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The Failure of Alpha-Methyl-Para-Tyrosine to Produce Supersensitivty in 6-OHDA Lesioned Rats

GREGORY L. WILLIS[1] AND GEORGE SINGER

Department of Psychology, LaTrobe University, Bundoora, Australia 3083

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WILLIS, G. L. AND G. SINGER. *The failure of alpha-methyl-para-tyrosine to produce supersensitivity in 6-OHDA lesioned rats.* PHARMAC. BIOCHEM. BEHAV. 12(3) 371–376, 1980.—When rats with lateral hypothalamic lesions are pretreated with alpha-methyl-p-tyrosine (AMPT) serious behavioral impairment can be avoided. It has been suggested that the rapid behavioral recovery is due to an AMPT-induced supersensitivity of catecholamine receptors. This experiment attempted to test an alternative hypothesis which suggests that the reduction of monoamine accumulation by AMPT could also account for the shortened period of reduced food and water intake. Eight $\mu g/\mu l$ concentrations of 6-hydroxydopamine (6-OHDA) were injected into the lateral hypothalamus (LH) in order to achieve a more specific depletion of catecholamines and to produce monoamine accumulation. The behavioral deficits displayed in animals pretreated with AMPT were not significantly different from those occurring in rats pretreated with saline. Although histochemical evaluation revealed that the levels of monoamine accumulation were unaffected by the AMPT pretreatment, there was a direct relationship between the severity of behavioral deficit and the volume and intensity of monoamine accumulation. Depletion of the nigro-striatal dopamine system was in no way related to the severity of behavioral impairment. From this work it seems unlikely that post-synaptic supersensitivity to catecholamines can account for the return of behavior after hypothalamic damage.

Lateral hypothalamus Nigro-striatal dopamine Caudate nucleus Post-synaptic supersensitivity
Monoamine accumulation Eating and drinking deficits

WHEN depletion of forebrain catecholamines occurs the eating and drinking behavior of 6-OHDA treated animals is greatly reduced [10, 17, 20]. Although damage to forebrain catecholamines is permanent [3, 7, 8], the ability to regulate consummatory behavior gradually returns. Behavioral recovery is believed to be mediated by the development of supersensitivity in monoamine receptors [11,17].

An alternative to the depletion-supersensitivity hypothesis suggests that it is the buildup of monoamines in severed axons which causes the observed deficits in behavior and that the reduction of accumulation over time results in behavioral recovery [13]. If monoamine accumulation is responsible for producing these deficits than alpha-methyl-p-tyrosine could be used in conjunction with 6-hydroxydopamine to further test this hypothesis. Pretreatment with AMPT would reduce the catecholamine content of central adrenergic nerves before the administration of 6-OHDA and thereby decrease the resulting accumulation of monoamines. Consequently, the severe impairments in consummatory behavior which usually result from such trauma should be avoided. Decreases in the severity of behavioral deficits have been reported when AMPT preceded the placement of radiofrequency lesions in the lateral hypothalamus [5]. Since only biochemical changes were assessed using a fluorometric assay technique, it was impossible to evaluate degree of monoamine accumulation. The results from this study can be interpreted in two ways: the AMPT treatment may have led to supersensitivity of monoamine receptors or alternatively, the treatment may have resulted in less monoamine accumulation. Fluorescent histochemical examination should make it possible to differentiate between these two hypotheses. In the present experiment consummatory behavior and body temperature were observed following injections of 6-OHDA with and without AMPT pretreatment [5]. Monoamine accumulation and depletion were assessed using fluorescent histochemical analysis.

METHOD

Animals

Thirty-three male Wistar-derived rats ranging in weight from 325 to 425 g at the time of surgery were used. Animals were housed individually in wire mesh cages (20×23×40 cm) in a room with a 12 hr light/dark cycle. The temperature of this room was maintained constantly at 72° ± 2°F. Animals were allowed ad lib access to Clark King crushed food and tap water. Food was made available in dishes specially designed to minimize spillage ($\leq$1 g/day) while water could be obtained from calibrated cylinders attached to the front of each cage (leakage $\leq$1 ml/day). Rectal temperature was measured daily with a SMEC-10 electronic thermometer.

Procedure

Surgery. Chronically indwelling cannulae were bilaterally

[1]Reprint requests to the first author at Monash Department of Psychological Medicine, Prince Henry's Hospital, Melbourne, Australia.

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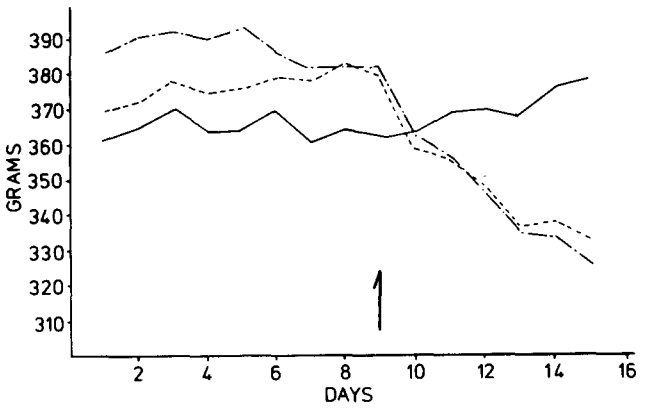

FIG. 1. The effects of intracerebral 6-OHDA on body weight when animals were pretreated with AMPT (— - —) or saline (----). Control animals pretreated with AMPT before intracerebral saline are represented by the solid black line. As previously reported a slight decrease in body weight occurs on days seven through nine in response to AMPT injection [5]. Arrow indicates day of intracerebral injection.

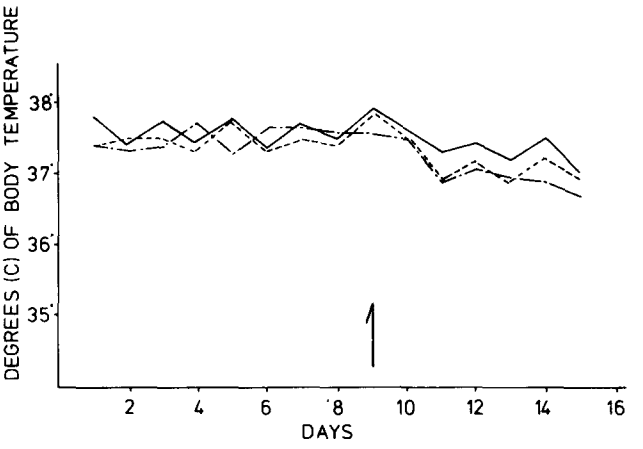

FIG. 2. The effects of intracerebral 6-OHDA on body temperature when animals were pretreated with AMPT (— - —) or saline (----). Control animals pretreated with AMPT before intracerebral saline are represented by the solid black line. Arrow indicates day of intracerebral injection.

implanted in animals anesthetized with a chloral hydrate/nembutal mixture administered into the peritoneum. Implants were aimed at the posterior lateral hypothalamus (PLH coordinates, A–P=−0.8 mm, L=±1.9 mm, D=−8.0 mm). All coordinates are relative to bregma and in the plane of Pelligrino and Cushman (1967). Animals were allowed at least 10 days to recover before the experiment commenced.

Drug solutions. Alpha-methyl-para-tyrosine was obtained from Sigma Chemicals and used for intraperitoneal injections. Solutions were prepared in distilled water in a concentration of 100 mg/ml and administered in a volume of 0.5 ml, in line with previous reports [5]. IP control injections were made with 0.5 ml of isotonic sodium chloride solutions.

6-Hydroxydopamine hydrobromide (6-OHDA-SIGMA) was mixed in 8 μg/μl solutions for intracranial injection. The drug was dissolved in distilled water containing 2 mg/ml of ascorbic acid in order to prevent rapid oxidation. Isotonicity of this solution was achieved by adding the appropriate amount of sodium chloride. Placebo injections were made with isotonic saline-ascorbic acid solutions. The injection procedure was the same as that described previously [18].

Behavioral measurements. Body weight, body temperature and food and water intake were monitored for at least 5 days preceding the intraperitoneal injection of AMPT or placebo. This was done in order to obtain baseline conditions for each animal. All measures were performed approximately 2 hr after the onset of the light cycle.

Three days before the intracranial administration of 6-OHDA two groups of 11 rats were injected IP with 100 mg/kg of AMPT in a volume of 0.5 ml. Each 100 mg/kg dose was administered twice daily for 2 days and then once only on the third day (10 a.m. and 5 p.m. on Day 1 and 2; 10 a.m. only on Day 3). The remaining 11 rats served as controls and were injected with 0.5 ml of isotonic saline on the same schedule.

On the day following the last day of drug administration intracranial injections of 6-OHDA were made. One group of 11 animals pretreated with AMPT and the control group pretreated with saline were injected as described previously [18] with 2 μl of 8 μg/μl of 6-OHDA. The 11 remaining AMPT treated animals were injected with 2 μl of isotonic saline.

If animals exhibited a complete cessation in eating and drinking behavior until death they were classified as moribund. If only a temporary decrease in consummatory behavior occurred animals were classified as transient aphagics. The remaining unaffected animals were described as displaying no change in behavior. The behavioral criteria for these 3 classifications have been described previously [13].

All measures were continued for 6 days after these intracerebral injections. At the end of this period all animals were decapitated, their brains extracted and prepared for histochemical examination using a modified version of the Falck-Hillarp method [18].

RESULTS

As illustrated in Fig. 1, a considerable decrease in body weight for both 6-OHDA injected groups occurred whether they were pretreated with saline or AMPT. To analyse these postinjection changes in body weight and other parameters measured, the method of planned comparisons was used [19]. Both of the aforesaid 6-OHDA injected groups dropped significantly compared to the animals injected intracranially with placebo at three days post 6-OHDA ($F=7.85$; $\nu_1=1$, $\nu_2=60$, $p<0.01$). Although both groups dropped significantly compared to control, they did not differ significantly from each other ($F=0.004$, $\nu_1=1$, $\nu_2=60$, $p<0.01$). Even at six days after the injection of 6-OHDA the body weight of both saline and AMPT pretreated groups were not significantly different ($F=0.07$, $\nu_1=1$, $\nu_2=20$, $p<0.01$).

Figures 2 to 4 illustrate the changes in body temperature and food and water intake occurring in saline and AMPT pretreated 6-OHDA injected animals. The body temperature of these animals did not differ significantly from controls at 3 days postinjection ($F=0.04$, $\nu_1=1$, $\nu_2=60$, $p<0.01$). The body temperatures of both saline and AMPT pretreated groups did not differ significantly from each other at either 3 days

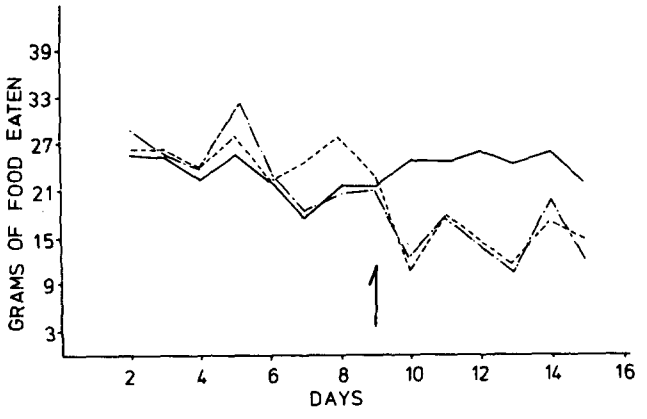

FIG. 3. The effects of intracerebral 6-OHDA on food intake when animals were pretreated with AMPT (- — --) or saline (----). Control animals pretreated with AMPT before intracerebral saline are represented by the solid black line. Arrow indicates day of intracerebral injection.

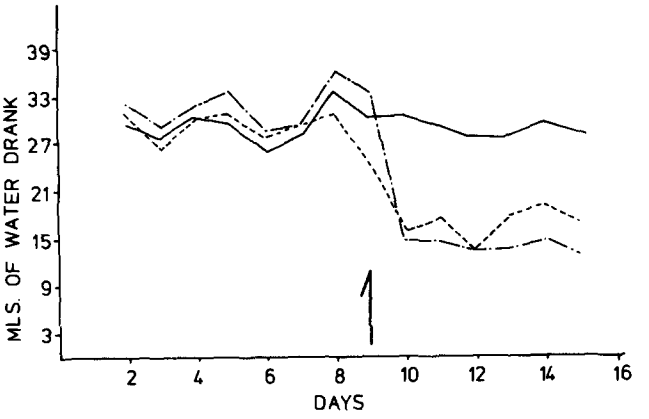

FIG. 4. The effects of intracerebral 6-OHDA on water intake when animals were pretreated with AMPT (- — -) or saline (----). Control animals pretreated with AMPT before intracerebral saline are represented by the solid black line. Arrow indicates a day of intracerebral injection.

TABLE 1

NO CHANGE, TRANSIENT AND MORIBUND ANIMALS SHOWING HYPOTHALAMIC AND STRIATAL DEPLETION AFTER 6-OHDA

	Amount of depletion		
	No depletion observed	Slight to moderate depletion	Severe depletion
Severity of aphagia and adipsia			
No change			
Hypothalamic	10	0	0
Striatal	8	2	0
Transient			
Hypothalamic	8	3	1
Striatal	8	4	3
Moribund			
Hypothalamic	1	5	2
Striatal	1	6	1

Assessment of 3 animals was impossible due to tissue damage during processing.

(F=0.07, $\nu_1=1$, $\nu_2=60$, $p<0.01$) or 6 days (F=2.91, $\nu_1=1$, $\nu_2=20$, $p<0.01$) post 6-OHDA.

Food and water intake decreased significantly 3 days after intracranial injection in both saline and AMPT pretreated groups (food intake: F=11.8; water intake: F=62.9, $\nu_1=1$, $\nu_2=60$, $p<0.01$ for both cases). However decreases in food and water intake for both groups were not significantly different from each other at 3 (respectively: F=0.05, F=0.010 $\nu_1=1$, $\nu_2=60$, $p<0.01$ for both cases) or 6 days (respectively: F=0.36, F=0.007; $\nu_1=1$, $\nu_2=20$, $p<0.01$ for both cases) after intracranial injection of 6-OHDA.

After histochemical evaluation depletion of noradrenergic (see Table 1; Fig. 5) and dopaminergic structures (see Table 1; Fig. 6) occurred. However, depletion of either dopamine or noradrenaline in saline or AMPT pretreated animals differed little between the two groups (see Table 2; Fig. 6). Complete denervation of the hypothalamus or corpus striatum was never seen. Large areas of normal innervation always remained in animals classified as either transient or moribund. In addition, the striatum and hypothalamus of many animals classified as transient aphagics appeared normally innervated (see Table 1; Figs. 5 and 6). Monoamine accumulation was also unaffected by pretreatment with AMPT. Peripheral AMPT did not significantly reduce the volume of accumulation occurring after the injection of 6-OHDA (F=0.14, $\nu_1=1$, $\nu_2=18$, $p<0.01$, see Table 4).

When animals were classified as moribund, transient or showing no change, a three-way analysis of variance revealed that the volume of accumulation which occurred after intracranial injection correlated significantly with the degree of behavioral impairment (F=18.2, $\nu_1=2$, $\nu_2=27$, $p<0.01$; see Fig. 7; Table 4).

The intensity of accumulation of all moribund animals was classified as being very intense whilest most transient aphagics had accumulation of a slight to moderate intensity. Animals showing no change in consummatory behavior also had areas of accumulation classified as slight to moderate in intensity (see Table 3).

DISCUSSION

Contrary to previous reports [5] pretreatment with AMPT does not decrease the severity of deficits in consummatory behavior after catecholamine destruction. The discrepancies between previous work [5,6] and our results may be due to the different methods employed to obtain destruction to catecholamine-containing fibres in the hypothalamus. While the experiments in question used radiofrequency lesions this study obtained a high degree of catecholamine-specific destruction with injections of 6-OHDA [18]. Consequently, the large areas of non-specific damage which radiofrequency lesions produce were not seen. It is possible that destruction to systems other than those containing catecholamines would be responsible for the resulting behavioral impairment, although unlikely in consideration of the placement of the lesions [5,6].

Although the results from this study cannot clearly attri-

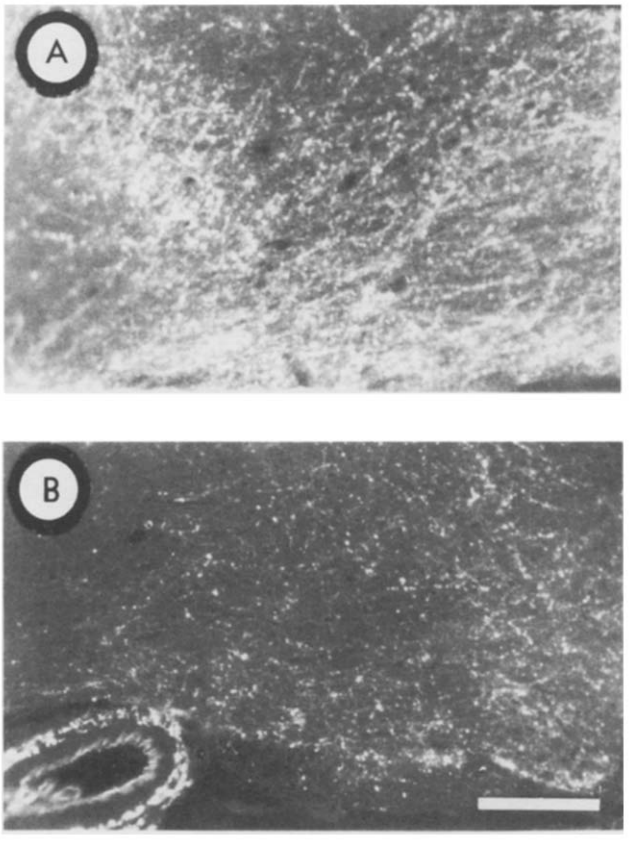

FIG. 5. Fluorescence micrographs of perifornical-lateral hypothalamic innervation in saline and AMPT pretreated 6-OHDA injected animals. Although depletion sometimes occurred in both of these drug groups (B), many of the animals showed normal hypothalamic innervation (A). Both depleted (B) and normally innervated hypothalamus (A) was seen in transient and moribund animals. (Calibration bar=100 μ).

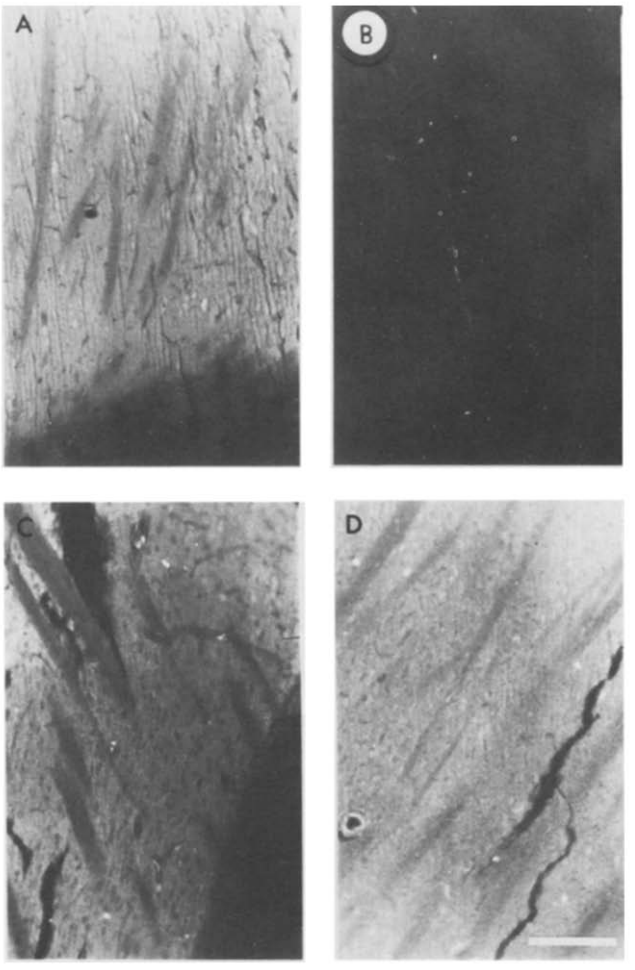

FIG. 6. Fluorescence micrographs of corpus striatal innervation in saline and AMPT pretreated 6-OHDA injected rats. Normal striatal fluorescence was often observed in placebo (D) and AMPT pretreatment groups (A,C). However, depletion of this structure, as in (B), also occurred in both drug treatment groups. The most severely impaired animals did not show the greatest striatal depletion. Transient aphagics sometimes showed more pronounced depletion (B) than those classified as moribund (C). (Calibration bar=100 μ).

bute behavioral impairment to the production of monoamine accumulation, our interpretation of these results sheds doubt upon the previous interpretation that supersensitivity of residual catecholamine neurons is responsible for the return of behavior after lateral hypothalamic damage. In previous work the administration of AMPT preceding catecholamine destruction was performed in order to produce supersensitivity before electrolytic trauma occurred. This, in turn, was reported to facilitate recovery from lesion-imposed deficits. By substituting 6-OHDA injections for radiofrequency lesions in our experiment, a more specific involvement of catecholamines was accomplished [18]. Under these conditions further evidence for the supersensitivity hypothesis would be obtained if AMPT treatment initiated recovery of 6-OHDA induced behavioral impairment. However, in view of our attempt to maximize the probability of catecholamine involvement and failure to initiate behavioral recovery with AMPT, the interpetation that supersensitivity of catecholamine nerves facilitates behavioral recovery is an unlikely possibility.

In addition to failing to produce these previously reported behavioral effects due to an AMPT and 6-OHDA interaction, neurochemical parameters were also unaffected on a long-term basis. Our attempt to decrease the area and intensity of accumulation by pretreatment with AMPT were not successful and were probably due to the depleting properties of AMPT. The reduction of catecholamines seen after employing this regimen of injections is typically of only a short duration and it affects only a limited portion of the existing catecholamine neurons [1,14]. The amount of catecholamine depletion and volumes of accumulation observed in both saline and AMPT pretreated groups 7 days after AMPT injections were similar (Fig. 6; Table 2). Examination of rats receiving only peripheral AMPT further supports this in that they showed normal hypothalamic and striatal fluorescence 7 days after injection (Figs. 5 and 6).

However futile the attempt to decrease the intensity and volume of monoamine accumulation with AMPT, this experiment lends further support to our earlier findings [13]. That is, the intensity of monoamine accumulation as well as the volume of tissue over which it extends, gives a better indication of the severity of behavioral deficits than does depletion

TABLE 2

THE NUMBER OF ANIMALS SHOWING STRIATAL AND HYPOTHALAMIC DEPLETION AFTER AMPT OR SALINE PRETREATMENT WHICH PRECEDED INTRAHYPOTHALAMIC INJECTIONS OF 6-OHDA. THE VOLUME OF TISSUE OVER WHICH ACCUMULATION EXTENDS FOR EACH ANIMAL WITHIN THE TWO DRUG TREATMENT GROUPS IS ALSO EXPRESSED

	AMPT pretreated			Saline pretreated		
	Striatal depletion	Hypothalamic depletion	Volumes of accumulation ($\times 10^6 \mu^3$)	Striatal depletion	Hypothalamic depletion	Volumes of accumulation ($\times 10^6 \mu^3$)
Slight depletion	5	2	798.0 0.00025 0.00025	1	2	219.0 126.0 54.6
Moderate depletion	2	4	976.5 0.00025 0.0005	7	4	0.00025 300.0 54.00025
Severe depletion	0	0	630.0 432.0 90.0	0	0	2,360.0 339.0 108.0
No depletion observed	3	4	1,050.0	2	4	910.0

In some cases (depletion, n=3; accumulation, n=2) it was not possible to assess biochemical changes due to tissue damage during processing.

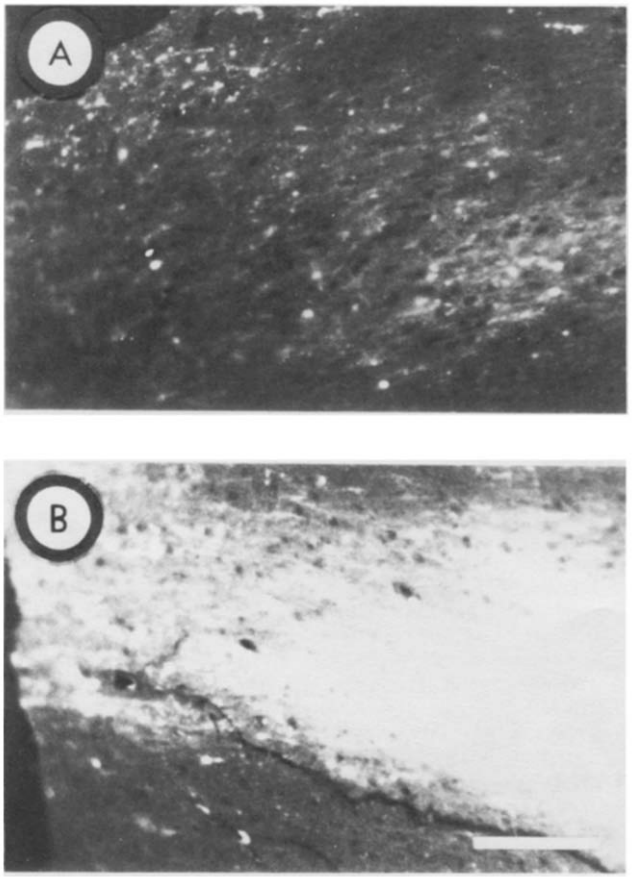

FIG. 7. Fluorescence micrographs of monoamine accumulation in saline and AMPT pretreated 6-OHDA injected rats. The most severely affected animals always showed considerably more accumulation of a brighter intensity (B) than animals displaying only transient aphagia and adipsia (A). (Calibration bar=100 μ).

TABLE 3

BRIGHTNESS INTENSITY RATINGS FOR AREAS OF MONOAMINE ACCUMULATION IN MORIBUND, TRANSIENT AND THOSE ANIMALS SHOWING NO CHANGE IN CONSUMMATORY BEHAVIOR

	Intensity of accumulation		
	No accumulation	Slightly to moderately intense	Very intense
Severity of aphagia and adipsia			
No change	4	6	0
Transient	1	11	0
Moribund	0	0	8

Assessment of 3 animals was impossible due to tissue damage during processing.

TABLE 4

VOLUME OF TISSUE ($\times 10^6 \mu^3$) OVER WHICH ACCUMULATION EXTENDED IN MORIBUND, TRANSIENT AND ANIMALS SHOWING NO CHANGE IN CONSUMMATORY BEHAVIOR

Moribund	Transient	No change
798.0	0.00025	0.00025
976.5	0.00025	0.0
630.0	0.00050	0.0
432.0	90.0	0.0
1,050.0	219.0	96.0
2,360.0	126.0	36.0
339.0	546.0	48.0
910.0	0.00025	0.0
	300.0	0.0
	54.00025	81.0
	108.0	

Evaluation of 4 brains was impossible due to damage during procesing.

of catecholamine-containing structures (see Fig. 7). It was often observed that animals showing the most severe depletion of forebrain catecholamines exhibited only transient behavioral impairment when compared to those animals exhibiting total cessation of eating and drinking until death (see Fig. 6; Table 1). Further to this, the finding that moribund animals die 6 days after the injection of 6-OHDA when much of the corpus striatum remains intact is hard to explain using the catecholamine receptor supersensitivity model [5,17].

In an attempt to elucidate the mechanism by which the observed build up of monoamines in severed axons may be producing its inhibitory effect upon behavior, the effects of endogenous catecholamine release on body temperature deserves consideration. As previously reported, long term changes in the ability to regulate body temperature after catecholamine destruction do not typically occur. Only temporary reductions of body temperature occurring 1–3 hr after intracerebral 6-OHDA are reported. The immediate displacement of catecholamines from storage vesicles is suggested to be responsible for producing this immediate decrease in body temperature [9,12]. It is interesting to note, however, that a drastic decrease in body temperature can also occur when 6-OHDA injections are placed in an area which has relatively few fluorescent catecholamine-containing nerve terminals, and yet may be traversed by fibres of passage which contain catecholamines [13, 17, 18]. The uptake and transport of 6-OHDA to nerve terminals where the displacement of thermoactive catecholamines can occur, would take far more time than that which would be required to initiate the catecholamine-induced drop in body temperature [9, 12, 13]. Alternatively, in view of both the relatively short period of time required for the buildup of fluorescent monoamines to develop and the relationship between the amount of buildup and degree of thermoregulatory deficit [13], it is reasonable to suggest that thermoactive neurotransmitters are released endogenously from axons severed by 6-OHDA rather than nerve terminals distant from the site of injection. In addition, when the severity of 6-OHDA induced hypothermia clearly predicts the severity of aphagia and adipsia which will occur many days later [13], it is possible that release of thermoactive catecholamines from axotomized nerves may also cause long term behavioral impairment which is common after electrolytic lesions or 6-OHDA. The suggestion that axons severed by 6-OHDA can release neuroactive substances is not a novel one. It has been shown to occur in degenerating peripheral nerves having a pronounced effect upon physiological processes [16]. It should be clearly understood that this is only a suggested mode of action for accumulation. Until further experimentation is carried out in an attempt to discover what behavior-altering properties the accumulation of monoamines may possess, the role remains speculative. It is, however, important that the individual effects of catecholamine depletion and accumulation be clearly defined if the participation of the central catecholamine systems in behavior is to be more clearly understood.

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