*Pharmacology Biochemistry & Behavior*, Vol. 16, pp. 17–21, 1982. Printed in the U.S.A.

# Differential Effects of Diazepam (Valium) on Brain Stimulation Reward Sites

MAURO CAUDARELLA,[1] K. A. CAMPBELL AND N. W. MILGRAM

*Department of Psychology, Scarborough College*
*University of Toronto, West Hill, Canada M1C 1A4*

Received 17 January 1981

CAUDARELLA, M., K. A. CAMPBELL AND N. W. MILGRAM. *Differential effects of diazepam (Valium) on brain stimulation reward sites.* PHARMAC. BIOCHEM. BEHAV. **16**(1) 17–21, 1982.—The effect of diazepam on electrical self-stimulation of the dorsolateral hippocampus was tested in 19 male rats. Two mg/kg of diazepam significantly suppressed lever pressing rates at both high and low current intensities, whereas 1 mg/kg did so only at the low current intensity. The lowest dose (0.5 mg/kg) of diazepam had no significant effect. The second experiment tested the generality of the effect of diazepam on self-stimulation using electrodes in the lateral hypothalamus. A significant increase in lever pressing rates was observed at all dose levels.

Diazepam Self-stimulation Hippocampus Hypothalamus

## EXPERIMENT 1

Electrical self-stimulation (SS) of the brain is a technique that has often been used to test the effects of drugs on brain reward mechanisms. Although most mild tranquillizers produce decreases in SS behavior, Olds [13] reported that diazepam markedly increased lever pressing rates with stimulating electrodes in either the anterior or posterior hypothalamus. In that study, one possible explanation discussed was the anticonvulsant properties of the durg: an increase in SS rates could be due to suppression of brain seizure activity that might otherwise accompany the brain stimulation and interfere with SS behavior. This explanation is consistent with other reports of facilitation of SS by anticonvulsant drugs such as sodium pentobarbital (e.g., [10]).

One brain site where SS is frequently accompanied by seizure activity is the hippocampus (HPC). Although hippocampal SS is a reliable and stable phenomenon, the interfering effects of convulsive activity may account for the slow learning and relatively low lever pressing rates observed in rats with hippocampal placements [3]. If diazepam facilitates hypothalamic SS behavior by suppressing the relatively mild interfering effects of seizure activity at this site, then this drug should facilitate hippocampal SS to an even greater degree since the hippocampus is more prone to seizures than the hypothalamus. The first experiment to be reported here tested this hypothesis by examining the effect of three doses of diazepam on lever press responding reinforced by electrical stimulation of the dorsolateral HPC. Two current intensities were used to determine any interaction between stimulation intensity and drug dose.

### METHOD

#### *Subjects and Surgery*

Thirty male hooded rats of the long Evans strain, 3–5 months of age, were implanted stereotactically with a single electrode in the dorsolateral hippocampus (target: cell field CA3; coordinates: 3.8 mm posterior to Bregma, 4.2 mm lateral and 3.3 mm below the dura, with skull level). Two types of electrodes were implanted: a single strand of 220 $\mu$ diameter 80% platinum-20% iridium wire insulated with clear vinyl for monopolar stimulation or a bipolar electrode made of two twisted strands of 225 $\mu$ diam. Nichrome wire insulated with Formvar. If the electrode was of the single, monopolar type, the skull anchor screws served as the indifferent electrode. For EEG recording, a similar indifferent skull-screw electrode was used along with a depth bipolar electrode. One additional animal was implanted with a bipolar electrode in each HPC so that one HPC could be stimulated while the EEG was recorded from the contralateral HPC. The surgery was carried out under Na pentobarbital (60–80 mg/kg) anesthesia and atropine sulfate (0.5 cc of 1% solution). The animals were allowed at least two weeks to recover from surgery before behavioral testing began.

#### *Testing Procedure*

In a Plexiglas Skinner box (30×18×62 cm high) inside a sound-attenuating chamber the animals were trained to deliver 0.5 sec of 30 $\mu$A (rms) sine wave current to their brain by pressing a small lever. The same current intensity was

[1]Present address: Laboratoire de Psychophysiologie, Institut de Biologie Animale, Université de Bordeaux I, Avenue des Facultés, 33405 Talence Cedex, France.

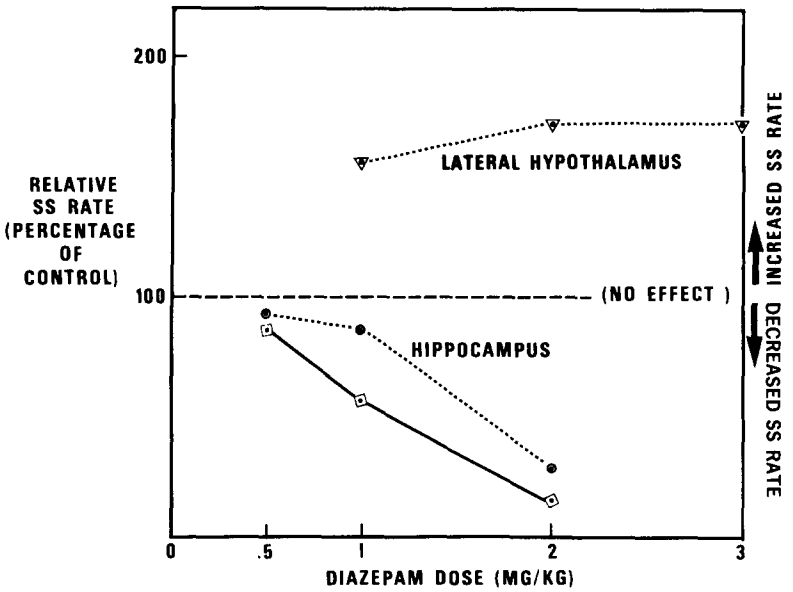


FIG. 1. (A, Lower Part) Effect of three doses of diazepam (independent samples) on hippocampal lever pressing rates at two current intensities (repeated measures). The means are expressed as a percentage of average control (vehicle) rates. Each point is based on 3 drug and 3 vehicle tests. (B, Upper Part) Effect of diazepam on two hypothalamic SS placements. Within each group, the data constitute repeated measures and are expressed as a percentage of average control (vehicle) rates.

used throughout initial training for all animals. Nineteen self-stimulators (10 with monopolar electrodes, 9 with bipolar electrodes) were selected and randomly divided into three groups, each receiving a different dose of diazepam (undiluted Valium injection), *viz.*, 0.5 mg/kg (n=7), 1 mg/kg (n=6), or 2 mg/kg (n=6), and a different volume of vehicle solution (40% propylene glycol+10% ethanol, in distilled water) equal to the volume of drug administered to the same animal. The same dosage of drug, or vehicle, was administered intraperitoneally 5 min before testing on three successive days. The sequence of three daily drug tests and three daily vehicle tests (one week apart) took place at each of two current intensities, 10 $\mu$A and 50 $\mu$A, approximately four weeks apart. The order of presentation of drug and vehicle and of low and high current intensity was counterbalanced and randomly determined. Each rat, then, was tested at both current intensities but at only one dose level of diazepam. Daily 20-min tests were conducted seven days a week and the number of lever presses and stimulations received by the animal was automatically recorded. Before drug testing began, the rats were switched from the initial training current of 30 $\mu$A to 10 or 50 $\mu$A (depending on counterbalancing) and allowed 14 days at this new intensity to allow lever pressing rates to stabilize. In three cases, when the current intensity was switched, 10 $\mu$A was found to be below the animal's threshold for stable lever pressing and was therefore raised to 15–25 $\mu$A for all test sessions.

*EEG Recording*

To find out whether seizure activity is associated with HPC self-stimulation, sample EEG records were taken during self-stimulation from four animals with Nichrome bipolar electrodes and the one animal with bilateral HPC electrodes. These animals were tested in a Plexiglas Skinner box housed in a shielded outer chamber which was otherwise identical to the boxes used during drug tests. EEG's were recorded only before or after the drug phase of the experiment. The animal was simply left to press the lever ad lib while the EEG was recorded for 10–20 min. The current intensity was 30 $\mu$A or less. EEG recordings were made through the stimulating electrode (4 *S*s) or the contralateral HPC electrode (1 *S*), fed through a Brush differential amplifier and displayed on a Brush oscillograph.

*Histology*

Upon completion of the experiment each animal was killed with an overdose of Na pentobarbital or $CO_2$. The brain was perfused with 10% Formalin by intracardial injection, removed from the cranium and stored in a 10% Formalin solution. A 1–2 cm segment of brain was frozen and sliced in an Ames Cryostat microtome. Sections were stained with cresyl violet to show both cells and fibers. All electrode tips were located in regio inferior (CA3) of the dorsolateral hippocampus or immediately adjacent fimbria. Photographs of similar electrode placements have been previously published [3].

## RESULTS

The number of lever presses in 20 min were averaged across the last three stabilization days: at 10 $\mu$A, the mean SS rate was 124.2 ($\pm$12.8 S.E.M.); at 50 $\mu$A, the mean SS

rate was 129.3 (±13.2 S.E.M.). Thus, a change in intensity did not significantly alter baseline SS rate ($p>0.05$, Wilcoxon Matched-Pairs Test [19]). Since the type of electrode (monopolar or bipolar) also did not produce a significant difference in SS rates, electrode configuration was ignored for all other analyses.

The numbers of lever presses made in 20 min were averaged across the three administrations of the diazepam doses at each current intensity and are presented in Table 1 and shown in Fig. 1 (lower part) expressed as a percentage of control (vehicle) SS rates. The highest dose (2 mg/kg) of diazepam produced a significant suppression of SS rates ($p<0.05$, Wilcoxon test) at both current intensities; the 1 mg/kg dose produced a significant suppression ($p<0.05$) only at the low current intensity; the difference between the low dose and vehicle control was not significant at either current intensity, even though at the low intensity 5 out of 7 animals showed a slight decrease in SS rate when drugged.

TABLE 1

MEAN NO. OF LEVER PRESSES (±S.E.M.) MADE IN 20 MIN BY THREE GROUPS OF RATS WITH HIPPOCAMPAL ELECTRODES

| | Dose (mg/kg) | | |
|---|---|---|---|
| | 0.5 (n=7) | 1.0 (n=6) | 2.0 (n=6) |
| Low Intensity | | | |
| Diazepam | 92 ± 17 | 85 ± 33 | 19 ± 13 |
| Vehicle | 106 ± 12 | 144 ± 21 | 119 ± 25 |
| High Intensity | | | |
| Diazepam | 134 ± 21 | 167 ± 13 | 32 ± 20 |
| Vehicle | 145 ± 7 | 194 ± 19 | 112 ± 20 |

Each group was tested with only one dose of diazepam at each of two current intensities. These data are presented in the lower part of Fig. 1 as a percentage of vehicle response rates.

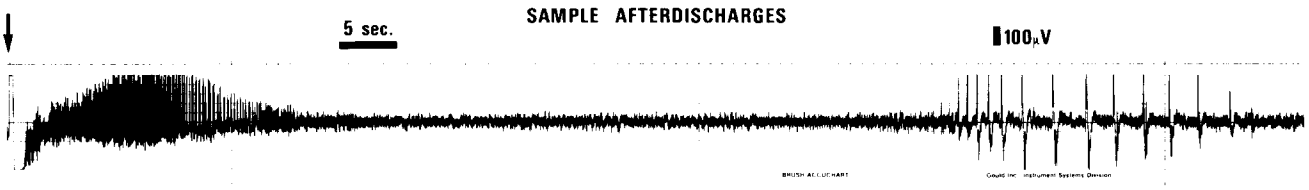


FIG. 2. EEG recorded from stimulating electrode in HPC after initial 0.5 sec, 30 μA stimulation (arrow). The continuous record shows both primary and secondary afterdischarges.

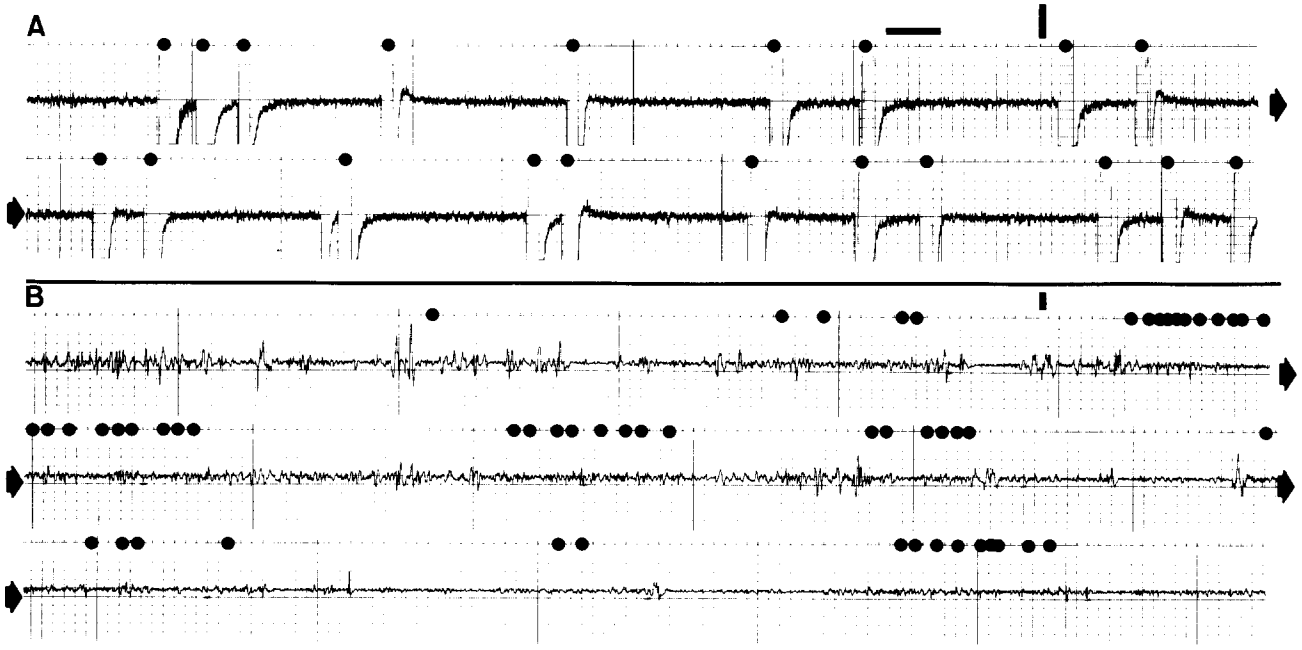


FIG. 3. (A) EEG recorded from stimulating electrode in HPC during SS session in a rat that had received one stimulation 5 min before testing. Vertical bar: 100 μV. Horizontal bar: 5 sec. (B) EEG recorded from contralateral HPC during SS session. Black dots indicate 0.5 sec stimulations through the ipsilateral (stimulating) hippocampal electrode. Heavy vertical bar: 200 μV.

To some extent, the suppression of SS rates was associated with the sedative and ataxic effects of the drug. At 2 mg/kg animals were inactive when left alone and showed some lack of coordination when handled. At the lowest dose (0.5 mg/kg) there were no obvious effects. When one of the higher doses resulted in inactivity during the testing session, priming stimulation had a surprisingly small effect: it did not arouse the animal, nor did it trigger lever pressing.

*EEG Recordings and Convulsive Activity*

Samples of hippocampal EEG activity recorded through the stimulating electrode in undrugged animals showed that local epileptiform afterdischarges were evoked by the first stimulation of the testing session in a large majority of trials; subsequent stimulations (during a 10–20 min SS session) rarely elicited seizure activity. Two animals that received 0.5 sec of HPC stimulation five minutes before a SS test showed no epileptiform afterdischarges during SS (Fig. 3A). A sample EEG recorded from the contralateral HPC during SS in the rat with bilateral electrodes is shown in Fig. 3B. The afterdischarge threshold for this animal was 80 $\mu$A and the SS session was conducted using a current intensity of 30 $\mu$A. No seizure activity was seen in the contralateral HPC during the entire 15-min session; at the same time, the rat pressed the lever 96 times.

Lever pressing rates did not appear to be related to the occasional occurrence of seizure activity except for a decrease in SS rate immediately following a convulsion. On days when no seizures occurred, lever pressing rates (30–50 responses per 10-min period) and patterns of responding did not differ noticeably from those observed on days when a seizure did occur.

Diazepam (2 mg/kg) failed to block local afterdischarges elicited by the first self-stimulation of the testing day (three animals), although generalized motor convulsions were suppressed.

## EXPERIMENT 2

In Experiment 1, treatment with diazepam produced a dose-dependent suppression of SS behavior. Since diazepam has sedative properties, it is not clear whether the dose-dependent decrease is attributable to a change in the reward value of the stimulation. Motor and other performance effects of diazepam seem unlikely, however, since other investigators have reported that lever pressing for hypothalamic stimulation is actually increased by diazepam [13, 14, 15]. In the present experiment, a group of self-stimulators with electrodes in the lateral hypothalamus was also tested with diazepam in order to replicate this latter finding using testing conditions similar to those followed in Experiment 1.

### METHOD

Four adult male hooded rats were implanted with a single bipolar Nichrome-wire electrode in the lateral hypothalamus using target coordinates: 0.8 mm posterior to Bregma, 1.75 mm lateral and 8.6 mm below the skull, with the incisor bar 5 mm above the interaural line. After recovery from surgery, the rats were trained to press a lever for brain stimulation in a Skinner box. In the first 30 min session, the current was varied from 0–60 $\mu$A (rms) while the rat was shaped to approach and press the lever. Daily training sessions continued until stable lever pressing rates were observed. Each animal then received 1, 2, and 3 mg/kg of undiluted Valium injection and an equal volume of vehicle, with the four treatments administered in counterbalanced order. Each treatment was repeated on each of three successive days with at least four days between treatments. Intraperitoneal injections were made five min before testing. The current intensity was individually set for each subject at a level (20–40 $\mu$A) which yielded self-stimulation rates of approximately 400–600 lever presses per 15 min. Lever presses were recorded after 15 and 30 min of SS, and were averaged across the three treatment days.

TABLE 2

MEAN NO. OF LEVER PRESSES (±S.E.M.) FOR STIMULATION OF THE LATERAL HYPOTHALAMUS

| Diazepam Dose (mg/kg) | Lever Pressing Rates | |
|---|---|---|
| | first 15 min | second 15 min |
| 0 | 530 ± 108 | 498 ± 78 |
| 1 | 820 ± 108 | 776 ± 110 |
| 2 | 846 ± 107 | 855 ± 143 |
| 3 | 709 ± 74 | 857 ± 158 |

The drug data are presented in the upper part of Fig. 1 as a percentage of vehicle (0 mg/kg) response rates.

### RESULTS

The mean SS rates during the first 15 min period are presented in Fig. 1 (upper part) expressed as percentage of control (vehicle) rates. Self-stimulation rates increased significantly ($p<0.05$, at each dose, sign test [19]) with all doses of diazepam. Similar results were obtained during the second 15-min period. Both are shown in Table 2.

At the highest doses, animals appeared to be sedated but were immediately aroused and began to press the lever when they were given two or three priming stimulations at the beginning of the session.

## DISCUSSION

In the first experiment, responding for electrical stimulation of the hippocampus was suppressed by diazepam. This finding was unexpected: previous research had indicated that diazepam facilitated lever pressing for brain stimulation of the hypothalamus, and this observation was confirmed in the second experiment. It is therefore apparent that the effect of diazepam on brain stimulation depends upon the neural locus stimulated.

Behaviorally, diazepam administered at the moderate doses used in these experiments has sedative and ataxic effects which might be expected to interfere with lever pressing behavior. Although the absence of a suppressive effect on hypothalamic SS would appear to contradict this suggestion, it is possible that hypothalamic but not hippocampal stimulation may have facilitatory effects which counteract the sedative effects of diazepam. This hypothesis is suggested by observations of the animals' behavior during the second experiment: priming stimulation of the hypothal-

amus was sufficient to arouse the animals and instigate vigorous lever pressing, although in the undrugged state priming was not required. It has also been previously established that hypothalamic stimulation has facilitatory effects on motor performance [11]. Indeed, there is even evidence that such stimulation can overcome the inactivity induced by partially-paralyzing doses of curare and sub-anaesthetic doses of barbiturates [5].

A quite different interpretation of Experiment 1 may be advanced based on suggestions that SS of anterior limbic brain structures may be dependent on abnormal seizure activity [2, 12, 17]. Since diazepam has well-known anticonvulsant effects (e.g., [6]), it may be suggested that the drug suppresses hippocampal SS because it removes the seizure activity responsible for maintaining the behavior. However, this interpretation is not consistent with the results of the present EEG sampling which showed a dissociation of SS behavior and EEG seizure activity. In addition, in Experiment 1, diazepam did not block local afterdischarges even at a dose which produced almost total suppression of self-stimulation. The lack of effect of diazepam on local seizure activity has also been reported by Racine [18]. Therefore, on the basis of the present results, it cannot be concluded that the suppression of hippocampal SS is causally related to suppression of seizure activity.

A third possible explanation for these findings relates to the possibility that diazepam has selective neurophysiological effects on different neuronal circuits. Diazepam may affect neuronal processing differently in the HPC than in the hypothalamus, which may give rise to the differential effect of diazepam on the behavioral consequences of electrical stimulation at these sites. In this regard, it is interesting to note that recent *in vitro* and *in vivo* receptor-binding studies in mice found that the density of benzodiazepine-specific receptor sites was much higher in the HPC than in the hypothalamus [4]. Moreover, there is evidence that diazepam suppresses the electrical activity of the HPC [1, 9, 16, 20], probably by potentiating GABA-mediated recurrent inhibition on HPC pyramidal cells [20]. Therefore, in disrupting HPC self-stimulation, diazepam may be acting by blocking activity in the HPC circuits that are directly stimulated through the electrode.

The direct effect of diazepam on hypothalamic activity is not known. However, there is evidence that HPC activity has an inhibitory effect on SS of the hypothalamus: response rates are increased by HPC lesions [7] and inhibited by dorsal HPC stimulation [8]. Thus, it is possible that the facilitatory effect of diazepam on hypothalamic SS is also attributable to a suppression of hippocampal activity by diazepam.

ACKNOWLEDGMENTS

This research was supported by N. R. C. Grant A7659 to N. W. Milgram. We thank Hoffman-La Roche (Canda) Inc. for supplying the Valium.

## REFERENCES

1. Adamec, R. E., B. McNaughton, R. Racine and K. E. Livingston. Effects of diazepam (Valium) on hippocampal excitability in the rat: action in the dentate area. *Epilepsia* 1981, in press.
2. Bruner, A. Self-stimulation in the rabbit: an anatomical map of stimulation effects. *J. comp. Neurol.* **131:** 615–630, 1966.
3. Campbell, K. A. and N. W. Milgram. Potentiating hippocampal stimulation facilitates acquisition of lever-pressing for stimulation but not food. *Physiol. Behav.* **24:** 1115–1118, 1980.
4. Duka, T., V. Hollt and A. Herz. In vivo receptor occupation by benzodiazepines and correlation with the pharmacologic effect. *Brain Res.* **179:** 147–156, 1979.
5. Gallistel, C. R., P. Shizgal and J. S. Yeomans. A portrait of the substrate for self-stimulation. *Psychol. Rev.* **88:** 228–273, 1981.
6. Hernandez-Peon, R., J. A. Rojas-Ramirez, J. J. O'Flaherty and A. L. Mazzuchelli-O'Flaherty. An experimental study of the anticonvulsive and relaxant actions of Valium. *Int. J. Neuropharmac.* **3:** 405–412, 1964.
7. Jackson, F. The effects of posterior hippocampal lesions upon intracranial self-stimulation rates under satiation and deprivation conditions. Unpublished Ph.D. thesis, Rutgers-The State University, New Brunswick, New Jersey, 1967.
8. Jackson, W. J. and E. L. Gardner. Modulation of hypothalamic ICSS by concurrent limbic stimulation. *Physiol. Behav.* **12:** 177–182, 1974.
9. Matthews, W. D. and J. D. Connor. Effects of diphenylhydantoin and diazepam on hippocampal evoked responses. *Neuropharmacology* **15:** 181–186, 1976.
10. Mogenson, G. J. Effects of sodium pentobarbital on brain self-stimulation. *J. comp. physiol. Psychol.* **58:** 461–462, 1964.
11. Murphy, J. P. and E. Gellhorn. The influence of hypothalamic stimulation on cortically induced movements and on action potentials of the cortex. *J. Neurophysiol.* **8:** 341–364, 1945.
12. Newman, B. L. and S. M. Feldman. Electrophysiological activity accompanying intracranial self-stimulation. *J. comp. physiol. Psychol.* **57:** 244–247, 1964.
13. Olds, M. E. Facilitatory action of diazepam and chlordiazepoxide on hypothalamic reward behavior. *J. comp. physiol. Psychol.* **62:** 136–140, 1966.
14. Olds, M. E. Effectiveness of morphine and ineffectiveness of diazepam and phenobarbital on the motivational properties of hypothalamic self-stimulation behavior. *Neuropharmacology* **15:** 117–131, 1976.
15. Olds, M. E. and M. Gardner. Effects of diazepam and phenobarbital on self-stimulation in posterior hypothalamic and preoptic regions and on the thermoregulatory responses to rewarding brain stimulation. *Neuropharmacology* **15:** 103–115, 1976.
16. Olds, M. E. and J. Olds. Effects of anxiety-relieving drugs on unit discharges in hippocampus, reticular midbrain, and preoptic area in the freely moving rat. *Int J. Neuropharmac.* **8:** 87–103, 1969.
17. Porter, R. W., D. G. Conrad and J. V. Brady. Some neural and behavioral correlates of electrical self-stimulation of the limbic system. *J. exp. Analysis Behav.* **2:** 43–55, 1959.
18. Racine, R., K. E. Livingston and A. Joaquin. Effects of procaine hydrochloride, diazepam and diphenylhydantoin on seizure development in cortical and subcortical structures in rats. *Electroenceph. clin. Neurophysiol.* **39:** 261–271, 1975.
19. Siegel, S. *Nonparametric Statistics for the Behavioral Sciences.* New York: McGraw-Hill, 1956.
20. Wolf, P. and H. L. Haas. Effects of diazepines and barbiturates on hippocampal recurrent inhibition. *Naunyn-Schmiedeberg's Arch. Pharmac.* **299:** 211–218, 1977.