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GABAergic and Glycinergic Mechanisms Within the Substantia Nigra: Pharmacological Specificity of Dopamine-Independent Contralateral Turning Behavior and Interactions With Other Neurotransmitters

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Abstract. The pharmacological specificity of the GABA agonist muscimol-induced contralateral turning behavior after unilateral injection into substantia nigra pars reticulata (SNR) has been studied. Muscimol-induced turning was antagonized by intranigral bicuculline methochloride (BMC) and picrotoxin, whereas antagonists of glycine, morphine, dopamine, noradrenaline, and serotonin were ineffective. Glycine induced a qualitatively similar turning behavior which was strychnine-sensitive but relatively BMC and picrotoxin-insensitive. Other drugs, including substance P, kainic acid, clonidine, oxymetazoline, serotonin, and carbachol, induced turning that could be dissociated from the effect of muscimol. Muscimol-induced turning was dopamine-independent, indicated by resistance to haloperidol (1 mg/kg), to pretreatment with reserpine (7.5 mg/kg) plus α-methyl-*p*-tyrosine (200 mg/kg), to haloperidol injections into the SNR, striatum and nucleus accumbens, and finally to kainic acid lesions of the striatum. 6-Hydroxydopamine lesions increased the efficacy of intranigral muscimol, while kainic acid lesions of the SNR antagonized muscimol. Muscimol-induced turning was inhibited by oxotremorine (0.25 mg/kg), by intranigral carbachol, and by apomorphine (0.1 – 0.5 mg/kg), but only moderately by intranigrally injected apomorphine. These data suggest specificity of GABA-agonist-induced contralateral turning and indicate an interaction between nigral GABA and other neurotransmitters, particularly dopamine and acetylcholine.

Key words: Muscimol – GABA – Substantia nigra – Turning behavior – Glycine – Dopamine – Apomorphine – Noradrenaline – Acetylcholine – Substance P

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It has become evident that the substantia nigra (SN) plays a much more complex role in the control of posture than previously thought. The SN has mainly been considered to be a structure serving as part of a feedback loop regulating the nigrostriatal dopaminergic pathway, possibly via GABAergic inhibition of SN neurones (Andén and Stock, 1973; Tarsy et al., 1975; Racagni et al., 1977; for references see Dray and Straughan, 1976; Chéramy et al., 1978a). In this context, turning behavior has been discussed primarily as the consequence of imbalance between the two nigrostriatal (Glick et al., 1976) and/or limbic dopaminergic systems (Kelly and Moore, 1977; Pycock and Marsden, 1978).

However, recent work from several laboratories has indicated that pharmacological manipulation of SN mechanisms produces dramatic behavioral effects independent of the integrity of dopaminergic neurons. Injections of GABA and the GABA agonist muscimol into the SN, pars reticulata (SNR) induces a vigorous contralateral turning behavior and intense stereotypies following uni- or bilateral injections, respectively (Scheel-Krüger et al., 1977; Olianas et al., 1978a; Oberlander et al., 1977; Olpe et al., 1977). Furthermore, the neurotoxin kainic acid produced similar, but chronic effects (Di Chiara et al., 1977; Olianas et al., 1978b). These and other results have suggested that the well-known striatonigral GABAergic pathway (Fonnum et al., 1974, 1978; Brownstein et al., 1977) is the output pathway in mediation of dopamine receptor stimulation in the forebrain (Garcia-Munoz et al., 1977; Olianas et al., 1978a; Scheel-Krüger et al., 1979).

Stimulation of other putative nigral neurotransmitter receptors has also been implicated in contralateral turning behavior although generally weaker effects have been observed: Substance P (James and Starr, 1977; Olpe and Koella, 1977), glycine (Mendez et al., 1976), noradrenaline (McG Donaldson et al., 1976), opiates (Iwamoto and Way, 1977), and cholinergic drugs (Wolfarth et al., 1978). However, De Montis et al.

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(1978a), contrarily, found ipsilateral turning after intranigral carbachol.

The neuronal complexity of the SN (for review, see Dray and Straughan, 1976), together with the broad classes of drugs inducing grossly analogous effects, makes it very difficult to interpret turning behavior after intranigral injections in terms of pharmacological specificity. As this animal model is a sensitive in vivo test for quantitative evaluation of GABA agonists (Arnt et al., 1978, 1979; Scheel-Krüger et al., 1979), we decided to study the specificity of dopamine-independent behavioral stimulation. In addition, some possible interactions between nigral GABAergic system(s) and other nigral and striatal/limbic neurotransmitter systems were investigated.

Materials and Methods

Surgery. Male Wistar SPF rats (180–200 g) were anesthetized with pentobarbital (50–55 mg/kg, i.p.) and immobilized in a Kopf stereotaxic frame. Stainless steel cannulas were inserted vertically above the SN, aimed at coordinates A 1.6–1.9, L 2.1, and V ÷ 2.8 according to the atlas of König and Klippel (1963). The cannulas were fixed with Cyanolite acrylic resin and Simplex Rapid dentate acrylic plastic. Guide cannulas protruded 6 mm below the surface of the skull (2 mm dorsal to SN). In some experiments, guide cannulas were aimed at the rostral SN (coordinates A 2.2–2.6, L 2.1, V ÷ 2.8).

For experiments with combined intranigral and intrastriatal or accumbens injections the following coordinates were used: center of caudate A 8.4, L 2.6, V 0.4; posterior caudate A 7.2, L 2.6, V 0.4; nucleus accumbens A 9.4, L 1.2, V ÷ 1.0. For striatum, guide cannulas were implanted vertically, whereas for nucleus accumbens a lateral angle of 10° was used to avoid diffusion to the ventricle.

To check if destruction of cerebral tissue above the SN by the guide cannulas was a prerequisite for muscimol-induced behavioral effects, control experiments with angled injections were made: 20° lateral injection, and 20° anterior-posteriorly angled injection with the needle passing through the thalamus.

6-Hydroxydopamine (6-OHDA) lesions were made in the medial forebrain bundle using the coordinates A 3.0, L 1.2, V ÷ 2.5. 6-OHDA was dissolved in cold saline containing 0.2 mg/ml ascorbic acid and injected in an amount of 8 µg/4 µl at a rate of 1 µl/min. The needle (0.4 mm outer diameter) was held in position for a further 2 min. Vehicle was similarly injected on the contralateral side. These animals were implanted 10–12 days later with guide cannulas for intranigral injections essentially as described above.

Kainic acid lesions were made in the caudal SNR, central or posterior striatum respectively, using the same stereotaxic coordinates described above except for SNR, where the vertical coordinate was only V ÷ 2.5–2.6. For the striatum, the dose was 2 µg/µl (made up in water) during 1 min, and for the SNR, 0.75–1 µg/µl during 1 min with the needle (0.25 mm outer diameter) held in position for a further 1 min.

Turning Behavior. Unilateral intranigral injections were performed at least 6 days after operation into hand-restrained, awake rats. The rats had free access to food and water until the experiments. All drugs were injected in a volume of 0.5 µl, if not otherwise stated, during 20–30 s using a 31 g Hamilton microsyringe (outer diameter 0.25 mm). The syringe was held in position for a further 15–20 s. Immediately after injection the rats were placed in a open field (50 × 75 cm) for continuous behavioral observation. Turning behavior was manually recorded. For experiments using only a single injection, maximum turning frequency was used to characterize the effect. For experiments with muscimol or glycine combined with a second injection of other drugs, each rat was used as its own control. That is, inhibition of turning behavior was expressed in percent change in turning rate following the second injection. This method could be used since muscimol and, to some extent, glycine induced stable turning frequencies during prolonged periods. Statistical comparisons were made using the Mann-Whitney U-test (Siegel, 1956).

Uptake of ^{3}H-Dopamine. Striata from rats with 6-OHDA and nigral kainic acid lesions were dissected and ^{3}H-dopamine uptake was measured in a crude synaptosomal fraction according to Tuomisto et al. (1974). The lesioned side was always compared to the vehicle-injected contralateral side.

Histology. After the experiments the rats were cardially perfused with 50 ml 4% formaldehyde solution. The brains were stored in buffered formaldehyde for at least 1 week. Serial sections around the needle track were cut at 40 µ and stained with cresylviolet. Only successful injections within the anatomical region described (see Surgery) were included in the results.

Drugs. Muscimol, kindly provided by Dr. P. Krogsgaard-Larsen; glycine (Analar); bicuculline methochloride (BMC), kindly provided by Dr. G. A. R. Johnston, Canberra; picrotoxin; serotonin (Fluka); kainic acid (Sigma); 6-hydroxydopamine, HCl (6-OHDA) (A/B Hässle); methysergide hydrogenmaleate; LSD, (Lysergide®, Sandoz); oxymetazoline; clonidine (Boehringer); oxotremorine sesquifumarate (Aldrich); cis-Z-flupenthixol 2 HCl (H. Lundbeck and Co.); substance P (Beckman); apomorphine, HCl; phenylephrine, HCl; Carbachol, HCl; strychnine, HNO_3 (Mecobenzon); phentolamine (Regitin®, CIBA); haloperidol (Serenase®, Janssen Pharmaceutical); Naloxone® Endo Lab.). All doses were expressed in terms of the salts. Systemic injections were given in a volume of 1 ml/kg body weight whenever possible. Drugs for intracerebral injections were dissolved in bidistilled water and, if necessary, adjusted to neutral pH.

Results

Glycine, GABA, and Muscimol-Induced Contralateral Turning Behavior. In Table 1, effects of unilateral intranigral injections of muscimol, GABA, and glycine are presented. All three compounds induced a qualitatively similar, dose-dependent contralateral turning behavior beginning immediately after injection. Muscimol was by far the most potent whereas glycine was marginally more potent than GABA and of longer duration. The range of turning frequencies and action duration with the 50 µg dose of glycine showed large variations. Four of 13 rats developed very intense turning activity of 35–40 turns/min with a duration of about 2 h while four rats showed much less intense and short-lasting effects after the same dose. All injections were histologically verified to be within the SNR.

The glycine-induced contralateral turning was independent of dopamine receptor activity as shown by insensitivity to haloperidol pretreatment (1 mg/kg; 1 h). Furthermore, the marked catalepsy produced by this high dose of haloperidol was immediately antagonized. Similar results were obtained with intranigral muscimol; turning behavior was not abolished by haloperi-

Table 1. Contralateral turning behavior after unilateral intranigral injection of GABA, glycine, and muscimol in normal rats and rats pretreated with haloperidol (1 mg/kg, s.c.; 1 h prior to muscimol or glycine) or the combination of reserpine (7.5 mg/kg, s.c.; 24 h) and α-methyl-tyrosine (H44/68) (200 mg/kg, i.p.; 4 h prior to muscimol)

Drugs	Dose (μg)	Turning behavior		
		No. of rats turning	Maximal frequency (turns/min)	Duration (min)
GABA	10	3/6	4 ± 2	7 ± 2
	50	7/7	10 ± 1	16 ± 3
	100	7/7	22 ± 5	21 ± 5
Glycine	5	3/6	3 ± 1	13 ± 6
	25	5/5	8 ± 1	36 ± 9
	50	13/13	19 ± 4	73 ± 14
Haloperidol (1 mg/kg, s.c.; 60 min) + glycine	50	4/4	23 ± 3	100 ± 10
Muscimol	0.01	5/5	20 ± 3	70 ± 2
	0.025	15/15	24 ± 2	100 ± 10
Haloperidol (1 mg/kg, s.c.; 60 min) + muscimol	0.025	6/6	13 ± 2	116 ± 7
Reserpine (7.5 mg/kg, s.c.; 24 h) + α-MT (200 mg/kg, i.p.; 4 h) + muscimol	0.01	5/5	13 ± 4	100 ± 15

dol or by previous catecholamine depletion induced by combined treatment with reserpine (7.5 mg/kg, s.c.) and α-methyl-*p*-tyrosine (200 mg/kg, i.p.; 4 h) (Table 1). The contralateral turning was less intense but this could be attributed to the bad appearance of animals in the latter group (akinesia and hypothermia).

GABA and Glycine Antagonists on Glycine-Induced Turning. The contralateral turning behavior induced by intranigral glycine with a dose of 50 μg was highly significantly reduced within 5 min after injection of strychnine (1 μg given 10 min after glycine) into the same area of SNR (Fig. 1). Lower doses of strychnine were less effective (data not shown). Strychnine (1 μg injected before 25 μg glycine was similarly effective in reducing effects of glycine (data not shown). Strychnine in itself did not induce significant turning behavior following unilateral intranigral injection of 1 μg (n = 8) or 2 μg (n = 5), although the rats showed slight contralateral posture asymmetry. Furthermore, these rats appeared sedated.

Intranigral injections of GABA antagonists bicuculline methochloride (BMC; 0.1 μg) and picrotoxin (0.25 μg) did not block the effect of 50 μg glycine (Fig. 1). There was a tendency towards antagonism of glycine but this effect developed slowly and did not reach statistical significance.

GABA and Glycine Antagonists on Muscimol-Induced Turning. The contralateral turning behavior induced by muscimol (25 ng) was completely blocked by intranigral BMC (0.1 μg) or picrotoxin (0.25 μg) injected 25 min after muscimol, whereas strychnine was without effects (Fig. 2).

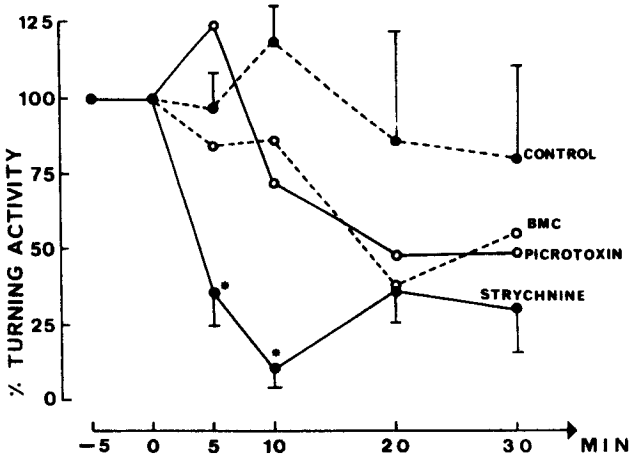

Fig. 1. Effects of intranigral GABA and glycine antagonists on glycine-induced contralateral turning after injection into the SN. Glycine (50 μg in 0.5 μl) was injected 10 min before strychnine (1 μg in 0.5 μl), bicuculline methochloride (*BMC*) (0.1 μg in 0.5 μl), picrotoxin (0.25 μg in 0.5 μl), or control (0.5 μl H_2O). *Abscissa:* min after injection of antagonist. *Ordinate:* Percent of turning frequency (rpm) obtained before antagonist or control injection. 100% = 7–18 rpm. Each value represents the mean SEM of 4–6 experiments. * $P < 0.01$ with respect to the glycine control group

The blocking action of BMC (0.1 μg) was seen within 3 min and persisted for 25–30 min. Central stimulation (sniffing, head movements) induced by muscimol was similarly antagonized by BMC. Picro-

toxin (0.25 µg) produced a slowly developing antagonism of muscimol-induced turning behavior with maximal effects seen 30 min after injection, at which time the muscimol-induced contralateral turns were reversed into ipsilateral ones (Fig. 2).

Antagonism of muscimol by GABA antagonists after intranigral injection was clearly dose-dependent as shown in Table 2. BMC (0.025 and 0.05 µg)

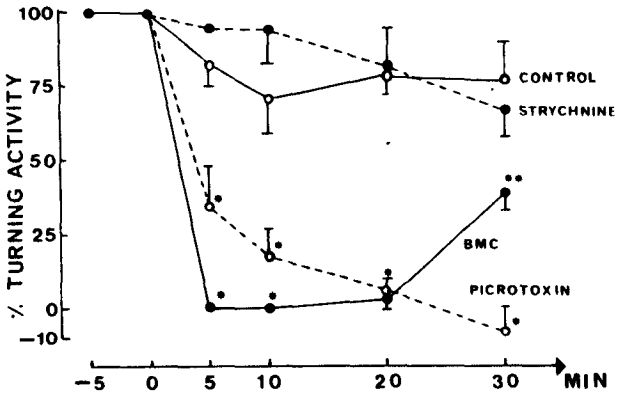

Fig. 2. Effects of intranigral GABA and glycine antagonists on muscimol-induced contralateral turning after injection into the SN. Muscimol (25 ng in 0.5 µl) was injected 25 min before bicuculline methochloride (*BMC*) (0.1 µg in 0.5 µl), picrotoxin (0.25 µg in 0.5 µl), strychnine (1 µg in 0.5 µl), or control (0.5 µl H_2O). *Abscissa:* min after injection of antagonist. *Ordinate:* Percent of turning frequency (rpm) obtained before antagonist or control. 100% = 14–21 rpm. Each value represents the mean ± SEM of 4–5 experiments. $^*P < 0.01$ $^{**}P < 0.05$ with respect to muscimol control group

significantly antagonized muscimol (25 ng), whereas picrotoxin was less potent. Furthermore, if muscimol was given in a lower dose of 10 ng, only 0.05 µg BMC was sufficient to block completely the muscimol-induced turning (data not shown), suggesting a specific interaction between the agonist/antagonist. If BMC (0.25 µg) was injected 10 min before muscimol (25 ng), the muscimol-induced turning was antagonized for about 40 min after BMC injection (not shown) indicating that the order of injection of agonist/antagonist was of minor importance. When injected alone into the SNR, BMC and picrotoxin induced weak turning behavior as previously reported (Scheel-Krüger et al., 1977). Doses up to 0.1 µg induced weak and transient ipsilateral turning whereas the higher dose (0.25 µg) induced short-lasting turning in both directions, mainly dependent on the exact site of injection within the SNR, i.e., within the rostral area of the SNR for contralateral turning whereas injection of BMC within the caudal SNR induces ipsilateral turning. Systemically injected GABA antagonists also inhibited the muscimol-induced contralateral turning as shown for bicuculline (2 mg/kg; s.c.) and picrotoxin (4 mg/kg, i.p.) in Table 3. The antagonism was not complete but it was not possible to increase doses of BMC and picrotoxin due to occurrence of general convulsions.

Effects of Systemically Injected Drugs on Intranigral Muscimol. The effects of GABA and dopamine antagonists have been reported above. Involvement of opiate

Table 2. Effects of intracerebrally administered drugs on contralateral turning induced by intranigral muscimol (25 ng in 0.5 µl). Second injection was given 25 min after muscimol. The results are expressed as percent of the stable turning frequency obtained before the second injection. 100% = 14–20 turns/min

Drug	(µg)	Site	*N*	% Change in turning frequency 10 min after injection	Duration of inhibition (min)
H_2O	0.5 µl	SNR	5	70 ± 11	–
BMC	0.025	SNR	4	36 ± 10*	15
	0.05	SNR	7	25 ± 10*	25–30
	0.1	SNR	4	0**	30–40
Picrotoxin	0.1	SNR	7	43 ± 10*	> 40
	0.25	SNR	4	17 ± 10**	> 50
Strychnine	1.0	SNR	4	95 ± 13	–
Haloperidol	2.5	SNR	5	78 ± 6	–
	2.5	Ipsilateral Striatum	4	74 ± 9	–
	2 × 2.5	Accumbens	7	65 ± 13	–
Apomorphine	2.5	SNR	6	70 ± 11	–
	10	SNR	5	36 ± 6*	30
	20/1 µl	SNR	4	31 ± 5*	30
Carbachol	2.5	SNR	4	90 ± 14	–
	5	SNR	4	56 ± 9	–
	10	SNR	6	12 ± 5**	20

$^*P < 0.05$; $^{**}P < 0.01$ with respect to H_2O injected animals

Other intranigral drugs not affecting muscimol-induced turning were methysergide (2 µg, $N = 4$), cis-Z-flupenthixol (5 µg, $N = 4$), phentolamine (10 µg, $N = 4$), and 5-hydroxytryptamine (25 µg, $N = 3$)

Table 3. Effects of systemically administered drugs on contralateral turning induced by intranigral muscimol (25 ng in 0.5 µl). Systemic injection was given 20–25 min after muscimol. The results are expressed as percent of the stable turning frequency before the systemic injection. 100% = 15–20 turns/min

Pretreatment	Dose	*N*	% Change in turning frequency		Duration of inhibition
	(mg/kg)		10 min	20 min	(min)
Bicuculline	2 (s.c.)	6	43 ± 13*	47 ± 9*	30
Picrotoxin	4 (i.p.)	5	40 ± 12*	21 ± 11**	40
Oxotremorine	0.25 (s.c.)	5	30 ± 11**	10 ± 4**	20–30
Apomorphine	0.1 (s.c.)	6	49 ± 11**	43 ± 8	15–20
Apomorphine	0.5 (s.c.)	5	3 ± 3**	7 ± 5**	25–30
Naloxone	2 (i.p.)	5	85 ± 21	89 ± 12	–
None	–	7	95 ± 13	75 ± 5	–

* $P < 0.05$; ** $P < 0.001$ with respect to untreated animals

receptors in muscimol-induced contralateral turning behavior seems unlikely since naloxone (2 mg/kg, i.p.) had no observable effect (Table 3). On the other hand, the cholinomimetic oxotremorine significantly reduced the muscimol-induced turning after a resonably low dose (0.25 mg/kg, s.c.). The rats injected with oxotremorine were pretreated with methylscopolamine (1 mg/kg, s.c.; 5 min) to counteract the peripheral effects of oxotremorine.

The dopaminergic stimulant apomorphine dose-dependently antagonized intranigral muscimol after doses of 0.1–0.5 mg/kg injected s.c. 25 min after muscimol. The antagonism was observed 5–10 min after injection and had a time course corresponding to the duration of action in untreated rats (Table 3). It may be emphasized that apomorphine antagonism of muscimol was not due to the occurrence of stereotyped behavior since very little of this behavior was observed after the low dose of 0.1 mg/kg. It has been observed that control injections of saline into the SNR resulted in ipsilateral turning after systemic administration of apomorphine (De Montis et al., 1978a). To exclude the possibility of artifact involvement in the apomorphine antagonism of muscimol, due to unspecific tissue damage, similar experiments were performed with an additional simultaneous control injection of 0.5 µl H_2O in the SNR contralateral to the muscimol injection. However, the muscimol-induced contralateral turning was counteracted to a similar extent as that without vehicle injection in the opposite SNR after apomorphine.

Effects of Intracerebral Drugs on Intranigral Muscimol. Haloperidol and cis-Z-flupenthixol had no effects on muscimol-induced turning when the neuroleptics were injected into three different areas: SNR (haloperidol, 2.5 µg; flupenthixol, 1–5 µg), ipsilateral striatum (haloperidol, 2.5 µg) and bilateral nucleus accumbens (haloperidol, 2.5 µg) (Table 2). Furthermore, intranigral injections of 5-HT (25 µg), the 5-HT antagonist methysergide (2 µg), and the α-antagonist phentolamine (5–10 µg) did not modify the muscimol-induced turning. Consistent with the systemic effects of oxotremorine, intranigral injection of carbachol dose-dependently antagonized muscimol-induced turning although relatively large doses were necessary (Table 2). After the highest dose of carbachol, turning was almost blocked but all rats still showed contralateral body asymmetry.

Intranigral apomorphine also attenuated muscimol-induced turning, but to a much smaller degree than was found after systemic apomorphine which may indicate that brain areas other than the SNR also participate in the dopamine-dependent depression of muscimol-induced turning (Tables 2 and 3). The effects of GABA and glycine antagonists have been described in a separate section above.

Effects of 6-OHDA and Kainic Acid Lesions on Intranigral Muscimol. Previous destruction of dopaminergic cell bodies with 6-OHDA did not attenuate the contralateral turning following intranigral muscimol. Interestingly, a significant increase in maximum turning frequency was observed as shown in Table 4. The 6-OHDA-lesioned rats were selected on the basis of intense contralateral turning after systemically injected apomorphine (0.50 mg/kg) and of ipsilateral turning after amphetamine (2.5 mg/kg). Measurement of ^{3}H-dopamine uptake in striatal synaptosomes indicated a depletion by 93% (range 83–99%). Lesions of striatal cell bodies by infusion of 2 µg kainic acid into the ipsilateral striatum (centrally or posteriorly) or bilaterally into the center of the striatum did not antagonize the effects of intranigral muscimol (Table 4). No differences were found in the mean frequency of

Table 4. Effects of 6-OHDA and kainic acid lesions on the contralateral turning induced by intranigral muscimol (25 ng in 0.5 µl). Muscimol was injected 3 weeks after 6-OHDA lesion of the MFB and 9 – 12 days after kainic acid lesions

Lesion	*N*	Max. turning (turns/min)		Duration (min)	
		Lesion side	Control side	Lesion side	Control side
6-OHDA (8 µg/4 µl) MFB	7	32 ± 8*	21 ± 5	111 ± 10	102 ± 12
Kainic acid (2 µg/1 µl) Central striatum, unilateral	8	12 ± 2	12 ± 2	106 ± 15**	78 ± 8
Kainic acid (2 × 2 µg/1 µl) Central striatum, bilateral	5	15 ± 2	–	129 ± 18	–
Kainic acid (2 µg/1 µl) Posterior striatum, unilateral	8	13 ± 2	13 ± 3	79 ± 9	75 ± 9
Kainic acid (0.75 – 1 µg/0.5 µl) SN reticulata, unilateral	9	5 0 4 10 ± 2**	20 ± 2	78 ± 13	90 ± 8

* $P < 0.05$; ** $P < 0.005$ with respect to control side

tendency towards longer duration of muscimol action was found when the kainic acid lesion was localized in the center of corpus striatum, either uni- or bilaterally (Table 4). No changes were found after kainic acid lesions in the posterior striatum although the muscimol-induced turning in some rats was remarkably weak. Furthermore, the sniffing behavior and fast, repetitive head movements characteristic of intranigral muscimol were reduced. In contrast, slow up/down head movements were prominent. The acute behavioral effects of kainic acid showed close similarities to those previously reported by Coyle et al. (1977) and were also used to select the rats for the intranigral muscimol treatment. On the other hand, when kainic acid was injected intranigrally (0.75 – 1.0 µg) at the same site as muscimol, the contralateral turning after intranigral muscimol was dramatically decreased (Table 4). In five animals turning was absent and in the remaining four it was of low and unstable intensity. Quiet periods alternated with bursts of turning behavior, the animals being very sensitive to sensory stimuli. Muscimol was injected 10 – 12 days after kainic acid when spontaneous turning, resulting from the kainic acid lesion, had disappeared (see Table 5). The lesion was characterized by extensive degeneration of cell bodies within the SNR and was followed by a moderate decrease in striatal ^{3}H-dopamine uptake (45% decrease). Under our conditions, presynaptic terminals were also moderately affected, suggested by a small decrease in the SN of glutamic acid decarboxylase (GAD) by 20 – 40% (Braestrup, personal communication).

Regional Specificity of Intranigral Muscimol-Induced Turning. To verify that muscimol-induced contralateral turning specifically involves the SNR, a number of control experiments have been performed; i.e., 20° angled injections of muscimol, either laterally or anterior-posteriorly directed ($n = 5$). No differences in the effect of muscimol were found, indicating that turning behavior is independent of damage to overlying structures caused by the guide cannula. Furthermore, injections into the zona compacta of the SN (0.7 mm dorsal to SNR injections) of muscimol induced no contralateral turning. In fact, a slight ipsilateral posture asymmetry was observed in some rats, but in no case did this develop into spontaneous turning.

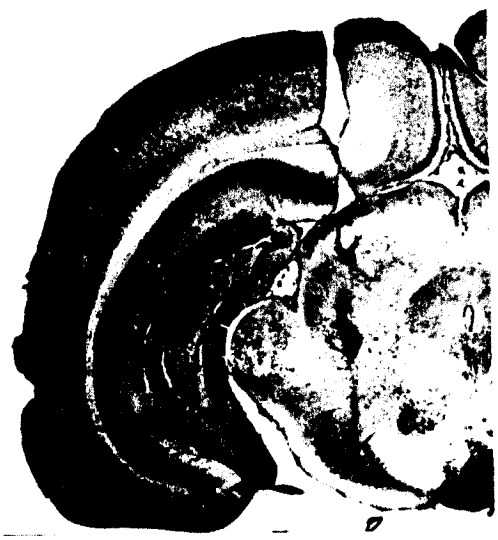

Fig. 3. Histological section showing localization of injection site in the ventral part of the caudal SNR

An example of correct localization of the injection into the caudal part of the SNR is shown in Fig. 3. Note

Table 5. Turning behavior induced by unilateral intranigral injection of various putative transmitter agonists. If not stated, injections were restricted to coordinates A 1.6–2.0, L 1.7–2.5, V + 2.6–3.0

Drug	Dose (μg)	Turning behavior			
		No. of rats turning	Direction	Max. freq. (turns/min)	Duration (min)
Substance P	2.5	10/11	C	10 ± 2	8 ± 2
	2.5[a]	9/10	I	5 ± 6	15 ± 3
Clonidine	10	4/4	C	9 ± 2	22 ± 7
Oxymetazoline	1	1/4	C	8	10
	10	6/7	C	9 ± 2[b]	> 300
Phenylephrine	10	0/5	–	–	–
Kainic acid	0.75–1	18/18	C	10 ± 2[c]	> 4 days
LSD	0.1	0/4	I[d]	–	–
5-HT	25	3/6	I	1–2	35 ± 5
		2/6	C	3; 12	35 ± 5
Apomorphine	10	0/4	–	–	–
Carbachol	2.5–5–10	13/16	C[d]	–	–
	2.5–10[a]	4/7	I	8 ± 1	12 ± 3

I = ipsilateral; C = contralateral
[a] Rostral SNR (A 2.2–2.8)
[b] Maximal turning developed slowly
[c] Measured 24 h after injection (after 2–10 h, ipsilateral asymmetry was observed)
[d] Posture asymmetry

the presence of nucleus ruber as a landmark for caudal SNR.

Unilateral Intranigral Injection of Other Putative Transmitter Agonists. Substance P induced qualitatively the same contralateral turning behavior as muscimol after injection into the caudal SNR (Table 5), but had an extremely short duration of action (less than 10 min after injection of 2.5 μg). After injection into the rostral SNR the opposite effect, namely ipsilateral turning, was found (Table 5). This differentiation, dependent on the injection site, has not been observed after muscimol.

The glutamate analogue kainic acid induced, in agreement with other reports, an initial ipsilateral asymmetry and body-rolling upon recovery from pentobarbital anesthesia, whereas typical head-tail contralateral turning began about 20 h after injection (Table 5). This behavior persisted for at least 4 days and was accompanied by aphagia and adipsia. A high mortality was present among these rats.

Stimulation of noradrenergic receptors did not induce strong postural effects. The α-agonist phenylephrine, acting preferentially on postsynaptic receptors, had no effect, whereas the preferentially presynaptic-acting α-agonists clonidine and oxymetazoline had some effects after high doses (10 μg) (Table 5). Furthermore, the long-lasting effect of oxymetazoline developed slowly during the 1st h. Stimulation of serotonergic receptors within the SNR by 5-HT (25 μg) and LSD (0.1 μg) induced inconsistent turning never reaching more than few turns/min. Fifty percent of rats injected with 5-HT and all rats given LSD showed an ipsilateral body asymmetry and a tendency towards decreased activity (Table 5).

The cholinergic drug carbachol did not produce strong and consistent turning behavior. However, body posture was changed either to the ipsi- or contralateral side and spontaneous turning was only episodically observed (Table 5). The more caudally localized injections induced mainly contralateral posture asymmetry, whereas the ipsilateral turning was found in rats injected into the rostral SNR.

Discussion

The neuronal organization of the SN is complex and the possible interaction among various neurotransmitters present in this nucleus is largely unknown (Dray and Straughan, 1976). In this study, we have investigated the role of GABA with respect to the regional and pharmacological specificity related to the previously described contralateral turning behavior (Scheel-Krüger et al., 1977; Arnt et al., 1979). The regional specifi-

city was evidenced by the negative effect of the GABA agonist muscimol after injection just posterior to the SN, in the reticular formation dorsal to the SN, or in the zona compacta of the SN (Scheel-Krüger et al., 1977). The turning response was not dependent on tissue damage in overlying structures since analogous effects were found after injections at two different angles. However, a chemical lesion of SNR cells, induced by kainic acid (Coyle et al., 1977; Schwarcz and Coyle, 1977), antagonized maximally induced turning. The area within the SNR is important since injection of GABA antagonists within the rostral area of the SNR induces contralateral turning but injection within the caudal SNR induces ipsilateral turning (Scheel-Krüger et al., 1977). Contralateral turning is seen immediately and strongest when muscimol and other GABA agonists are injected within the caudal SNR; when injected within the rostral SNR, the turning is most often of weaker intensity and develops after a certain time lag, possibly due to diffusion (Scheel-Krüger et al., 1977).

Contralateral turning after injection of a drug into the SN is not sufficient criterion to classify the drug as a GABAergic compound since several types of drugs can induce this effect (Table 5). Some drugs, i.e., substance P or opiates, can be differentiated from GABA agonists by regional differences within the SNR. Substance P induces contralateral turning (James and Starr, 1977; Olpe and Koella, 1977) when injected into the caudal SNR but, in contrast to GABA agonists, substance P induces ipsilateral turning or asymmetry when injected into the rostral SNR (Table 5). Electrophysiologically it has been shown that substance P induces excitation in the area of the SNR while GABA or glycine induce depression of the neurones (Dray et al., 1976; Davies and Dray, 1976). Opiates were not included in this study, but from the detailed investigation by Iwamoto and Way (1977) it is clear that opiate-induced turning differs from that of the GABA agonists in two respects: It is dopamine-dependent, and contralateral turning is induced by injection into the rostral SNR. Recently, Bache and Møller-Nielsen (1978) found ipsilateral turning after injection of opiates into the caudal SNR.

Among other drugs which induced contralateral turning after injection into the SNR, we found an effect after clonidine and oxymetazoline which act mainly on presynaptic α-noradrenaline receptors (Berthelsen and Peetinger, 1977), but no effect after phenylephrine which mainly acts on postsynaptic α-receptors. The effect after clonidine or oxymetazoline was only seen after very high doses, compared to those used in other models (e.g., Broekkamp and van Rossum, 1972) and the effect of oxymetazoline was slow in onset in contrast to the immediate effect of GABA agonists. No effect corresponding to muscimol was found after local injection of LSD, serotonin, apomorphine, or carbachol. Qualitative differences thus exist with respect to turning behavior after intranigral injection and, in support of specificity concerning muscimol turning, we have found that it is specifically antagonized by GABA antagonists (see below), but not influenced by selective antagonists of glycine, α-noradrenaline, serotonin, dopamine, or opiate receptors (Tables 2 and 3).

The chronic contralateral turning induced by kainic acid needs a special comment since there is evidence that the cells destroyed by kainic acid are the same as those inhibited by local injection of GABA agonists (Di Chiara et al., 1977, De Montis et al., 1978a, b; Olianas et al., 1978b). In support of this hypothesis, it is seen that chronic contralateral turning caused by kainic acid develops with a time course corresponding to its neurotoxic action (Coyle et al., 1977; Olianas et al., 1978b) and that the effect of muscimol is reduced after injection into the site of the SNR previously lesioned with kainic acid (Table 4)]. The initial effects of kainic acid differ completely from a GABA agonist-like action since ipsilateral asymmetry is prominent, possibly due to excitation of neurons normally inhibited by GABA.

The muscimol- and glycine-induced contralateral turning initiated from the caudal SNR could be differentiated using selective antagonists. Muscimol was dose-dependently antagonized by intranigrally applied BMC and picrotoxin, but not affected by the glycine antagonist strychnine (Fig. 2, Table 2). Systemic administration of bicuculline or picrotoxin also reduced muscimol-induced turning (Table 3). Another line of evidence supporting the specificity of GABA agonist-induced contralateral turning comes from the fact that it is stereospecifically induced by the optical isomers of 5′-methylmuscimol and the comparative potency of a large number of GABA agonists, in the turning model, are in reasonable agreement with the affinity of these drugs to GABA receptors in vitro (Arnt et al., 1979). The strong effect after intranigrally injected glycine may also indicate a functional role of this amino acid in the SN. Its neurotransmitter function seems well-established (Johnston, 1978) and the SN contains relatively high amounts of glycine (Perry et al., 1971; Dray and Straughan, 1976). Presently it is assumed that glycine is present in interneurones in the SN (Dray et al., 1977; Chéramy et al., 1978b). Most neurons in the SN are easily depressed by glycine, the SNR being the most sensitive area (Dray et al., 1976). In our study, the glycine-induced contralateral turning was blocked by strychnine, but not markedly affected by GABA antagonists (Fig. 1). The specificity of strychnine and the partial selectivity of GABA antagonists has also been found in electrophysiological studies (Hill et al., 1976). Biochemically, Chéramy et al. (1978a, b) have shown differences between glycine and GABA on the nigrostriatal dopamine system. Our pharmacological studies

have shown that muscimol- or glycine-induced contralateral turning is not dependent on activation of striatal dopamine receptors; the effect was not abolished following blockade of dopamine receptors by an extremely high dose of haloperidol (Table 1). Depletion of brain dopamine (Table 1) by combined treatment with reserpine and α-methyl-tyrosine (reducing the dopamine level to less than 5% of the control level; Scheel-Krüger, unpublished data) or chemical lesion of the nigrostriatal dopamine system with 6-OHDA (Table 4) did not antagonize muscimol. On the contrary, facilitation of turning behavior was observed after 6-OHDA. Olianas et al. (1978a) also found no inhibition of muscimol-induced turning in 6-OHDA lesioned rats, but they observed facilitation after lower doses of systemic-injected haloperidol. The turning induced by intranigrally injected muscimol was also not antagonized after local injection of the dopamine antagonist haloperidol into the striatum or the nucleus accumbens (Table 2).

Finally, destruction of cell bodies in the striatum with kainic acid, which destroy dopamine receptors related to dopamine-sensitive adenyl cyclase (McGeer et al., 1976; Schwarcz and Coyle, 1977), did not inhibit the effect of intranigral muscimol (Table 4). Interestingly, an apparent prolongation of muscimol-induced turning was found after kainic acid lesions in the striatum (Table 4). This effect may be attributed to degeneration of the well-known striatonigral GABA system (Fonnum et al., 1974, 1978) after intrastriatally injected kainic acid (Coyle et al., 1977), a treatment which may cause denervation supersensitivity of GABA receptors in the SNR, or influence and reduce the uptake of intranigrally injected muscimol into GABA nerve terminals (Johnston et al., 1978).

All these data clearly indicate that muscimol-induced turning is different from the apomorphine-induced contralateral turning seen after unilateral lesion of the nigrostriatal dopamine system, since this latter turning is dependent on activation of dopamine receptors in the striatum and nucleus accumbens (Kelly and Moore, 1977). This fact is also clearly emphasized by our results showing that muscimol-induced contralateral turning is dose-dependently antagonized by peripheral administration of the dopaminergic agonist apomorphine. Olianas et al. (1978a) found a similar result. Intranigrally injected apomorphine in high doses also antagonized muscimol.

From biochemical data it was expected that stimulation of dopamine receptors should increase the efficacy of muscimol, since dopamine in vitro increases GABA release in nigral slices (Reubi et al., 1977), possibly by action via the dopamine-sensitive adenyl cyclase very likely present in GABA terminals (Spano et al., 1977; Philipson et al., 1977). Other experiments in our laboratory have shown that local injection of apomorphine into the ipsilateral striatum or bilaterally injected into the nucleus accumbens also antagonizes intranigral muscimol (Arnt and Scheel-Krüger, in press). Presently, we cannot interpret the mechanism of action underlying these results indicating a functional balance between GABA and dopamine. The muscimol-induced contralateral turning was also subject to inhibitory control by acetylcholine. Peripheral administration of the muscarinic agonist oxotremorine in a low dose effectively antagonized muscimol. The intranigral injection of carbachol had a similar dose-dependent inhibitory effect, suggesting the presence of a GABA-acetylcholine balance within the SNR, in close agreement with results recently reported by De Montis et al. (1979). Other data also suggest a functional role of acetylcholine in the SN (Aghajanian and Bunney, 1975; Wolfarth et al., 1978). These GABA-dopamine-acetylcholine interactions may have clinical relevance, since disturbances in these systems have been reported in parkinsonism and Huntington's disease (McGeer and McGeer, 1976; Lloyd et al., 1975). The effects of GABA agonists in these diseases would, therefore, be of great theoretical interest.

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References

Aghajanian, G. K., Bunney, B. S.: Dopaminergic and non-dopaminergic neurons of substantia nigra: Differential responses to putative transmitters. In: Neuropsychopharmacology, J. R. Boissier, H. Hippius, P. Pichot, eds., pp. 444–452. Amsterdam: Excerpta Medica 1975

Andén, N. E., Stock, G.: Inhibitory effect of gammahydroxybutyric acid and gammaaminobutyric acid on the dopamine cells in substantia nigra. Naunyn-Schmiedebergs Arch. Pharmacol. **279**, 89–92 (1973)

Arnt, J., Scheel-Krüger, J., Magelund, G., Krogsgaard-Larsen, P., Hjeds, H.: Muscimol analogues injected into the substantia nigra: A valuable new in vivo model for GABAergic drugs. In: Amino acids as chemical transmitters, F. Fonnum, ed., pp. 323–326. New York: Plenum 1978

Arnt, J., Scheel-Krüger, J., Magelund, G., Krogsgaard-Larsen, P.: Muscimol and related GABA agonists: The potency of GABAergic drugs in vivo determined after intranigral injection. J. Pharm. Pharmacol. (in press, 1979)

Bache, S., Møller-Nielsen, I.: Turning behavior in rats after intranigral injection of GABA agonists and antagonists and opiate agonists and antagonists. Abstract 1543, Seventh Int. Congress of Pharmacology, Paris (1978)

Berthelsen, S., Pettinger, W. A.: A functional basis for classification of α-adrenergic receptors. Life Sci. **21**, 595–606 (1977)

Broekkamp, C., van Rossum, J. M.: Clonidine induced hypothalamic stimulation of eating in rats. Psychopharmacologia (Berl.) **25**, 162–168 (1972)

Brownstein, M. J., Mroz, E. A., Tappaz, M. L., Leeman, S. E.: On the origin of substance P and glutamic acid decarboxylase (GAD) in substantia nigra. Brain Res. **135**, 315–323 (1977)

Chéramy, A., Nieoullon, A., Glowinski, J.: GABAergic processes involved in the control of dopamine release from nigrostriatal dopaminergic neurones in the cat. Eur. J. Pharmacol. **48**, 281–295 (1978a)

Chéramy, A., Nieoullon, A., Glowinski, J.: Inhibition of dopamine release in the cat caudate nucleus by nigral application of glycine. Eur. J. Pharmacol. **47**, 141–147 (1978b)

Coyle, J. T., Schwarcz, R., Bennett, J. P., Campochiaro, P.: Clinical, neuropathologic and pharmacologic aspects of Huntington's disease: Correlates with a new animal model. Prog. Neuro-Psychopharmacol. **1**, 13–30 (1977)

Davies, J., Dray, A.: Substance P in the substantia nigria. Brain Res. **107**, 623–627 (1976)

De Montis, G. M., Olianas, M. C., Serra, G., Tagliamonte, A., Scheel-Krüger, J.: Evidence that a nigral GABAergic-cholinergic balance controls posture. Eur. J. Pharmacol. **53**, 181–190 (1979)

De Montis, G. M., Olianas, M. C., Rawlow, A., Tagliamonte, A., Scheel-Krüger, J.: GABA receptor binding in the substantia nigra following local injection of kainic acid or 6-OHDA. Eur. J. Pharmacol., submitted publication (1978b)

Di Chiara, G., Olianas, M., Del Fiacco, M., Spano, P. F., Tagliamonte, A.: Intranigral kainic acid is evidence that nigral non-dopaminergic neurones control posture. Nature **268**, 743–745 (1977)

Dray, A., Straughan, D. W.: Synaptic mechanisms in the substantia nigra. J. Pharm. Pharmacol. **28**, 400–405 (1976)

Dray, A., Gonye, T. J., Oakley, N. R.: Caudate stimulation and substantia nigra activity in the rat. J. Physiol. (Lond.) **259**, 825–849 (1976)

Dray, A., Fowler, L. J., Oakley, N. R., Simmonds, M. A., Tanner, T.: Regulation of nigro-striatal dopaminergic neurotransmission in the rat. Neuropharmacology **16**, 511–518 (1977)

Fonnum, F., Grofova, I., Rinvik, E., Storm-Mathiesen, J., Walberg, F.: Origin and distribution of glutamate decarboxylase in substantia nigra of the cat. Brain Res. **71**, 77–92 (1974)

Fonnum, F., Gottesfeld, Z., Grofova, I.: Distribution of glutamate decarboxylase, choline acetyltransferase and aromatic amino acid decarboxylase in the basal ganglia of normal and operated rats. Evidence for striatopallidal, striatoentopeduncular and striatonigral GABAergic fibers. Brain Res. **143**, 125–138 (1978)

Garcia-Munoz, M., Nicolaou, N. M., Tulloch, I. F., Wright, A. K., Arbuthnott, G. W.: Feedback loop or output pathway in striatonigral fibers? Nature **265**, 363–365 (1977)

Glick, S. D., Jerussi, T. P., Fleisher, L. N.: Turning in circles: The neuropharmacology of rotation. Life Sci. **18**, 889–896 (1976)

Hill, R. G., Simmonds, M. A., Straughan, D. W.: Antagonism of γ-aminobutyric acid and glycine by convulsants in the cuneate nucleus of cat. Br. J. Pharmacol. **56**, 9–19 (1976)

Iwamoto, E. T., Way, E. L.: Circling behavior and stereotypy induced by intranigral opiate microinjections. J. Pharmacol. Exp. Ther. **203**, 347–359 (1977)

James, T. A., Starr, M. S.: Behavioral and biochemical effects of substance P injected into the substantia nigra of the rat. J. Pharm. Pharmacol. **29**, 181–182 (1977)

Johnston, G. A. R.: Neuropharmacology of amino acid inhibitory transmitters. Annu. Rev. Pharmacol. Toxicol. **18**, 269–289 (1978)

Johnston, G. A. R., Kennedy, S. M. E., Lodge, D.: Muscimol uptake, release and binding in rat brain slices. J. Neurochem. **31**, 1519–1523 (1978)

Kelly, P. H., Moore, K. E.: Mesolimbic dopamine neurons: Effects of 6-hydroxydopamine induced destruction and receptor blockade on drug-induced rotation of rats. Psychopharmacology **55**, 35–41 (1977)

König, J. F. R., Klippel, R. A.: The rat brain: A stereotaxic atlas of the forebrain and lower parts of the brain stem. Baltimore: Williams and Wilkins 1963

Lloyd, K. G., Möhler, H., Heitz, P. H., Bartholini, G.: Distribution of choline acetyltransferase and glutamate decarboxylase within the substantia nigra and in other brain regions from control and parkinsonian patients. J. Neurochem. **25**, 789–795 (1975)

McGeer, P. L., McGeer, E. G.: Enzymes associated with the metabolism of catecholamines, acetylcholine and GABA in human controls and patients with Parkinson's disease and Huntington's chorea. J. Neurochem. **26**, 65–76 (1976)

McGeer, E. G., Innanen, V. T., McGeer, P. L.: Evidence on the cellular localization of adenyl cyclase in the neostriatum. Brain Res. **118**, 356–358 (1976)

McG Donaldson, I., Dolphin, A., Jenner, P., Marsden, C. D., Pycock, C. J.: The roles of noradrenaline and dopamine in contralateral circling behavior seen after unilateral electrolytic lesions of the locus coeruleus. Eur. J. Pharmacol. **39**, 179–191 (1976)

Mendez, J. S., Finn, B. W., Dahl, K. E.: Rotatory behavior induced by glycine injected into the substantia nigra of the rat. Exp. Neurol. **50**, 174–179 (1976)

Oberlander, C., Dumont, C., Boissier, J. R.: Rotational behavior after unilateral intranigral injection of muscimol in rats. Eur. J. Pharmacol. **43**, 389–390 (1977)

Olianas, M. C., De Montis, G. M., Mulas, G., Tagliamonte, A.: The striatal dopaminergic functions is mediated by the inhibition of a nigral, nondopaminergic neuronal system via a strio-nigral GABAergic pathway. Eur. J. Pharmacol. **49**, 233–241 (1978a)

Olianas, M. C., De Montis, G. M., Concu, A., A., Tagliamonte, A., Di Chiara, G.: Intranigral kainic acid: Evidence for nigral non-dopaminergic neurons controlling posture and behavior in a manner opposite to the dopaminergic ones. Eur. J. Pharmacol. **49**, 223–232 (1978b)

Olpe, H. R., Koella, W. P.: Rotatory behavior in rats by intranigral application of substance P and an eledoisin fragment. Brain Res. **126**, 576–579 (1977)

Olpe, H. R., Schellenberg, H., Koella, W. P.: Rotational behavior induced in rats by intranigral application of GABA-related drugs and GABA-antagonists. Eur. J. Pharmacol. **45**, 291–294 (1977)

Perry, T. L., Berry, K., Hansen, S., Diamond, S., Mok, C.: Regional distribution of amino acids in human brain obtained at autopsy. J. Neurochem. **18**, 513–519 (1971)

Philipson, O. T., Emson, P. C., Horn, A. S., Jessell, T.: Evidence concerning the anatomical location of the dopamine stimulated adenylate cyclase in the substantia nigra. Brain Res. **136**, 45–58 (1977)

Pycock, C. J., Marsden, C. D.: The rotating rodent: A two component system? Eur. J. Pharmacol. **47**, 167–175 (1978)

Racagni, G., Bruno, F., Cattabeni, F., Maggi, A., Di Guilio, A. M., Parenti, M., Groppetti, A.: Functional interaction between rat substantia nigra and striatum: GABA and dopamine interrelation. Brain Res. **134**, 353–358 (1977)

Reubi, J. C., Iversen, L. L., Jessell, T. M.: Dopamine selectively increases ^{3}H-GABA release from slices of rat substantia nigra in vitro. Nature **268**, 652–654 (1977)

Scheel-Krüger, J., Arnt, J., Magelund, G.: Behavioral stimulation induced by muscimol and other GABA agonists injected into the substantia nigra. Neurosci. Letters **4**, 351–356 (1977)

Scheel-Krüger, J., Arnt, J. Bræstrup, C., Christensen, A. V., Magelund, G.: Development of new animal models for GABAergic actions using muscimol as a tool. In: GABA-neurotransmitters, P. Krogsgaard-Larsen, J. Scheel-Krüger, H.

Kofod, eds., pp. 447–464. Copenhagen: Munksgaard, New York: Academic Press 1979
Schwarcz, R., Coyle, J. T.: Striatal lesions with kainic acid: Neurochemical characteristics. Brain Res. **127**, 235–249 (1977)
Siegel, S.: Non-parametric statistics for the behavioral sciences. New York: McGraw-Hill 1956
Spano, P. F., Trabucchi, M., Di Chiara, G.: Localization of nigral dopamine-sensitive adenylate cyclase on neurons originating from corpus striatum. Science **196**, 1343–1345 (1977)
Tarsy, D., Pycock, C., Meldrum, B. S., Marsden, C. D.: Rotational behavior induced in rats by intranigral picrotoxin. Brain Res. **89**, 160–165 (1975)
Tuomisto, L., Tuomisto, J., Smissman, E.: Dopamine uptake in striatal and hypothalamic synaptosomes: Conformational selectivity of the inhibition. Eur. J. Pharmacol. **25**, 351–361 (1974)
Wolfarth, S., Dulska, E., Golembiowska-Nikitin, K., Vetulani, J.: A role of the polysynaptic system of substantia nigra in the cholinergic-dopaminergic equilibrium in the central nervous sytem. Naunyn-Schmiedebergs Arch. Pharmacol. **302**, 123–131 (1978)

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