Pharmacology Biochemistry & Behavior, Vol. 9, pp. 43–47. Printed in the U.S.A.

Differential Behavioral Activities from Anterior and Posterior Hypothalamic Lesions in the Rat

DANIEL B. RONDEAU,[1] FRANCOIS B. JOLICOEUR,[2] FERDINAND BELANGER AND ANDRE BARBEAU[3]

Department of Neurobiology, Clinical Research Institute of Montreal
110 Pine Avenue West, Montreal Quebec, Canada, H2W 1R7

(Received 24 April 1978)

RONDEAU, D. B., F. B. JOLICOEUR, F. BELANGER AND A. BARBEAU. *Differential behavioral activities from anterior and posterior hypothalamic lesions in the rat.* PHARMAC. BIOCHEM. BEHAV. 9(1) 43–47, 1978.—Bilateral 6-hydroxydopamine injections into the anterolateral (AL) or posterolateral (PL) portions of the hypothalamus produced hypokinesia, catalepsy, rigidity and severe weight losses due to aphagia and adipsia. Subcutaneous administration of apomorphine, 1 mg/kg, 48 hr after 6-OHDA injections reversed temporarily the hypokinesia in both AL and PL 6-OHDA groups. However, qualitative and quantitative differences in the behavioral responses to the drug were observed. Motor activity as measured by photocell counts was significantly greater in AL 6-OHDA rats. Apomorphine induced stereotyped behavior in both groups; however, the predominant behavioral responses were oral stereotypies in PL 6-OHDA animals and sniffing in AL 6-OHDA rats.

6-Hydroxydopamine Apomorphine Motor activity Anterolateral hypothalamus
Posterolateral hypothalamus Stereotyped behavior Catalepsy Rigidity

DESTRUCTION of catecholamine containing neurons in the CNS is reliably produced by administration of 6-hydroxydopamine (6-OHDA), an isomer of norepinephrine, into the lateral ventricules or cerebral tissue [13]. Since at least some of the symptoms of Parkinson's disease are convincingly linked to a biochemical deficiency in dopamine (DA) and loss of cell bodies in the substantia nigra [10], unilateral and bilateral microinjections of 6-OHDA into the substantia nigra and/or along the dopaminergic nigrostriatal pathway have been employed to create animal models of Parkinsonism [21,22]. Recently, an experimental model of the hypokinesia of Parkinson's disease based on hypothalamic 6-OHDA administration has been proposed [17]. Loss of active avoidance response, loss of visual placing and hypokinesia under certain stimulus conditions have been observed following microinjections of 6-OHDA in the anterolateral hypothalamus (AL) [14, 17, 18]. It has been recently demonstrated that the hypokinesia resulting from such injections is temporarily reversed following the administration of several drugs used in the treatment of parkinsonism, such as L-DOPA in combination with peripheral decarboxylase inhibitor Ro 4-4602, piribedil and bromocriptine; the putative dopamine receptor agonist apomorphine and the amino acid m-tyrosine were also effective in reversing the hypokinesia [3, 8, 9].

Microinjections of 6-OHDA into the anterolateral portion of the hypothalamus have been reported to produce catecholamine denervation, as determined by fluorescent microscopy, in the neocortex, hippocampus, limbic forebrain, anteromedial striatum and anterolateral hypothalamus [8]. Rats administered 6-OHDA into the posterolateral region of the hypothalamus (PL), which showed more severe motor deficiencies than AL 6-OHDA treated animals [18], had these same areas denervated in addition to denervation of the entire striatum, amygdala, thalamus and posterolateral hypothalamus [8]. Differential catecholamine denervation appears to result from the interruption, at two different sites, of both dopaminergic nigrostriatal and mesolimbic pathways as well as of the noradrenergic pathway [8]. Animals which received AL or PL hypothalamic microinjections of 6-OHDA presented different behavioral responses to L-DOPA. Administration of L-DOPA, 50 mg/kg, produced locomotor activity, running and rearing, in AL 6-OHDA rats. Since injections of this dose of the drug had no behavioral effect in vehicule injected animals, behavioral responses to L-DOPA were presented as different forms of behavioral supersensitivity determined by the pattern and/or extent of catecholamine denervation [8]. The purpose of the present experiment was first to compare the behavioral effects of 6-OHDA treated rats to the administration of apomorphine, 1 mg/kg. Results demonstrate prevalence of motor activity over stereotypy in AL 6-OHDA rats.

[1]Supported by Medical Research Council of Canada.
[2]Supported by Conseil de la Recherche en Santé du Québec.
[3]Requests for reprints should be mailed to: Dr. André Barbeau, Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7.

Apomorphine produced sniffing in the latter animals and bit ing, a higher component of stereotyped behavior, in PL 6-OHDA rats.

METHOD

Animals

Male Sprague-Dawley rats obtained from Canadian Breeding Farm (St. Constant, P. Quebec) were used. They were housed in a colony under constant conditions of temperature, humidity and light cycles. Food (Purina Rat Chow) and water were available ad lib. Rats weighed 275–325 g at surgery.

Surgery

Operations were carried out using a David Kopf stereotaxic instrument in rats anesthetized with sodium pentobarbital (60 mg/kg). Each microinjection consisted of 4 μl of distilled water containing 6-hydroxydopamine (Sigma Chemicals), 6.5 μg/μl, and ascorbic acid, 0.4 μg/μl. Injection solutions were mixed just prior to operation and chilled until loaded into a 10 μl Hamilton syringe (No. 701) fitted with a shortened (3 cm) needle; rate of injections was 1 μl/min. Stereotaxic coordinates of the microinjections of 6-OHDA were based on the atlas by de Groot [7]. Rats were injected bilaterally in either the anterolateral hypothalamus (AL), 7.0 mm anterior to the interaural line, 2.0 mm lateral to the sagittal sinus and 8.0 mm ventral to the dural surface, or the posterolateral hypothalamus (PL), A5.0, L2.0, V8.0. The coordinates for these bilateral hypothalamic injections are identical to the ones used by others [8,18]. Behavioral tests were performed 48 hr after 6-OHDA administration.

Procedure

Behavioral tests were designed to evaluate catalepsy, rigidity and general motor activity in both AL and PL 6-OHDA treated animals. Following these tests, general motor activity and stereotyped behavior in response to administration of apomorphine, 1 mg/kg, were assessed quantitatively in the same animals. The intensity of catalepsy was measured by a modification of the simple bar and cork used by Simon *et al.* [15]. The simple bar test involved placing the front paws of the rat on a horizontal metal bar (1.2 cm in dia.) suspended 10 cm above the table. The number of seconds the animal remained in such posture was calculated, the maximum allowable duration being 60 sec. The modified cork test entailed placing a front paw on a 9 cm-high cylindrical piece of wood (2.2 cm in dia.) glued to a wooden platform. Maximum duration of this test was 120 sec or 60 sec for each front paw. The intensity of rigidity was measured by three different tests, for each of which the number of seconds an animal retained a given position was calculated up to a maximum of 60 sec. In the parallel bars test previously described by Simon *et al.* [15], the front paws and the hind legs of a rat are placed on two metal bars (1.2 cm in dia.) of different height, 11 cm and 3 cm, respectively, held approximately 20 cm apart. Similarly in the suspension test the anterior and posterior limbs of the animal are placed on two 0.5 cm-wide and 11 cm-high pieces of wood, also maintained approximately 20 cm apart. The latter test is a modification of the bridge test used by Bloom *et al.* [1]. In the grasping test, the rat's front paws grasp a small metal rod (0.5 cm in dia.) held by the experimenter about 50 cm above the table. The response to this test has been associated with muscle rigidity in rats [19]. The animals were tested for catalepsy and rigidity six times at 30 min intervals. The average duration in seconds of these six trials was then recorded for each separate test.

Motor activity was measured using a circular (70 cm in dia.) activity meter (Lehigh Valley Electronics) comprising 6 photoelectric cells. Activity scores were recorded for periods of 5 min at 10 min intervals. During these 10 min intervals animals were removed from the apparatus and placed singly in circular wiremesh cages. Activity scores were obtained on three occasions before the administration of apomorphine, i.e., at T=0–5, 15–20 and 30–35. Immediately prior to the fourth test session in the activity meter, each animal received a subcutaneous injection of 1 mg/kg apomorphine (Apomorphine HCl, MacFarlan-Smith Ltd). Motor activity was then recorded for seven 5 min test sessions, i.e., at T=45–50, 60–65, 75–80, 90–95, 105–110, 120–125 and 135–140. Upon removal from the apparatus after completion of a test session, animals were placed in individual wire-mesh cages and observed for stereotypy during the following 5 min. Stereotyped behavior was rated according to the scale used by Butterworth *et al.* [2]: 0—no gnawing or sniffing; 1—moving around and continuous sniffing; 2—continuous sniffing and occasional biting and gnawing; 3—little moving around and frequent biting and gnawing; 4—no moving around, continuously and intensively biting and clinging teeth around the wires.

Immediately or up to 48 hr after the behavioral testing was completed, rats were perfused with isotonic saline (0.9%) followed by Formalin (10%). Brains were removed, placed in Formalin (10%) for at least 24 hr and then sectioned (40 μ) on a freezing microtome. Needle tracks were clearly visible indicating the site of 6-OHDA injection. Data collected on animals for which the site of 6-OHDA injection did not match the stereotaxic coordinates mentioned above were discarded.

RESULTS

Results are based on data obtained in a group of 12 rats which received microinjections of 6-OHDA in the anterolateral portion of the hypothalamus (AL group) and a second group of 12 rats injected in the posterolateral region (PL group). Both AL and PL 6-OHDA injections produced aphagia and adipsia. The AL 6-OHDA rats suffered a mean body weight loss of 45.0 g during the 48 hr period following the bilateral hypothalamic injections. During the same period mean weight of the PL 6-OHDA rats decreased by 43.2 g. Data on the two catalepsy tests and on the three rigidity tests were respectively combined for each group in order to obtain single measures of the intensity of catalepsy and rigidity. Results expressed as the mean time in seconds for both measures in AL and PL 6-OHDA groups are presented in Fig. 1. Animals of both groups showed similar intensity of catalepsy and rigidity; there were no significant differences between the two groups for both measures ($p<0.025$). There is no doubt that the 6-OHDA injected animals were rigid and cataleptic in comparison to normal rats. Naive or sham injected rats do not display catalepsy and/or rigidity (data not shown). It appears that the grasping test is the only one for which such animals remain in position for a short period of time; results of naive rats to this test are much lower than those obtained with the AL and PL 6-OHDA groups. It should be mentioned that administration of apomorphine, 1 mg/kg, clearly reversed for a period of approximately 1 hr the catalepsy and rigidity resulting from the 6-OHDA injec-

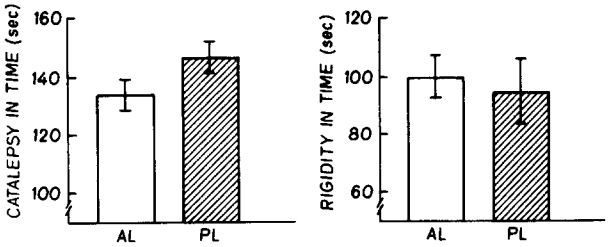

FIG. 1. Mean performance in seconds on catalepsy and rigidity tests in AL and PL 6-OHDA rats. Maximum duration: 180 sec. Catalepsy: Mean performance on simple bar + cork tests. Rigidity: Mean performance on parallel bars + suspension + grasping tests.

tions in both portions of the hypothalamus (data not shown).

Stereotyped behavior induced by administration of apomorphine, 1 mg/kg, was rated on seven occasions, each of which followed a test session in the activity meter. The mean stereotypy scores of both groups on these determinations are presented in Fig. 2. It is obvious that in both cases the onset of stereotyped activity was almost immediate and that it lasted approximately 1 hr. Stereotyped behavior in AL and PL 6-OHDA rats differed significantly especially from 20 to 50 min after administration of the drug. During this period of time animals in the PL 6-OHDA group almost invariably displayed what is generally referred to as the high-intensity component of stereotyped behavior [2], compulsive biting and gnawing with restricted locomotion while the AL 6-OHDA rats did so only rarely. Most of the latter animals displayed only sniffing.

Results on motor activity are based on the photocell counts obtained during the ten 5 min test sessions in the activity meter. A 2×10 repeated measures ANOVA [23] was carried out on the activity scores for each group of rats and for each test session. Significant differences were found between activity scores during the test sessions, $F(9,198)=59.31$, $p<0.01$, and more importantly between activity scores of the AL and PL 6-OHDA groups, $F(1,22)=17.99$, $p<0.01$. The groups of rats by test sessions interaction was also found to be significant, $F(9,98)=4.02$, $p<0.05$. An analysis of the simple main effects of the factor groups of rats revealed that activity scores were significantly different between AL and PL 6-OHDA animals for four test sessions, i.e., at T=60–65, 75–80, 90–95 and 105–110, $F(1,217)=14.7$, 24.0, 9.1 and 10.6, all $p<0.01$. These differences are illustrated in Fig. 3 in which mean activity scores of both groups are presented for each test session. An examination of the data obtained for the first three sessions indicated that AL and PL rats showed severe akinesia. This akinesia was reversed in both groups immediately following injection of apomorphine 1 mg/kg, and animals displayed motor activity up to 90 min after drug administration. As revealed by the statistical analysis, activity scores were much higher in the AL 6-OHDA rats for four consecutive sessions but similar scores were obtained for the two groups during the test session at T=120–125. When tested for the last time all but one AL animal showed akinesia, as it had been observed before apomorphine administration.

DISCUSSION

After bilateral 6-OHDA injections into the AL or PL regions of the hypothalamus rats exhibited hypokinesia, catalepsy and rigidity at similar intensity. A bilateral electrolytic lesion of the ascending dopaminergic fibres in the lateral hypothalamus has been reported to produce hypokinesia but only mild catalepsy [5]. However, serious catalepsy resulted from 6-OHDA injections into the area ventralis tegmenti but not from injections into the substantia

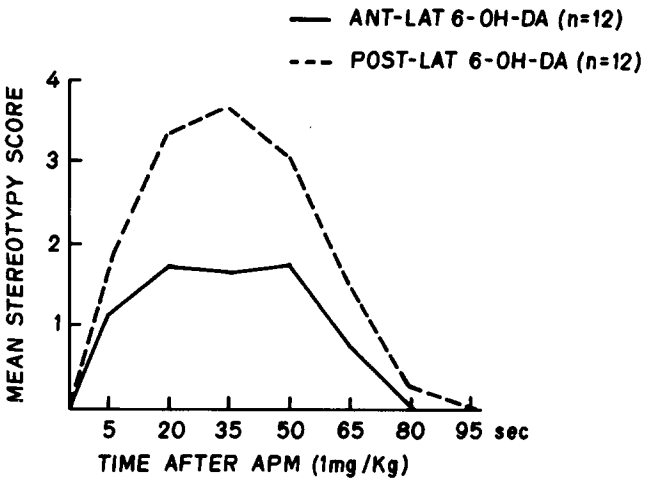

FIG. 2. Mean stereotypy scores of AL and PL 6-OHDA rats at various times following administration of apomorphine, 1 mg/kg.

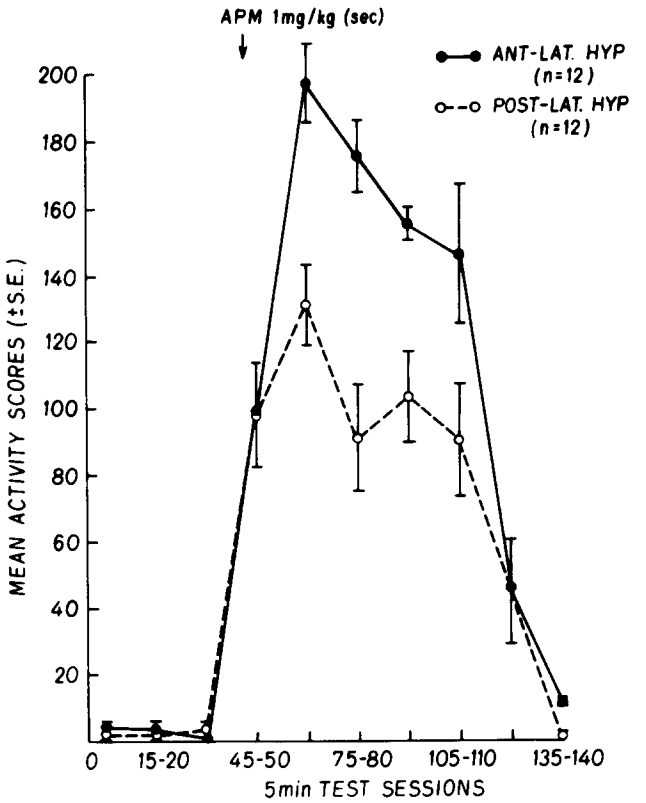

FIG. 3. Mean activity scores of AL and PL 6-OHDA rats for each 5 min test session in the activity meter. The bars are standard errors of the means. Arrow indicates apomorphine administration immediately prior to the test session.

nigra, suggesting that the occurrence of catalepsy is related to the extent of destruction of ascending dopaminergic axons [22]. Even though it appears that PL 6-OHDA treated rats show more severe motor disturbances than AL 6-OHDA animals [18], sufficient damage to the dopaminergic fibres as well as to the noradrenergic fibres originating in the pons and medulla resulted from 6-OHDA injections into these two hypothalamic sites to induce catalepsy and rigidity. The presence of rigidity in these animals was in agreement with earlier observations made by Ungerstedt *et al.* [21]. Administration of apomorphine, 1 mg/kg, 48 hr following microinjections of 6-OHDA induced stereotyped behavior and motor activity at different intensities in the two groups. PL 6-OHDA treated rats displayed mainly the high components of stereotyped behavior, gnawing and biting of limbs or wires, while AL 6-OHDA animals, who showed mostly sniffing, obtained lower stereotypy scores. These latter scores are comparable to those reported for naive animals injected with this dose of apomorphine [2]. Although the hypokinesia or akinesia was reversed in both groups immediately following apomorphine administration, motor activity was much greater in AL 6-OHDA rats from 15–60 min after the drug treatment. Locomotion was frequently ob served in these animals while PL 6-OHDA rats remained in the same area of the activity meter and displayed gnawing and/or biting.

Different behavioral responses to L-DOPA after AL or PL hypothalamic injections of 6-OHDA have been described. A same dose of L-DOPA produced running and rearing in AL 6-OHDA treated rats and oral stereotypies, grooming and biting of limbs or objects, in PL 6-OHDA animals [8]. Similarly, in the present experiment locomotion and stereotyped sniffing behavior were the predominant responses to the putative dopamine agonist apomorphine in AL 6-OHDA rats and oral stereotypies prevailed over motor activity in the PL 6-OHDA group. The responses to L-DOPA in AL and PL 6-OHDA treated rats were presented as examples of behavioral supersensitivity to the drug [8]; behavioral supersensitivity to L-DOPA and apomorphine has been described following unilateral catecholamine denervation produced by 6-OHDA administration [20]. Catecholamine denervation of various brain areas in AL or PL 6-OHDA injected rats has been studied by fluorescent microscopy. The neocortex, hippocampus, limbic forebrain and anteromedial striatum were denervated in both groups but the PL 6-OHDA animals presented in addition denervation of the thalamus, amygdala and entire striatum [8]. Moreover, it has been reported that changes in dopamine and norepinephrine concentrations in various regions of the brain were similar after AL or PL 6-OHDA injections with the exception of caudate dopamine which decreased less in AL than in PL 6-OHDA rats [16,18]. In regard to these differences in denervation and changes in catecholamine concentrations, the differential behavioral responses of AL and PL 6-OHDA rats to L-DOPA [8] and apomorphine (present experiment) would tend to suggest a predominant role of limbic forebrain structures in motor activity and sniffing, a low component of stereotyped behavior, and a predominant role of the striatum in oral stereotyped behaviors, such as gnawing and biting of limbs or objects (Fig. 4). If it is assumed that dopamine receptors in the above-mentioned areas were stimulated by apomorphine, the emergence of oral stereotypies in PL 6-OHDA treated rats can be seen as having precluded the full expression of motor activity as

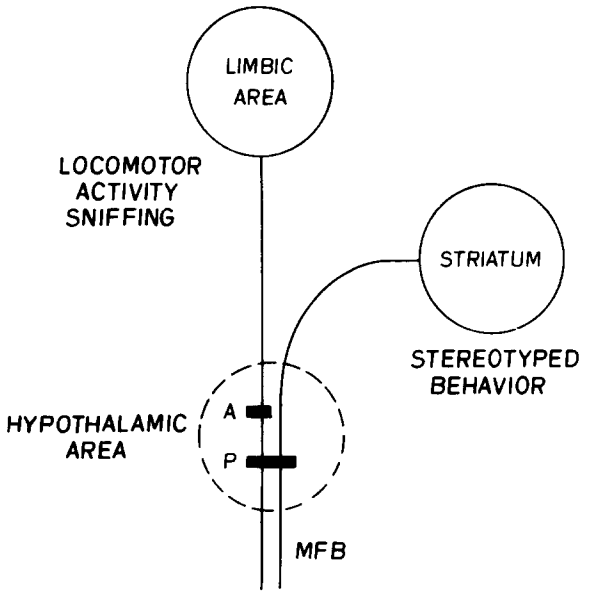

FIG. 4. Schematic representation of the differential activities elicited by AL or PL 6-OHDA lesions.

displayed by AL 6-OHDA animals. In this respect, it should be mentioned that potentiation of stereotyped behavior from sniffing to biting in response to a low dose of apomorphine, 0.1 mg/kg, has been previously observed in rats injected with 6-OHDA into the lateral hypothalamus at slightly different coordinates from the PL site studied in the present experiment [6]. Moreover, rats which received bilateral 6-OHDA injections into the caudate nucleus or the nucleus accumbens septi exhibited differential supersensity to apomorphine; the nucleus accumbens septi 6-OHDA treated rats showed enhanced locomotor activity and the caudate 6-OHDA group enhanced stereotyped behavior in response to apomorphine 1.0 mg/kg [11,12]. Contradictory findings related to brain nuclei and brain pathways involved in apomorphine induced locomotor activity and stereotyped behavior are numerous; the necessity of a detailed differentiation, both qualitatively and quantitatively, of the distinct behavioral effects of apomorphine has been emphasized recently [4].

The observations made in the present study are limited since fluorescence histochemistry or pharmacological assays were not used to substantiate the effects of 6-OHDA administration in the AL and PL regions of the hypothalamus. Such data have been obtained by others [8, 16, 18]. The findings that AL and PL 6-OHDA rats presented different behavioral responses to L-DOPA and apomorphine could have a serious implication for the pathophysiology of parkinsonism, and choreic disorders.

REFERENCES

1. Bloom, F., D. Segal, N. Ling and R. Guillemin. Endorphins: Profound behavioral effects suggest new etiological factors in mental illnes. *Science* **194:** 630–632, 1976.
2. Butterworth, R. F., J. C. Poignant and A. Barbeau. Apomorphine and piribedil in rats: biochemical and pharmacologic studies. In: *Advances in Neurology*, Vol. 9, edited by D. B. Calne, T. N. Chase and A. Barbeau. New York: Raven Press, 1975, pp. 307–325.
3. Butterworth, R. F., F. Bélanger and A. Barbeau. Hypokinesia produced by anterolateral hypothalamic 6-hydroxydopamine lesions and its reversal by some antiparkinson drugs. *Pharmac. Biochem. Behav.* **8:** 41–45, 1978.
4. Colpaert, F. C., W. F. M. Van Bever and J. E. M. E. Leysen. Apomorphine: Chemistry, Pharmacology, Biochemistry. In: *Int. Rev. Neurobiol.*, Vol. 19, edited by C. C. Pfeiffer and J. R. Smythies. New York: Academic Press, 1976, pp. 225–268.
5. Costall, B. and R. J. Naylor. The role of telencephalic dopaminergic systems in the mediation of apomorphine-stereotyped behavior. *Eur. J. Pharmac.* **24:** 8–24, 1973.
6. Costall, B., C. D. Marsden, R. J. Naylor and C. J. Pycock. Stereotyped behaviour patterns and hyperactivity induced by amphetamine and apomorphine after discrete 6-hydroxydopamine lesions of extrapyramidal and mesolimbic nuclei. *Brain Res.* **123:** 89–111, 1977.
7. De Groot, J. The rat brain in stereotaxic coordinates. *Trans. Roy. Netherl. Acad. Sci.* **52:** 1–38, 1959.
8. Ervin, G. N., J. S. Fink, R. C. Young and G. P. Smith. Different behavioral responses to L-DOPA after anterolateral or posterolateral hypothalamic injections of 6-hydroxydopamine. *Brain Res.* **132:** 507–520, 1977.
9. Fink, J. S. and G. P. Smith. Apomorphine, but not clonidine, repairs deficits in locomotor and investigatory exploration after ablation of dopamine afferents to limbic forebrain and anteromedioventral striatum. *Neurosci. Abst.* **3:** 441, 1977.
10. Hornykiewicz, O. Neurochemistry of parkinsonism. In: *Handbook of Neurochemistry*, Vol. 7, edited by A. Lajtha. New York: Plenum Press, 1972, pp. 465–501.
11. Iversen, S. D., P. H. Kelly, R. J. Miller and P. Seviour. Amphetamine and apomorphine responses in the rat after lesion of mesolimbic or striatal dopamine neurones. *Br. J. Pharmac.* **54:** 244P, 1975.
12. Kelly, P. H., P. W. Seviour and S. D. Iversen. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Res.* **94:** 507–522, 1975.
13. Kostrzewa, R. M. and D. M. Jacobwitz. Pharmacological actions of 6-hydroxydopamine. *Pharmac. Rev.* **26:** 199–288, 1974.
14. Sechzer, J. A., G. N. Ervin and G. P. Smith. Loss of visual placing in rats after lateral hypothalamic microinjections of 6-hydroxydopamine. *Expl Neurol.* **41:** 723–737, 1973.
15. Simon, P., R. Langwinski and J. R. Boissier. Comparaison de différents tests d'évaluation de la catalepsie chez le rat. *Thérapie* **24:** 985–995, 1969.
16. Smith, G. P. The neuropharmacology of thirst. In: *The Neuropsychology of Thirst*, edited by A. N. Epstein, H. Kissileff and E. Stellar. Washington: H. V. Winston & Sons, 1973, pp. 231–241.
17. Smith, G. P. and R. C. Young. A new experimental model of hypokinesia. In: *Advances in Neurology*, Vol. 5, edited by F. H. McDowell and A. Barbeau. New York: Raven Press, 1974, pp. 427–432.
18. Smith, G. P., B. E. Levin and G. N. Ervin. Loss of active avoidance responding after lateral hypothalamic injections of 6-hydroxydopamine. *Brain Res.* **88:** 483–498, 1975.
19. Steg, G. Efferent muscle innervation and rigidity. *Acta Physiol. Scand.* **61:** Suppl. 225, 5–53, 1964.
20. Ungerstedt, U. Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiol. Scand.* **82:** Suppl. 367, 69–93, 1971.
21. Ungerstedt, U., T. Ljungberg and G. Steg. Behavioral, physiological and neurochemical changes after 6-hydroxydopamine-induced degeneration of the nigro-striatal dopamine neurons. In: *Advances in Neurology*, Vol. 5, edited by F. H. McDowell and A. Barbeau. New York: Raven Press, 1974, pp. 421–26.
22. Ungerstedt, U., A. Avemo, E. Avemo, T. Ljungberg and C. Ranje. Animal models of parkinsonism. In: *Advances in Neurology*, Vol. 3, edited by D. B. Calne. New York: Raven Press, 1973, pp. 257–271.
23. Winer, B. J. *Statistical Principles in Experimental Design*. Second edition. New York: McGraw-Hill, 1971.