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Effect of 6-Hydroxydopamine-Induced Lesions of A10 Dopaminergic Neurons on Aggressive Behavior in Rats[1]

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PUCIŁOWSKI, O., W. KOSTOWSKI, A. BIDZIŃSKI AND M. HAUPTMANN. *Effect of 6-hydroxydopamine-induced lesions of A10 dopaminergic neurons on aggressive behavior in rats.* PHARMAC. BIOCHEM. BEHAV. **16**(4) 547–551, 1982.—The present study evaluated the effects of microinjections of 6-hydroxydopamine into the ventral mesencephalic tegmental area (nucleus A10) on aggressive behavior in rats. This treatment resulted in a reduction in foot-shock-induced fighting but failed to influence muricide (mouse-killing) behavior in chronically isolated rats. The general activity of animals tested in the open field was significantly increased two weeks after lesions. These behavioral changes were accompanied by a significant depletion of forebrain dopamine, with no difference between lesioned and sham-lesioned rats in norepinephrine and 5-hydroxyindole acetic acid levels.

Aggressive behavior A10 region Brain dopamine Mesocorticolimbic system Muricide behavior

THE function of the mesocorticolimbic dopaminergic system has been recently the subject of much research interest. The cell bodies of dopamine (DA) containing neurons which form this system lie within the ventral mesencephalic tegmentum (VMT) (nucleus A10, according to Dahlström and Fuxe [4]). Their axonal fibres project to limbic and cortical forebrain areas, including nucleus accumbens, tuberculum olfactorium, medial frontal and sulcal cortical areas [4,18]). The physiological role of this system is still not well understood. There if evidence that DA neurons reaching limbic and cortical areas are involved in locomotion [6, 13, 17, 20], self-stimulation [7,22], acquisition of avoidance response [6, 7, 17] and probably can be related to antipsychotic action of neuroleptic drugs [11]. Injection of 6-hydroxydopamine (6-OHDA), the neurotoxin which causes a selective degeneration of catecholaminergic neurons [16], has been found to produce behavioral changes such as locomotor hyperactivity and deficits in the passive avoidance learning [6,13]. Moreover, the hyperactivity after lesioning in the VMT was positively correlated with a decrease in DA concentrations in the cortical and limbic areas [6].

Some observations suggest that DA mesocorticolimbic system is involved in mechanisms of aggressive behavior. Electrical stimulation of the VMT elicited biting attack in cats [3,5]. However, lesioning of this region may also produce similar effect. Nakamura and Nakamura found that direct administration of 6-OHDA into the A10 area caused aggressive behavior, including "irritable" aggression and mouse-killing behavior, in rats. The effect was however, accompanied by a decrease in norepinephrine (NE) content in the cortex and fall in 5-hydroxyindole acetic acid (5-HIAA) concentrations in numerous cerebral regions, but there was no change in the DA content in the mesolimbic olfactory tubercle [19].

The role of mesocorticolimbic DA system in aggressive behavior remains therefore unclear and more information is needed. The present study was performed in order to re-evaluate the influence of 6-OHDA-induced lesions of A10 area on aggressive behavior in rats. Aggressiveness (of the affective type) can be related to the increase in activity and reactivity of experimental animals so we also planned to investigate the general behavior of rats in the open field.

METHOD

Animals

Male Wistar rats weighing 180–190 g at the beginning of experiment were housed under normal light conditions, at 20±2°C, 60–65% humidity, with ad lib access to standard granulated diet and water. Behavioral testing was always done between 10 and 12 a.m. Details of housing and experimental design are described in Table 1.

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TABLE 1
EXPERIMENTAL PROCEDURE AND GROUPS

Group 1		
	Lesion or sham-lesion ↓	Evaluation of muricide ↓
Isolation 6 weeks	Continued isolation 3 weeks	Histology
Group 2		
Lesion or sham-lesion ↓		Evaluation of muricide ↓
	Isolation 6 weeks	Histology
Group 3		
	Open field ↓ test	
Lesion or sham-lesion ↓	↓Shock-induced ↓↓fighting ↓↓	Shock-induced ↓ fighting ↓
14 days	7 days	Histology Biochemistry

Lesions of the VMT

For selective lesions of the DA neurons within the VMT (A10) the intracerebral injections of 6-OHDA were performed under chloral hydrate anesthesia (400 mg/kg IP). In order to protect the NE neurons all animals, including controls, were pretreated with desipramine, (desipramine hydrochloride, Ciba-Geigy, Switzerland) 25 mg/kg IP 45 min before stereotaxic injection. Animals were fixed in a Stoelting stereotaxic instrument with the incisors base angled 5° downward to the horizontal. The injection needles of Hamilton microsyringe were positioned bilaterally in the A10 cell group according to coordinates: A=2.0 mm, L=0.6 mm and H=−2.8 mm [14]. Rats were injected with freshly prepared 6-OHDA (ICN—K & K Laboratories, Inc., USA) solution (4 μg in 2 μl) on each side with 1 μl/min rate. Control sham-lesioned rats received an equal volume of the solvent (0.2 mg/ml ascorbic acid in 0.9% NaCl).

Evaluation of Aggressive Behavior

Mouse-killing (muricide) behavior. Details of the procedure were described elsewhere [15]. Briefly, animals were housed in single cages and divided into two experimental groups. The first group was tested every other day for 6 weeks to assess fixed behavioral pattern (killer or non-killer), then lesioned (or sham-lesioned) and for the following 3 weeks retested every other day to check the effect of lesion (see Table 1, group 1). The second group was first lesioned (or sham-lesioned), then isolated and tested every other day for 6 weeks, then the effect of isolation on lesioned animals was determined (Table 1, group 2). Swiss albino male mice were used as the prey. It should be mentioned that although rats were tested every other day for muricide behavior only the final result, i.e., scored on the last day of isolation, was analyzed. Lesioned groups were compared with sham-lesioned using the Fisher's exact test.

Shock-induced fighting. Male Wistar rats were housed 5–6 to a cage and tested for aggression 12–14 days after surgery (Table 1, group 3). Animals were retested one week later (3 weeks after surgery). Rats were paired lesioned-lesioned or sham-lesioned-sham-lesioned (never from the same home-cage). Each pair was stimulated for 5 min with DC square wave current (3 mA, 0.1 sec, 2 Hz) applied to the grid floor. Number of aggressive postures, attacks (boxing) as well as latency of the first aggressive encounter (posture or attack) was noted. The behavioral scoring was done for the pair (not for the experimental animal) and only pairs with both animals lesioned correctly were analyzed statistically (Mann-Whitney U test, two-tailed).

General Behavior in the Open Field

All animals from experimental group 3 (see Table 1) were submitted to this procedure. The open field testing was done 2 weeks after lesioning on the day preceding shock-induced aggression test. Each rat was put in the open field 60×60 cm divided into 16 squares (12 "peripheral" and 4 "central") then the number of entries into peripheral squares, central squares, rearings and immobility time during 5 min observation was noted. Data were analyzed with the two tailed Student's *t*-test.

Biochemical Analysis and Histology

After the behavioral testing was completed rats were killed by decapitation, their brains removed and cut anterior to the hypothalamus in two parts: forebrain and brain stem. Brain stems were checked histologically for lesions placement and size after fixation in 10% Formalin and staining with hematoxillin and eosin.

Forebrains from all the animals in group 3 were assayed for DA, NE and 5-HIAA content after removing striopaldium which is known to contain high concentrations of DA originating from the cells located within the substantia nigra. The extraction and fluorimetric determinations of amines was carried out according to Haubrich and Denzer [10].

RESULTS

Location of 6-OHDA-Induced Lesions

Histological examination showed that lesions were mainly restricted to the A10 area (see Fig. 1). In some rats lesions were not accurately positioned and involved other structures such as nucleus interpeduncularis or nucleus ruber. These animals were excluded from the statistical analysis of results.

Biochemical Determinations

Rats with bilateral lesions of A10 area showed significant ($p<0.001$) depletion of DA (by 56%) in the forebrain. The levels of NE and 5-HIAA did not differ significantly in lesioned and control groups (Table 2).

Aggressive Behavior

Lesions of the A10 area failed to influence the fixed behavioral pattern of killer rats. When the animals were

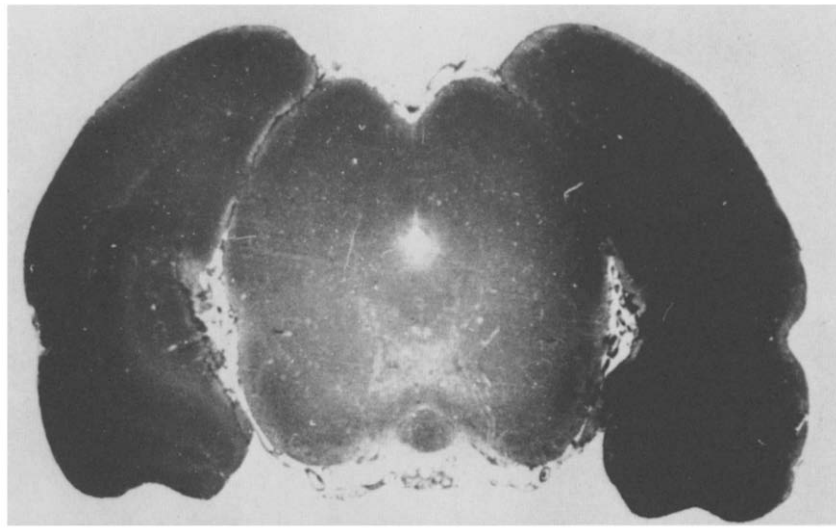

FIG. 1. Microphotograph showing a typical localization and size of 6-OHDA-induced lesion within the ventral tegmental area (10× actual size).

TABLE 2

EFFECT OF 6-OHDA MICROINJECTIONS INTO A10 REGION ON BIOGENIC AMINE FOREBRAIN* CONCENTRATIONS

	Control n=8	Operated n=14
DA	415.5 ± 41.1	181.4 ± 29.7†
NE	444.0 ± 12.8	443.0 ± 11.5 (NS)
5-HIAA	979.7 ± 83.2	900.4 ± 53.8 (NS)

Data are expressed as ng/g of tissue and represent the mean ± standard error of mean. Statistical significance in parentheses: †$p<0.001$ vs control, NS=nonsignificant. *=before the assay the corpus striatum was removed.

TABLE 3

EFFECT OF A10 LESION ON FIXED MURICIDE BEHAVIOR IN ISOLATED KILLERS

Experimental group	n	Behavioral pattern			
		Before lesion		After lesion	
		K	NK	K	NK
1. Sham-lesioned	12	4	8	4	8
A10 lesioned	19	10	9	9	10
				After 6 weeks of isolation	
2. Sham-lesioned	15			3	12
A10 lesioned	10			2	8

There were no significant differences between killer (K) and non-killer (NK) rats and no difference in groups 1 "before lesion" and "after lesion" according to the Fischer's exact test. n=number of animals.

lesioned prior to isolation they behaved similarly to sham-lesioned when tested for muricide behavior (Table 3).

The foot-shock-induced fighting was significantly reduced in rats with lesions of the A10. This effect occurred both 2 and 3 weeks after surgery (Fig. 2). Lesioned animals showed prolonged latency of attacks/postures, as well as decreased number of aggressive postures and attacks when measured during 5 min stimulation.

Behavior in the Open Field

Animals with lesions of the A10 area were significantly more active than controls during testing session (2 weeks after lesion). They moved more frequently through the peripheral and central squares, the immobility time was also shorter in this group (Fig. 3).

DISCUSSION

Brain DA is believed to play an important role in defence behavior (affective aggression) [9,21]. Infusion of this catecholamine into the lateral ventricle increases shock-induced fighting in rats [8]. Also administration of apomorphine, an agonist of dopaminergic receptors, is known to produce affective aggression in laboratory animals [9,23]. Moreover, it was shown that rats exhibiting spontaneous interspecific aggression had significantly higher DA content in the hypothalamus than non-aggressive ones. No such differences were observed between killers and non-killers [2]. Our results indicate that direct administration of 6-OHDA into the VMT (A10 cell group) decreased foot-shock-induced aggression and this was accompanied by a selective fall in DA forebrain content. This effect can be attributed to loss of dopaminergic input to some limbic and/or cortical areas involved in the mechanism of emotional behavior. Attenuation of shock-induced fighting after chemical destruction of DA cells in the A10 nucleus supports the hypothesis that DA mesocorticolimbic neurons play a facilitatory role in this type of behavior [21].

The present results differ from that obtained by Naka-

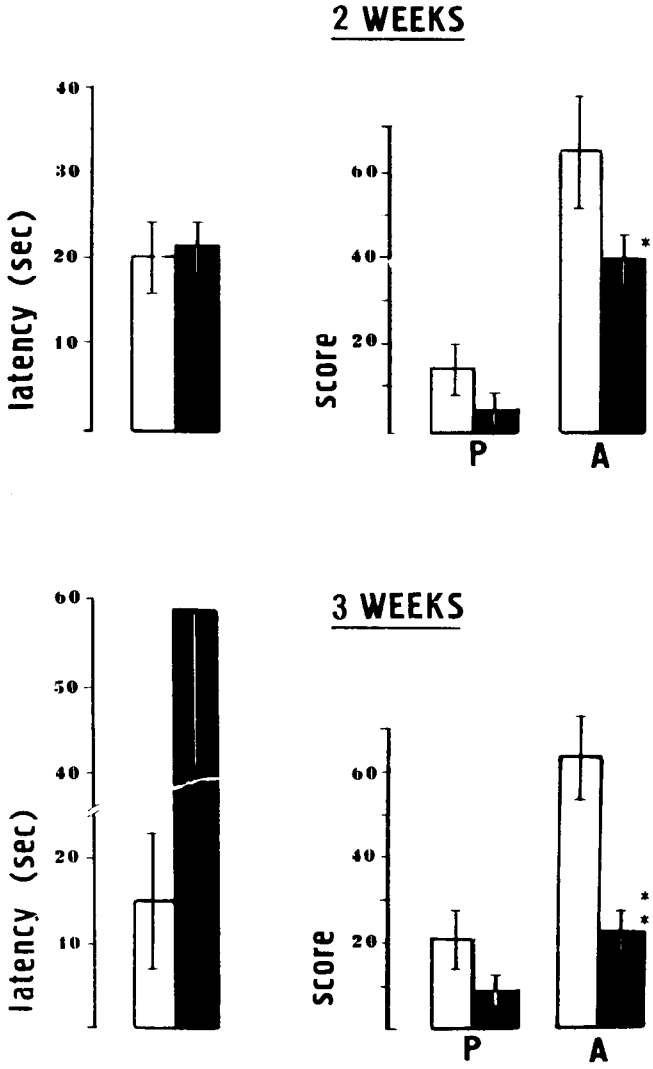

FIG. 2. Effect of lesion of A10 nucleus on shock-induced fighting in rats. White columns=sham-operated rats; Black columns=A10 lesioned animals (mean values from n=4 and 6 pairs, respectively, ±SE). Abbreviations: P=aggressive postures; A=attacks. Vertical scale—latency of the first aggressive encounter in sec and score of aggression (number of postures and attacks during 5 min observation). *=$p<0.05$ and ⁑=$p<0.02$ (Mann-Whitney U test, two-tailed).

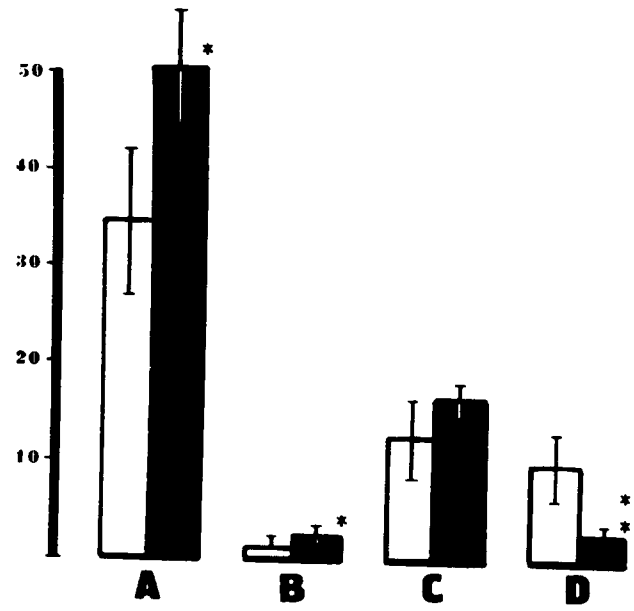

FIG. 3. Effect of lesion of A10 nucleus on behavior of rats in the open field. White columns=sham-lesioned; Black columns=A10 lesioned rats (mean values from n=8 and 14 rats, respectively, ±SE). Abbreviations: A=the number of entries into peripheral squares, B=the number of entries into central squares, C=the number of rearings, D=the number of immobility periods (each 5-second period was calculated). *=$p<0.01$ and ⁑=$p<0.001$ (Student's *t*-test, two-tailed).

mura and Nakamura who reported that A10 lesions by 6-OHDA induced signs of aggession in rats [19]. Lesioned animals showed dominant aggressive behavior if handled, and displayed mouse-killing attitude. However, lesions described by these authors failed to decrease significantly DA content in mesolimbic as well as cortical areas. It seems, therefore, that the behavioral phenomena described in their paper were due to destruction of other than DA neurons passing through the VMT, the cause being probably the high dose of neurotoxin used (10 μg bilaterally).

Much less information was gathered about the role of DA in predatory aggression. Experiments employing pharmacological methods (administration of dopaminergic drugs such as N-n-propyl-norapomorphine, amphetamine, L-DOPA) seems to suggest that DA plays rather inhibitory role in this type of behavior [1,21]. In our study destruction of DA neurons in the VMT did not influence the fixed behavioral pattern of isolated rats. Table 3 shows only final results, yet it should be mentioned that we did not observe any change in behavioral pattern of killers and non-killers throughout the experiment. Our results seem to indicate that A10 dopaminergic neurons are not directly involved in the regulation of isolation-induced muricidal behavior. We found recently that bilateral lesion of the ventral noradrenergic bundle (VB) decreased shock-induced fighting while it did not change muricidal behavior in isolated killers [15]. Fibres of this system and DA mesocorticolimbic system pass together through the medial forebrain bundle and reach various limbic structures including hypothalamus [4,18]. Taking together these results with the ones obtained presently, we conclude that destruction of either noradrenergic VB or dopaminergic mesocorticolimbic system failed to influence muricidal behavior while substantially suppressed affective aggression. Interestingly, when both NE and DA neurons of the medial forebrain bundle terminating in the lateral hypothalamus are destroyed, this treatment results in the impairment of predatory aggression (frog-killing) [12]. This suggests that physiological balance between DA and NE rather than a single transmitter is of importance in this type of aggression.

When tested two weeks after surgery animals with lesions of the A10 region were hyperactive in the open field. This is compatible with results of some other authors who have also observed hyperactivity after destruction of the VMT area. On this basis the inhibitory role of the A10 area in locomotion have been suggested [6,17]. On the other hand drugs that activate DA neurons or receptors e.g., amphetamine and

apomorphine, are known to produce locomotor excitation [13,20]. However, in animals with lesion in the VMT these drugs paradoxically reduce hyperactivity produced by this lesion [17]. Interpretation of these data is presently rather difficult, yet it should be remembered that A10 system is not functionally and anatomically homogenous. For instance, it was shown that locomotion is dependent mainly on activity of "mesocortical" part of A10 dopaminergic system, i.e., on neurons that reach frontal cortical areas. Destruction of A10 region leads to decreased ^{3}H-DA uptake only in frontal cortex but not in the nucleus accumbens or tuberculum olfactorium [24]. Very interesting observation was reported by Thierry *et al.* who studied the effect of foot-shock stress on DA metabolism in discrete brain regions. DA levels in frontal cortical areas of stressed rats fell by 60%, however, no significant changes were observed in other structures belonging to the mesocorticolimbic system [25]. The authors conclude that only cortical portion of this system seems to be activated under stressful situation. In conclusion, the role of dopaminergic neurons forming A10 system in the regulation of locomotion appears to be very complex and requires further investigation.

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