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Reactions to Apomorphine and Spiroperidol of Rats with Striatal Lesions: The Relevance of Kind and Size of the Lesion

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WOLFARTH, S. *Reactions to apomorphine and spiroperidol of rats with striatal lesions: the relevance of kind and size of the lesion.* PHARMAC. BIOCHEM. BEHAV. 2(2) 181–186, 1974. – The stereotypogenic action of apomorphine and cataleptogenic action of spiroperidol were studied in the rats with large electrolytical or mechanical lesions of neo– and/or paleostriatum. Regardless of time interval between the surgery and the test and the extent of the electrolytical lesion the lesion of neostriatum changed the character of response of the rat to apomorphine (preferential inhibition of licking on the expense of gnawing) and potentiated it. Lesions of paleostriatum did not change the response of rats to apomorphine, whereas mechanical lesions placed on the border of neo– and paleostriatum inhibited stereotypy. The lesions in general do not affect the cataleptogenic action of spiroperidol. It is suggested that the responsibility for the development of stereotypy bears a small area on the border of neo– and paleostriatum, whereas the more rostral part of neostriatum exerts an inhibitory effect.

Striatal lesions Apomorphine stereotypy Spiroperidol and striatal lesions

MONOAMINERGIC mechanisms play an important role in the functions of the central nervous system [2]. One of the most investigated monoamine tracts is the dopaminergic nigro-neo-striatal pathway [3]. Upon stimulation the nerve endings of this pathway release dopamine in the striatum [6, 19, 22]. Dopamine inhibits the inhibitory projections of the striatum [23] and this effect is believed to cause stereotyped behaviour and locomotor stimulation.

Various forms of stereotyped behaviour might be induced either by direct stimulation of dopaminoceptive receptors in the striatum, or by systemic administration of apomorphine (APO) [15,16] and intrastriatal implantations of dopa or dopamine [11] or by indirect stimulation of these receptors by amphetamine [13, 14, 16, 21] or by intranigral injections of cholinomimetics [19,24]. The stereotyped behaviour induced by stimulation of dopaminergic neurons in the striatum is antagonized by neuroleptics of phenothiazine and butyrophenone series [15, 23, 24]. Those compounds, given at large doses, bring about catalepsy, which, in turn, is antagonized by the stimulants of the dopaminergic brain structures [23].

Amsler reported [1] that surgical removal of the striatum abolished the APO-induced stereotypy in several animal species. Similarly Fog [14] has reported that amphetamine does not produce stereotypy in rats with the striatum removed by suction. Recently, however, McKenzie [17] and Divac [9], have reported independently that large lesions of the striatum do not change the stereotypy induced by high doses of APO.

As the findings of McKenzie [17] and Divac [9] seem to contradict the general view on the role of the striatum as the target point of action of compounds producing stereotyped behaviour, the studies on the relevance of the striate body for the action of APO and spiroperidol were undertaken.

The striatum was lesioned in two ways: either electrolytically, aiming to destruct more or less equal proportion of nerve endings and receptor cells, or by mechanical cutting of the striatum, to destruct preferentially the afferent nerve fibers, particularly of the nigro-neostriatal pathway, with little damage to the cell bodies in the striatum.

METHOD

Animals and Surgery

The experiments were carried out on 168 male Wistar rats, weighing approximately 250 g at the time of surgery. The lesions were placed stereotaxically [18] under chloral hydrate (PPH POCh, Gliwice) anesthesia (400 mg/kg i.p.).

According to the kind and size of the lesion the rats were divided into following groups: electrolytical lesions of

60% of the striatum (E_I), 70% (E_{II}), 80% (E_{III}), 90% (E_{IV}), electrolytical lesions of frontal cortex and subcortical fibers (E_V), electrolytical lesions of fronto-parietal cortex and radiatio corporis callosi (E_{VI}), electrolytical lesions of globus pallidus (in 70%) (E_{VII}), and mechanical lesions of the border of paleo– and neostriatum (M_I and M_{II}) and of neostriatum (M_{III}).

Electrolytical lesions. Anodic current was passed through a platinum electrode, 0.4 mm in diameter, isolated with the exception of the 0.5 mm tip. The current was adjusted to obtain maximal destruction of the lesioned structure with possibly minimal damage to the neighboring structures. The neutral electrode was placed on the edge of the surgical wound.

In Groups E_I–E_{III} the lesioning electrode was introduced 5 times in each striatum (A = +2.0, L = 2.5, H = +2.5; A = +2.0, L = 3.5, H = +1.0, H = +2.0; A = +1.5, L = 2.5, H = +2.5; A = +1.0, L = 4.0, H = +0.75), in Group E_{IV} 7 times (A = +2.5, L = 2.5, H = +2.0; A = +2.0, L = 2.5, H = +1.5, H = +3.0; A = +2.0, L = 3.4, H = +2.0; A = +1.5, L = 2.5, H = +2.7; A = +1.5, L = 3.5, H = +0.4, H = +1.3; A = +1.0, L = 3.0, H = +3.0; A = +1.0, L = 3.0, H = +2.0). In the rats of Group E_V the lesions of the same size as in the Group E_I were placed 2.0 mm anteriorly, and in the Group E_{VI} 1.5 mm anteriorly, but otherwise similarly as in the Group E_I. In Group E_{VIII} the electrode was placed once (A = 0.0, L = 3.0, H = +1.0).

Mechanical lesions. The mechanical cuts of the striatum were accomplished by bilateral introduction of a stainless steel knife 0.15 mm thick. The knife was introduced in the sagittal plane. In Groups M_I and M_{II} the knife width was 2.5 mm and its end was placed stereotaxically [18] at A = +0.5, L = 3.0, H = +7.0. In Group M_{III} only neostriatum was destroyed, whereas in Groups M_I and M_{II} the rostral edge of globus pallidus and neighboring dorsal and lateral part of the neostriatum were destroyed.

All lesioned groups were accompanied by appropriate sham operated groups. For groups with electrolytical lesions the procedure was the same, but current was not passed through the electrodes. The sham operations in the groups with mechanical lesions consisted of introduction of the knife to the level of the lower border of the radiatio corporis callosi (A = +0.5, L = 3.0, H = +3.2 for sham operated M_I and M_{II}, and A = +1.0, L = 3.0, H = +3.2 for sham operated M_{III}).

Following the surgery the rats were kept on diet consisting of chocolate dissolved in condensed milk, to counteract adypsia and aphagia. In spite of that about 30% of lesioned animals (especially in Groups E_{III} and E_{IV}) died within 1–5 days.

Histology

After completion of pharmacological experiments the rats were killed by cervical dislocation and their brains were removed and placed for 24 hr in 10% formaldehyde solution. The degree of electrolytical lesion was assessed by the slightly modified method of Carpenter *et al.* [5]. Instead of the calculation of the percentage of destructed area on the cross section of each consecutive 25 μ thick slide, the destruction was assessed in each fourth slide 100 μ wide. At least six slides were examined. Preliminary comparisons indicated that the results do not differ from those obtained by the original method. The percentage of the destruction of each right and left striatum was calculated separately and the symmetry of the lesion was checked. The animals with lesions placed asymmetrically, in wrong position or of size different from that which was aimed to, were discarded.

In rats with mechanical lesions the surface of the lesion was not measured. A typical picture of the mechanical cut of the striatum and of sham operation is presented in Fig. 1.

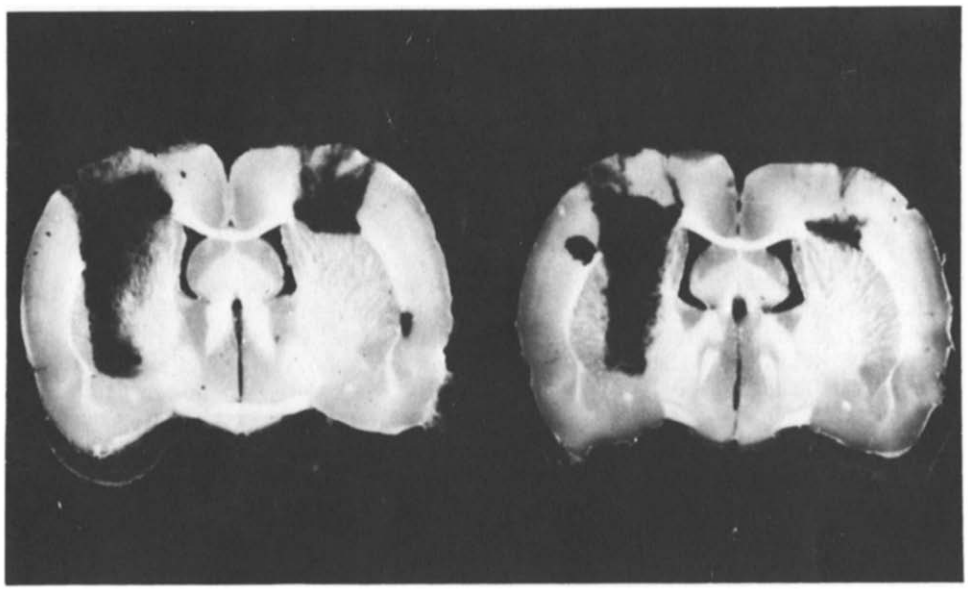

FIG. 1. Two consecutive frontal sections of the rat brain (100 μ) demonstrating the mechanical lesions. The lesions were made with a 3.5 mm wide knife on the level of the rostral edge of globus pallidus. Left side: lesion, right side: sham lesion (in the experiments reported in the text lesions or sham lesions were placed bilaterally).

Procedure

The stereotypy was tested on Day 2, 8 or 14 after the electrolytical lesion, or on Day 3 after the mechanical cut. The rats received APO (apomorphine hydrochloride, McFarlane-Smith) at a dose of 1.0 mg/kg s.c. (in one experiment a dose of 5.0 mg/kg s.c. was used) and the degrees of stereotypy was scored at 10 or 15 min intervals according to the scale of Janssen *et al.* [16] either for 1 hr, or till the disappearence of stereotyped behaviour. Two days after test with APO the cataleptogenic action of spiroperidol (Spiperone, gift of Janssen Pharmaceutica, Ltd.) was measured according to the method of Delini-Stula and Morpurgo [8]. The measurments were usually taken at 30 or 60 min intervals, in some experiments only once, 2 hr after the injection of spiroperidol. The compound was administered usually at a dose of 0.3 mg/kg i.p., in some experiments at doses 0.5 and 1.0 mg/kg.

APO was administered as solution in saline, spiroperidol – as a suspension in 3% Tween 80 solution in saline.

Statistical analysis carried out using the two-sample Wilcoxon's test, and the median results are presented.

RESULTS

Reactions to Apomorphine

Electrolytic lesions of the neostriatum produced invariably, regardless to the degree of lesion (60–90%) and the interval between the surgery and the test (2–14 days), potentiation or prolongation of the stereotyped behaviour brought about by the administration of 1 mg/kg of APO (Fig. 2). The stereotypy was also more intensive in one group with sham lesioned neostriatum (sham lesioned E_{IV}, Fig. 2D). It seems that in this group the neostriatum was damaged mechanically by placing the electrode 7 times.

The character of stereotypy changed with the extent of the damage. The larger was the lesion, the less frequent were the symptoms of licking, and the more often there appeared a sharp change from stereotyped sniffing to intensive gnawing. In rats with neostriatum destroyed in 90% the licking did not appear at all. In the neostriatum-lesioned groups a second difference in the course of stereotypy could also be observed: the stereotypy recurred several times after the first disappearance of the symptoms, whereas in intact or sham operated controls the symptoms of stereotypy after reaching the maximum declined progressively. This recurrent stereotypy was mostly expressed in the groups with large lesions. In the group with 90% destruction of the neostriatum (E_{IV}) the period of stereotypy was followed by the period of elevated explorative activity, lasting for 20–30 min i.e. present 90–120 min after APO administration. This was never observed in intact or sham operated controls.

The cuts on the level of the rostral edge of globus pallidus (Groups M_I and M_{II}), inhibited the stereotypy produced by 1 mg/kg of APO (Fig. 3A), although did not affect the stereotypy caused by a higher dose (5 mg/kg of APO, Fig. 3B). The more anterior cuts (Groups M_{III}), destroying only the neostriatum, increased the response to APO (Fig. 3C) similarly as large electrolytical lesions did (Fig. 2), but did not produce a change of the character of the stereotypy.

Specific lesions of globus pallidus (Group $E_{V\,III}$) did not affect the stereotypy (Fig. 4A), whereas the lesions of frontal cortex and subcortical fibers (Group E_V) inhibited the abnormal behaviour (Fig. 4B). Slightly caudal lesions (Groups $E_{V\,I}$) moderately elevated the stereotypy (Fig. 4C).

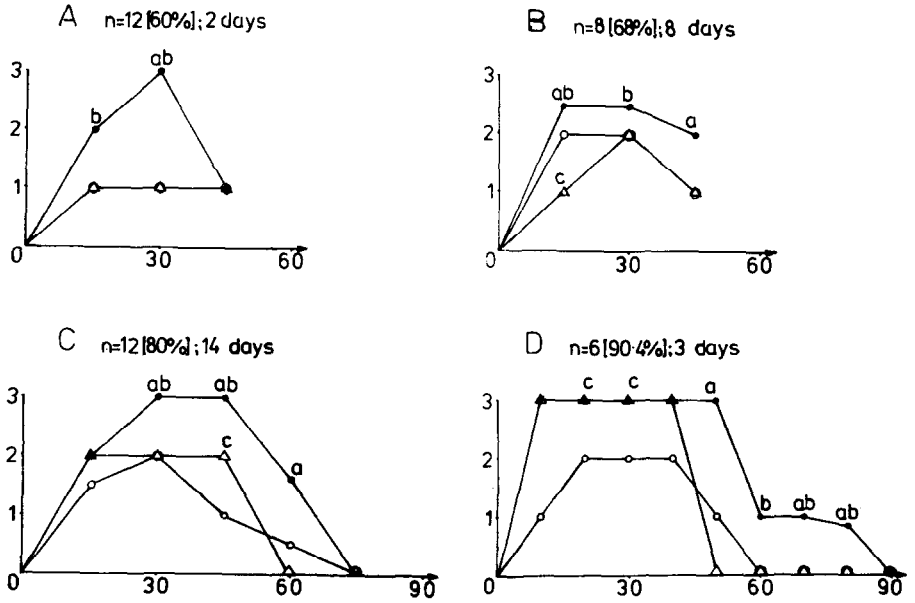

FIG. 2. The effect of various electrolytic lesions of caudate-putamen on apomorphine (1 mg/kg) induced stereotypy. n: size of the operated group; percentage in brackets: the extent of the lesion; interval between surgical procedure and test given in days. Abscissa: time in min, ordinate: the degree of stereotypy. Full circles: lesioned rats, open triangles: sham lesioned rats, open circles: intact rats. a: significant difference between lesioned and sham operated group, b: significant difference between lesioned and intact rats, c: significant difference between sham operated and intact rats ($p<0.05$, Wilcoxon's two-sample test). Each point represents the median value.

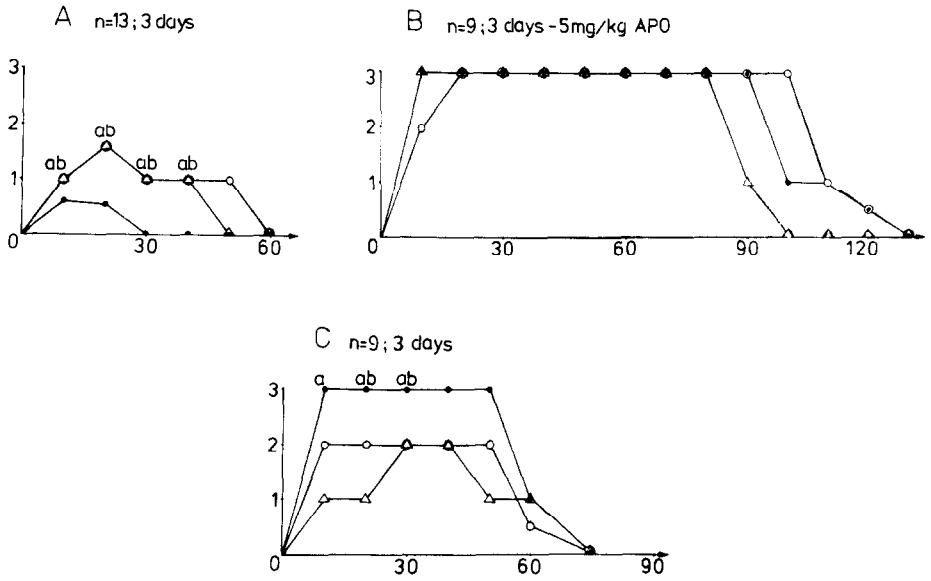

FIG. 3. The effect of mechanical cut of the striatum on apomorphine-induced stereotypy. Groups A and C received 1 mg/kg of APO, Group B – 5 mg/kg of APO. Groups A and B – cuts on the level of rostral edge of the pallidum (M_I and M_{II}), C – cuts in the neostriatum (M_{III}). For other explanations see Fig. 2.

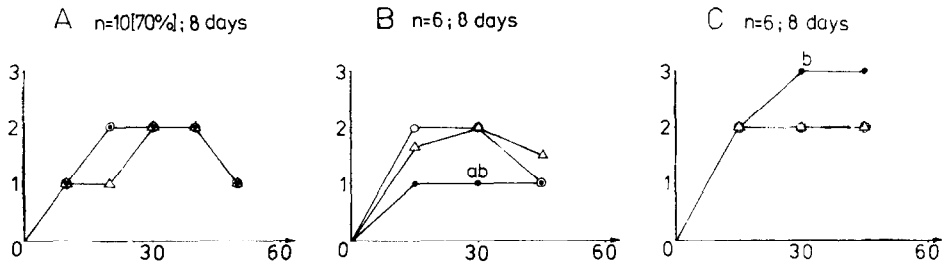

FIG. 4. The effect of electrolytical lesions of rat brain on apomorphine (1 mg/kg) induced stereotypy. A: lesions of 70% of globus pallidus (E_{VII}), B: lesion of frontal cortex and subcortical fibers (E_V), C: lesion of fronto-parietal cortex and radiatio corporis callosi (E_{VI}). For other explanations see Fig. 2.

Reactions to Spiroperidol

Destruction of 80% of neostriatum (Group E_{III}) 16 days before administration of 0.3 mg/kg of spiroderidol produced more rapid onset of the catalepsy without changing its maximal intensity (Fig. 5A). In the group with 90% of neostriatum destroyed 5 days earlier (Group E_{IV}) spiroperidol did not produce catalepsy at all (Fig. 5B). In animals with mechanical lesions spiroperidol (0.3 mg/kg) produced the effects similar as in the controls (Fig. 6). In other groups with electrolytical lesions in paleo– (E_{VII}) or neostriatum (E_I and E_{II}) or with cortical lesions (E_V and E_{VI}) spiroperidol produced the effects similar as in the controls (as measured only once, 2 hr after the injection of the neuroleptic).

DISCUSSION

The results indicating the potentiation of the stereotypy produced by a low dose of APO in rats with large electrolytical lesions of caudatus-putamen are opposite to those reported by Amsler [1], who described the cessation of APO-induced stereotypy following surgical removal of the striated body (Streifenhügeln). McKenzie [17] and Divac [9] have reported no effect of the removal of the striatum by suction on APO stereotypy, but they did not find any potentiation. As the method used by Amsler, McKenzie and Divac differ from those used in the present study, it has been initially supposed that the produces of decomposition of electrolytically necrotized brain tissue may influence the results. The volume of necrotized tissue remaining in the

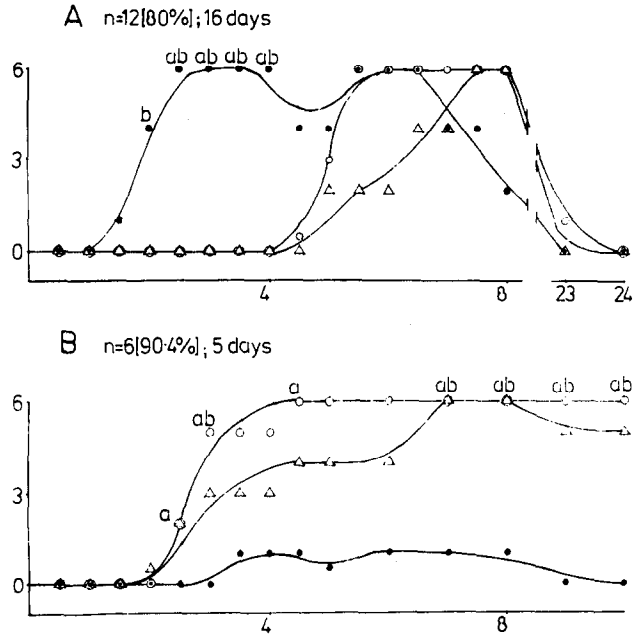

FIG. 5. The effect of electrolytical lesions of caudate-putamen on spiroperidol (0.3 mg/kg) induced catalepsy. Ordinate: degree of catelepsy (according to the method of Delini-Stula and Morpurgo [6] and multiplied by 2); abscissa: time in hr. For other explanations see Fig. 2.

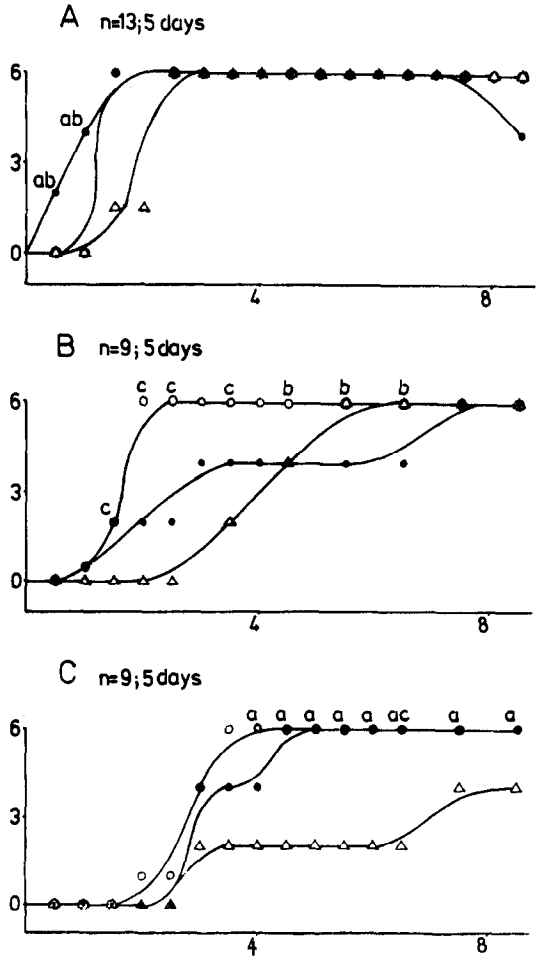

FIG. 6. The effect of mechanical cuts of the striatum on spiroperidol (0.3 mg/kg) induced catalepsy. In Groups A and B (M_I and M_{II}) the lesions were placed on the rostral border of pallidum; in Group C (M_{III}) in the neostriatum. For other explanations see Fig. 2.

brain after extirpation of the striatum is undoubtedly smaller than in the case of electrolytical lesions. The possibility of the involvement of the products of decomposition seems, however, to be unlikely, as the effect does not depend on the length of the interval between the operation and pharmacological test (2–14 days). Moreover similar results were produced by knife cutting the neostriatum. In this case the volume of necrotized tissue was small. The comparison of the above mentioned results regarding different time intervals as well as different degrees of lesions could be questionable as the degeneration of nerve fibers and sensitivities of receptors may be different. The sameness of the results let us make such a comparison though.

It could be postulated that changes in apomorpine-induced stereotypy after various striatal lesions were due to accidental damage to associated areas. However, the medial parts of septal nuclei and of capsula interna were the only other areas where damages were consistently observed, but these damages in all cases were minimal (the animals with lesions placed in a wrong position were discarded); minimal damage to other associated areas was rarely observed. It would thus appear improbable that the effects were due to such damage, although the possibility cannot be entirely excluded.

In the experiments reported the dose of APO was lower than that used by others [9,17]. It seemed that doses of 2 or 5 mg/kg are too high to allow the observation of possible potentiating effects. In fact the lack of influence of cutting the rostral edge of globus pallidus on the action of a high (5 mg/kg) dose of APO, but its clear effect on the action of a low dose (1 mg/kg) suggest that the dose of 5 mg/kg of APO is too high. It seems, therefore, obvious that neither McKenzie [17], nor Divac [9] could observe the potentiation of the action of APO in rats with the striatal lesions.

Amsler [1] removed the whole striate body, paleostriatum including. In attempt to assess the role of paleostriatum we destroyed the pallidum only, but this lesion did not affect the action of APO.

It seems that the difference between the results presented here and those of Amsler [1], McKenzie [17] and Divac [9] could be explained by assumption that the trigger zone for stereotyped behaviour lies in a small part of the striate body on the border of caudatus-putamen and pallidum, whereas the more rostrally placed part of the neostriatum exerts an inhibitory effect. It does not seem likely that the possible denervation supersensitivity of the striatal dopamine receptor [22] plays a role in the potentiation of the APO effects. If it were so, the cutting of the neostriatum, which damages the nerve fibers, but only slightly, if at all the receptors, would promote much greater potentiation of the action of APO than the electrolytical

lesions did (which destroy both nerve fibers and receptors), whereas the opposite effect was observed. We also may suppose that some presynaptic apomorphine effects [7] can be responsible for the results presented here. This possibility seems unlikely, for the apomorphine action is primary postsynaptic [4, 10, 12, 20].

The assumption that the target area for the stereotypogenic action of APO is confined to a small area on the border of neo– and paleostriatum agrees with the results of Ernst [11], who studied the effects of implantation of crystalls of dopa, APO and amphetamine into various parts of the striatum. The assumption seems also reconcile the results given in this paper with those of Amsler [1] and McKenzie [17] and Divac [9]. Amsler removed all the striate body (Streifenhügeln) in unanesthetized animals, during the action of APO. It seems likely that using this procedure the simultaneous destruction of the stereotypy triggering area and structures inhibiting the stereotyped behaviour can result eventually in the cessation of stereotypy. It is also possible that the stereotype disappeared as the result of post-traumatic shock.

Large electrolytic lesions (90%) of caudatus-putamen changed the character of stereotypy, namely prevented completely the reaction of licking. It may suggest the existence of various anatomical substrates for the three components of the stereotyped behaviour: sniffing, licking and gnawing.

This view agrees with the results obtained previously in the rabbit [24], in which intranigral injection of cholinomimetics produces a stereotypy characterized by intensive sniffing and gnawing, but infrequent licking.

In general most lesions do not affect the cataleptogenic action of spiroperidol. After the most extensive lesions (80% and 90%) the responses to spiroperidol were, however, changed. Lesions of 80% of the neostriatum accelerate the development of catalepsy, whereas the lesions of 90% inhibit it almost completely. The elucidation of this discrepancy and of the lack of parallelism between the effects of neostriatal lesions on APO-induced stereotypy and spiroperidol-promoted catalepsy requires further studies. It seems probable however, that APO acts on different brain structures than spiroperidol does.

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