Br. J. Pharmac. (1983), **79**, 599–610

Differential anatomical location of [^{3}H]-N,*n*-propylnorapomorphine and [^{3}H]-spiperone binding sites in the striatum and substantia nigra of the rat

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1 Specific [^{3}H]-spiperone and [^{3}H]-N,*n*-propylnorapomorphine (NPA) binding was measured in striatum and substantia nigra of the rat following unilateral 6-hydroxydopamine (6-OHDA) lesions of the medial forebrain bundle, kainic acid lesions of the substantia nigra or striatum, and following decortication.

2 Binding sites labelled by [^{3}H]-spiperone in striatum were found to lie on striatal cell bodies and on the terminals of cortico-striate glutamate fibres, but not on presynaptic dopamine terminals. In contrast, binding sites labelled by [^{3}H]-NPA were demonstrated on striatal cell bodies and on the terminals of nigro-striatal dopamine fibres, but not on cortical afferents.

3 In substantia nigra, specific [^{3}H]-spiperone binding sites were found only on non-dopamine cell bodies. No clear evidence was found for their existence on dopamine cell bodies, the terminals of strio-nigral fibres or the terminals of cortico-nigral fibres. In contrast, specific binding sites for [^{3}H]-NPA were found on dopamine cell bodies and the terminals of strio-nigral fibres. Localization on non-dopamine cell bodies or on cortico-nigral fibres was not demonstrated.

4 These studies support the concept of differential localization of agonist and antagonist binding sites.

Introduction

Dopamine receptors are located at a number of different anatomical sites within the strio-nigral complex. In striatum, post-synaptic dopamine receptors are located, at least in part, on the cell bodies or dendrites of cholinergic interneurones where dopamine acts to inhibit acetylcholine release (Stoof, Thieme, Vrijmoed-de-Vries & Mulder, 1979). Others may lie also on striatal GABAergic neurones. Dopamine receptors are found on the terminals of cortico-striate glutamate fibres where dopamine exerts inhibiting control of glutamate release (Roberts & Anderson, 1979). Other dopamine receptors (autoreceptors) are thought to exist on presynaptic dopamine terminals where dopamine acts to control its own synthesis and release (Chéramy, Nieoullon, Michelot & Glowinski, 1977). In substantia nigra, dopamine receptors are located on dopamine-containing cell bodies and dopamine exerts an inhibitory effect on nigral cell firing at this locus (Bunney, Walters, Roth & Aghajanian, 1973; Aghajanian & Bunney, 1977). There is evidence also for dopamine receptors on the terminals of strio-nigral γ-aminobutyric acid (GABA) terminals controlling GABA release (Reubi, Iversen & Jessell, 1977) and for dopamine receptors on the cell bodies of nigro-thalamic fibres which alter firing in these pathways (Dray & Straughan, 1976; Ruffieux & Schultz, 1980).

Dopamine receptors not only have differing anatomical locations within the strio-nigral complex but multiple forms may exist. Dopamine receptors may be divided into those linked to adenylate cyclase (D-1) and those which act independently of this enzyme (D-2) (Kebabian & Calne, 1979). Sub-classification of adenylate cyclase independent dopamine receptors has been proposed (D-2, D-3, D-4) involving differences in agonist and antagonist affinity. At present there are two such classifications namely that of Seeman (1980) and that of Sokoloff, Martres & Schwartz (1980). In the present study, the scheme of Sokoloff & colleagues has been adopted. According to their classification D-2 receptors (high affinity for agonists and antagonists) lie mainly on striatal cell bodies, D-3 receptors (high agonist affini-

ty but low antagonist affinity) are predominantly found on dopamine fibre terminals and D-4 receptors (high antagonist affinity but low agonist affinity) are located on the terminals of cortico-striate glutamate fibres. The major distinction between this scheme and that of Seeman (1980) is that the latter author reverses the definition of D-2 and D-4 sites.

Receptor binding data following lesioning of pathways within the strio-nigral complex has confirmed the existence of binding sites at some of these locations (Table 1). These studies have led to two areas of dispute. There is no agreement over the nature of dopamine receptors located on presynaptic dopamine terminals in striatum, nor whether agonist ligands, (such as [^{3}H]-apomorphine or [^{3}H]-N,*n*-propylnorapomorphine (NPA)) differentially label binding sites within the strio-nigral complex compared to antagonist ligands, (such as [^{3}H]-haloperidol or [^{3}H]-spiperone) (compare for example Titeler, List & Seeman, (1979) with Leysen, (1979)). One reason for these discrepancies may lie in the fact that few comprehensive studies have been undertaken. Most investigations have looked at the effect of one lesion on the binding of several ligands, or the effect of a number of different lesions on the binding of a single ligand. In the present study we have investigated the effect of lesioning input and output pathways to the striatum and substantia nigra on the binding of both [^{3}H]-spiperone and [^{3}H]-N,*n*-propylnorapomorphine. We find a differential anatomical location of agonist and antagonist binding sites in these two brain areas.

Methods

Lesioning techniques

Female Wistar rats (150–175 g; Charles Rivers Ltd.) were anaesthetized with chloral hydrate (300 mg/kg i.p., BDH Ltd.). The following lesions were performed using a Kopf stereotaxic frame: unilateral kainic acid lesions of striatum (A 7.8, L 2.6, V + 1.1) (de Groot, 1959) were made with 1 μg kainic acid (Sigma Chemical Co. Ltd.) dissolved in 1 μl 0.9% w/v NaCl solution (saline) and buffered to pH 6.5 using dilute NaOH solution. Injections were made with a 10 μl Hamilton syringe and Luer needle (o.d. 0.33 mm; i.d. 0.18 mm) at a rate of 0.2 μl per min.

Unilateral kainic acid lesions of the substantia nigra (A 1.5, L 2.0, V–3.5) (de Groot, 1959) were made with 0.25 μg kainic acid in 0.25 μl saline buffered to pH 6.5 and injected at a rate of 0.05 μl per min.

In both cases animals were pretreated immediately before surgery and upon recovery with 0.35 mg/kg i.p. clonazepam (Rivotril; Roche Products Ltd.) to prevent seizures and to limit damage to fibre bundles.

Unilateral 6-hydroxydopamine (6-OHDA) lesions of the medial forebrain bundle (A 4.5, L 1.9, V–3.0) (de Groot, 1959) were made using 6-hydroxydopamine hydrobromide (8 μg in 3 μl saline containing 2 μg ascorbic acid; Sigma Chemical Co. Ltd.) injected with a 10 μl Hamilton syringe and Luer needle (o.d. 0.33 mm; i.d. 0.18 mm) at a rate of 1 μl per min. The effectiveness of the 6-OHDA lesion was tested 5 days following surgery by assessment of contraversive rotation in response to the administration of apomorphine hydrochloride (0.5 mg/kg s.c. 15 min previously; MacFarlan Smith).

Following kainic acid or 6-OHDA lesioning, animals were allowed 21 days to recover and to allow for neuronal degeneration before biochemical and histological examination. This timing was chosen to be consistent with those employed previously and found to alter ligand binding (Schwarcz, Creese, Coyle & Snyder, 1978).

Unilateral cortical ablations were performed using a Stoelting stereotaxic frame. Following removal of the overlying calvarium, the parietal and frontal cortex were removed by shallow knife cuts to the level of the corpus callosum. Following surgery, bleeding was controlled by implantation of absorbable gelatin sponge (Sterispon No.2; Allen & Hanbury Ltd.). Following surgery a 5 day period was allowed for neuronal degeneration before biochemical and histological examination. This time had previously been demonstrated to be sufficient to cause a reduction of striatal [^{3}H]-haloperidol binding (Schwarcz *et al.*, 1978).

Histological examination

Animals from each lesion group were randomly selected for histological verification of lesion sites. The animals were killed by cervical dislocation and decapitation, the brains removed and placed in 40% formaldehyde/glacial acetic acid/methanol (1:1:8) for at least one week. Following fixation, brains were processed to remove water and embedded in paraffin wax. Serial sections were cut at 12 μm in the case of kainic acid and 6-OHDA lesions, and 20 μm in the case of decortications, using a sledge microtome. Sections were stained either with phosphotungstic acid haematoxylin and luxol fast blue, or with cresyl fast violet and luxol fast blue. Sections were examined using a projecting microscope (Projectina) and Zeiss Photomicroscope II.

Determination of specific [^{3}H]-spiperone binding

Ten rats from each lesion group were killed by cervical dislocation and decapitation. The brains were rapidly removed and placed on ice. The substantia

Table 1 The effect of lesions of the nirgro-striatal system on agonist and antagonist binding in rat striatum and substantia nigra

^{3}H-ligand	*Receptor type*	*Lesion site*	*Change in* Bmax	*Reference*
A. Striatum				
Effects of 6-hydroxydopamine lesions				
[^{3}H]-haloperidol	D-2	MFB	+50%	1
	D-2	MFB	+28%	2
	D-2	SN	+38%	3
[^{3}H]-spiperone	D-2	MFB	+ 3%	4
	D-2	MFB	+25%	5
	D-2	MFB	+30%	6
	D-2	MFB	+ 3%	7
	D-2	SN	+63%	3
[^{3}H]-apomorphine	D-3	MFB	−47%	8
	D-3	SN	−18%	9
[^{3}H]-ADTN	D-2	MFB	+27%	10
Effects of kainic acid lesions of striatum				
[^{3}H]-haloperidol	D-2	ST	−36%	11
[^{3}H]-spiperone	D-2	ST	−49%	12
	D-2	ST	−45%	10
	D-2	ST	−48%	13
	D-2	ST	−38%	6
	D-2	ST	−48%	7
	D-2	ST	−62%	14
	D-2	ST	−53%	15
[^{3}H]-*cis*-flupenthixol	D-1	ST	−54%	15
	D-1	ST	−74% (not *Bmax*)	16
[^{3}H]-apomorphine	D-2	ST	−64%	9
	D-3	ST	0%	13
	D-2	ST	−71%	14
[^{3}H]-ADTN	D-2	ST	−90%	10
Effects of decortication				
[^{3}H]-haloperidol	D-4	ST	−32%	11
[^{3}H]-spiperone	D-4	ST	−41%	6
	D-4	ST	−22%	7
[^{3}H]-ADTN	D-2	ST	+15%	10
B Substantia nigra				
Effects of 6-hydroxydopamine lesions				
[^{3}H]-spiperone		MFB	− 9%	8
		MFB	−40%	5
		SN	−36%	17
[^{3}H]-apomorphine		MFB	−76%	8
Effects of kainic acid lesions of striatum				
[^{3}H]-spiperone		ST	+11%	5
		ST	+ 9%	17

1 Creese, Burt & Snyder, 1977.
2 Mishra, Wong, Varmuza & Tuff, 1978.
3 Thal, Mishra, Gardner, Horowitz, Varmuza & Makman, 1979.
4 Leysen, 1979.
5 Reisine, Nagy, Fibiger & Yamamura, 1979.
6 Spano, Memo, Stefanini, Fresia & Trabucchi, 1980.
7 Theodorou, Reavill, Jenner & Marsden, 1981.
8 Nagy, Lee, Seeman & Fibiger, 1978.
9 Fujita, Saito, Iwatsubo, Hirata, Noguchi & Yoshida, 1980.
10 Fuxe, Hall & Köhler, 1979.
11 Schwarcz, Creese, Coyle & Snyder, 1978.
12 Minneman, Quik & Emson, 1978.
13 Weinreich & Seeman, 1980.
14 Leysen, Gommeren & van Gompel, 1982.
15 Cross & Waddington, 1981.
16 Leff, Adams, Hyttell & Creese, 1981.
17 Quik, Emson & Joyce, 1979.

nigra and striatum from the lesioned and intact sides of the brain were dissected out and placed separately in ice-cold Tris-HCl buffer (50 mM, pH 7.6). The striatal and nigral tissue from the ten animals was pooled for subsequent study of [^{3}H]-spiperone binding. At least 3 such tissue pools were studied for each lesion experiment (except in the case of the effect of decortication on nigral binding; see Tables 4 and 5). The small amount of nigral tissue available (approximately 4 mg wet weight for each nigra) allowed only the determination of binding at a single ligand concentration, (approximately 4.0 nM) which was previously determined by saturation studies in tissue from untreated animals. Specific [^{3}H]-spiperone binding (defined using 10^{-5} M (−)-sulpiride) represented approximately 25% of total nigral binding in control tissue at this ligand concentration.

The preparation of washed tissue preparations and the determination of specific [^{3}H]-spiperone (21 Ci/mmol; Amersham International) binding was carried out using a modification of the method of Leysen, Gommeren & Laduron (1978). Specific binding of [^{3}H]-spiperone (0.1–4.0 nM) (defined in the presence and absence of 10^{-5} M (−)-sulpiride; Delagrange), which represented 65–75% of total binding, was determined in triplicate on pooled striatal tissue preparations from the lesioned and intact forebrain in a final incubation buffer (pH 7.5) containing 50 mM Tris-HCl and 120 mM NaCl on at least three separate occasions. Specific binding of [^{3}H]-spiperone was determined three times in triplicate in tissue preparations from substantia nigra at a single saturating ligand concentration (approximately 4.0 nM).

Determination of specific [^{3}H]-N,n-propylnorapomophine binding

Specific [^{3}H]-N,*n*-propylnorapomorphine ([^{3}H]-NPA) (60 Ci/mmol; New England Nuclear) binding was determined by a modification of the technique of Leysen & Gommeren (1981) for [^{3}H]-apomorphine (Hall, Jenner & Marsden, 1981). Pooled tissue from the substantia nigra or striatum from the lesioned and intact sides of the brains from ten rats was homogenized in ice-cold Tris-HCl (15 mM) buffer containing disodium edetate (EDTA. Na_2, 1 mM), pH 7.8 (Tris-EDTA). This buffer was used throughout the experimental procedure for [^{3}H]-NPA binding. Specific binding of [^{3}H]-NPA (defined using 10^{-6} M (±)-6,7-ADTN; Wellcome Research Laboratories) which represented 60–68% of total striatal binding, was determined in triplicate at a range of concentrations (0.05–2.0 nM) on washed striatal tissue preparations from the lesioned and intact forebrain on at least three separate tissue pools from animals from the same supplier. Specific [^{3}H]-NPA binding was determined also at a single saturating ligand concentration (approximately 2.0 nM) on substantia nigra preparations on three separate occasions. Specific [^{3}H]-NPA binding (defined using 10^{-6} M (±)-ADTN) represented approximately 30% of total nigral binding at this ligand concentration.

Statistical analysis

Receptor binding data from striatal preparations was examined using Scatchard analysis. Where a range of concentrations was used, data were subjected to

Table 2 Effect of lesions on the number of binding sites (*Bmax*) and equilibrium dissociation constants (K_D) for specific striatal [^{3}H]-spiperone (0.1–4.0 nM)

Lesion	Bmax *(pmol/g wet wt. tissue)*		K_D *(nM)*	
	Intact	*Lesioned*	*Intact*	*Lesioned*
Unlesioned	20.4 ± 1.13	—	0.15 ± 0.02	—
6-OHDA lesion of MFB	19.5 ± 1.52	26.7 ± 1.2* (137%)	0.19 ± 0.03	0.20 ± 0.03 (105%)
Kainic acid lesion of SN	18.5 ± 2.3	25.1 ± 2.3* (136%)	0.15 ± 0.02	0.19 ± 0.02 (127%)
KA lesion of striatum	19.2 ± 1.1	10.5 ± 0.7* (55%)	0.12 ± 0.02	0.13 ± 0.03 (108%)
Decortication	21.4 ± 1.5	15.9 ± 1.3* (74%)	0.13 ± 0.02	0.16 ± 0.03 (123%)

Results are expressed as the mean (± 1 s.e.mean) of the estimated of *Bmax* and K_D from tissue pools of 10 animals performed in at least 3 independent experiments. The values in parentheses represent the data for lesioned hemisphere expressed as a percentage of values obtained from intact forebrain. Tissue from lesioned and intact forebrains from the same animals was always compared on the same occasion and in strict parallel.

MFB = medial forebrain bundle; SN = substrantia nigra.

*$P < 0.01$ compared with striatal preparations from the intact forebrain using a two tailed Student's *t* test.

linear regression analysis to define the number of binding sites (*Bmax*) and equilibrium dissociation constant (K_D) and comparison of data from the separate experiments on lesioned and intact brain tissue was carried out using an unpaired two-tailed Student's *t* test. Binding data from the individual experiments on substantia nigra was pooled from the three separate determinations and comparison of data from lesioned and intact brain tissue was made using an unpaired two-tailed Student's *t* test.

Results

Specific [^{3}H]-spiperone binding to striatal tissue preparations

Unilateral lesioning of the medial forebrain bundle with 6-OHDA 21 days previously caused a 37% increase in *Bmax* for specific [^{3}H]-spiperone binding to striatal preparations from the lesioned hemisphere. There was no change in the dissociation constant (K_D) compared to the intact forebrain (Table 2) in agreement with previous findings (Reisine, Nagy, Fibiger & Yamamura, 1979; Spano, Memo, Stefanini, Fresia & Trabucchi, 1980).

Similarly, unilateral kainic acid lesions of substantia nigra 21 days beforehand caused a 36% increase in *Bmax* for specific [^{3}H]-spiperone binding to striatal preparations from the lesioned forebrain compared to the intact side (Table 2). Again K_D was unchanged.

Unilateral kainic acid lesions of striatum 21 days previously caused a 45% decrease in the number of specific [^{3}H]-spiperone binding sites (*Bmax*) in the lesioned striatum compared to the intact side (Table 2). The dissociation constant (K_D) was unaltered. These results are in agreement with many previous studies on the effect of kainic acid lesions on [^{3}H]-spiperone binding (Table 1).

Unilateral removal of the frontal and parietal cortex 5 days previously caused a 26% decrease in *Bmax* for [^{3}H]-spiperone binding with no change in K_D when compared to the unlesioned striatum (Table 2). This alteration confirms the results in a previous report (Theodorou, Reavill, Jenner & Marsden, 1981).

Specific [^{3}H]-N,n-propylnorapomorphine (NPA) binding to striatal tissue preparations

Unilateral 6-OHDA lesions of the medial forebrain bundle 21 days previously caused a 40% decrease in the number of specific [^{3}H]-NPA binding sites (*Bmax*) in striatal tissue preparations from the lesioned forebrain compared to the intact side (Table 3). The dissociation constant (K_D) was decreased by 31% by the 6-OHDA lesion.

Unilateral kainic acid lesions of substantia nigra 21 days beforehand also caused a 41% decrease in *Bmax* for specific [^{3}H]-NPA binding, and K_D was decreased by 34% when compared to the intact forebrain (Table 3).

Similarly, unilateral kainic acid lesions of the striatum 21 days previously also caused a fall (29%) in *Bmax* for [^{3}H]-NPA binding but K_D was unchanged in comparison with the intact striatum (Table 3).

Unilateral removal of frontal and parietal cortex 5 days previously had no effect on *Bmax* or K_D for

Table 3 The effect of lesions on the number of binding sites (*Bmax*) and equilibrium dissociation constants (K_D) for specific striatal [^{3}H]-N,*n*-propylnorapomorphine (0.05–2.0 nM) binding

Lesion	Bmax *(pmol/g wet wt. tissue)*		K_d *(nM)*	
	Intact	*Lesioned*	*Intact*	*Lesioned*
Unlesioned	17.4±2.0	—	1.10±0.10	—
6-OHDA lesion of MFB	18.9±2.6	11.4±1.3* (60%)	1.08±0.11	0.74±0.07* (69%)
Kainic acid lesion of SN	17.3±1.9	10.4±2.4* (59%)	0.88±0.10	0.58±0.13* (66%)
Kainic acid of striatum	16.3±1.7	11.6±1.4* (71%)	0.94±0.10	0.90±0.07 (96%)
Decortication	18.2±2.2	15.0±2.0 (82%)	1.06±0.14	1.05±0.21 (99%)

Results are expressed as the mean (±1 s.e.mean) of the estimates of *Bmax* and K_D from tissue pools of 10 animals performed in at least 3 independent experiments. The values in parentheses represent the data for lesioned hemisphere expressed as a percentage of values obtained from intact forebrain. Tissue from lesioned and intact forebrains from the same animals was always compared on the same occasion and in strict parallel. Abbreviations as in Table 2.

*$P < 0.01$ compared with striatal preparations from the intact forebrain using a two-tailed Student' *t* test.

Table 4 The effects of lesions on specific [^{3}H]-spiperone (4.0 nM) binding to tissue preparations of substantia nigra from lesioned and intact forebrain

Lesion	*Specific binding* (pmol/g wet wt. tissue) *Intact*	*Lesioned*	
6-OHDA lesion of MFB	2.11	3.77	(179%)
	2.32	4.05	(175%)
	2.76	4.90	(177%)
	2.40 ± 0.19	4.24 ± 0.34*	(177%)
Kainic acid lesion of SN	1.89	0.59	(31%)
	2.70	1.06	(39%)
	3.68	1.12	(30%)
	2.76 ± 0.52	0.92 ± 0.17*	(33%)
Kainic acid lesion of striatum	2.69	4.54	(169%)
	2.60	3.79	(146%)
	2.42	3.74	(155%)
	2.57 ± 0.08	4.02 ± 0.26*	(156%)
Decortication	3.09	4.23	(137%)
	2.62	3.45	(132%)
	2.86	3.84	(134%)

Results given are from three individual experiments; also shown are the mean (± 1 s.e.mean) for the binding to substantia nigra (SN) from lesioned and intact forebrain. Each determination was the mean of triplicate estimates on pooled substantia nigra tissue from 10 animals per experiment. For decortication, only two experiments were performed. The values in parentheses represent the data from lesioned hemispheres expressed as a percentage of values obtained for the intact forebrain.

*$P < 0.05$ compared with substantia nigra preparations from intact forebrain using a two-tailed Student's t test.

Table 5 The effects of lesions on specific [^{3}H]-N,*n*-propylnorapomorphine (2.0 nM) binding to tissue preparations of substantia nigra from lesioned and intact forebrain

Lesion	*Specific binding* (pmol/g wet wt. tissue) *Intact*	*Lesioned*	
6-OHDA lesion of MFB	5.78	2.56	(44%)
	4.87	2.77	(57%)
	5.45	3.64	(67%)
	5.37 ± 0.27	2.99 ± 0.33*	(56%)
Kainic acid lesion of substantia nigra	4.72	2.74	(58%)
	4.71	3.37	(72%)
	5.74	3.72	(65%)
	5.06 ± 0.34	3.28 ± 0.29*	(65%)
Kainic acid lesion of striatum	5.16	3.06	(59%)
	5.04	2.99	(59%)
	6.05	3.65	(60%)
	5.42 ± 0.32	3.23 ± 0.21*	(60%)
Decortication	5.97	5.87	(98%)
	5.00	4.68	(94%)
	5.49	5.28	(96%)

Results given are from three individual experiments; also shown are the mean (± 1 s.e.mean) for binding to substantia nigra from lesioned and intact forebrain. Each determination was the mean of triplicate estimates on pooled substantia nigra tissue from 10 animals per experiment. For decortication, only two experiments were performed. The values in parentheses represent the data from lesioned hemispheres expressed as a percentage of values obtained for the intact forebrain.

*$P < 0.05$ compared with substantia nigra preparations from intact forebrain using a two-tailed Student's t test.

specific [^{3}H]-NPA binding in striatal preparations from the lesioned forebrain compared to the intact side.

Effect of lesions on specific [^{3}H]-spiperone binding to substantia nigra

Unilateral destruction of the medial forebrain bundle with 6-OHDA 21 days beforehand caused a 77% increase in specific nigral [^{3}H]-spiperone (4.0 nM) binding compared to the intact forebrain (Table 4).

Unilateral kainic acid lesions of substantia nigra 21 days previously caused a 67% decrease in specific nigral [^{3}H]-spiperone binding by comparison to the unlesioned nigra (Table 4).

Unilateral lesioning of the striatum with kainic acid 21 days previously produced a 56% increase in specific nigral [^{3}H]-spiperone binding when compared to the unlesioned side (Table 4).

Unilateral removal of the frontal and parietal cortex 5 days previously increased specific nigral [^{3}H]-spiperone binding by about 34% compared to the intact forebrain (Table 4).

Effect of lesions on specific [^{3}H]-N,n-propylnorapomorphine binding to substantia nigra

Unilateral 6-OHDA lesioning of the medial forebrain bundle 21 days previously reduced specific [^{3}H]-NPA (2.0 nM) binding to nigral preparations by 44% compared with the intact forebrain (Table 5). Unilateral kainic acid lesions of the substantia nigra 21 days previously also caused a 35% decrease in specific nigral [^{3}H]-NPA binding compared to the intact substantia nigra (Table 5).

Unilateral kainic acid lesions of the striatum 21 days previously similarly reduced specific nigral [^{3}H]-NPA binding by 40% compared to the unlesioned forebrain (Table 5).

Unilateral removal of the frontal and parietal cortex 5 days beforehand had no effect on specific nigral [^{3}H]-NPA binding compared to the intact forebrain (Table 5).

Histological examination of lesion sites

6-Hydroxydopamine lesions of the medial forebrain bundle caused an almost complete loss of the large, darkly staining cell bodies in the pars compacta of the substantia nigra on the lesioned side. Kainic acid lesions of striatum caused neuronal cell loss and gliosis in lateral and ventral areas of the anterior striatum (Figure 1). In many cases there was also some damage to the frontal cortex, lateral aspects of the nucleus accumbens, globus pallidus and areas of the ventral forebrain.

Kainic acid lesions of substantia nigra were confined to more caudal aspects of this body, and were characterized in particular by the loss of neuronal perikarya in the pars reticulata. Gliosis was evident around the needle tract throughout the pars reticulata, pars compacta and the area just dorsal to the caudal substantia nigra. Some loss of neuronal

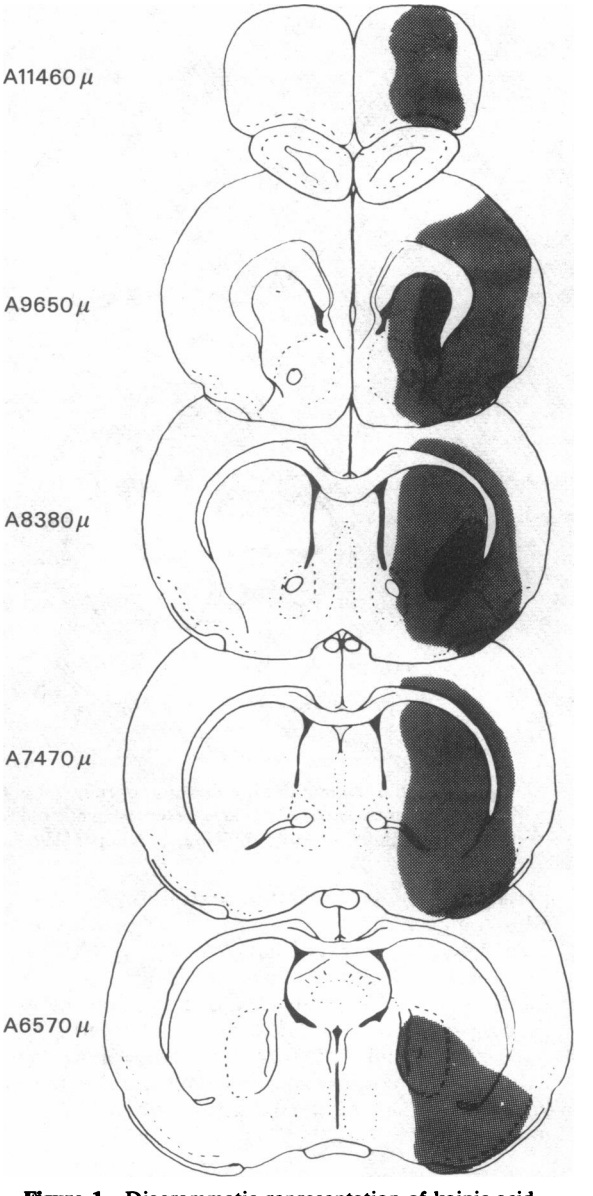

Figure 1 Diagrammatic representation of kainic acid lesions of the striatum. The hatched areas represent the most extensive area of damage observed, while black areas represent the area of damage common to all animals. Levels according to the atlas of König & Klippel (1963).

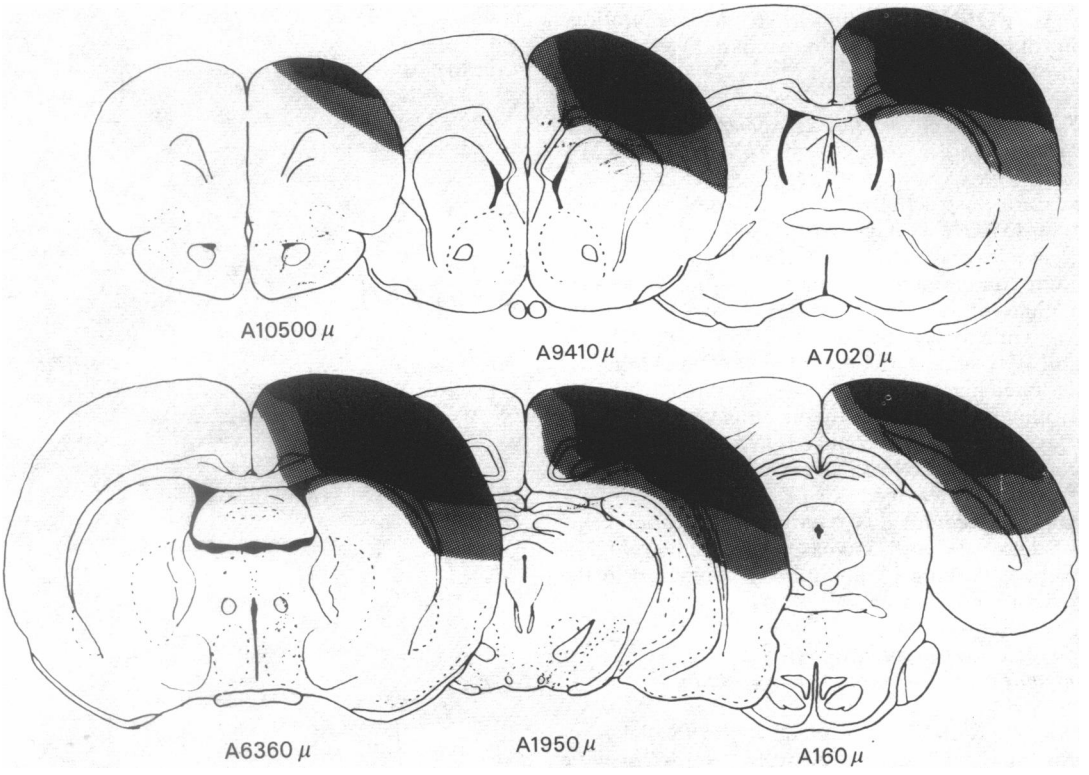

Figure 2 Diagrammatic representation of removal of the frontal and parietal cortex. The hatched areas represent the most extensive area of damage observed, while black areas represent the area of damage common to all animals. Levels according to the atlas of König & Klippel (1963).

perikarya was evident in the central aspects of the pars compacta, although damage was not as widespread as in the pars reticulata.

Unilateral decortication removed large areas of the frontal and parietal cortex, damage typically extending forward to A 12120 and caudally to P 100 (König & Klippel, 1963). In many cases damage was evident to the corpus callosum, while damage to the striatum was slight and confined to the most dorsal aspects (Figure 2).

Discussion

Lesioning of the striatum and substantia nigra has shown the existence of a number of different anatomical sites at which dopamine receptors labelled by [^{3}H]-spiperone or [^{3}H]-NPA are found. The data also show that agonist and antagonist binding sites have a differential distribution within the striatum and substantia nigra.

Specific binding of [^{3}H]-spiperone defined by (−)-sulpiride in striatum labels adenylate cyclase-independent D-2 dopamine receptor binding sites (Jenner, Clow, Reavill, Theodorou & Marsden, 1980). Alterations in specific [^{3}H]-spiperone binding in striatum caused by the lesions were in good agreement with the previous reports on the localization of D-2 receptors (see Table 1). Thus, the decrease in specific [^{3}H]-spiperone binding following destruction of striatal cells by kainic acid concurs with the finding of many other groups and supports the hypothesis of D-2 sites located on these cells. Similarly, 6-OHDA-induced destruction of the medial forebrain bundle causing loss of striatal presynaptic dopamine terminals did not decrease but rather increased the number of [^{3}H]-spiperone binding sites. This confirmed the findings of many others, although some workers have been unable to demonstrate any change in [^{3}H]-spiperone binding (see Table 1). The increase in receptor numbers, presumably resulting from denervation-induced proliferation of binding sites, again demonstrates a postsynaptic location for D-2 receptors. This conclusion is confirmed by the almost identical change induced by the destruction of ascending dopamine fibres following injection of kainic acid into substantia nigra.

Extensive removal of frontal and parietal cortex

caused a decrease in specific [^{3}H]-spiperone binding confirming the presence of D-2 receptors on the presynaptic terminals of cortico-striate glutamate fibres (Schwarcz *et al.*, 1978).

The localization and characterization of the [^{3}H]-NPA binding site appears to be more complex. We have already discussed the variable effect lesions have been reported to have on striatal agonist binding sites. We find [^{3}H]-NPA binding sites to be located both on presynaptic dopamine terminals and on striatal cell bodies. Thus, both 6-OHDA- and kainic acid-induced destruction of the medial forebrain bundle reduced [^{3}H]-NPA binding suggesting a presynaptic location. This would agree with the previous studies of others using [^{3}H]-apomorphine (Nagy, Lee, Seeman & Fibiger, 1978). However, kainic acid lesions of striatum also reduced [^{3}H]-NPA binding, also suggesting a postsynaptic location. In contrast, decortication had no statistically significant effect on [^{3}H]-NPA binding suggesting that few if any, agonist binding sites lie on presynaptic glutamate terminals in striatum.

Why do the results of lesions give such variable changes in agonist binding in different studies? The major consideration would appear to be the wide variation in tissue preparation and assay condition. The binding of agonist ligands ([^{3}H]-NPA, [^{3}H]-apomorphine, [^{3}H]-ADTN, [^{3}H]-dopamine) has proved notoriously difficult, particularly because of the high degree of non-specific binding that may occur to tissue. Weinreich & Seeman (1980) have suggested that various assay conditions enable specific labelling of different receptors. Thus the properties of the postsynaptic agonist site (D-2) may differ sufficiently from those of the presynaptic site (D-3) so as to make small change in assay procedure of critical importance.

The small amount of nigral tissue available allowed the use of only a single saturating ligand concentration which was determined by saturation analysis in tissue from untreated rats. The rationale was to detect changes in *Bmax* rather than any change in K_D that might occur. Our findings on the effect of lesions on [^{3}H]-spiperone binding in nigral tissue are not in agreement with previous reports. The 75% increase in specific [^{3}H]-spiperone binding occurring after destruction of dopamine cell bodies using 6-OHDA contrasts with the 43% and 50% reduction in [^{3}H]-spiperone binding reported by Reisine *et al.*, (1979) and Quik, Emson & Joyce (1979) respectively, and also with the lack of effect on [^{3}H]-spiperone reported by Nagy and colleagues (1978). However, there are differences between these studies and the present work which might explain the discrepancy. We used a saturating concentration of [^{3}H]-spiperone (4.0 nM) to detect changes in *Bmax*. In contrast, studies showing a decrease or no change in specific binding used sub-saturating ligand concentrations (<0.5 nM). Differences in the time allowed following the lesion for neuronal adaptation might be of importance. Thus, studies showing decreased binding were performed 7 or 14 days after surgery, whereas we allowed 21 days since this had previously been shown effective in striatum (Schwarcz *et al.*, 1978). It is conceivable that the 6-OHDA lesion did cause a loss of [^{3}H]-spiperone binding sites on nigral cell bodies but that this was compensated for by proliferation of dopamine receptors at other sites within nigra due to loss of nigral dopamine release. The alternative explanation is that a pattern of change in dopamine receptor numbers and dissociation constant occurs within nigra with time as demonstrated in striatum following 6-OHDA lesions. Whatever the explanation, the present results would suggest that considerable numbers of dopamine receptors in nigra are to be found at locations other than on dopamine cell bodies.

This suggestion is supported by the dramatic decrease in specific [^{3}H]-spiperone binding caused by kainic acid lesions of substantia nigra. Such lesions will destroy the dopamine containing cell bodies of zona compacta as well as non-dopamine cell bodies in both zona compacta and reticulata. It is difficult to dismiss the possibility that some loss of [^{3}H]-spiperone binding sites may be due to dopamine receptors on dopamine cell bodies but since we observed no decrease after 6-OHDA lesions the conclusion must be that the majority of these binding sites are located on non-dopamine containing cell bodies. These might either be the cell bodies of GABA containing outflow pathways to the thalamus (Straughan, Macleod, James & Kilpatrick, 1980), superior colliculus (Kilpatrick, Collingridge & Starr, 1982) and reticular formation (Graybiel & Sciascia, 1975), or GABA interneurones within substantia nigra (Kilpatrick, Starr, Fletcher James & Macleod, 1980; Ribak, Vaughan & Roberts, 1980).

Specific [^{3}H]-NPA binding in nigra showed a different pattern of change following the various lesions. Destruction of the medial forebrain bundle using 6-OHDA caused a loss of specific [^{3}H]-NPA binding which would be consistent with the location of agonist labelled dopamine receptors on dopamine neurones. Kainic acid lesions of substantia nigra caused a similar loss of specific [^{3}H]-NPA binding. This may also represent loss of agonist sites on dopamine cell bodies, but the possibility that some sites may lie on non-dopamine cell bodies in nigra cannot be ignored. Other [^{3}H]-NPA binding sites would appear to lie on the terminals of strio-nigral fibres for kainic acid lesions of striatum caused a loss of specific [^{3}H]-NPA binding. In contrast, decortication failed to alter [^{3}H]-NPA binding in nigra, again suggesting that dopamine agonist receptors do not lie

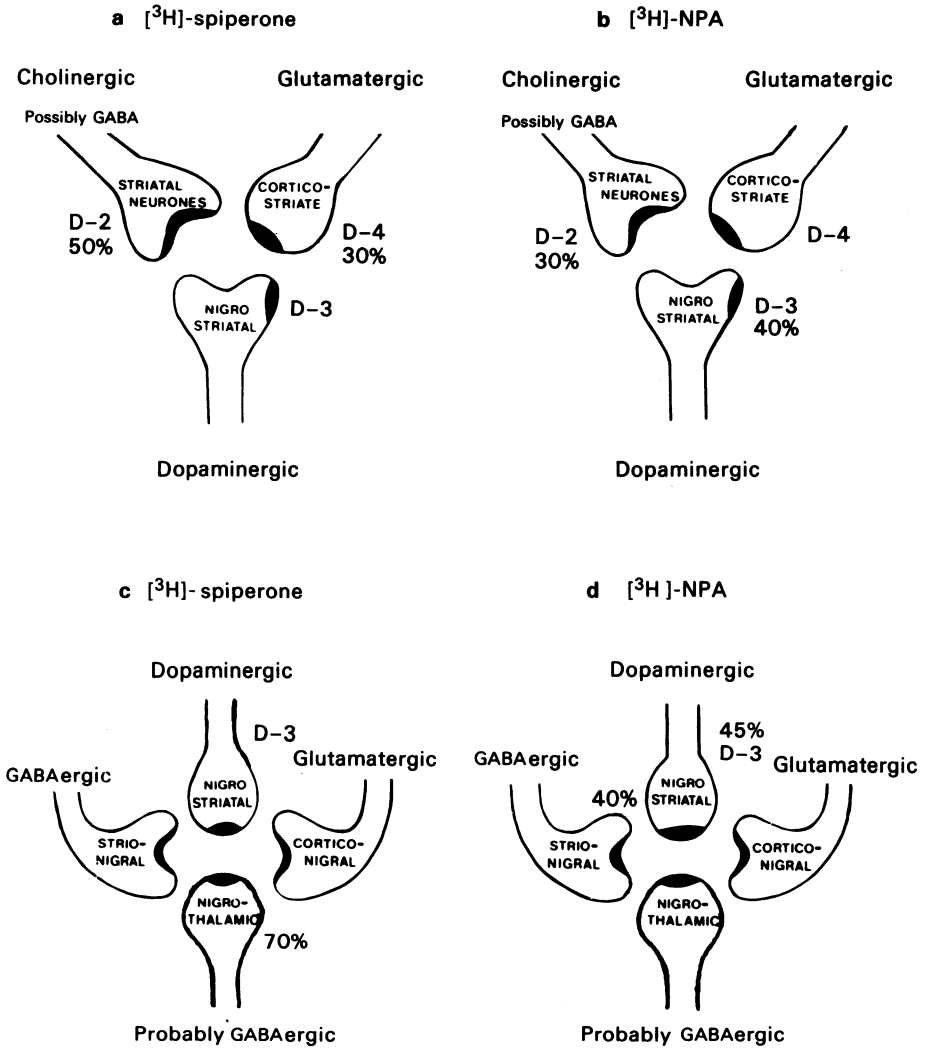

Figure 3 Summary diagram of the possible anatomical location of [^{3}H]-spiperone and [^{3}H]-N,*n*-propylnorapomorphine ([^{3}H]-NPA) binding sites in rat striatum (a and b) and in rat substantia nigra (c and d) as determined from the binding data.

on the terminals of cortico-nigral fibres removed by our surgical technique.

In conclusion, lesions of the substantia nigra and striatum have shown that agonist and antagonist binding sites, as measured by binding techniques, do have different anatomical locations within the strio-nigral complex (Figure 3). In addition, we have provided further evidence for the anatomical location of dopamine receptors within these two brain regions showing that receptors are found at a number of sites in each area. Lastly, the effects of lesions on the number of binding sites may not necessarily reflect only the removal of the particular neuronal element for, as pointed out by Hattori & Fibiger (1981), adaptive changes may occur so as to mask the true effect on the receptor population.

This study was supported by the Medical Research Council, The Wellcome Trust and the Research Funds of King's College Hospital and the Bethlem Royal and Maudsley Hospitals. M.D.H. and E.K. are M.R.C. Scholars. Correspondence to C.D.M., please.

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(Received December 28, 1982
Revised January 28, 1983.)