing a lesser role [6, 7, 14, 18].

In the RF method, enumeration analysis [32] is not necessary and parametric analysis can be used. Of course the subjectivity of the rater(s) is another problem that disappears with the use of an automated activity measuring instrument.

The RF apparatus led to another, somewhat surprising, finding, namely, that saline treated animals had less activity at 8–9 Hz and at 4–5 Hz than they did prior to injection of APO. This decrease in the 8–9 Hz signal was prevented by cLG. A decrease in activity is thought to be a pre-synaptic effect of APO. DA agonists stimulate pre-synaptic receptors and stimulate a negative feedback signal to decrease DA synthesis and/or DA release. But this is reported to occur only at very low doses of APO between 0.02 and 0.2 mg/kg (IP) [27] whereas our dose of APO, 0.50 mg/kg, was considerably larger. Moreover, this pre-synaptic effect of low doses of APO has been observed to regulate locomotor activity (mesolimbic) whereas we observed decreases in the 8–9 Hz signal which represents sniffing (nigrostriatal). Therefore the effect we observed may not be due to that particular mesolimbic, pre-synaptic mechanism.

To further support this premise, we note that it has been reported that in experiments using a similar apparatus, saline treated animals underwent a reduction in activity at these frequency bands. Those data were interpreted as being due to habituation [12]. In any event, our finding appears to be opposite to one previous report [31] in which it was observed that cLG actually potentiated the low dose, locomotor-activity reducing, effect of APO.

In conclusion, we have reported a novel method for the simultaneous detection and quantification of different stereotypies known to be mediated by striatal D-2 DA receptors. We have further shown, using this method, that the peptide cLG can prevent the development of behavioral supersensitivity that is induced by haloperidol. cLG may therefore be of interest in various clinical disorders that are thought to involve hyperdopaminergic states such as tardive dyskinesia.

ACKNOWLEDGEMENTS

This study was supported by grants from the Veterans Administration.

REFERENCES

1. Bhargava, H. N. Enhanced striatal [3H]spiroperidol binding induced by chronic haloperidol treatment inhibited by peptides administered during the withdrawal phase. *Life Sci* **34:** 873–879, 1984.
2. Bhargava, H. N. and R. F. Ritzmann. Inhibition of neuroleptic induced dopamine receptor supersensitivity by cyclo(Leu-Gly). *Pharmacol Biochem Behav* **13:** 633–636, 1980.
3. Bjorkman, S. and H. Sievertsson. On the optimal dosage of Pro-Leu-Gly-NH_2 (MIF) in neuropharmacological tests and clinical use. *Naunyn Schmiedebergs Arch Pharmacol* **298:** 79–81, 1977.
4. Chiu, P., G. Rajakumar, S. Chiu, R. L. Johnson and R. K. Mishra. Mesolimbic and striatal dopamine receptor supersensitivity: Prophylactic and reversal effects of L-prolyl-L-leucyl-glycinamide (PLG). *Peptides* **6:** 179–184, 1985.
5. Chiu, S., C. S. Paulose and R. K. Mishra. Neuroleptic drug-induced dopamine receptor supersensitivity: Antagonism by L-Prolyl-L-Leucyl-Glycinamide. *Science* **214:** 1261–1262, 1981.
6. Costall, B. and R. J. Naylor. Mesolimbic and extrapyramidal sites for the mediation of stereotyped behavior patterns and hyperactivity by amphetamine and apomorphine in the rat. In: *Cocaine and Other Stimulants*, edited by E. H. Ellinwood, Jr. and M. M. Kilbey. New York: Plenum Press, 1977, pp. 47–76.
7. Costall, B. and R. J. Naylor. Extrapyramidal and mesolimbic involvement with the stereotypic activity of d- and l-amphetamine. *Eur J Pharmacol* **25:** 121–129, 1974.
8. Ellinwood, E. H., Jr., D. W. Molter and K. A. Stauderman. An assessment of spectral analysis of amphetamine-induced behavior. *Pharmacol Biochem Behav* **15:** 627–631, 1981.
9. Ellinwood, E. H., Jr. and M. M. Kilbey. Alteration in motor frequencies with dopamine agonists and antagonists. *Psychopharmacol Bull* **15:** 49–50, 1979.
10. Ernst, A. M. Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. *Psychopharmacologia* **10:** 316–323, 1967.
11. Fog, R. On stereotypy and catalepsy: Studies on the effect of amphetamines and neuroleptics in rats. *Acta Neurol Scand [Suppl]* **50:** 1, 1972.
12. Gonzalez, L. P. Quantitative analysis of physostigmine-induced changes in behavior. *Pharmacol Biochem Behav* **21:** 551–554, 1984.
13. Gonzalez, L. P. and E. H. Ellinwood, Jr. Cholinergic modulation of stimulant-induced behavior. *Pharmacol Biochem Behav* **20:** 397–403, 1984.
14. Joyce, J. Multiple dopamine receptors and behavior. *Neurosci Biobehav Rev* **7:** 227–256, 1983.
15. Kobayashi, R. M., J. Z. Fields, R. E. Hruska, K. Beaumont and H. I. Yamamura. In: *Animal Models in Psychiatry and Neurology*, edited by I. Hanin and E. Usdin. Oxford: Pergamon, 1977, pp. 405–409.
16. Lee, J. M., J. Z. Fields and R. F. Ritzmann. Cyclo(Leu-Gly) attenuates the striatal dopaminergic supersensitivity induced by chronic morphine: Agonist binding to D-2 dopamine receptors correlates with stereotypic behavior. *Life Sci* **33:** Suppl 1, 405–408, 1983.
17. Lee, J. M., R. F. Ritzmann and J. Z. Fields. Cyclo(Leu-Gly) has opposite effects on D-2 dopamine receptors in different brain areas. *Peptides* **5:** 7–10, 1984.
18. Ljungberg, T. and U. Ungerstedt. A rapid and simple behavioral screening method for simultaneous assessment of limbic and striatal blocking effects of neuroleptic drugs. *Pharmacol Biochem Behav* **23:** 479–485, 1985.
19. Mackenzie, R. G. and M. J. Zigmond. Chronic neuroleptic treatment increases D-2 but not D-1 receptors in rat striatum. *Eur J Pharmacol* **113:** 159–165, 1985.
20. Mishra, R. K., S. Chiu, P. Chiu and C. P. Mishra. Pharmacology of L-Prolyl-L-Leucyl-Glycinamide (PLG): A Review. *Methods Find Exp Clin Pharmacol* **5:** 203–233, 1983.
21. Plotnikoff, N. P., F. N. Minard and A. J. Kastin. DOPA potentiation in ablated animals and brain levels of biogenic amines in intact animals after Prolyl-leucylglycinamide. *Neuroendocrinology* **14:** 271–279, 1974.
22. Ritzmann, R. F. and H. N. Bhargava. Effects of cyclo(Leu-Gly) on chemical denervation supersensitivity of dopamine receptors induced by intracerebroventricular injection of 6-hydroxydopamine in mice. *Life Sci* **27:** 2075–2080, 1980.
23. Ritzmann, R. F., J. M. Lee and J. Z. Fields. Modification of morphine induced changes in striatal [3H]spiroperidol binding and stereotype behavior by cyclo(Leu-Gly). *Life Sci* **30:** 1573–1580, 1982.
24. Ritzmann, R. F., J. M. Lee and J. Z. Fields. Peptide inhibition of morphine-induced dopaminergic supersensitivity. *Life Sci* **31:** 2287–2290, 1982.
25. Ritzmann, R. F., J. M. Lee and J. Z. Fields. Effect of peptides on morphine induced tolerance and physical dependence. *Psychopharmacol Bull* **19:** 321–324, 1983.
26. Ritzmann, R. F., R. Walter and H. N. Bhargava. Blockade of narcotic induced dopamine receptor supersensitivity by cyclo(Leu-Gly). *Proc Natl Acad Sci USA* **76:** 5997–5998, 1979.

27. Schwartz, J. C., J. Costentin, M. P. Martres, P. Protais and M. Baudry. Modulation of receptor mechanisms in the CNS: Hyper- and hypo-sensitivity to catecholamines. *Neuropharmacology* **17:** 665–685, 1978.
28. Seeman, P. Brain dopamine receptors. *Pharmacol Rev* **32:** 229–313, 1981.
29. Tarsy, D. and R. Baldessarini. Behavioral supersensitivity to apomorphine following chronic treatment with drugs which interfere with synaptic functions of catecholamines. *Neuropharmacology* **13:** 927–940, 1974.
30. Tarsy, D. and R. J. Baldessarini. Tardive dyskinesia. *Annu Rev Med* **35:** 605–623, 1984.
31. Xiao, X., H. D. Veldhius and J. M. Van Ree. Neuropeptides related to neurohypophyseal hormones interfere with apomorphine-induced behavioral changes. *Neuropeptides* **4:** 237–245, 1984.
32. Zar, J. H. *Biostatistical Analysis*. Englewood Cliffs, NJ: Prentice Hall, 1974, pp. 41–109.