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Self-stimulation and Local Injections of 6-Hydroxydopamine into the Rat Brain: Enhanced Behavioral Depressive Effects of α-Methylparatyrosine[1]

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STINUS, L., A. M. THIERRY AND B. CARDO. *Self-stimulation and local injections of 6-hydroxydopamine into the rat brain: enhanced behavioral depressive effects of α-methylparatyrosine.* PHARMAC. BIOCHEM. BEHAV. 3(1) 19–23, 1975. – The effects of intracerebral injections of 6-OHDA on self-stimulation were examined. Small amounts of 6-OHDA were injected either in the area ventralis tegmenti (AVT) or laterally in the pedonculus cerebellaris superior (PCS), then all rats were implanted in the AVT. In spite of marked depletion of brain catecholamines, self-stimulation learning was not altered by PCS 6-OHDA injections, whereas, AVT 6-OHDA injections produced a small perturbance. The administration of low doses of AMPT which had no observable effect in control rats, produced a severe depression of self-stimulation rates in 6-OHDA pretreated rats. The depressive effect of AMPT is always more important in rats injected with 6-OHDA in the AVT than those injected at the level of PCS. The respective role of noradrenergic and dopaminergic neurons in AVT self-stimulation are discussed.

Self-stimulation Catecholamines 6-Hydroxydopamine AMPT

FOR several years, a catecholaminergic (CA) hypothesis has been postulated for self-stimulation behavior [20, 25, 26, 32, 33, 34]. Recently, the central administration of 6-hydroxydopamine (6-OHDA) has been shown to produce prolonged reduction of brain CA, due to the destruction of dopaminergic and noradrenergic systems [3, 5, 41]. However, important chronic deficits did not occur after intraventricular injection of 6-OHDA [6, 10, 11, 13, 16, 18]; the only permanent behavioral modification that has been observed is an increasing irritability [22,36]. Thus, 60% of brain CA depletion produced by intraventricular 6-OHDA injection, reduced self-stimulation rates to 40 or 50% only [4,31]. However, as Cooper *et al.* [10,11] showed, 6-OHDA treated rats, showing no deficits in performance of several behavioral tasks, can be distinguished from control rats by an increased sensitivity to the behavioral depressant effects of α-methylparatyrosine (AMPT) or reserpine. In our study, we have tested the effect of central 6-OHDA administration upon self-stimulation produced by electrodes implanted in the area ventralis tegmenti (AVT), not by producing intraventricular injections which produced a general depletion of central CA, but by injecting locally 6-OHDA at the level of catecholaminergic structures which can be critical for self-stimulation. Two injection sites have been selected: the AVT that contains the ventral noradrenergic bundle and the mesolimbic dopaminergic cell bodies (A_{10}) and laterally to the pedunculus cerebralis superior (PCS) which is both the pathway of the dorsal noradrenergic bundle and of part of the ventral noradrenergic bundle [35,39]. These two injection sites could allow us to analyse the part of the A_{10} dopaminergic group reached only by AVT injection and to dissociate the respective effects of the

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two ascending noradrenergic bundles. Finally, we have tested the depressive effect of low doses of AMPT on the self-stimulation performed by these animals.

METHOD

Animals

Twenty-nine male Sprague Dawley rats weighing initially 370 g were used in this study. During the experiments, rats were individually housed and fed ad lib with food pellets given at the end of the day (7 p.m.).

Procedure

Surgical methods. Operations were made in two steps:

(1) 6-hydroxydopamine injections: Operations were performed with a Stoelting apparatus (Krieg Model Nr. 51.200). Chemical lesions were made bilaterally under penthotal anaesthesia (80 mg/kg) by stereotaxic microinjections of 6-OHDA hydrochloride (6-OHDA, AB Biotec, H88/32) (2 μg in 1 μl of isotonic saline solution containing 1 mg/ml of ascorbic acid adjusted to pH 4). The fluid was delivered at a speed of 1 μl/5 min, with the help of a Hamilton syringe. In these experimental conditions, most of the preliminary estimations revealed that the 6-OHDA injected diffused in a sphere of 2 mm diameter [2]. Ten rats received 6-OHDA injections in the area ventralis tegmenti (AVT group) (posterior to the bregma: P 4.4 mm; lateral to the midline: L ± 0.5 mm; below the calvarium surface: H 8.3 mm). Nine rats received 6-OHDA injections laterally to the pedonculus cerebralis superior (PCS group) (P 6 mm; L ± 2 mm; H 7.7 mm); the 10 other rats were injected with the 6-OHDA free medium either in the AVT (5 rats) or laterally to the PCS (5 rats) (control group).

(2) Electrode implantations: One week after the chemical lesions, two bipolar electrodes were stereotaxically implanted in the AVT (one on each side) at stereotaxic coordinates slightly different from those used for the 6-OHDA chemical injection (P 4 mm; L ± 0.4 mm; H 8.5 mm) (bilateral implantations).

Behavioral methods. Ten days after the second surgery, the self-stimulation learning was started as described previously [33].

(1) Self-stimulation learning. Animals were submitted daily to 15 min training session using each AVT electrode during an 11 day period (between the 20th and 31st day after 6-OHDA treatment); for each rat, these 2 daily training sessions (right and left electrode) were separated by 5 hr. The successive intensities applied during learning were: 0, 0, 10, 10, 20, 20, 30, 30, 40, 60, and 80 μA peak to peak. Electrodes unable to elicit self-stimulation rates over 500 presses within 15 min at 60 μA were not utilized.

(2) α-methylparatyrosine treatments. For pharmacological experiments, electrodes showing the higher rates responses intensity (60 μA) during the self-stimulation learning were selected from among those of the 23 rats trained. Within these groups of animals, no statistical significant differences could be found in the rate of self-stimulation (control group: 9 electrodes, 1719 ± 130 presses in 15 min; PCS group: 11 electrodes, 1781 ± 132 presses in 15 min; AVT group: 13 electrodes, 1501 ± 126 presses in 15 min). Pharmacological experiments took place between the 40th and the 53rd day after 6-OHDA treatment. On the day of the experiment, rats were allowed to self-stimulate during 10 sessions of 30 min each, alternating with rest periods of 30 min. At the beginning of the second self-stimulation session, the animals received an intraperitoneal injection (ip) of AMPT (50 mg/kg or 75 mg/kg) (AMPT, AB Biotec, H44/68). Some animals were studied in the two pharmacological situations; in this case, a 6 day period separates two successive drug tests.

Biochemical methods. One hundred-twenty days after 6-OHDA treatment, rats were killed by decapitation and their brains were immediately dissected with glass manipulators under a dissecting microscope at 4°C; tissues were weighed and homogenized in 10 ml of an ethanol-water solution (74:16 V/V) with an ultraturax apparatus, catecholamines were isolated from the supernatant, after centrifugation of homogenates, by ion exchange chromatography on amberlite CG50 [14]. Norepinephrine (NE), dopamine (DA) and serotonin were estimated on 0.5 ml aliquots of the amberlite eluates; using the spectrofluorimetric methods of Von Euler and Lishajko [12] and Laverty and Taylor [17]. All values were corrected for the respective recoveries.

Histological control. Only rats unable to perform self-stimulation (with one or both electrodes) were controlled; these electrod tips were not localized in the AVT (4 electrodes localized below the brain, 2 in the red nucleus and 2 in the lemniscus medialis). This histological control has further revealed the absence of necrotic lesions in 6-OHDA injection sites.

Statistics. In behavioral as well as in biochemical studies, the significance of differences observed between two groups were estimated by the Student's t test [30].

RESULTS

Effects of 6-OHDA Lesions on Self-stimulation Learning

The general evolution of the self-stimulation behavior during learning was not markedly altered by the 6-OHDA chemical lesions. Indeed, the thresholds of self-stimulation were found with current intensities of 10 to 20 μA in all rats. Moreover, rates of self-stimulation of the PCS animals were similar to those observed in control rats. However, the rates of self-stimulation of AVT rats were lower than those of control or PCS animals, these differences were statistically significant between 20 and 40 μA (Fig. 1). It should be mentioned that 6-OHDA chemical lesions did not affect the weight of the animals.

Effects of AMPT on Self-stimulation Behavior following 6-OHDA Lesions

AMPT 50 mg/kg ip (AMPT 50). AMPT 50 treatment slightly reduced the rate of self-stimulation in control rats (20%). However a similar small effect has been observed in a previous experiment after the injection of saline [34]. Both 6-OHDA lesions enhances the depressing effect of AMPT on self-stimulation rate. This effect was particularly striking in rats which had received 6-OHDA in the AVT area; whereas the differences of PCS group when compared with control values have not statistical significance (except for the 5th self-stimulation session).

AMPT 75 mg/kg ip (AMPT 75). At this dose, AMPT rapidly reduced the rate of self-stimulation in sham operated rats. In this case, AMPT was much more effective in both groups of 6-OHDA lesioned animals. For instance self-stimulation which was only reduced by 52% in control rats

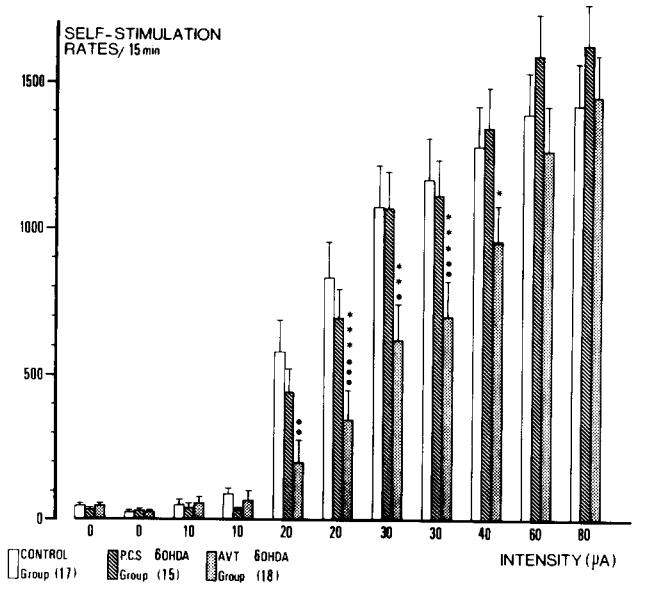

FIG. 1. Effects of local 6-OHDA injections upon self-stimulation learning. Abscissa: intensity (μA) of daily 15 min self-stimulation sessions. Ordinate: the mean (±SEM) of lever pressing in 15 min. Statistical significance: AVT group when compared with control group: •$p<0.02$; ••$p<0.01$; •••$p<0.001$; AVT group when compared with PCS group: *$p<0.05$; **$p<0.02$; ***$p<0.01$. In brackets, the number of AVT electrodes tested for each group.

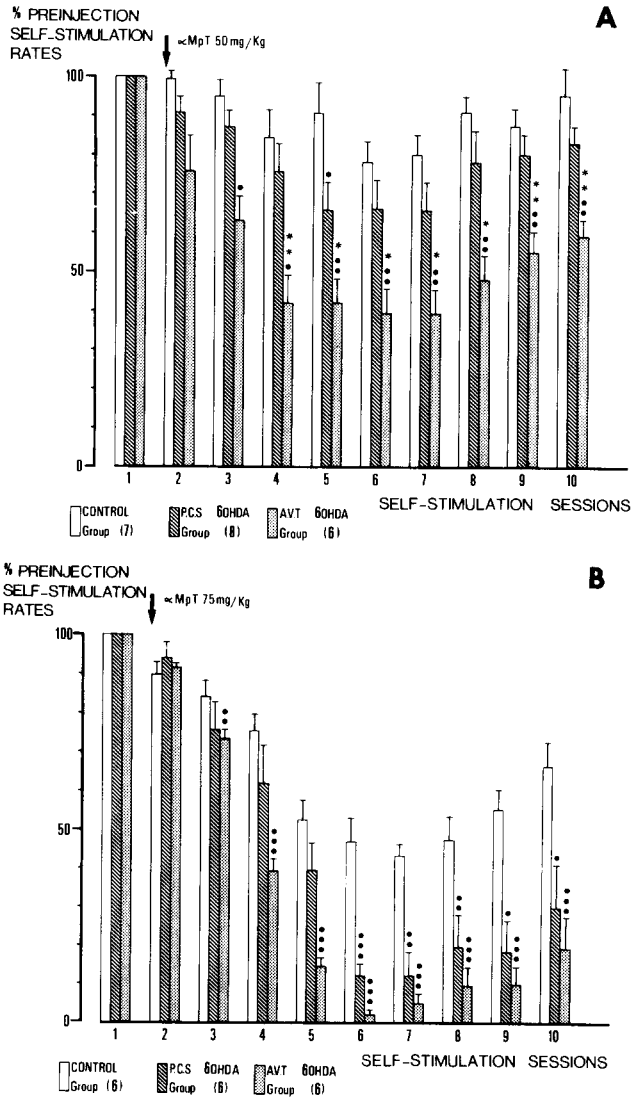

FIG. 2. (A) Effect of AMPT 50 mg/kg ip on self-stimulation behavior following 6-OHDA treatments. Abscissa: the 10 self-stimulation sessions of 30 min each alternating with rest periods of 30 min. Ordinate: the mean (±SEM) of lever pressing is expressed in percentage of the first session score. Statistical significance: 6-OHDA groups when compared with control group: •$p<0.05$; ••$p<0.01$; •••$p<0.001$; AVT group when compared with PCS group: *$p<0.05$; **$p<0.01$. In brackets the number of AVT electrodes tested for each group. (B) Effect of AMPT 75 mg/kg ip on self-stimulation behavior following 6-OHDA treatments: abscissa and ordinate: see Fig. 2A. Statistical significance: 6-OHDA groups when compared with control group: •$p<0.02$; ••$p<0.01$; •••$p<0.001$. In brackets the number of AVT electrodes for each group.

was decreased by more than 86% in PCS and AVT 6-OHDA groups during the 6th session. However, the rates of self-stimulation were always more depressed in AVT lesioned rats than in PCS lesioned animals (Fig. 2), but these differences were not statistically significant.

Biochemical results. As with previous results [35], 6-OHDA injections made in the PCS produced a preferential reduction of NE levels in structures mainly innervated by the dorsal NE pathway, whereas those made in the AVT preferentially affected structures innervated by the ventral NE pathway, although they also affect structures such as the cortex and the hippocampus which are mainly innervated by the dorsal ascending NE pathway. DA levels were significantly reduced in the neostriatum and in the olfactory tubercules only in 6-OHDA AVT rats (Table 1). Besides, biochemical analysis shows that local injections of 6-OHDA either at the level of AVT or PCS do not perturb serotoninergic systems (cortical serotonin in μg/g; control group: 0.225 ± 0.023; AVT group: 0.230 ± 0.022; PCS group: 0.235 ± 0.038).

DISCUSSION

These results suggest the following observations:

(1) 6-OHDA injection does not produce alone self-stimulation important deficits whatever the injection site either AVT or PCS. The perturbances observed are lower than those produced by a 6-OHDA intraventricular administration [4,31]; moreover, they are slighter than those produced by AMPT intraperitoneal injection, which is sufficient to inhibit CA synthesis at 80% [33]. In this last case, nearly all the CA neurons activity is blocked; on the contrary, after a local 6-OHDA injection, only a few neurons have degenerated, while others do not seem to have taken up enough 6-OHDA and therefore are undamaged [15]. The remaining intact CA neurons are able to compensate the functional activity of the neurons destroyed, either by enhanced synthesis [1] or by supersensitivity of receptors [21, 37, 40] or by the sprouting of new terminals [24]. However that may be, this result confirms the reward system redundancy already observed after electrolytical lesions [19, 23, 38, 42].

TABLE 1

NOREPINEPHRINE AND DOPAMINE LEVELS IN VARIOUS BRAIN STRUCTURES AFTER DIFFERENT 6-HYDROXYDOPAMINE TREATMENTS. 6-OHDA (2 μg/1 μl) WAS INJECTED BILATERALLY, EITHER IN AVT, OR LATERALLY IN THE PCS. PARENTHESES SHOW THE NUMBER OF ANIMALS OF EACH GROUP. RESULTS ARE THE MEAN ±SEM OF CONTROL VALUES (SALINE INJECTION).

	control (μg/g) (7)		6-OHDA lesion percentage of control			
			NE %		DA %	
Brain structures	NE	DA	PCS Group (8)	AVT Group (8)	PCS Group (8)	AVT Group (8)
Brain stem	0.426 ± 0.019	0.137 ± 0.007	95 ± 3	103 ± 4	97 ± 2	91 ± 6
Hypothalamus	1.79 ± 0.12	0.710 ± 0.100	37 ± 2‡	74 ± 4†	69 ± 8	66 ± 5
Hippocampus	0.454 ± 0.025		49 ± 4‡	54 ± 5‡		
Olfactif lobs	0.464 ± 0.029	1.81 ± 0.21	73 ± 5†	97 ± 7	98 ± 3	63 ± 5*
Caudate nucleus	0.517 ± 0.031	6.25 ± 0.30	59 ± 3‡	64 ± 5‡	106 ± 5	60 ± 6‡
Cortex	0.234 ± 0.011	0.141 ± 0.014	27 ± 2‡	39 ± 4‡	103 ± 16	106 ± 17

*$p<0.02$, †$p<0.01$, ‡$p<0.001$

(2) The self-stimulation behavior is only perturbed after AVT injection of 6-OHDA, PCS injection has no effect. Similarly, the injection of low doses of AMPT is more effective on 6-OHDA AVT injected rats than in PCS injected ones. Biochemical analysis shown that, the main differences between the two 6-OHDA groups are that mesolimbic DA neurons, a larger part of the ventral noradrenergic pathway, and part of the dorsal one, are destroyed in the AVT group; whereas PCS group is characterized by a nearly total lesion of the dorsal noradrenergic pathway and of the ventral one. We can notice too, that for all structures located rostrally to the 6-OHDA injected sites, the NE decreases are always more important after PCS lesions than after AVT ones, although differences are often slight.

So the higher perturbations induced by AVT lesions can be explained by a lesion of the mesolimbic DA system (A10 group), this is in agreement with other workers [8] and with the fact that several dopamine β-hydroxylase inhibitors are unable to inhibit AVT self-stimulation (unpublished data).

The existence of perturbations in PCS group after low doses of AMPT, and the fact that in a previous study [34] we have shown that AMPT inhibition of self-stimulation elicited by AVT electrodes could be reversed by dl-dihydroxyphenylserine a precursor which leads only to the formation of NE, show that also a noradrenergic link is implicated in AVT self-stimulation. It is, at present time, difficult to state precisely about the origin of this NA link, however several hypothesis can be made: first, this NA link could be formed by the ventral NE pathway, since it lies along the AVT, however Clavier *et al.* [7] failed to show self-stimulation from electrodes closely localized to the cell groups of origin of this pathway; second, as Crow [9] obtained self-stimulation with electrodes localized in the locus coeruleus, it is possible to think that electrodes implanted in AVT activate interneuronally this NE cell group, which could represent the noradrenergic link of AVT self-stimulation. However, other workers failed to obtain locus coeruleus self-stimulation [29]. Finally our results could be explained by the existence of a new NE neuron group not yet described, since it has been shown that self-stimulation can be obtained with electrodes located in the nucleus raphe medialis a serotoninergic cell group [28], whereas this behavior can be inhibited by AMPT while parachlorophenylalanine does not (Simon, unpublished data), this shows a catecholaminergic link in a serotoninergic structure.

(3) At last our results show the limits of the capacities of vicariance of the analyzed system, since a partial inhibition of CA synthesis is sufficient to reduce self-stimulation rates by 50% in AVT injected rats. As has been noticed in a previous study [33], the newly synthesized CA are preferentially released in self-stimulation. However, after synthesis has been blocked, the CA stocked can compensate temporarily this deficit. In the present experiment CA stores are likely not to be sufficient to compensate the effects of the synthesis partial inhibition. Such a situation can be compared with the behavioral effect of low doses of AMPT on reserpine pretreated rats [27].

REFERENCES

1. Agid, Y., F. Javoy and J. Glowinski. Hyperactivity of remaining dopaminergic neurons after partial destruction of the nigro striatal dopaminergic system in the rat. *Nature* **245:** 150–151, 1973.
2. Agid, Y., F. Javoy, J. Glowinski, D. Bouvet and C. Sotelo. Injection of 6-hydroxydopamine into the substantia nigra of the Rat. II. Diffusion and specificity. *Brain Res.* **58:** 291–301, 1973.
3. Bloom, F. E., S. Algeri, A. Groppetti, A. Revuelta and E. Costa. Lesions of central norepinephrine terminals with 6-OHDA: Biochemistry and fine structure. *Science* **166:** 1284–1286, 1969.
4. Breese, G. R., J. L. Howard and J. P. Leahy. Effect of 6-hydroxydopamine on electrical self-stimulation of the brain. *Br. J. Pharmac.* **43:** 255–257, 1971.

5. Breese, G. R. and T. D. Traylor. Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: evidence for selective degeneration of catecholamine neurons. *J. Pharmac. exp. Ther.* **174:** 413–420, 1970.
6. Burkard, W. P., M. Jalfre and J. Blum. Effect of 6-hydroxydopamine on behaviour and catecholamines content in Rats. *Experientia* **25:** 1295–1296, 1969.
7. Clavier, R. M. and A. Routtenberg. Ascending monoamine-containing fiber pathways related to intracranial self-stimulation: histochemical fluorescence study. *Brain Res.* **72:** 25–40, 1974.
8. Crow, T. J. Catecholamine-containing neurons and electrical self-stimulation. 1. A review of some data. *Psychol. Rec.* **2:** 414–421, 1972.
9. Crow, T. J., P. J. Spear and G. W. Arbuthnott. Intracranial self-stimulation with electrodes in the region of the locus coeruleus. *Brain Res.* **36:** 275–287, 1972.
10. Cooper, B. R., G. R. Breese, J. L. Howard and L. D. Grant. Enhanced behavioral depressant effects of reserpine and α-methyltyrosine after 6-hydroxydopamine treatment. *Psychopharmacologia* **27:** 99–110, 1972.
11. Cooper, B. R. , L. D. Grant and G. R. Breese. Comparison of the behavioral depressant effects of biogenic amine depleting and neuroleptic agents following various 6-hydroxydopamine treatments. *Psychopharmacologia* **31:** 95–109, 1973.
12. Euler, Von U. S. and F. Lishajko. Improved technic for the fluorometric estimation of catecholamines. *Acta physiol. scand.* **51:** 348–355, 1961.
13. Evetts, K. D., N. J. Uretsky, L. L. Iversen and S. D. Iversen. Effects of 6-hydroxydopamine on CNS catecholamines, spontaneous motor activity and amphetamine induced hyperactivity in rats. *Nature* **225:** 961–962, 1970.
14. Javoy, F., M. Hammon and J. Glowinski. Disposition of newly synthesized amines in cell bodies and terminals of central catecholaminergic neurons. (1). Effects of amphetamine and thioproperazine on the metabolism of CA, in the caudate nucleus, the substantia nigra and the ventro-medial nucleus of the hypothalamus. *Eur. J. Pharmac.* **10:** 178–188, 1970.
15. Jonsson, G. and C. Sachs. Degenerative and non-degenerative effects of 6-hydroxydopamine on adrenergic nerves. *J. Pharmac. exp. Ther.* **180:** 625–635, 1972.
16. Laverty, R. and P. J. Arnott. Recovery of avoidance behaviour in rats following intraventricular injection of 6-hydroxydopamine. *Proc. Univ. Otago med. Sch.* **48:** 18–20, 1970.
17. Laverty, R. and K. M. Taylor. The fluorometric assay of catecholamines and related compounds, improvements on extensions to the hydroindol technique. *Analyt. Biochem.* **22:** 269–279, 1968.
18. Lidbrink, P. and K. Fuxe. Effects of intracerebral injection of 6-hydroxydopamine on sleep and waking in rats. *J. Pharm. Pharmac.* **25:** 84–87, 1973.
19. Lorens, S. A. Effect of lesions in the central nervous system on lateral hypothalamic self-stimulation in the rat. *J. comp. physiol. Psychol.* **62:** 256–262, 1966.
20. Margules, D. L. Noradrenergic rather than serotoninergic basis of reward in the dorsal tegmentum. *J. comp. physiol. Psychol.* **67:** 32–35, 1969.
21. Nadeau, R. A., J. De Champlain and G. M. Tremblay. Supersensitivity of the isolated rat heart after chemical sympathectomy with 6-hydroxydopamine. *Can. J. Physiol. Pharmacol.* **49:** 36–44, 1971.
22. Nakamura, K. and H. Thoenen. Increased irritability: a permanent behavior change induced in the rat by intraventricular administration of 6-hydroxydopamine. *Psychopharmacologia* **24:** 359–372, 1972.
23. Olds, M. E. and J. Olds. Effects of lesions in the medial forebrain bundle on self-stimulation behavior. *Am. J. Physiol.* **217:** 1253–1264, 1969.
24. Pickel, V. M., H. Krebbs and F. E. Bloom. Proliferation of norepinephrine containing axons in the rat cerebellar cortex after peduncle lesions. *Brain Res.* **59:** 169–179, 1973.
25. Poschel, B. P. H. Mapping of rat brain for self-stimulation under monoamine oxidase blockade. *Physiol. Behav.* **4:** 325–331, 1969.
26. Poschel, B. P. H., F. W. Ninteman and S. C. Stana. Concerning the excitatory effect of alpha-methylmetatyrosine on hypothalamic self-stimulation. *Life Sci.* **4:** 53–56, 1965.
27. Rech, R. H., L. A. Carr and K. E. Moore. Behavioral effects of α-methyltyrosine after prior depletion of brain catecholamines. *J. Pharmac. exp. Ther.* **160:** 326–335, 1968.
28. Simon, H., M. Le Moal and B. Cardo. Mise en évidence du comportement d'autostimulation dans le noyau raphé médian du Rat. *C. r. hebd. Séanc. Acad. Sci. Paris* **277:** 591–593, 1973.
29. Simon, H., M. Le Moal and B. Cardo. Self-Stimulation in the dorsal pontine tegmentum in the rat. *Behav. Biol.* (submitted for publication, 1974).
30. Snedecor, T. W. and W. G. Cochran. *Statistical Methods.* Ames: Iowa State University Press, 1967.
31. Stein, L. and C. D. Wise. Possible etiology of schizophrenia: progressive damage of the noradrenergic reward mechanism by endogenous 6-hydroxydopamine. *Science* **171:** 1032–1036, 1971.
32. Stinus, L., M. Le Moal and B. Cardo. Résultats comparatifs d'une étude pharmacologique de l'autostimulation dans les régions hypothalamiques et mésencéphaliques ventrales. *J. Physiol. (Paris)* **2:** 97A, 1971.
33. Stinus, L., M. Le Moal and B. Cardo. Autostimulation et catécholamines. I. Intervention possible de deux compartiments (compartiment fonctionnel et compartiment de réserve). *Physiol. Behav.* **9:** 175–182, 1972.
34. Stinus, L. and A. M. Thierry. Self-stimulation and catecholamines. II. Blockade of self-stimulation by treatment with alpha-methylparatyrosine and reinstatement by catecholamine precursors adminstration. *Brain Res.* **64:** 189–198, 1973.
35. Thierry, A. M., L. Stinus, G. Blanc and J. Glowinski. Some evidence for the existence of dopaminergic neurons in the rat cortex. *Brain Res.* **50:** 230–234, 1973.
36. Thoa, N. B., B. S. Eichelman and Y. Ng. Shock induced aggression: effect of 6-hydroxydopamine and other pharmacological agents. *Brain Res.* **43:** 467–476, 1972.
37. Trendelenburg, U. and K. Wagner. The effect of 6-hydroxydopamine on the cat's nictitating membrane; degeneration contraction and sensitivity to (−)noradrenaline. In: *6-Hydroxydopamine and Catecholamine Neurons,* edited by T. Malmfors and H. Thoenen. Amsterdam: North-Holland Publishing Co., 1971, pp. 215–223.
38. Umemoto, M. Self-stimulation of the lateral hypothalamus after electrolytic injury of the medial forebrain bundle in the cat. *Brain Res.* **11:** 325–335, 1968.
39. Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiol. scand. Suppl.* **367:** 1–47, 1971.
40. Ungerstedt, U. Postsynaptic sensitivity after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta physiol. scand. Suppl.* **367:** 69–93, 1971.
41. Uretsky, N. J. and L. L. Iversen. Effects of 6-hydroxydopamine on catecholamines neurons in the rat brain. *J. Neurochem.* **17:** 269–278, 1970.
42. Valenstein, E. S. The anatomical locus of reinforcement. *Progress in Physiol. Psychol.* **1:** 149–190, 1966.