Pharmacology Biochemistry & Behavior, Vol. 8, pp. 441–445. Printed in the U.S.A.

An Analysis of Dorsal and Median Raphe Self-Stimulation: Effects of Para-Chlorophenylalanine

DEREK VAN DER KOOY, HANS C. FIBIGER AND ANTHONY G. PHILLIPS[1]

Department of Psychology and Division of Neurological Sciences, Department of Psychiatry
University of British Columbia, Vancouver, Canada

(Received 20 September 1977)

VAN DER KOOY, D., H. C. FIBIGER AND A. G. PHILLIPS. *An analysis of dorsal and median raphe self-stimulation: effects of para-chlorophenylalanine.* PHARMAC. BIOCHEM. BEHAV. 8(4) 441–445, 1978. – The role of serotonergic systems in intracranial self-stimulation (ICSS) of the dorsal and median raphe nuclei of rats was investigated. Intragastric administration of 400 mg/kg of parachlorophenylalanine (PCPA) depressed ICSS rates in the group with dorsal raphe electrode placements over a similar time course to the depletion of brain serotonin which results from treatment with PCPA. An intrasessional analysis of these behavioral changes on the fourth day after PCPA revealed that dorsal raphe ICSS was depressed over both halves of the 2 hr test session, whereas a significant depression in median raphe ICSS occurred only in the last hr of the session. The data from these studies suggest that brain serotonin systems contribute to the phenomenon of brain-stimulation reward in the dorsal and median raphe nuclei. The involvement of multiple neurochemical substrates of brain stimulation reward is discussed.

Brain-stimulation reward Serotonin Dorsal raphe Median raphe PCPA

EXPLANATIONS of the neurochemical mediation of reinforcement have focused on monoaminergic mechanisms [9,32] but the role of serotonin (5–HT) has proved particularly controversial. Reports of serotonergic punishment receptors [32] contrast with studies demonstrating increased 5–HT release by highly rewarding brain stimulation [11]. Recent investigations of the involvement of 5–HT in ICSS have examined the effect of the 5–HT synthesis inhibitor PCPA on ICSS obtained from electrode placements in several brain regions containing high concentrations of 5–HT terminals or cell bodies. Thus, a PCPA–induced decrease in ICSS elicited from the caudate [21] and hippocampus [31] has pointed to a role for 5–HT in maintaining ICSS of these forebrain structures.

The 5–HT systems in the forebrain arise from cell bodies located primarily in the midbrain raphe nuclei [2,30]. There appears to be some discrepancy as to the effect of PCPA on ICSS at sites in these raphe nuclei, as PCPA has been reported to increase ICSS in the dorsal raphe [27] while decreasing ICSS in the median raphe [18]. In an attempt to shed more light on this issue, the following experiment was designed to examine the effects of PCPA treatment on both dorsal and median raphe ICSS.

METHOD

Animals

The animals were male Wistar rats weighing 280–330 g at the time of surgery. They were housed in individual cages throughout the experiment with free access to food and water.

Surgery and Histology

Animals were anaesthetized with sodium pentobarbital (50 mg/kg IP), placed in a Kopf stereotaxic apparatus, and small dia. bipolar nichrome electrodes (Plastic Products Co., MS 303–0, .005 in.), were chronically implanted according to standard procedures. The coordinates for the dorsal raphe placements were AP–8.0 mm posterior to bregma, L – 0 mm from the midline and DV–6.2 mm below the dura, with the head level between lambda and bregma. The coordinates for the median raphe placements were AP – 8.0 mm, L – 0 mm, and DV – 7.9 mm, again with the head level between lambda and bregma.

At the completion of the experiment, all animals were asphyxiated with CO_2 and their brains rapidly removed and stored in 10% Formalin. Brains were frozen, sectioned at 30 μ and sections containing electrode tracts were mounted and stained with Luxol fast blue and counterstained with thionin.

Procedure

Following recovery from surgery, animals were tested for ICSS in 5 identical Plexiglas chambers (46 cm × 30 cm × 24 cm). Depression of a small 2.5 cm wide bar activated

[1] Send reprint requests to: Dr. A. G. Phillips, Department of Psychology, University of British Columbia, Vancouver, B.C. V6T 1W5, Canada.

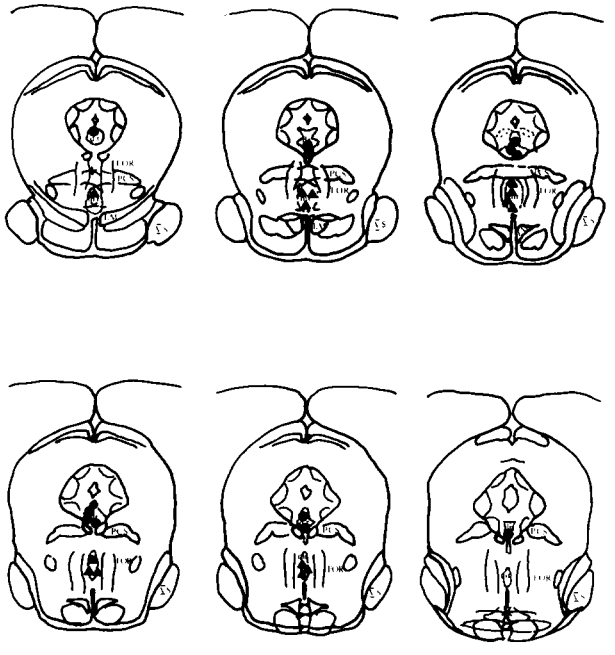

FIG. 1. Location of electrode tips in dorsal raphe (•) and median raphe (▲).

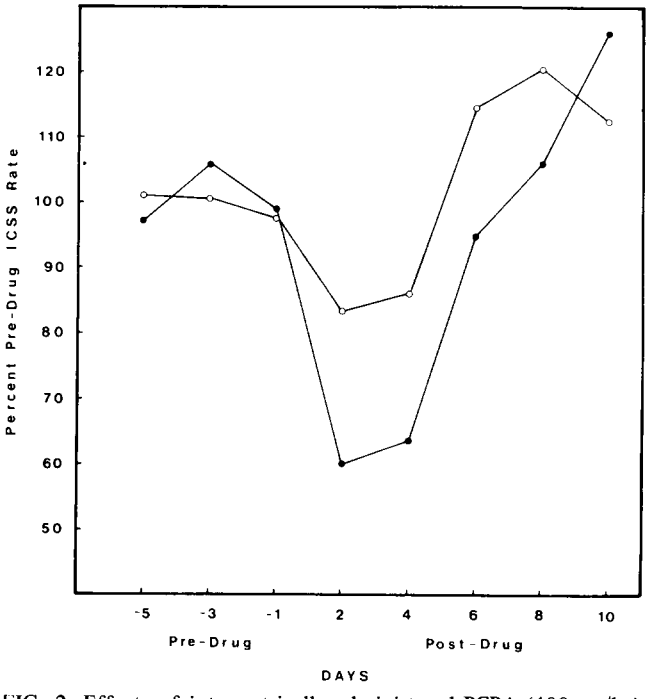

FIG. 2. Effects of intragastrically administered PCPA (400 mg/kg) on dorsal raphe (•) and median raphe (○) ICSS. Data points depicted are group means for 2 hr test sessions expressed as percentages of predrug rates. The predrug ICSS rates were a) dorsal raphe group, $\bar{X}$ = 3460/2 hr ± 623; b) median raphe group, $\bar{X}$ = 2912/2 hr ± 670.

an AC constant current stimulator which delivered a variable intensity (1–150 μA) 60 Hz sine wave stimulus of fixed duration (0.2 sec) through a flexible cable, to the chronic electrode assembly. Animals were shaped to the bar on their first day in the ICSS chambers until the bar pressing response was acquired. After 7 days, rats not reaching a criterion of 50 bar-presses/15 min were rejected from the experiment. Stimulation intensities were individually adjusted to elicit stable submaximal ICSS rates. Thirteen animals with dorsal raphe placements and 14 with median raphe electrodes were selected for the experiment. These rats were then tested on alternate days over a 14 day period to ensure stable performance. The duration of the test sessions was 2 hr per day. When animals had displayed stable ICSS rates, they were placed under light ether anaesthesia prior to intragastric intubation of a 400 mg/kg dose of PCPA, as a suspension in H_2O. Post-drug tests for ICSS began 24 hr later and continued for 2 hr per day over a 10 day period.

RESULTS

All dorsal and median raphe animals that acquired the bar pressing response usually did so on the first day of training although they usually stabilized at lower rates than those obtained on the first day. The ICSS behavior often involved in very vigorous biting of the bar. Kindled seizures were never observed after stimulation of the raphe nuclei.

The electrode placements of all animals in both dorsal and medial raphe groups are shown in Fig. 1. It is important to note that similar predrug barpress rates were obtained in both dorsal ($\bar{X}$ = 3,460/2 hr) and median (2,912/2 hr) raphe groups.

Figure 2 shows the effect of intragastric injections of PCPA on ICSS in these two groups. ICSS in the dorsal raphe group was temporarily disrupted following PCPA treatment. A one-way analysis of variance on the raw data from the dorsal raphe group revealed a significant main effect of days, $F(5,60) = 4.06$, $p<0.01$. Moreover, a trend analysis of this effect demonstrated a significant quadratic component, $F(1,12) = 11.94$, $p<0.01$. Thus dorsal raphe ICSS was depressed by PCPA treatment over a time period comparable to that over which whole brain 5–HT is depleted by intragastric administration of PCPA [31]. Analysis of the raw data from the median raphe group did not reveal a significant effect of days, $F(5,65) = 2.20$, $p>0.05$.

A more complete analysis of the PCPA effects is revealed by examining the changes in ICSS rates which occurred within sessions. Figure 3 compares the percent change in ICSS during each 1 hr period within the 2 hr test session on Day 4 post-PCPA, with scores obtained in comparable 1 hr periods on the baseline day prior to PCPA. This intrasessional analysis on Day 4 post-PCPA (the day of maximal 5–HT depletion) [31] showed that dorsal raphe ICSS was significantly depressed during both the first hr ($p<0.05$, t-test) and second hr ($p<0.05$) of the test session, whereas median raphe ICSS was significantly decreased during the second hr ($p<0.05$) but not the first hr ($p>0.20$) of the session. This latter finding probably accounts for the failure to observe a statistically significant decrease in ICSS for the total 2 hr score of the median raphe group.

DISCUSSION

The present experiment confirms and extends previous reports of ICSS in the dorsal [25,27] and median raphe nuclei [18,26]. Miliaressis *et al.* [18] suggested that test sessions of 2 hr duration are more sensitive to the disruptive effects of PCPA and this appears to be the case. When the ICSS data for the median raphe group were analysed on the basis of two 1 hr periods on Day 4 post-PCPA a significant inhibition was only observed in the last hour of the session.

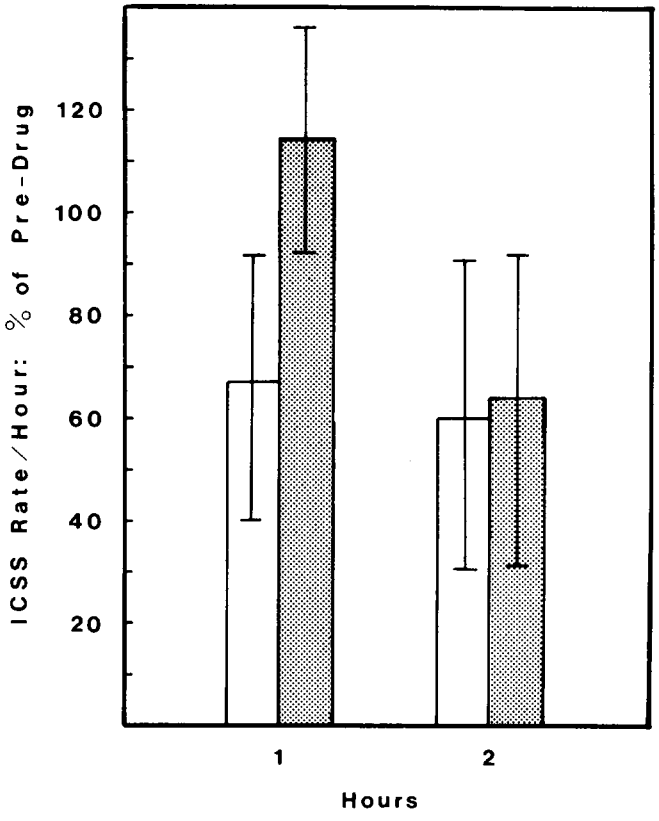

FIG. 3. Intrasessional analysis of ICSS responding on Day 4 post-PCPA in dorsal raphe (open bars) and median raphe (filled bars) animals. Data represent group means (± SEM) for each of the two 1 hr periods comprising the 2 hr test sessions, expressed as percentage of rates obtained on the day immediately prior to PCPA treatment.

Similar within session effects on median raphe ICSS have been reported both 24 and 48 hr after treatment with PCPA [18]. A preliminary study, employing 15 min tests failed to detect any significant effect of PCPA on median raphe ICSS, whereas similar treatment inhibited ICSS in the dorsal raphe when the rate was less than 1000 presses/15 min (van der Kooy, unpublished observations). In the present study ICSS in the dorsal raphe was depressed equally in both halves of the 2 hr test. These locus specific differences in the effect of PCPA within the test session are noteworthy because similar effects have been observed with caudate and hippocampal electrode placements. The suppression of caudate-ICSS is evident at the beginning of the test session and continues throughout [21], thus resembling the pattern seen in the dorsal raphe. In contrast, the decrease in hippocampal ICSS is observed primarily in the later part of the test [31] as is ICSS in the median raphe [18]. It is probably no coincidence that a 5-HT system from the dorsal raphe projects to the caudate-putamen [12,17], whereas there is evidence of a second 5-HT projection from the median raphe to the hippocampus [12,19].

The gradual decrease in median raphe ICSS within the test session has been attributed to the progressive disappearance of remaining brain 5-HT, by electrical stimulus of the brain [18]. However, there is no independent evidence that electrical stimulation further depletes 5-HT levels in neurons in which synthesis has already been inhibited by PCPA. Furthermore, if one pursues this line of reasoning, it is necessary to postulate differential effects of PCPA on separate 5-HT systems in the brain. This is an intriguing possibility but again there is no evidence to support it.

The finding that dorsal raphe ICSS is suppressed by PCPA is in contrast to the recent report of a significant increase in ICSS rate after PCPA treatment [27]. These contradictory findings may reflect procedural differences as the animals in the previous study [27] were selected for their high bar-pressing rates. It is interesting to note that the two animals in that study with the lowest ICSS rates (i.e., 926 and 1279 presses/30 min) showed very little change in responding after PCPA. Another important difference between these studies is the dose of PCPA and the route of administration. Simon *et al.* [27] gave two IP injections of PCPA (350 mg/kg), 48 hr apart. In an attempt to minimize the non-specific peripheral effects of PCPA injections which can be problematic in the interpretation of behavioral experiments [29], we have consistently employed intragastric intubation [21,31]. Biochemical analysis has confirmed that this procedure produces a maximal depletion of whole brain and regional 5-HT (20% of control) on Day 4 post PCPA, with levels returning to baseline 12 days after PCPA treatment (see Table 2 and Fig. 2, ref. [31]). Thus the time course of 5-HT depletion and recovery after intragastric intubation is very similar to that found after IP injections of comparable doses of PCPA [15]. It is also important to note that the temporal pattern of ICSS suppression after PCPA is highly correlated with the inhibition of 5-HT synthesis (compare Fig. 2, present experiment with Fig. 2, ref. [31]). Furthermore, the differential effects of PCPA observed in the present study and in previous experiments [21,31] argue against attributing the suppression of dorsal raphe ICSS to the disruption of sensory and/or motor function following treatment with PCPA.

There is now a large body of data implicating 5-HT in brain-stimulation reward, but unfortunately much of it appears contradictory. This is especially true if one focusses on the hypothalamus. At this site, PCPA has been reported to inhibit [10,28], facilitate [5, 21, 23, 31] or to have no effect [4] on ICSS. Neurotoxic lesions of 5-HT systems in the brainstem with 5,6 dihydroxytryptamine [24] produced an increase in responding for ICSS in the LH suggesting an inhibitory role.

A similar pattern of conflicting results emerges from the consideration of studies which have examined the effects of increasing 5-HT levels at the synapse by treating animals with biochemically specific 5-HT reuptake inhibitors or precursors of 5-HT such as l-tryptophan (Trp) or 5-hydroxytryptophan (5-HTP). Systemic injections of the 5-HT reuptake inhibitor Lilly 110140 (fluoxetine hydrochoride), produced a dose-related reduction in ICSS in the medial forebrain bundle that was partially reversed by 5-HT receptor blocker methysergide [13]. Atrens *et al.* [1] have also reported a decrease in running for hypothalamic brain-stimulation in a shuttlebox after injections of the 5-HT reuptake blocker LU 5-003. However, these authors attributed the decrease in response initiation to the rewarding effect of increases in 5-HT levels that were not contingent on the animal's behavior. Small doses of 5-HTP (5-10 mg/kg) have been shown to facilitate LH-ICSS, whereas a high dose (300 mg/kg) depressed it [22]. Bose *et*

al. [6] also observed a decrease in ICSS following treatment with 5–HTP, but this effect was observed with both high and low doses (28, 52 and 115 mg/kg). In this latter study, pretreatment with the decarboxylase inhibitor RO4–4602 to counteract the effect of 5–HTP on peripheral levels of 5–HT had no influence on the inhibition of ICSS. Again, a subsequent experiment provides the opposite result [20]. In this study 5–HTP inhibited ICSS through hypothalamic electrodes in cats, but this effect was accompanied by drowsiness. In cats pretreated with the peripheral decarboxylase inhibitor RO 4–4602, injections of both 5–HTP and Trp produced a significant increase in ICSS, suggesting a facilitatory role for 5–HT in brain-stimulation reward.

Elsewhere we have argued that multiple neurochemical systems subserve ICSS in different regions of the brain [21,31] and this approach would seem to provide an obvious resolution of the paradox described above. Two of the 5–HT pathways which course through the hypothalamus appear to have a facilitatory role. One of these pathways originates in the dorsal raphe and termintes in the neostriatum [12,17] and ICSS in both regions is disrupted by treatment with PCPA ([21], present study). A second pathway arises primarily in the median raphe and projects to the hippocampus [12,19]. Self-stimulation in the cell bodies and axon terminal regions of this system is also suppressed by the inhibition of 5–HT synthesis ([18,31], present study) although this system appears less sensitive to the effect of PCPA. Finally, there would appear to be a third 5–HT system which has an antagonistic effect on ICSS, in view of the frequently described facilitation of hypothalamic ICSS rates following injections of PCPA [5, 21, 23, 31]. These results might be related anatomically to groups of 5–HT cell bodies recently observed in the hypothalamus, which are independent of the hypothalamic projections from the dorsal and medial raphe nuclei [7].

In describing the possible role of separate 5–HT systems in brain stimulation reward, it is important to emphasize that they are not viewed as the sole neurochemical substrate of this phenomenon. In fact, it seems inappropriate to attribute the neural substrate of reinforcement to any exclusive transmitter system. Like most complex functions in the brain, it is undoubtedly dependent on the interaction of a variety of neurochemical systems, the nature of which we have only begun to discern.

ACKNOWLEDGEMENTS

The excellent technical assistance of F. G. LePiane is gratefully acknowledged. Supported by the Medical Research Council of Canada. A. G. Phillips is a Killam Senior Research Scholar.

REFERENCES

1. Atrens, D. M., U. Ungerstedt and T. Ljungberg. Specific inhibition of hypothalamic self-stimulation by selective reuptake blockade of either 5-hydroxytryptamine or noradrenaline. *Psychopharmacology* **52:** 177–180, 1977.
2. Anden, N. E., A. Dahlström, K. Fuxe, K. Larsson, L. Olson and U. Ungerstedt. Ascending monoamine neurons to the telencephalon and diencephalon. *Acta physiol. scand.* **67:** 313–326, 1966.
3. Beaudet, A. and L. Descarries. A serotonin-containing nerve cell group in rat hypothalamus. *Neurosci. Abstr.* **2:** 479, 1976.
4. Black, W. C. and B. R. Cooper. Reduction of electrically-rewarded behavior by interference with monoamine synthesis. *Physiol. Behav.* **5:** 1405–1409, 1970.
5. Blum, K. and I. Geller. Facilitation of brain stimulation with parachlorophenylalanine. *Fedn Proc.* **28:** 794, 1969.
6. Bose, S., P. T. Bailey, N. B. Thoa and S. N. Pradham. Effects of 5-hydroxytryptophane on self-stimulation in rats. *Psychopharmacologia* **36:** 255–262, 1974.
7. Dahlström, A. and K. Fuxe. Evidence for the existence of monoamine containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta physiol. scand.* **62:** Suppl. 232: 1–55, 1965.
8. Fuxe, K. and G. Jonsson. Further mapping of central 5-hydroxytryptamine neurons: Studies with the neurotoxic dihydroxytryptamines. *Adv. biochem. Psychopharmac.* **10:** 1–12, 1974.
9. German, D. C. and D. M. Bowden. Catecholamine systems as the neural substrate for intracranial self-stimulation. *Brain Res.* **73:** 381–419, 1974.
10. Gibson, S., E. G. McGeer and P. L. McGeer. Effect of selective inhibitors of tyrosine and tryptophane hydroxylase on self-stimulation in the rat. *Expl Neurol.* **27:** 283–290, 1970.
11. Holloway, J. A. Norepinephrine and serotonin: specificity of release with rewarding electrical stimulation of the brain. *Psychopharmacologia* **42:** 127–134, 1975.
12. Jacobs, B. L., W. D. Wise and K. M. Taylor. Differential behavioral and neurochemical effects following lesions of the dorsal or median raphe nuclei in rats. *Brain Res.* **79:** 353–361, 1974.
13. Katz, R. J. and B. J. Carroll. Intracranial reward after Lilly 110140 (Fluoxetine HCl): Evidence for an inhibitory role for serotonin. *Psychopharmacology* **51:** 189–193, 1977.
14. Kent, D. and J. R. Sladek. Localization of serotonin neurons in the rat hypothalamus. *Neurosci. Abstr.* **2:** 493, 1976.
15. Koe, B. K. and A. Weissman. P-chlorophenylalanine: A specific depletor of brain serotonin. *J. Pharmac. exp. Ther.* **154:** 499–516, 1966.
16. Konig, J. F. T. and R. A. Klippel. *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem.* Baltimore: Williams and Williams, 1963.
17. Miller, J. J., T. L. Richardson, H. C. Fibiger and H. McLennan. Anatomical and electrophysiological identification of a projection from the mesencephalic raphe to the caudate-putamen in the rat. *Brain Res.* **97:** 133–139, 1975.
18. Miliaressis, E., A. Bouchard and D. M. Jacobowitz. Strong positive reward in median raphe: specific inhibition by para-chlorophenylalanine. *Brain Res.* **98:** 194–201, 1975.
19. Moore, R. Y. and A. E. Halaris. Hippocampal innervation by serotonin neurons of the midbrain raphe in the rat. *J. comp. Neurol.* **164:** 171–184, 1975.
20. Patinka, N. A. and I. P. Lapin. Effect of serotoninergic drugs on positive and negative reinforcing systems in cats. *Pharmac. Biochem. Behav.* **5:** 241–245, 1976.
21. Phillips, A. G., D. A. Carter and H. C. Fibiger. Differential effects of parachlorophenylalanine on self-stimulation in caudate-putamen and lateral hypothalamus. *Psychopharmacology* **49:** 23–27, 1976.
22. Poschel, B. P. H. and F. W. Nineteman. Excitatory effects of 5–HTP on intracranial self-stimulation following MAO blockade. *Life Sci.* **5:** 11–16, 1968.

23. Poschel, B. P. H. and F. W. Ninteman. Intracranial reward and forebrain's serotonergic mechanism: Studies employing parachlorophenylalanine and parachloroamphetamine. *Physiol. Behav.* 7: 39–46, 1971.
24. Poschel, B. P. H., F. W. Ninteman, J. R. McLean and D. Potoczah. Intracranial reward after 5,6 dihydroxytryptamine: Further evidence for serotonin's inhibitory role. *Life Sci.* **15:** 1515–1522, 1975.
25. Saint-Laurent, J., R. Leclerc and M. Mitchel. Autostimulation des noyaux du raphe et exploration diffuse. *J. Physiol.* **66:** 87–92, 1973.
26. Simon, H., M. Le Moal and B. Cardo. Mise en evidence du comportement d'autostimulation dans le noyan raphe median du rat. *C.R. Acad. Sci.* **277:** 591–593, 1973.
27. Simon, H., M. Le Moal and R. Cardo. Intracranial self-stimulation from the dorsal raphe nucleus of the rat: effects of the injection of parachlorophenylalanine and of alpha-methylparatyrosine. *Behav. Biol.* **16:** 353–364, 1976.
28. Stark, P., R. W. Fuller, L. W. Hartley, R. J. Schaffer and J. A. Tuch. Dissociation of the effects of p-chlorophenylalanine on self-stimulation and on brain serotonin. *Life Sci.* **9:** 41–48, 1970.
29. Thornburg, J. E. and K. E. Moore. Stress-related effects of various inhibitors of catecholamine synthesis in the mouse. *Archs int. Pharmacodyn.* **194:** 158–167, 1971.
30. Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the brain. *Acta physiol. scand.* Suppl. 367: 1–48, 1971.
31. van der Kooy, D., H. C. Fibiger and A. G. Phillips. Monoamine involvement in hippocampal self-stimulation. *Brain Res.* **136:** 119–130, 1977.
32. Wise, C. D., B. D. Berger and L. Stein. Evidence of alpha-noradrenergic reward receptors and serotonergic punishment receptors in the rat brain. *Biol. Psychiat.* **6:** 3–21, 1973.