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Muscarinic Receptor Binding and Behavioral Effects of Atropine Following Chronic Catecholamine Depletion or Acetylcholinesterase Inhibition in Rats[1]

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SCHALLERT, T., D. H. OVERSTREET AND H. I. YAMAMURA. *Muscarinic receptor binding and behavioral effects of atropine following chronic catecholamine depletion or acetylcholinesterase inhibition in rats.* PHARMAC. BIOCHEM. BEHAV. 13(2) 187–192, 1980.—Rats were subjected to one of two experimental treatments: (1) intraventricular infusion of the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA), known to permanently reduce brain dopamine and norepinephrine levels, or (2) chronic administration of the irreversible acetylcholinesterase inhibitor diisopropylfluorophosphate (DFP). Both treatments are believed to produce relative overactivity of cholinergic systems and to suppress forward locomotion. The anticholinergic agent atropine sulfate yielded excessive forward walking in otherwise chronically akinetic 6-OHDA-treated rats, whereas atropine slightly decreased locomotion in controls. The hypothesis that such supersensitivity to atropine may be related to a reduction in the density of muscarinic cholinergic receptors was not supported: First, ^{3}H-quinuclidinyl benzilate (QNB) binding to membrane preparations was not decreased in the 6-OHDA-treated rats; secondly, atropine did not induce excessive forward locomotion in the DFP-treated rats in which ^{3}H-QNB binding was decreased. There were other changes in the DFP-treated rats consistent with muscarinic receptor alteration, including tolerance to the locomotor suppressive effects of DFP, cross tolerance to the cholinergic agonist pilocarpine, and exaggerated atropine-induced increases in core temperature and stereotypy. It is concluded that 6-OHDA and DFP produce different long-term changes in cholinergic brain systems and atropine-sensitive behaviors.

Muscarinic receptor binding Anticholinergic Locomotion Anticholinesterase 6-Hydroxydopamine Chronic DFP Animal model of parkinsonism Catecholamine depletion Dopamine receptor binding Thermoregulation Stereotypy Supersensitivity Subsensitivity

IN analyzing an animal analog of parkinsonism, it was found that the muscarinic anticholinergic agent atropine sulfate reversed severe akinesia in rats which had been previously treated intraventricularly with the irreversible catecholamine-depleting neurotoxin 6-hydroxydopamine (6-OHDA) [26, 27, 28, 39]. In some cases, several thousand turns of an activity wheel were recorded over an 8 hr atropine session. Such enormously excessive activity is particularly surprising because the animals were otherwise totally akinetic and, when given atropine, did not run but walked with a parkinsonian (small step) gait. This supersensitivity to anticholinergics developed only gradually over the several weeks following 6-OHDA treatment and was correlated with the post-operative duration of akinesia and extent of catecholamine depletion in the striatum and cortex [26, 27, 39].

The mechanisms underlying this phenomenon are not yet known. Behavioral supersensitivity to catecholamine agonists, such as apomorphine, following denervation of catecholaminergic terminals is well documented [4, 13, 30, 32, 34, 37] and is thought to be related to an increase in dopaminergic receptors to which the agonists bind [4,5]. However, because atropine is an *antagonist* (at muscarinic

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cholinergic receptors), supersensitivity to atropine is not readily explained by this type of process.

The anticholinergic/supersensitivity phenomenon may be related to the well-known mutually antagonistic interaction between catecholaminergic and cholinergic systems in the brain [2, 6, 11, 14, 20]. For example, following 6-OHDA treatment, cholinergic activity may be chronically released from its normal catecholaminergic control, an effect that could alter the characteristics of the cholinergic receptors with which atropine presumably interacts [8, 15, 21]. We therefore compared behavioral and physiological effects of 6-OHDA with the effects of diisopropylfluorophosphate (DFP), an irreversible acetylcholinesterase inhibitor which chronically increases cholinergic activity in the brain and thereby reduces muscarinic cholinergic receptors [9, 18, 19, 23, 29, 35]. We analyzed locomotor responses to atropine and assayed muscarinic cholinergic receptor binding to relevant brain membrane preparations.

METHOD

Animals

Male Sprague-Dawley rats from Holtzman stock were housed in the laboratory for at least one week prior to the beginning of the experiments, at which time they were at least 70 days old (300 g). They were housed individually in a temperature-controlled room (23°C) under a 12 hr light-dark cycle. All testing was carried out during the light part of the cycle. The rats used in the 6-OHDA and DFP studies were drawn from different populations.

Apparatus

Activity was recorded as the number of turns in activity wheels similar to those described previously [26]. In some cases the number of line crossings in a rectangular open field chamber, 60×30 cm, with 10×10 cm squares, was additionally determined.

6-OHDA Administration

After one week of daily handling, rats were divided into a control group of seven rats and an experimental group of seven rats. On the first day of the experiment a baseline recording of locomotor activity in an open field chamber was taken. Subsequently, the experimental animals received bilateral infusions of 6-OHDA using procedures described previously [26]. Briefly, pargyline (50 mg/kg, IP) was injected 30 min prior to 6-OHDA. The 6-OHDA, dissolved in artificial CSF, was a 14 μg/μl solution infused into each lateral ventricle at a rate of 3 μl/min over a 5 min period (210 μg total per ventricle).

Locomotor activity in the open field was assessed again at 1, 8, and 15 days after the intraventricular administration of 6-OHDA. During this period four of the 6-OHDA-treated rats were severely aphagic and adipsic [31,36]; consequently, they were intubated two or three times daily to maintain their body weight above 85% of the preoperative level [33]. Three of the control rats were given a limited food supply during this period so that their weight was reduced to match the levels of the 6-OHDA-treated rats. This weight reduction had no influence on the behavioral effects of atropine or on muscarinic binding.

The animals were given a 2 hr baseline session in the activity wheel 15 days after the lesion. Two or three days later they then received an injection of atropine sulfate 15 min before being placed in the activity wheel for a second 2 hr session. The dose (50 mg/kg, IP) was based on previous dose-response analyses of central effects [26,38], although it remains to be determined whether the effects are due to anticholinergic action. The animals were sacrificed by decapitation at least 48 hr after the atropine administration (at least 38 hr after the behavioral effects ceased), and the brains were rapidly removed and dissected on ice. The hippocampi, striatum, and frontal cortex were stored at −20°C until the receptor binding assays were conducted.

DFP Administration

Animals were assigned to a control group of three rats and an experimental group of four rats. These rats were taken from a locally bred population of Holtzman rats. To parallel previous DFP studies [17], on the first day of the experiment baseline rectal temperatures were taken. A thermistor probe attached to a telethermometer (Yellow Springs Instruments) was inserted 7 cm into the rectum. Based on pilot work and previous experiments [17,18], animals were injected intramuscularly with either 1 mg/kg of DFP in an arachis oil vehicle or 1 ml/kg of the vehicle alone (control). Temperatures were taken again 4 hr later. Behavior in activity wheels was examined for 2 hr on the day (21–27 hr) after the first injection. Subsequent injections of 0.5 mg/kg DFP (or 0.5 ml/kg vehicle) were administered at 3-day intervals in order to maintain a reduced activity of acetylcholinesterase and to induce tolerance [16,17].

Temperatures were recorded again immediately before and 4 hr after the sixth injection of DFP or vehicle. A 2 hr session in the activity wheel and a 3 min session in the open field were given the day after this DFP injection.

Since the above tests indicated that tolerance to DFP had developed, a challenge with atropine sulfate (50 mg/kg, IP) was given to all animals approximately 24 hr after the eighth injection of the chronic regimen [22]. The rats were placed in the activity wheels immediately after the injection and remained there for one hour. They were then taken out, exposed to the open field for 3 min and returned to the wheel for a second hour. Body temperatures were also recorded.

To confirm that the DFP tolerance was comparable to that described in previous experiments [16,17], pilocarpine (a cholinergic agonist) was administered approximately 24 hr after the ninth injection of the chronic regimen. All animals first received an injection of methyl atropine (3 mg/kg, IP) to prevent the peripheral actions of pilocarpine, followed by pilocarpine hydrochloride (10 mg/kg, IP) 15 min later. Twelve min after this latter injection the animals were exposed to the open field for three min.

The animals were sacrificed approximately 24 hr after the tenth injection of the chronic regimen. The hippocampi were rapidly dissected and stored frozen at −20°C until the binding assays were carried out. Recent studies have demonstrated reductions in striatal, hippocampal, and cortical muscarinic receptors following chronic DFP administration [29].

Binding Assays

The tissues from the various brain regions were homogenized in 20 volumes of Tris HCl buffer (0.05 M, pH 7.4) centrifuged at 20,000×g for 10 min, washed in Tris buffer, centrifuged at 20,000×g for 10 min, and the final pellet

TABLE 1

ACTIVITY WHEEL REVOLUTIONS WITH AND WITHOUT ATROPINE AND ^{3}H-SPIROPERIDOL BINDING IN 6-OHDA-TREATED RATS AND THEIR CONTROLS (MATCHED FOR WEIGHT LOSS) AT 15–18 DAYS POSTSURGERY

	Activity (2 hr period)		^{3}H-Spiroperidol bound (fmol/mg protein)
	No atropine	Atropine	
Akinetic 6-OHDA	0 ± 0	364.5 ± 45.5*	80.87 ± 7.37†
Controls	252.3 ± 143.7	108.0 ± 53.4	58.89 ± 3.54
Hypokinetic 6-OHDA	5.2 ± 4.5	13.3 ± 6.6	61.44 ± 7.82
Controls	243.4 ± 126	125.7 ± 90.5	61.97 ± 0.60

Data are means ± SE. The control rats in this experiment were more active than in previous studies [26,27], possibly because they were somewhat younger. Striatal membrane preparations were incubated at 37°C for 30 min in 2 ml of 50 μM Tris HCl buffer containing ^{3}H-Spiroperidol in a final concentration of 100 pM, as described in METHOD.

*Significantly different from undrugged condition.

†Significantly different from control.

resuspended in 20 volumes of Tris buffer. The protein content of the tissue suspensions was determined by the Lowry method [12].

To assess muscarinic receptor binding, 2 ml assays containing Tris HCl buffer (0.05 M, pH 7.4), ^{3}H-Quinuclidinyl benzilate (QNB, 29.4 Ci/mmole, New England Nuclear) and aliquots of the tissue suspension were performed in duplicate and in the presence and absence of atropine (1 μM). Specific binding was defined as the difference between the binding in the presence and absence of atropine. After incubation at 37°C for 60 min, the reaction was terminated by rapid filtration, as described previously [40]. After addition of scintillation fluid, the filters were allowed to extract overnight before counting on a scintillation counter at an efficiency of 45%.

To assess dopamine receptor binding 2 ml assays containing Tris HCl buffer (0.05 M, pH 7.4), ^{3}H-Spiroperidol (25.4 Ci/mmole, New England Nuclear) and aliquots of the tissue suspension were performed in duplicate and in the presence and absence of (+) butaclamol (1 μM). Specific binding was defined as the difference between the binding in the presence and absence of (+) butaclamol. After incubation at 37°C for 30 min, the reaction was terminated by rapid filtration. After addition of scintillation fluid, the filters were allowed to extract overnight before counting on a scintillation counter at an efficiency of 45%.

RESULTS

6-OHDA-Treated Rats

Without atropine. The rats treated with 6-OHDA were akinetic or hypokinetic throughout the postoperative period. Behaviorally, the 6-OHDA-treated rats could be separated easily into two groups (confirmed later biochemically; see below). One group (3 rats) recovered some forward locomotion and ate dry food within 5 days after the lesion (hypokinetic group). Another group (4 rats) did not recover locomotion or eating, and they actively rejected [25, 33, 39] familiar wet cookie mash (akinetic group). As shown in Table 1, both the akinetic and hypokinetic 6-OHDA groups showed many fewer revolutions in the activity wheel than their control rats matched for body weight loss ($p<0.01$). In the open field test (used to assess degree of akinesia/hypokinesia) the average line crossing activity (M ± SEM) of the 6-OHDA-treated rats, as a group, was 1.0 ± 0.6 on Day 1, 3.2 ± 1.1 on Day 8, and 4.5 ± 2.3 on Day 15. These were significantly different from control values, which were 29.9 ± 1.4 ($p<0.05$), 17.7 ± 3.0 ($p<0.05$), and 12.9 ± 2.2 ($p<0.05$) for Days 1, 8, and 15 respectively.

Atropine. When given atropine sulfate on Day 18, the otherwise akinetic rats walked almost continuously during their 2 hr in the activity wheel. Despite taking very short steps (50–70% of controls), their activity was far in excess of the activity of atropinized hypokinetic or atropinized control groups (Table 1). The most striking feature of their behavior was the transformation from total akinesia to relentless forward locomotion. When placed in a 45° corner, however, they stopped walking and appeared completely trapped without scanning upward, turning around, or attempting to "escape" [26, 27, 39].

Binding. The ^{3}H-QNB binding data for the akinetic rats was virtually identical to that of the hypokinetic rats at all brain regions analyzed, and so the data were pooled. As shown in Table 2, there were no significant alterations in ^{3}H-QNB binding to membrane preparations taken from the 6-OHDA-treated rats. (Although we chose to analyze three of the most likely brain areas in which to expect changes, we cannot rule out alterations in muscarinic cholinergic receptors at regions other than striatum, frontal cortex or hippocampus, nor the role of other neurotransmitter receptors). Indeed, there was a slight but nonsignificant increase in binding in striatum and hippocampus.

In contrast, ^{3}H-Spiroperidol binding to membrane preparations in striatum was greatly increased in the akinetic 6-OHDA-treated rats compared with the hypokinetic 6-OHDA-treated rats and controls (Table 1).

DFP-Treated Rats

Without atropine. Twenty-four hr after the initial DFP injection, there was a clear hypokinesia, although it was not comparable to that seen in the hypokinetic 6-OHDA-treated rats (in pilot work, higher doses of DFP produced a degree of

TABLE 2

MUSCARINIC RECEPTOR BINDING TO MEMBRANE PREPARATIONS FROM RATS WITH INTRAVENTRICULAR 6-OHDA LESIONS

Brain region	^{3}H-QNB bound (fmoles bound/mg protein) 6-OHDA-lesioned	Control
Hippocampus	740.04 ± 25.89	718.73 ± 46.67
Corpus striatum	828.45 ± 36.96	791.69 ± 36.42
Frontal cortex	912.81 ± 45.96	913.42 ± 64.65

Data are mean ± SE. Brain membrane preparations were incubated at 37°C for 60 min in 2 ml of 50 mM Tris HCl buffer containing ^{3}H-QNB in a final concentration of 400 pM, as described in METHOD.

TABLE 3

ACTIVITY WHEEL REVOLUTIONS IN DFP-TREATED AND CONTROL RATS WITH AND WITHOUT ATROPINE 19 DAYS AFTER FIRST INJECTION

	No atropine	Atropine
DFP	100.5 ± 10.3	77.0 ± 10.3*
Control	76.3 ± 6.7	48.0 ± 24.2*

*Significantly different from undrugged condition.

akinesia severe enough to be more comparable quantitatively to the 6-OHDA-treated rats 24 hr postoperatively; however, animals so-treated rarely survived more than a day or two after the initial injection). Compared with the 56.3 ± 7.8 revolutions in control rats, there were 17.0 ± 8.6 revolutions in DFP-treated rats ($p<0.05$). This hypokinesia was attenuated over the next 6 injections of DFP; i.e., tolerance developed. Thus, in contrast to the 6-OHDA-treated rats, 19 days after the first injection the DFP-treated rats showed no akinesia or hypokinesia relative to their controls (Table 3).

Twenty-four hr after the initial DFP injection there was a decrease in body temperature of 3.05°C ± 0.09 relative to baseline, which was significantly different from that seen in vehicle control rats (0.07°C ± 0.07). Tolerance developed to this hypothermia-producing aspect of DFP [17]. Thus, after the sixth injection, instead of decreasing, body temperature showed a slight increase over baseline of 0.23°C ± 0.06. In controls there was a slight decrease over baseline (0.1°C ± 0.1).

Atropine. Following atropine, the DFP rats did not show the dramatic increase in forward locomotion, nor the short steps, seen in the akinetic 6-OHDA animals. As shown in Table 3, forward locomotion was in fact slightly decreased by atropine in the DFP and control animals ($p<0.05$). However, there was a very obvious behavioral difference between the DFP-treated and control animals in their response to atropine. Although both groups showed typical behavioral and pupillary signs of atropinization, the DFP-treated animals were more thigmotactic and displayed more continuous and vigorous scanning stereotypy of the type described previously [24] in otherwise intact animals given very high doses of atropine (75 mg/kg) or atropine plus low doses of amphetamine. Thus the DFP-treated animals engaged in virtually non-stop scanning, with the snout, up and down in a corner of the open field. The atropinized control animals showed less vigorous stereotyped behavior, which was occasionally interrupted by 5–15 sec periods of immobility or 10–30 sec periods of scanning activity away from the corner along the walls of the open field. Further analysis of this exaggerated atropine stereotypy of the DFP-tolerant rats should be carried out.

There was a supersensitivity to atropine with respect to core temperature. In DFP-tolerant animals, atropine caused a rise in temperature of 1.33°C ± 0.09 above baseline, which was significantly different from the slight increase seen in controls (0.6°C ± 0.06). Although this supersensitivity may be related to the exaggerated up-and-down scanning stereotypy described above in the atropinized DFP-tolerant rats, it also may reflect a compensatory change at the cholinergic receptor level in the same thermoregulatory system affected so dramatically in the opposite direction by the initial DFP injection.

The response of the DFP-tolerant rats to a standard pilocarpine challenge confirmed that the DFP-tolerant rats were subsensitive to cholinergic agonists and therefore were comparably tolerant to DFP-treated rats reported in other studies. Thus, while in control rats pilocarpine significantly reduced average open field activity from 64.3 ± 8.7 (undrugged) to 13.3 ± 4.2 (pilocarpine) ($p<0.02$), it non-significantly reduced such activity in DFP-tolerant rats from 59.8 ± 8.7 (undrugged) to 49.5 ± 5.7 (pilocarpine).

Binding. The hippocampal membrane preparations from the DFP-tolerant and control rats were incubated with ^{3}H-QNB at a final concentration of 231 pM. There were significant differences in the amount of specific ^{3}H-QNB bound. Specific ^{3}H-QNB bound for the control group was 609.37 ± 50.12 fmoles/mg protein, while specific ^{3}H-QNB bound for the DFP-tolerant rats was 464.83 ± 15.46 fmoles/mg protein, representing a 24% reduction ($p<0.05$).

DISCUSSION

Chronically akinetic 6-OHDA-treated rats showed hyperactive forward walking in an activity wheel when injected with the anticholinergic agent atropine sulfate [26]. The hypothesis that this supersensitivity to atropine reflects a reduction in the density of cholinergic receptors was not supported. Firstly, for the structures analyzed there was no decrease in muscarinic cholinergic receptor binding in the 6-OHDA-treated rats; in fact, there was a nonsignificant increase. Secondly, atropine did not induce hyperactive forward walking in the DFP-treated rats in which muscarinic receptor binding was decreased.

Recently it was reported that following unilateral 6-OHDA lesions of the substantia nigra, ^{3}H-atropine binding in striatum ipsilateral to the lesion was found to be decreased relative to control values in rats sacrificed about 4–12 days postoperatively [10]. As in the present study, no differences in binding were noted if the assays were done on membrane preparations from animals sacrificed after more than 15 days. Unfortunately, atropine/behavioral analyses were not carried out. It may be relevant that the time required for muscarinic receptor binding to return to normal in the unilateral nigra-lesioned rats was similar to the time required for the development of a strong supersensitive behavioral response to atropine in rats made akinetic by intraventricular

6-OHDA-treatment [26]. Of course it would be very surprising if the atropine supersensitivity reported here somehow depended on *recovery* from striatal muscarinic receptor changes, unless the critical variable involved an interaction with some other neural events.

In this regard, we did find an increase in dopamine receptor binding (also [21]). This is consistent with other work which has indicated that otherwise akinetic 6-OHDA-treated animals displaying hyperactive forward locomotion in response to anticholinergic agents were also supersensitive to dopamine agonists [26, 28; unpublished data]. The supersensitivity to dopamine agonists precedes by several days the onset of anticholinergic supersensitivity (Schallert and Whishaw, unpublished data). It is possible that supersensitive dopamine receptors indirectly permit a potentiated anticholinergic action in the absence of any decrease in cholinergic receptor density. In addition to blocking cholinergic receptors, anticholinergics are thought to inhibit slightly dopamine re-uptake in the striatum, which might increase stimulation of dopamine receptors [1,3]. Because cholinergic neurons in the striatum are under the inhibitory control of dopaminergic (possibly specifically D_2 [7]) receptors, *supersensitive* dopamine receptors may contribute significantly to the anticholinergic-induced hyperactivity phenomenon.

Partial, rather than total, involvement of an indirect mechanism such as this would be consistent with the following observations: In 6-OHDA-treated rats a DA agonist produces a qualitatively different form of locomotion than an anticholinergic agent; that is, the locomotor response to dopamine agonists is not exclusively in the forward direction and involves excessive circling and mouthing stereotypies [26,28]. Secondly, in intact rats, very high doses of atropine (e.g. 200 mg/kg) do not produce hyperactive forward locomotion (Whishaw and Schallert, unpublished data). Finally, selective denervation of dopaminergic neurons relative to noradrenergic neurons (using 6-OHDA plus desimpramine) yields akinesia that is only mildly affected by atropine; that is, the typically striking atropine-induced hyperactivity was not observed [27]. Thus, an increase in the density of dopamine receptors alone without alteration of muscarinic function elsewhere in the brain (possibly brain stem [27]) cannot adequately account for the excessive walking induced by atropine. The mechanism underlying the anticholinergic supersensitivity phenomenon remains to be understood fully.

Although the DFP-tolerant rats did not show atropine-induced excessive forward locomotion, there were other effects that appear to reflect the observed reduction in cholinergic receptor binding in these rats. These include cross tolerance to pilocarpine (agonist subsensitivity), and exaggerated atropine-induced increases in core temperature and stereotypy (antagonist supersensitivity). These findings are consistent with other behavioral observations demonstrating cholinergic agonist subsensitivity [16, 17, 18, 23] and anticholinergic supersensitivity [18,22] following chronic DFP.

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REFERENCES

1. Besson, M. J., A. Cheramy, C. Gauchy and J. Glowinski. In vivo spontaneous and evoked release of newly synthesized dopamine in the cat caudate nucleus. In: *Advances in Neurology, Vol. 5*, edited by F. McDowell and A. Barbeau. New York: Raven Press, 1974.
2. Butcher, L. L. *Cholinergic-Monoaminergic Interactions in the Brain*. New York: Academic Press, 1978.
3. Coyle, J. T. and S. H. Snyder. Antiparkinson drugs: Inhibition of dopamine uptake in the corpus striatum as a possible mechanism of action. *Science* **166:** 899–901, 1969.
4. Creese, I., D. R. Burt and S. H. Snyder. Dopamine receptor binding enhancement accompanies lesion-induced behavioral supersensitivity. *Science* **197:** 456–548, 1977.
5. Creese, I. and S. H. Snyder. Nigrostriatal lesions enhance striatal ^{3}H-apomorphine and ^{3}H-spiroperidol binding. *Eur. J. Pharmac.* **56:** 277–282, 1979.
6. Duvoisin, R. C. Problems in the Treatment of Parkinsonism. In: *Parkinson's Disease: Neurophysiological, Clinical, and Related Aspects*, edited by F. S. Messiha and A. D. Kenny. New York: Plenum Press, 1977.
7. Euvrard, C., J. Premont, C. Oberlander, J. R. Boissier and J. Bockaert. Is dopamine-sensitive adenylate cyclase involved in regulating the activity of striatal cholinergic neurons? *Archs Pharmac.* **309:** 241–245, 1979.
8. Gianutsos, G. and H. Lal. Alteration in the action of cholinergic and anticholinergic drugs after chronic haloperidol: Indirect evidence for cholinergic hyposensitivity. *Life Sci.* **18:** 515–520, 1976.
9. Glow, P. H., S. Rose and A. Richardson. The effect of acute and chronic treatment with diisopropyl fluorophosphate on cholinersterase activities of some tissues of the rat. *Aust. J. exp. biol. med. Sci.* **44:** 73–86, 1966.
10. Kato, G., S. Carson, M. L. Kemel, I. Glowinski and M. F. Giorguieff. Changes in striatal specific binding after unilateral 6-hydroxydopamine lesions of nigrostriatal dopaminergic neurons. *Life Sci.* **22:** 1607–1614, 1978.
11. Ladinsky, H., S. Consolo, S. Bianchi, R. Samanin and D. Ohezzi. Cholinergic-dopaminergic interaction in the striatum—Effect of 6-hydroxydopamine or pimozide treatment on increased striatal acetylcholine levels induced by apomorphine, piribedil and d-amphetamine. *Brain Res.* **84:** 221–226, 1975.
12. Lowry, O. H., N. J. Roserrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193:** 265–275, 1951.
13. Marshall, J. F. and U. Ungerstedt. Supersensitivity to apomorphine following destruction of the ascending dopamine neurons: Quantification using the rotational model. *Eur. J. Pharmac.* **41:** 361–367, 1977.
14. Mason, S. T. and H. C. Fibiger. Possible behavioral function for noradrenaline-acetylcholine interaction in brain. *Nature* **277:** 346–397, 1979.
15. Nomura, Y., H. Kajiyama, Y. Nakata and T. Segawa. Muscarinic cholinergic binding in striatal and mesolimbic areas of the rat: Reduction by 6-hydroxydopa. *Eur. J. Pharmac.* **58:** 125–131.
16. Overstreet, D. H., D. Haddick and R. W. Russell. Effects of amphetamine and pilocarpine on eating behavior in rats with chronically low acetycholinesterase levels. *Behav. Biol.* **7:** 217–226, 1972.
17. Overstreet, D. H., M. D. Kozar and G. S. Lynch. Reduced hypothermic effects of cholinomimetic agents following chronic anticholinesterase treatment. *Neuropharmacology* **12:** 1017–1032, 1973.

18. Overstreet, D. H., R. W. Russell, B. J. Vasquez and F. W. Dalglish. Involvement of muscarinic and nicotinic receptors in behavioral tolerance to DFP. *Pharmac. Biochem. Behav.* **2:** 45–54, 1974.
19. Overstreet, D. H. and H. I. Yamamura. Receptor alterations and drug tolerance. *Life Sci.* **25:** 1865–1878, 1979.
20. Racagni, G., D. L. Cheney, M. Trabucchi and E. Costa. In vivo actions of clozapine and haloperidol on turnover rate of acetylcholine in rat striatum. *J. Pharmac. exp. Ther.* **196:** 323–332, 1976.
21. Reisine, T. D., J. I. Nagy, K. Beaumont, H. C. Fibiger and H. I. Yamamura. The localization of receptor binding sites in the substantia nigra and striatum of the rat. *Brain Res.* **177:** 241–252, 1979.
22. Russell, R. W., B. J. Vasquez, D. H. Overstreet and F. W. Dalglish. Effects of cholinolytic agents on behavior following development of tolerance to low cholinesterase activity. *Psychopharmacologia* **20:** 32–41, 1971.
23. Russell, R. W., D. H. Overstreet, C. W. Cotman, V. G. Carson, L. Churchill, F. W. Dalglish and B. J. Vasquez. Experimental tests of hypotheses about neurochemical mechanisms underlying behavioral tolerance to the anticholinesterase, diisopropyl fluorophosphate. *J. Pharmacol. exp. Ther.* **192:** 73–85, 1975.
24. Schallert, T., M. De Ryck and P. Teitelbaum. Atropine stereotypy as a behavioral trap: A movement subsystem and electroencephalographic analysis. *J. comp. physiol. Psychol.* **94:** 1–24, 1980.
25. Schallert, T. and I. Q. Whishaw. Two types of aphagia and two types of sensorimotor impairment after lateral hypothalamic lesions: Observations in normal weight, dieted, and fattened rats. *J. comp. physiol. Psychol.* **92:** 720–741, 1978.
26. Schallert, T., I. Q. Whishaw, V. D. Ramirez and P. Teitelbaum. Compulsive, abnormal walking caused by anticholinergics in akinetic, 6-hydroxydopamine-treated rats. *Science* **199:** 1461–1463, 1978.
27. Schallert, T., I. Q. Whishaw, V. D. Ramirez and P. Teitelbaum. 6-Hydroxydopamine and anticholinergic drugs. *Science* **202:** 1216–1217, 1978.
28. Schallert, T., M. De Ryck, I. Q. Whishaw, V. D. Ramirez and P. Teitelbaum. Excessive bracing reactions and their control by atropine and L-Dopa in an animal analog of Parkinsonism. *Expl Neurol.* **64:** 33–43, 1979.
29. Schiller, G. D. Reduced binding of ^{3}H-Quinuclidinyl benzilate associated with chronically low acetylcholinesterase activity. *Life Sci.* **24:** 1149–1164, 1979.
30. Schoenfeld, R. and N. Uretsky. Altered response to apomorphine in 6-hydroxydopamine-treated rats. *Eur. J. Pharmac.* **19:** 115–118, 1972.
31. Stricker, E. M. and M. J. Zigmond. Recovery of function after damage to central catecholamine-containing neurons: A neurochemical model for the lateral hypothalamic syndrome. In: *Progress in Psychobiology and Physiological Psychology, Vol. 6*, edited by J. M. Sprague and A. N. Epstein. New York: Academic Press, 1976.
32. Tarsy, D. and Baldessarini. Behavioral supersensitivity to apomorphine following chronic treatment with drugs which interfere with synaptic function of catecholamines. *Neuropharmacology* **13:** 927–940, 1974.
33. Teitelbaum, P. and A. N. Epstein. The lateral hypothalamic syndrome: Recovery of feeding and drinking after lateral hypothalamic lesions. *Psychol. Rev.* **69:** 74–90, 1962.
34. Tye, N. C., L. Horsman, F. L. Wright and I. A. Pullar. Differential enhancement of locomotor activity by dopamine agonists following chronic neuroleptic treatment: Animal model of tardive dyskinesia. *Eur. J. Pharmac.* **55:** 103–107, 1979.
35. Uchida, S., K. Takeyasu, T. Matsuda and H. Yoshida. Changes in muscarinic acetylcholine receptors of mice by chronic administration of DFP and papaveine. *Life Sci.* **24:** 1805–1812, 1979.
36. Ungerstedt, U. Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigrostriatal dopamine system. *Acta physiol. scand., Suppl.* **367:** 95–122, 1971.
37. Ungerstedt, U. Brain dopamine neurons and behavior. In: *The Neurosciences Third Study Program*, edited by F. O. Schmitt and F. G. Worden. Cambridge: MIT Press, 1974.
38. Whishaw, I. Q., T. E. Robinson and T. Schallert. Intraventricular anticholinergics do not block cholinergic hippocampal RSA or neocortical desynchronization in the rabbit or rat. *Pharmac. Biochem. Behav.* **5:** 275–283, 1976.
39. Whishaw, I. Q., T. E. Robinson, T. Schallert, M. De Ryck and V. D. Ramirez. Electrical activity of the hippocampus and neocortex in rats depleted of brain dopamine and norepinephrine: Relations to behavior and effects of atropine. *Exp. Neurol.* **62:** 748–767, 1978.
40. Yamamura, H. I. and S. H. Snyder. Muscarinic cholinergic binding in rat brain. *Proc. natn. Acad. Sci. U.S.A.* **71:** 1725–1729, 1974.