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Enhanced muscimol-induced behavioral responses after 6-OHDA lesions: relevance to susceptibility for self-mutilation behavior in neonatally lesioned rats

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Abstract

Adult rats lesioned with 6-hydroxydopamine (6-OHDA), either as neonates or as adults, demonstrated increased turning, compared to unlesioned controls, when muscimol was unilaterally microinjected into the substantia nigra reticulata (SNR). At the higher doses of muscimol, the lesioned rats were so intensely lateralized that circling was impeded. These data suggest a functional supersensitivity of receptors associated with GABA function in the SNR of 6-OHDA-lesioned rats. When 30 ng muscimol was administered bilaterally into the SNR, self-mutilation behavior (SMB) was observed in 2/11 of the control unlesioned rats, in 0/8 adult 6-OHDA-lesioned rats, and in 11/11 of the neonatally-lesioned rats tested. The ability of muscimol to produce SMB in the rats lesioned as neonates was dose related. Behavioral observations indicated that behaviors associated with SMB (self-biting and taffy pulling) were present in neonatal, but not adult lesioned rats. Behavioral responses to dopamine agonist administration were also different between rats lesioned as neonates and those lesioned as adults with 6-OHDA. These data support the view that lesions of dopaminergic neurons cause an increased functional responsiveness of receptors acted upon by muscimol in the SNR, and that the increased susceptibility for SMB in neonatally lesioned rats is determined by neurons distal to the GABA receptor complex in the SNR.

Keywords

Muscimol; Substantia nigra reticulata; Self-mutilation behavior; 6-Hydroxydopamine-neonatal lesion; 6-Hydroxydopamine-adult lesion; Turning behavior; Behavior, muscimol-induced; SKF-38393; LY-171555; L-dopa; Monoamine metabolites

There is considerable evidence implicating dopaminergic mechanisms in motor function (Hornykiewicz 1973). In addition, GABA-containing neurons in the striatum also appear to play a significant role in motor control (Dray 1980; Graybiel and Ragsdale 1983; McGeer et al. 1984). These striatal GABAergic projections have been found to terminate in both segments of the globus pallidus as well as in the zona reticulata of the substantia nigra (SNR) (Fonnum

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etal. 1978; Preston et al. 1980; Scheel-Kruger et al. 1981). Unilateral administration into SNR of drugs which are associated with GABA receptor function all cause contralateral turning (Scheel-Kruger etal. 1977; Waddington 1978; Waddington and Cross 1979; McDevitt and Yunger 1982). In addition, GABA-mimetics microinjected bilaterally into the SNR or other terminal regions associated with striatal GABA efferents induce behaviors which resemble the effects induced by dopamine. These data led to the hypothesis that GABA efferents from the striatum act as secondary mediators of behaviors triggered by dopamine receptor stimulation (Scheel-Kruger et al. 1977, 1981; Waddington 1978a, b).

There are several observations suggesting that disruption of the function of GABA efferents from the striatum results in a change in receptor function in the SNR. For example, Pan et al. (1983) found that destruction of GABA efferents increased the number of muscimol binding sites in the SNR, suggesting that reduced GABA output from the striatum was responsible for this biochemical change in receptors associated with GABA function. Furthermore, Waddington and Cross (1978) found a high correlation between the increased binding of GABA in the SNR and the behavioral response to muscimol administration after lesioning GABA-containing neurons in the striatum. If the GABAergic neurons are acting as efferents for dopamine-stimulated output, it would seem reasonable to believe that destruction of dopamine-containing neurons would also influence the activity of the GABA-containing neurons in the striatum which terminate in the SNR. This reduced output of dopamine-containing neurons might be expected to increase the sensitivity of the SNR to GABAmimetics. In this regard, a unilateral lesion of dopamine-containing neurons has been reported to increase the circling induced by microinjecting muscimol into the SNR (Gale and Casu 1982). Further, Huffman and Ticku (1983) found that chronic haloperidol administration, which would antagonize dopamine actions, increased muscimol binding sites in the SNR but did not alter binding of ^{3}H-muscimol in other areas of brain. Pan et al. (1985) recently provided autoradiographic data on ^{3}H-muscimol binding after unilateral lesions of the nigrostriatal dopamine pathway, indicating that receptors for muscimol in the SNR increase after lesioning dopaminergic neurons. Finally, lesioning of dopamine-containing neurons as demonstrated by electrophysiological techniques increases the responsiveness of GABA iontophoresed onto cells in the SNR (Waszczak and Walters 1984).

Breese et al. (1984, 1985a, b) have demonstrated that rats given 6-hydroxydopamine (6-OHDA) as adults to lesion central catecholamine-containing neurons have differing behavioral responses when challenged with L-dopa than rats lesioned with 6-OHDA as neonates. In this regard, neonatally-6-OHDA-treated rats demonstrate an increased susceptibility for self-biting behavior when challenged with L-dopa, whereas adult-6-OHDA-treated rats do not (Breese et al. 1984). While the self-biting is associated with activation of supersensitive D_1-dopamine receptors (Breese et al. 1984,1985a, b), no alteration in receptor binding was found to account for the enhanced behavioral responsiveness (Breese et al. 1987).

Based on previous work suggesting that disruption of dopaminergic function will affect the function of the GABA receptor complex, we evaluated the functional sensitivity of receptors acted upon by muscimol in rats lesioned with 6-OHDA as neonates or as adults by measuring the turning response to unilaterally administered muscimol or the behavioral responses to bilateral administration of this drug into the SNR. This work was to determine whether the GABA-containing neurons coursing from the striatum might be contributing to the behavioral differences observed between neonatal and adult 6-OHDA-lesioned rats challenged with dopamine agonists (Breese et al. 1984).

Methods

General

All animals were derived from Sprague-Dawley rats purchased from Charles River Laboratories (Somerville, Mass). Female rats were bred in our laboratory. Approximately 1 week prior to delivery, pregnant females were individually housed in plastic cages with wood chip bedding. Delivered pups were treated intracisternally with 100 μg 6-OHDA in 10 μl saline (0.5% ascorbic acid) under ether anesthesia (Breese and Traylor 1972; Smith et al. 1973). Litters were limited to ten pups. Adult male and female rats (> 200 g) purchased from Charles River Laboratories received 200 μg 6-OHDA in 25 μl 30 min after pargyline (50 mg/kg) and 1 week later an additional dose of 6-OHDA (200 μg),(Breese and Traylor 1970). Other rats received the same volume of saline with ascorbic acid as those that received the 6-OHDA treatments (unlesioned controls).

Prior to any surgery, rats were screened with dopamine agonists to be certain that they were behaviorally supersensitive to dopamine agonists. Neonatal rats were given 100 mg/kg L-dopa 30 min after RO-4-4602 (to inhibit DOPA decarboxylase) and the presence or absence of self-mutilation behavior (SMB) observed (Breese et al. 1984). Rats demonstrating SMB were immediately given the D_1-dopamine antagonist, SCH-23390 (1 mg/kg; Iorio et al. 1983), and a sedating dose of pentobarbital (20 mg/kg) to prevent any further tissue damage (Breese et al. 1985a). These rats were subsequently given 3 mg/kg of the D_1-dopamine agonist SKF-38393 at weekly intervals to assure that maximal locomotor responses were observed (Breese et al. 1985b). Rats treated as adults with 6-OHDA were challenged with 0.3 mg/kg of the D_2-dopamine agonist LY-171555 (Tsuruta et al. 1981), and locomotor activity was measured (Breese et al. 1984). Only those lesioned rats with a locomotor response greater than 10,000 counts were used in subsequent experiments. Locomotor activity was recorded in circular-doughnut-shaped activity monitors as previously described (Hollister et al. 1979).

Drugs

Apomorphine hydrochloride (Merck & Co., Railway, NJ), administered subcutaneously (1 mg/kg), was dissolved in 0.05% ascorbic acid. L-Dihydroxylphenylalanine (L-dopa; Hoffmann-LaRoche, Nutley, NJ) was suspended in 0.5% methylcellulose and injected IP 60 min after RO-4-4602 (Hoffmann LaRoche). The 6-OHDA hydrobromide (Regis Chemical Co.), was dissolved in saline containing 0.5% ascorbic acid and administered intracisternally. Muscimol (Sigma Chemical Co., St. Louis, Mo.) was dissolved in saline so that it was in a volume of 0.5 μl and was micro-injected into substantia nigra and striatum through chronically implanted cannulae over a 5-min period (see below). The LY-171555 was a gift from Eli Lilly and Company (Indianapolis, Ind.) and the SKF-38393 a gift from Smith Kline and French Labs (Philadelphia, PA.). These latter drugs were dissolved in saline and given IP.

Surgical and microinjection procedure

Cannulae (26 gauge) were stereotaxically placed into brain of anesthetized rats (sodium pentobarbital, 45 mg/kg) and secured with stainless steel screws and acrylic dental cement. Coordinates for the substantia nigra were AP = 3.0; ML 2.0 and DV – 0.8 below the skull and for the striatum were AP = 0.92; ML = + 0.3; DV= −0.5 with the incisor bar set at −3 mm. Animals were allowed to recover for at least 7 days before receiving microinjection of muscimol or saline. These injections were unilateral as well as bilateral, depending on whether turning or behavior was measured, respectively. The solutions were administered in a volume of 0.5 μl over a 5-min period through a 33 gauge injection cannula which extended 1 mm below the guide tubing. During injection, the animals were hand held with minimal restraint. A 10 μl syringe driven by a Sage infusion pump (White Plains, NY) delivered the solution through the cannulae. The injector remained in place for 1 min after the end of the infusion. Rats

received no more than four injections at a site with at least 1 week between each treatment. Following the experimental procedures for the SNR, the brain was removed and the striatum and olfactory tubercles retained for biochemical assessments. The hind brain was placed on a cryostat chuck and frozen on dry ice. Each brain was sectioned to allow localization of the cannula tip. In the case of the rats with cannulae placed into the caudate, a portion of the striatum was punched from a cut section to allow determination of dopamine.

Behavioral evaluation

Following bilateral microinjection of muscimol or saline, rats were placed in clear plastic cages (23 × 44 cm) to which they had been habituated for 60 min. A high level of reliability was established for a single person who made all ratings. Periodic checks on ratings were made at arbitrary time periods by the primary author to assure that reliability was maintained. The observer was unaware of the treatments the rats had received. Behavior was observed 1 min every 10 min for 2 h (see Breese et al. 1984; Baumeister and Frye 1984, 1986). Each 1-min period was divided into four 15-s intervals and behaviors observed during each 15-s period were recorded. The proportion of the 15-s periods that each behavior occurred was determined (percent scoring interval). The behaviors monitored included sniffing, rearing, locomotion, licking, self-biting and skin laceration (Breese et al. 1984). The occurrence of skin laceration (SMB) was determined for each dose of muscimol. To prevent further damage after SMB was observed, rats received 30–40 mg/kg sodium pentobarbital.

Turning behavior

In some rats, muscimol or saline were administered unilaterally into the SNR. This results in contralateral turning (Scheel-Kruger et al. 1977; Arnt and Scheel-Kruger 1979). This rotation was quantified by visually counting the number of turns for 2 min every 15 min for 120 min. Accumulated counts and counts at each interval were statistically evaluated.

Monoamine assessments and histology

For determination of dopamine in striatum and olfactory tubercles, the method of Kilts et al. (1981) was used. After dissection, these brain areas were frozen on dry ice, weighed and stored at −70° C until assay (within 2 weeks). For the rats with cannulae aimed at the SNR, placements were determined by cutting frozen sections through the brain stem until the cannulae placements were localized. A similar approach was taken for localizing cannulae placed in the caudate nuclei. In this latter case, striatal tissue was punched from cut slices from an area not disturbed by the cannulae.

Statistical evaluation

The proportion of scoring intervals in which a behavior occurred was summed across the 12 1-min observation periods where this was possible. This yielded a single score ranging from 0 to 12 for each behavior. A one factor analysis of variance (ANOVA) was applied to these scores. For each ANOVA that yielded a significant *F*-Ratio, a Neuman-Keuls test was used to make all comparisons. ANOVA was used to evaluate turning data. Chi square analysis was used to assess the frequency of SMB in the various groups.

Results

Characteristics of 6-OHDA-treated rats

The first procedures undertaken were to establish that the lesioned rats exhibited the appropriate enhanced behavioral responses when challenged with a dopamine agonist. Results from these animals are presented in Table 1. Neonatally-lesioned rats exhibited SMB after 100 mg/kg L-dopa (with decarboxylase inhibitor) and showed an enhanced locomotion response to the fourth

dose of SKF-38393 (3 mg/kg), a D_1 dopamine agonist (Setler et al. 1978). Selected adult 6-OHDA-treated and unlesioned rats given L-dopa did not exhibit this behavior as previously reported (Breese et al. 1984). Adult 6-OHDA-lesioned rats demonstrated an elevated response to LY-171555, the D_2 dopamine agonist, when compared to unlesioned controls (Table 1). Rats demonstrating these supersensitive responses were the ones used for the microinjection of muscimol into the SNR or the caudate. In accord with previous results, both adult and neonatal-6-OHDA lesioned rats used in this investigation had dopamine and its acid metabolites reduced in striatum. In these groups, the neonatally-lesioned rats had a slightly greater depletion than those lesioned as adults (Table 1). In addition, the elevated serotonin content previously found in striatum of neonatally lesioned rats was present in the animals used in this investigation (Table 1; see Breese et al. 1984). Representative placements of the cannulae into caudata and SNR are presented in Fig. 1.

Rotation induced by unilateral muscimol administration into the SNR

Microinjection of muscimol into the SNR increased the rate of rotation in a dose-related manner in control, unlesioned rats (Table 2). Rotation in adult as well as neonatally 6-OHDA-treated rats was greater than in unlesioned rats at all doses administered, but no evidence of a dose-response relationship was observed in these treatment groups (Table 2). This lack of a dose-response relationship in the lesioned rats apparently reflects the intensity and laterality seen with the 15 and 30 ng doses of muscimol in these animals. A problem with these doses of muscimol in the 6-OHDA-treated rats was the induction of a turning posture so tight that it tended to block the rat's ability to turn. Therefore, the rates of rotation are likely lower than would be expected were it not for this compromised response.

Behavioral responses and incidence of SMB after bilateral administration of muscimol into the SNR

Investigators have reported stereotyped behavioral responses and self-biting after bilateral administration of GABAmimetics into the SNR (Scheel-Kruger et al. 1981; Baumeister and Frye 1984). In the present study various doses of muscimol (5, 15, 30 ng) were administered bilaterally into the SNR of 6-OHDA-lesioned and unlesioned rats and the incidence of SMB noted (Table 3). Muscimol produced no self-mutilation in adult 6-OHDA-lesioned rats at any of the doses administered. SMB was observed in two control rats late in the session (115 ± 7 min; *N*=2).In contrast to these findings in control and adult 6-OHDA lesioned rats, all doses of muscimol produced some incidence of SMB in neonatally 6-OHDA-lesioned animals (Table 3). At the 30 ng dose of muscimol all neonatally lesioned rats exhibited SMB. The latency for the occurrence of SMB was 44.3 ± 6.1 min for all neonatally 6-OHDA-treated rats demonstrating this behavior (*N*=16).

Because activation of dopamine receptors results in differing behaviors depending upon the age at which dopamine-containing neurons were destroyed, an additional study was undertaken to see what behaviors other than SMB were produced by muscimol when administered to unlesioned as well as to adult and neonatal 6-OHDA-lesioned rats. A major difficulty encountered with the neonatally 6-OHDA-treated rats was the rapid occurrence of SMB. Because any animal demonstrating this behavior was immediately anesthetized, it was difficult to accurately compare behaviors from the control and adult 6-OHDA-treated animals with those in the neonatally lesioned rats at the 30 and 15 ng doses of muscimol.

In spite of the limitation mentioned above, it is apparent that self-biting was more prominent after muscimol treatment in rats lesioned with 6-OHDA as neonates than in unlesioned controls after muscimol administration (Table 4). Adult 6-OHDA-treated rats did not exhibit this behavior. Since self-biting correlates with SMB, these data are consistent with data presented in Table 3. These data also demonstrate other responses which differed between the neonatal

and adult 6-OHDA-treated rats which included rearing, head nodding, grooming and taffy pulling. The neonatal 6-OHDA-lesioned rats exhibited several behaviors which differed from control at the 30 ng dose of muscimol. These included head nodding, taffy pulling and self-biting. The incidence of rearing in the adult-6-OHDA-lesioned rats varied from the response in unlesioned control rats (Table 4).

Behavioral responses following bilateral administration of muscimol into striatum

In order to determine the selectivity of the responses observed after muscimol administration, 30 ng muscimol was administered bilaterally into the caudate of neonatally 6-OHDA-treated rats and behavior and incidence of SMB measured. This treatment produced no major change in behavior. After 10 min, the animals remained immobile or asleep for the entire period of observation (data not shown).

Discussion

GABAergic neurons, forming the major output systems of the striatum (eg., striato-nigral pathway), are believed to mediate behaviors induced by dopamine receptor stimulation (Scheel-Kruger et al. 1981). It has been demonstrated that denervation of GABAergic projections from the striatum increases GABA and benzodiazepine binding in the SNR, a GABAergic terminal region (Waddington and Cross 1978; Gale and Casu 1982; Pan et al. 1983). This change in binding presumably reflects a compensatory adaptation to the denervation of GABA-containing neurons (Waddington and Cross 1978). Gale and Casu (1982) and Huffman and Ticku (1983) reported that chronic haloperidol increases ^{3}H-muscimol binding in the SNR. Thus, similar alterations in binding of ligands associated with the GABA receptor complex might be expected to occur in the 6-OHDA-lesioned rats as after chronic dopamine receptor blockade. In support of this view, Pan et al. (1985) found major differences in ^{3}H-muscimol binding in globus pallidus, entopenduncular nucleus, and SNR after a unilateral lesion of dopaminergic neurons. Whereas receptor number as measured with ^{3}H-muscimol was enhanced in the entopenduncular nucleus and SNR, ^{3}H-muscimol binding to GABA binding sites in the globus pallidus was reduced by 44%.

Because microinjection of muscimol into the SNR can induce turning (Waddington 1978; Scheel-Kruger et al. 1977), we examined whether the dose-response relationship for muscimol to induce turning would differ between lesioned and unlesioned rats. At all doses of muscimol administered, the 6-OHDA-lesioned rats were found to have elevated circling rates. However, no difference was observed in turning between adult and neonatal 6-OHDA-treated rats, in spite of the fact that the neonatally-lesioned rats had slightly greater depletion of dopamine than the adult lesioned animals, indicating that both treatments were sufficient to influence the response to muscimol when injected into the SNR. Thus, these data suggest a functional supersensitivity of receptors acted upon by muscimol, a finding consistent with the reported increase in ^{3}H-muscimol binding in the SNR after unilateral 6-OHDA lesions (Pan et al. 1985). Further, these findings are in agreement with the report by Gale and Casu (1982) who found elevated turning to intranigral administration of muscimol (15 ng) after unilateral destruction of dopaminergic neurons. However, Waddington and Cross (1979) did not observe potentiation of muscimol (100 ng)-induced turning after microinjection into SNR, but did find that turning induced by baclofen (Waddington and Cross 1979) and flurazepam (Waddington 1979) in 6-OHDA-treated rats was greater than in unlesioned controls. A possible explanation for the difference between the present work and that noted by Waddington and Cross (1979) could be the existence of a “ceiling effect” at this high dose of muscimol.

Breese et al. (1984) reported that self-biting and selfmutilation behavior after L-dopa is greater in rats lesioned neonatally with 6-OHDA than those lesioned as adults. In addition, behaviors other than SMB observed after dopamine agonist administration differed depending upon the

age at which dopaminergic neurons had been destroyed (Breese etal. 1984, 1985b). Previous reports have documented the differing behavioral responses between neonatal- and adult-lesioned rats after administration of L-dopa (Breese et al. 1984) as well as after D_1- or D_2-dopamine agonists (Breese et al. 1985a, b). These latter observations provide evidence that the adaptive mechanisms differ depending on the age at which dopaminergic neurons are destroyed. In the present work, bilateral administration of muscimol to neonatal and adult-6-OHDA-lesioned rats resulted in a differing incidence of SMB and of other behaviors observed when muscimol was microinjected bilaterally into the SNR. Thus, the differences in behavioral responses observed after administering dopamine agonists to neonatal and adult 6-OHDA-lesioned rats also extend to the responses observed after administering muscimol into the SNR.

At the present time, it is believed that muscimol acts on GABA receptors (Williams and Risley 1979). If this is the case, one would expect bicuculline, a GABA antagonist, to antagonize the SMB induced by muscimol microinjection into the SNR. In unlesioned rats, Baumeister et al. (1986; Baumeister and Fry 1984) found that bicuculline did not antagonize the SMB induced by muscimol, but did reduce the incidence of behaviors induced by GABA. Arnt and Scheel-Kruger (1980) previously observed that administration of bicuculline into the SNR produced a behavioral syndrome much like that induced by muscimol microinjected into this site. Given these results, it is difficult to understand the basis of the ability of muscimol to produce SMB (in regard to GABA receptor function), although considerable evidence has accumulated supporting a GABA-mimetic action of muscimol. However, resolution of these basic observations concerning the mechanism of action of these drugs associated with GABA receptor function should not detract from the finding that muscimol microinjected into the SNR produces SMB in the neonatally-lesioned rats but not those lesioned as adults. This finding clearly demonstrates the increased susceptibility of the neonatally lesioned rats for this behavior.

The increased susceptibility of the neonatally lesioned rat for SMB after muscimol suggests that not only dopamine but also other transmitters influencing dopaminergic function could initiate or modify this behavior. This concept could have importance for research attempting to find a means to block the SMB observed in Lesch-Nyhan disease – a syndrome with a deficiency of dopamine during development (Lloyd et al. 1981). In addition to the present finding, serotonin was found to be elevated in the 6-OHDA lesioned neonates, but not in adult-6-OHDA-lesioned rats (see Table 1). However, we have not been able to modify SMB induced by L-dopa by antagonizing serotonergic function (Towle, Mueller, Lauder and Breese, unpublished data). A more recent finding that substance P differs between adult and neonatally lesioned rats in the substantia nigra and striatum (Sivam, Breese, Mueller and Hong, unpublished data) is another intriguing finding that could allow additional insights into the mechanism responsible for the SMB induced by dopamine agonists in neonatally-lesioned rats. Further investigation will be required to determine if the reduced content of substance P in the substantia nigra has relevance to the SMB produced by microinjection of muscimol into this area of rats treated neonatally with 6-OHDA.

The present investigations are consistent with the view that dopaminergic neurons influence GABA efferents to the SNR. However, the present work suggests that the adaptive mechanism (s) responsible for the increased susceptibility for SMB in neonatally-lesioned rats and the behaviors different from those observed in adult-6-OHDA treated rats must involve a neural system(s) distal to the receptors acted upon by muscimol in the SNR. In contrast to the findings in the unlesioned rats, administration of muscimol into the SNR of neonatally 6-OHDA-treated rats was found to produce a dose-related increase in SMB, with the 30 ng dose of muscimol producing this behavior in all rats tested. Even though adult 6-OHDA-treated rats exhibited supersensitivity turning after unilateral administration of muscimol, SMB was not observed even at the highest dose of muscimol. Therefore, the overall sensitivity of the receptors acted

upon by muscimol does not appear to account for the differences in response betwen neonatally and adult-6-OHDA lesioned rats. In accord with previous results (Baumeister and Frye 1984; Scheel-Kruger etal. 1981), SMB was observed in some (2/11) unlesioned rats. The reduced incidence compared to that reported earlier by Baumeister and Fry (1984) may be due to the fact that the rats used in the present study received no anesthetic prior to microinjection of the muscimol into the SNR. Since neonatally-lesioned rats had an elevated susceptibility for SMB after muscimol administration into the SNR just as they do after receiving L-dopa, it is possible that the SNR is a critical output for the action of L-dopa to produce this behavior in neonatal 6-OHDA-lesioned rats. This view will be examined in future experiments.

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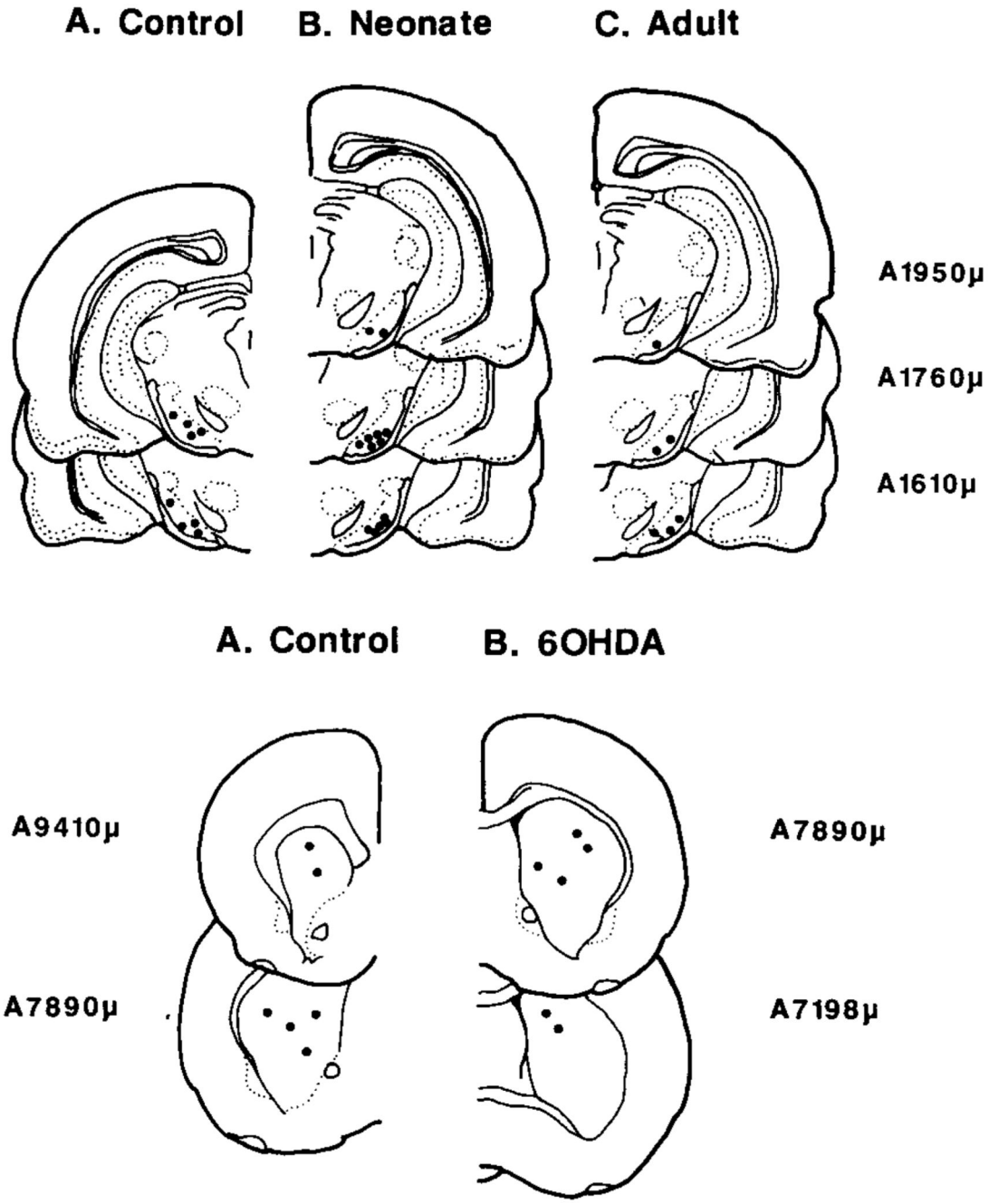

Fig. 1a, b.

Representative placements of injector tips in 6-OHDA-lesioned rats and unlesioned controls. Each symbol represents tip placement of bilateral injections. Upper figures illustrate placements in the SNR and lower figures illustrate placements in the striatum. Numbers to the right indicate anterior/posterior position in µm, according to Konig and Klippel (1963)

Table 1

Characteristics of control and 6-OHDA-lesioned rats used in the present investigation[a]

Measures	Groups [b]		
	Control	Adult 6-OHDA	Neonate 6-OHDA
A. Amine Content (ng/g striatum)			
DA	86.3±4.4	4.4±0.9*	0.6±0.3*
DOPAC	9.2±1.2	0.8±0.3*	0.8±0.2*
HVA	6.5±0.4	0.7±0.3*	N.D*
5-HT	8.2±1.2	6.5±0.7	14.0±0.73*
5-HIAA	5.0±0.5	3.7±0.5	7.4±0.7*
B. Response to SKF-38393 & LY-171555 [b] (Counts/150 min)			
SKF-38393 (3 mg/kg)	1,599±498	–	19,753±0.7*
LY-171555 (0.3 mg/kg)	3,671±863*	18,557±1,951*	5,865±1,657
C. Response to L-dopa[c] (Incidence of SMB)			
L-dopa (100 mg/kg)	0/6	0/6	26/26*

[a] Except for unlesioned and adult-6-OHDA-treated rats in “C”, there are at least ten determinations for each mean±SEM

[b] Saline responses for the various groups were 1,132±298 counts/150 min for unlesioned controls, 1,075±268 counts/150 min for adult 6-OHDA-lesioned rats (adult 6-OHDA) and 963±313 counts/150 min for neonate 6-OHDA-lesioned rats (neonate 6-OHDA) (*N* = at least 6)

[c] All neonates were screened for SMB with L-dopa (100 ng/kg) after receiving 50 mg/kg RO-4-4602, a decarboxylase inhibitor. Only six adult 6-OHDA-lesioned rats and six unlesioned rats were tested with L-dopa to confirm that these groups corresponded to ones previously described (Breese et al. 1984). The remaining animals in the adult 6-OHDA-treated group met the criteria for responses to LY-171555 (see B above)

* $P<0.05$ when compared to drug response in control group

Table 2

Effect of 6-OHDA lesions on turning induced by unilateral microinjection of muscimol into substantia nigra of adult rats[a]

Treatment	Dose (ng)	Control	Neonate 6-OHDA	Adult 6-OHDA
		(Turns/120 min)		
Saline	–	10±7	10±7	2±2
Muscimol	5	247±90*	945±307* **	1,283±375* **
Muscimol	15	375±187	915±150* **	796±97* **
Muscimol	30	517±97*	885±112* **	900±97* **

[a] Animals were habituated to the chamber for 60 min before receiving a unilateral microinjection of saline or muscimol (0.5 μl/5 min) into the SNR. (see Fig. 1 for placements). The turns were determined by multiplying each 2-min sample per 15-min times 7.5 to estimate the total for that period and then summing the score for each rat. Values are the mean±SEM turns of 6–15 determinations

* $P<0.05$ when compared to saline

** $P<0.05$ when compared to muscimol response in control rats

Table 3

Incidence of self-mutilation behavior (SMB) following intranigral administration of muscimol to control or 6-OHDA-lesioned rats[a]

Treatment	Sal	Muscimol Dose		
		5 ng	15 ng	30 ng
		Incidence of SMB (Positive/Total)		
Control	0/6	0/6	0/6	2/11*
Adult-6-OHDA	0/6	0/8	0/8	0/ 8
Neonate-6-OHDA	0/6	1/6	4/6**	11/11**

[a]Rats were habituated to the viewing chamber for 60 min before receiving the microinjection of muscimol (5, 15, or 30 ng) or saline (SAL) into the SNR. Behavior was assessed (see Table) as was the incidence of self-mutilation behavior (SMB). See Table 4 for behavioral responses

*$P<0.5$ when compared to saline administration

**$P<0.05$ when compared to control response at dose given

Table 4

Behavioral scores for control and 6-OHDA lesioned rats[a] after muscimol administration into the SNR

Behavior	Saline	Control			Adult-6-OHDA			Neonate 6-OHDA		
		5	15	30	5	15	30	5	15	30
Sniffing	3.1 ±0.51	6.88 ±1.28*	6.63 ±0.52*	6.97 ±0.63*	6.96 ±0.73*	7.03 ±0.75*	7.67 ±0.83*	6.6 ±0.72*	7.49 ±0.38*	5.61 ±0.44* **
Rearing	1.1 ±0.32	3.04 ±1.14	1.0 ±0.44	1.46 ±0.54	3.95 ±1.06*	2.16 ±1.1	3.97 ±1.10* **	2.47 ±1.94	2.26 ±0.58	1.90 ±0.53
Locomotion	0.51 ±0.14	3.58 ±1.17*	1.31 ±0.14	0.59 ±0.83	4.50 ±1.26*	2.25 ±0.54*	3.17 ±1.47	3.85 ±1.09*	2.44 ±0.97*	0.31 ±0.14
Headnod	0.0 ±0.0	0.58 ±0.32	0.38 ±0.21	2.66 ±0.87*	1.25 ±0.68*	0.35 ±0.14	5.39 ±1.31*	1.15 ±0.50*	0.41 ±0.21	0.65 ±0.26**
Digging	0.21 ±0.10	2.17 ±1.32*	1.14 ±0.47**	0.78 ±0.48	0.33 ±0.25**	0.0 ±0.0	0.29 ±1.24	0.16 ±0.10**	0.93 ±0.32	0.78 ±0.11
Grooming	1.07 ±0.20	0.29 ±0.11	0.27 ±0.20	0.15 ±0.09*	0.13 ±0.09	0.34 ±0.17	0.17 ±0.09*	0.38 ±0.11	0.32 ±0.10	0.0 ±0.0*
Taffy Pulling	0.0 ±0.0	0.08 ±0.08	0.1 ±0.07	0.27 ±0.27	0.12 ±0.09	0.0 ±0.0	0.0 ±0.0	0.25 ±0.25	0.86 ±0.32* **	0.72 ±0.21* **
Licking	0.02 ±0.02	1.13 ±0.69*	1.67 ±0.74*	1.14 ±0.62	0.21 ±0.08	0.03 ±0.02**	0.89 ±0.85	0.0 ±0.0	0.24 ±0.16**	0.25 ±0.11
Self Biting	0.0 ±0.0	0.0 ±0.0	0.18 ±0.11	0.43 ±0.21	0.09 ±0.09	0.0 ±0.0	0.0 ±0.0	0.5 ±0.5	1.28 ±0.36* **	4.32 ±0.38* **

[a]Muscimol and saline were microinjected into the SNR as described in Methods. Numbers below "groups" refer to dose of muscimol microinjected over a 5-min period (0.5 μl). See Table 1 for characteristics of rats treated with 6-OHDA

*$P<0.05$ when compared to saline response

**$P<0.05$ when compared to muscimol response in unlesioned controls