CHANGES IN [³H]MUSCIMOL BINDING IN SUBSTANTIA NIGRA, ENTOPEDUNCULAR NUCLEUS, GLOBUS PALLIDUS, AND THALAMUS AFTER STRIATAL LESIONS AS DEMONSTRATED BY QUANTITATIVE RECEPTOR AUTORADIOGRAPHY¹

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Abstract

A quantitative autoradiographic technique for measuring the binding of [³H]muscimol to central nervous system GABA receptors is described using tritium-sensitive film. [³H]Muscimol binding was studied in primary and secondary striatal projection areas of rat brain following kainic acid lesions of the striatum. Seven days after the lesion, binding affinities in the striatum and its projection areas were not altered significantly. There was a loss of [³H]muscimol receptors in the striatum. Receptors increased in numbers in the ipsilateral globus pallidus (19%), entopeduncular nucleus (22%), and substantia nigra pars reticulata (38%). [³H]Muscimol binding was decreased in the ipsilateral anteroventrolateral and ventromedial (8%) thalamic nuclei. [³H]Muscimol binding in other brain areas (layer IV of the cerebral cortex, central gray, superior colliculus, and stratum moleculare of hippocampus) was not affected.

The findings suggest that a loss of striatal innervation resulted in increased numbers of GABA receptors in striatal projection sites. It is further suggested that loss of inhibitory striatal inputs to neurons in the entopeduncular nucleus and substantia nigra pars reticulata may activate GABAergic projections to thalamus and thus result in decreased numbers of thalamic GABA receptors.

A major focus for neurochemists and neuropharmacologists in the past has been the study of nigrostriatonigral relationships. More recently, attention has been given to basal ganglia connections with thalamus. In various mammals, the major output of striatum is to lateral (LGP or globus pallidus, GP, in rodents and carnivores) and medial globus pallidus (MGP or entopeduncular nucleus, EPN, in rodents and carnivores) and substantia nigra pars reticulata (SN_r) (Nauta and Mehler, 1966; Johnson and Rosvold, 1971; Yoshida et al., 1972). The latter two project to thalamus (Carpenter and Strominger, 1967; Uno and Yoshida, 1975; Carter and Fibiger, 1978; Herkenham, 1979; van der Kooy and Carter, 1981) and to brainstem (Beckstead et al., 1979; Graybiel and Ragsdale, 1979; Garcia-Rill et al., 1981; Jackson and Crossman,

1981). Despite variations in the relative size of projections, the basic chemical and physiological characteristics of many of these pathways are conserved phylogenetically.

It is known that, in rat, striatal output mainly arises from medium-sized spiny neurons (Grofova, 1975; Bunney and Aghajanian, 1976; Somogyi et al., 1981). Many of these cells send axon collaterals to GP, EPN and SN_r (Preston et al., 1980). The bulk of these projections are GABAergic (Fonnum et al., 1978; Nagy et al., 1978), but there is also evidence for striatal output neurons containing enkephalin (particularly to GP) (Cuello and Paxinos, 1978; Sar et al., 1978; Pickel et al., 1980; DiFiglia et al., 1981), substance P (Brownstein et al., 1977; Jessel et al., 1978), cholecystokinin (CCK) (Emson et al., 1980), and angiotensin II (Arregui et al., 1979; Sirett et al., 1981). Output from EPN and/or SN_r to the anteroventrolateral (AVL) (Penney and Young, 1981), ventromedial (VM) (DiChiara et al., 1979; Kilpatrick et al., 1980; MacLeod et al., 1980), lateral habenular (LH) (Gottesfeld and Jacobowitz, 1978; Jones and Mogensen, 1980), and parafascicular (PF) (van der Kooy and Carter, 1981) nuclei of the thalamus and to the deep layer of the superior colliculus (SC) (Vincent et al., 1978) may be GABAergic as well. It has been shown that striatal output neurons make direct

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synaptic contact on neurons in SN_r, which project to VM (Somogyi et al., 1979). It is possible, therefore, that GABAergic striatal output inhibits GABA neurons in EPN and SN_r, which in turn project to thalamus and brainstem. Thus, striatal output might function to dis-inhibit the thalamus via sequential GABAergic neurons.

One approach to investigating the role of various neurotransmitters in basal ganglia function is to measure changes in neurotransmitter receptors in various regions of the brain after specific basal ganglia lesions. Several studies reporting increased numbers of GABA receptors in substantia nigra after lesions of striatonigral projections have appeared (Waddington and Cross, 1978, 1980; Guidotti et al., 1979). Rats with striatal kainic acid lesions have been proposed as a neurochemical model for Huntington's disease, a dominantly inherited, adult-onset striatal degeneration in humans (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976). In postmortem studies of Huntington's disease, GABA receptors have been found to be decreased in striatum and thalamus, whereas they are increased in globus pallidus and substantia nigra (Enna et al., 1976; Lloyd et al., 1977; Iversen et al., 1978; Penney and Young, 1982). In the rat striatal kainic acid model, GABA receptors have not been studied in regions other than the substantia nigra. The other areas of interest (GP, EPN, LH, PF, AVL, and VM) are small, and measurement of receptors in these areas would require pooling of tissue from many animals if traditional homogenate techniques were used.

Recently autoradiographic techniques have been developed which allow neurotransmitter receptors to be demonstrated both qualitatively (Young and Kuhar, 1979; Herkenham and Pert, 1981) and quantitatively (Palacios et al., 1981; Penney et al., 1981a, b; Rainbow et al., 1981). We report here an investigation of GABA receptors in striatum, GP, EPN, thalamus, and brainstem of rats with unilateral kainic acid lesions of striatum using quantitative receptor autoradiography.

Materials and Methods

Preparation of tissue and plastic standards. Paste was made from whole rat brain by repeated freezing and thawing followed by homogenization. Aliquots were thoroughly mixed with various amounts of [³H]quinuclidinyl benzilate (QNB) (33 Ci/mmol, New England Nuclear), frozen into cylinders, and mounted on microtome chucks. This procedure yielded tissue standards containing known but varying concentrations of radioactivity. Twenty-micron sections of the cylinders were thaw mounted onto subbed (0.5 gm/liter of chrome albumin) microscope slides, a sheet of Ultrofilm ³H (LKB) was apposed to the sections, and the slides were placed in an x-ray cassette along with commercial (Amersham) or prepared (see below) ¹⁴C standards. After a 3- to 30-day exposure at 4°C, the film was developed in D-19 for 5 min at 20°C, fixed, and dried. Film density was determined by microdensitometry (Dauth et al., 1981), and the relationship between the exposure produced by ³H in brain tissue and that produced by plastic ¹⁴C standards was determined. In all subsequent studies, ¹⁴C standards were routinely exposed along with the labeled tissue being studied.

Because the range of activities available in commercial

¹⁴C autoradiographic standards was found to be inadequate to cover the range of tissue ³H concentrations of interest, we constructed a set of 12 plastic standards with a 1000-fold range of ¹⁴C concentrations. Spurr low viscosity embedding plastic (Electron Microscopy Sciences) was mixed according to the manufacturer's instructions and divided into aliquots. The plastic was made radioactive by the introduction of [¹⁴C]succinic anhydride (Amersham) in trace volumes of benzene or toluene to the mixture before curing. Following thorough mixing and degassing, the plastic was then transferred to a mold and cured at 70°C.

Densitometry. Densitometry was performed using a spot densitometer developed by Dauth et al. (1981). A photographic enlarger was used to project an image of the autoradiogram onto a photosensitive diode located beneath a 1-mm aperture at the center of the enlarger's image plane. The enlarger was set to give a 10-fold magnification. Thus, 100- μ diameter spots could be measured. Readings of different spots were made by moving the autoradiographic film in the enlarger's film plane. Eight to 16 readings from randomly selected spots in each region of interest were averaged. The concentration of radioactivity in each region of the original section was determined by a computer-generated polynomial regression analysis which compared the film densities produced by the regions with those produced by the standards.

Lesions. Male Sprague-Dawley rats weighing 150 to 200 gm were anesthetized with 3.8 mg/kg of xylazine and 77 mg/kg of ketamine (i.p.). Kainic acid (Sigma), 0.1 M, dissolved in 0.01 M Tris adjusted to pH 7.0 with HCl was applied into the right striatum of these rats through 50- μ tip glass micropipettes using an adaptation of the electrophoretic method of Graybiel and Devore (1974) and Stephenson et al. (1981). Negative current (3 μ A) was passed in 500 msec on/500 msec off pulses for 30 min. Angled injections from 30° forward of vertical passing through prefrontal cortex were made using a David Kopf small animal stereotaxic device. The pipette tip was placed 2.0 mm anterior, 3.0 mm lateral, and 4.2 mm ventral to bregma with the incisor bar 5 mm above the ear bars (anteroposterior +8.0 mm, mediolateral +3.0 mm, dorsoventral +1.0 mm according to the atlas of König and Klippel, 1963).

Autoradiographic studies. One week after the striatal lesion, rats were decapitated, and the brains were quickly removed, mounted on microtome chucks with Lipshaw embedding medium, and frozen at -20°C. Twenty-micron coronal sections of the brains were cut in a cryostat (Harris) and thaw mounted onto detergent-cleaned, subbed microscope slides. Slides were kept frozen at -20°C until assayed. We found GABA receptors to be stable for at least 90 days under these conditions.

Mounted sections were subjected to three consecutive 5-min prewashes in 50 mM ice-cold Tris/citrate buffer, pH 7.0, to remove as much endogenous GABA as possible while retaining the integrity of the brain sections. Washed sections were then dried with a stream of cold air. Sections were immersed in various concentrations (3 to 150 nM) of [³H]muscimol (9.5 Ci/mmol, Amersham) with or without different concentrations of nonradioactive drugs. All chemicals were dissolved in 50 mM Tris/citrate buffer, pH 7.0. Bicuculline solutions were prepared

immediately prior to use. Nonspecific binding was determined in the presence of 0.1 mM GABA in adjacent sections. For kinetic studies, the sections were incubated for 0 to 60 min at 4°C. In equilibrium binding studies the sections were incubated for 30 min at 4°C. At the end of the incubation period, each section was subjected to three consecutive 3-sec rinses with ice-cold buffer and blown dry with cold air. Using these conditions, at 50 nM $[^3\text{H}]$ muscimol, approximately 5000 dpm were bound specifically/20- μ brain section. Nonspecific binding, determined in the presence of 0.1 mM GABA, was about 400 dpm per section. Thus, the signal to noise ratio was greater than 10:1. Slides were placed in x-ray cassettes along with appropriate standards and Ultrofilm ^3H (LKB) was apposed to the sections. After a 12-day exposure, the film was developed as described above. Regional isotope concentrations were determined by comparing densitometric readings from images of brain sections to those produced by the standards. After the exposure, the sections were fixed with paraformaldehyde vapor then stained with cresyl violet.

Additionally, the binding of $[^3\text{H}]$ quinuclidinyl benzilate, $[^3\text{H}]$ spiroperidol, and $[^3\text{H}]$ naloxone were examined. $[^3\text{H}]$ QNB binding was carried out as described previously (Fisher et al., 1981). Slides for studying $[^3\text{H}]$ spiroperidol binding were prewashed twice for 5 min in ice-cold 0.17 M Tris·HCl buffer containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 , 0.001% ascorbic acid, and 1 μM pargyline, pH 7.7. Slides were then incubated with 4 nM $[^3\text{H}]$ spiroperidol (40 Ci/mmol, Amersham) in the buffer at 20°C for 1 hr with or without 100 μM dopamine and/or 100 μM serotonin and subsequently washed with two 5-min buffer rinses at 4°C, a brief dip rinse in 4°C distilled water, and dried. $[^3\text{H}]$ Naloxone binding was studied by a modification of a previously described technique (Lewis et al., 1981). Slides were prewashed for two consecutive 5-min washes in 50 mM Tris·HCl, pH 7.4, at 4°C. Sections were then incubated with 2 nM $[^3\text{H}]$ naloxone (45 Ci/mmol, Amersham) in 50 mM Tris·HCl containing 100 mM NaCl, pH 7.4, at 4°C for 1 hr and were

subjected to six 20-sec washes in 50 mM Tris·HCl at 4°C. Autoradiographic exposure times were 14 days for $[^3\text{H}]$ QNB and 21 days for $[^3\text{H}]$ spiroperidol and $[^3\text{H}]$ naloxone.

Results

Quantitative autoradiography using Ultrofilm ^3H . Film density and tissue ^3H concentration were found to be sigmoidally related over the range of radioactivity levels and exposure times studied. Log-logit transformation of the data from each film yielded a linear plot as demonstrated in Figure 1. Equivalent tissue ^3H values were assigned to individual plastic standards by interpolation using the densities of the ^3H brain paste images from the same film. This procedure yielded calibration values for the ^{14}C plastic standards expressed in terms of tissue ^3H concentration which were independent of exposure time over a range of 3 to 30 days (Table I).

$[^3\text{H}]$ Muscimol binding to rat brain. Scatchard analysis of equilibrium saturation data of $[^3\text{H}]$ muscimol binding (3 to 150 nM) to layer IV of the cortex reveals a linear plot suggesting one population of receptor binding sites (Fig. 2). The plot gives a K_D of 56 nM and a B_{max} of 1.00 pmol/mg of tissue. Competitive studies of $[^3\text{H}]$ muscimol binding using various agents was consistent with the pharmacology observed in other studies (Table II). At a concentration of 100 μM , GABA was the most potent displacer, causing complete displacement. The GABA antagonist (+)bicuculline followed in potency, giving 75% displacement at 100 μM , whereas picrotoxin was totally ineffective at the same concentration. One hundred micromolar strychnine, a glycine antagonist, caused 45% displacement. The GABA uptake blockers, 2,4-diaminobutyric acid and β -alanine, as well as α -alanine, taurine, serine, proline, and glycine, had little effect at 100 μM .

Histology. The striatum, globus pallidus, and substantia nigra were atrophied on the side of the lesion. A loss of neuronal nuclei and Nissl substance was observed in the striatum, with an increase in astrocytic and microglial nuclei (Fig. 3). Lesions typically involved the dorsal and

Figure 1. Relationship between ^3H tissue concentration and voltage output from spot densitometer using LKB Ultrofilm. Densitometer voltage is proportional to film density. The logit transformation produces a linear plot of the sigmoidal relationship between the log of ^3H in brain paste standards and film density.

TABLE I

Tissue ^3H calibration values of ^{14}C plastic standards

^{14}C concentration is expressed in dpm/ml of uncured plastic as determined by liquid scintillation spectroscopy. ^3H values for the standards are expressed in dpm/ μg of protein and represent the mean $\pm$ SEM of repeated determinations under varied exposure conditions (see the text).

Standard	^{14}C	^3H	n
1	8.21×10^3	4.11 ± 0.35	5
2	1.71×10^4	7.40 ± 0.33	8
3	3.10×10^4	14.0 ± 0.6	11
4	6.61×10^4	28.6 ± 0.9	11
5	9.37×10^4	44.3 ± 1.2	12
6	2.09×10^5	97.4 ± 3.3	12
7	3.21×10^5	157 ± 5	12
8	7.43×10^5	397 ± 19	9
9	1.21×10^6	720 ± 43	9
10	2.17×10^6	1290 ± 90	9
11	3.88×10^6	2070 ± 100	7
12	6.79×10^6	3350 ± 280	6

Figure 2. Representative Scatchard analysis of $[^3\text{H}]$ muscimol binding to layer IV of the cerebral cortex. Twenty-micron serial sections of a frozen normal rat brain were prewashed, incubated in 3 to 150 nM $[^3\text{H}]$ muscimol with or without 100 μM GABA, and processed as described in the text. Densitometric readings of layer IV of the cortex were taken. The resultant Scatchard plot is linear with a correlation coefficient of 0.98 and yields a K_D of 56 nM and a B_{max} of 1.00 pmol/mg of tissue. Each point is the mean of 16 determinations. The experiment has been repeated three times.

lateral striatum from the level of the rostral end of the corpus callosum to that of the anterior commissure. In some animals the lesion extended into layer VI of overlying cortex. Distant symmetric neuronal loss was seen occasionally in the basal nuclei of the amygdala and in the pyramidal cell layers of region CA₃ of the hippocampus and the pyriform cortex on both sides of the brain. The density of neuronal and microglial nuclei was increased in the globus pallidus, entopeduncular nucleus, and substantia nigra pars reticulata. There were no changes in ventral lateral nucleus of thalamus or substantia nigra pars compacta.

$[^3\text{H}]$ Muscimol binding in rats 7 days after unilateral striatal kainate lesion. Scatchard analyses of the striatum and its projection areas suggested that the receptor numbers, not affinities, were changed. $[^3\text{H}]$ Muscimol binding was decreased to $83 \pm 4\%$ that of the unlesioned striatum (ST) (Fig. 4A) at the lesion site. Ipsilateral to the lesion $[^3\text{H}]$ muscimol binding was increased by $19\% \pm 3$ in GP (Fig. 4B), $22\% \pm 7$ in EPN (Fig. 4C), and $38\% \pm 8$ in SN_r (Fig. 4D). There was $8\% \pm 2$ decrease in

binding in ipsilateral AVL/VM (Fig. 4C). Representative Scatchard analyses are shown in Figure 5. Table III summarizes the changes in binding properties. Although some of the changes in receptor numbers were small, they were seen consistently in all animals studied and proved statistically significant. Some side-to-side differ-

TABLE II

Pharmacology of $[^3\text{H}]$ muscimol binding

Autoradiograms were generated as described in the text. Eight densitometric readings of the cerebral cortex, layer IV, were taken from each section in each experiment. The amounts of $[^3\text{H}]$ muscimol bound in sections co-incubated with displacers are expressed as percentages of those incubated with $[^3\text{H}]$ muscimol only. The values represent the mean $\pm$ SEM of two to five experiments.

Compound	Displacement (% Control Binding)	n
No displacer	100 ± 4	5
10 μM GABA	6 ± 3	2
100 μM GABA	0 ± 3	5
100 μM bicuculline	25 ± 3	5
100 μM strychnine	55 ± 9	5
100 μM β -alanine	77 ± 3	5
100 μM 2,4-diaminobutyric acid	78 ± 4	2
100 μM α -alanine	88 ± 4	4
100 μM taurine	91 ± 5	5
100 μM picrotoxin	99 ± 5	4
100 μM serine	100 ± 5	5
100 μM glycine	105 ± 6	5
100 μM proline	107 ± 6	5

Figure 3. Histology of 7-day-old kainate-lesioned rat striatum. The drawing indicates the site of the lesion. The inset at the lower left is a cresyl violet-stained section showing the normal striatal architecture. The inset at the lower right shows a loss of neuronal nuclei and Nissl substance with an increase in microglial nuclei. The inset at the upper right shows the preservation of pyriform cortex after microiontophoretic injections of kainate.

Figure 4. Autoradiograms of $[^3\text{H}]$ muscimol binding in coronal sections of rat brain 7 days after unilateral kainate lesion of the striatum. Binding data were obtained from these autoradiograms as described in the under "Materials and Methods." *A*, Section through the striatum which shows a loss of $[^3\text{H}]$ muscimol binding in the lesion. *B*, Section through globus pallidus (*GP*) which shows increased receptor binding on the side ipsilateral to the lesion. *C*, Section through entopeduncular nucleus (*EPN*) and anteroventrolateral and ventromedial thalamic nuclei (*AVL/VM*) demonstrating increased binding in the former and decreased binding in the latter structure on the side ipsilateral to the lesion without change in the lateral habenula (*LH*). *D*, Section through substantia nigra pars reticulata (*SN_r*) showing increased receptor binding on the side ipsilateral to the lesion without change in the deep layers of the superior colliculus. The experiment has been replicated 6 to 12 times.

ences were seen occasionally in binding affinity, but these changes were not seen consistently and were not found to be statistically significant. No significant differences in binding were found in PF, LH, or SC. No differences in binding were observed in other brain areas (Table III).

Binding of other ligands. Autoradiograms of sections incubated with $[^3\text{H}]$ QNB, $[^3\text{H}]$ spiroperidol, and $[^3\text{H}]$ naloxone are shown in Figure 6, and densitometric readings are shown in Table IV. No differences in binding were found between right and left *SN_r* for $[^3\text{H}]$ QNB or $[^3\text{H}]$ naloxone, whereas a 12% increase in $[^3\text{H}]$ spiroperidol bound was found in *SN_r*.

Discussion

Methods for quantitative autoradiography of tissue employing densitometric analysis of x-ray film have been described previously utilizing ^{131}I - (Freygang and Soko-

loff, 1958) and ^{14}C -labeled tracers (Reivich et al., 1969). Previous reports from this laboratory (Penney et al., 1981a, b) and others (Faraco-Cantin et al., 1980; Alexander et al., 1981; Palacios et al., 1981; Rainbow et al., 1981) describe adaption of the autoradiographic method to detection of ^3H -labeled tracers using LKB Ultrofilm. The present study confirms previous reports (Alexander et al., 1981; Penney et al., 1981a, b) that densitometric evaluation of Ultrofilm images can be used to quantify regional ^3H concentrations in brain tissue. Furthermore, we have demonstrated that ^{14}C -labeled plastic standards can serve to control for variability in exposure and development conditions in ^3H autoradiography. Thus, unlike methods employing ^3H reference standards, no correction for isotope decay with storage of the standards is necessary.

Using quantitative autoradiography, saturation, ki-

Figure 5. Representative Scatchard plots of data obtained from autoradiograms shown in Figure 4. Receptor numbers (B_{max}) were altered significantly as a result of the unilateral lesion, whereas binding affinities (K_D) were not changed. At the site of lesion, there was a 17% decrease in number of [^3H] muscimol binding sites. B_{max} on the side ipsilateral to the lesion increased by 19% in globus pallidus (GP); 22% in entopeduncular nucleus (EPN); and 38% in substantia nigra pars reticulata (SN_r). However, there was a decrease in B_{max} in anteroventral and ventromedial thalamic nuclei on the side ipsilateral to the lesion. The experiment has been replicated 6 to 12 times.

TABLE III

[^3H]Muscimol binding to rat brain 1 week after striatal kainate lesions

Receptor numbers (B_{max}) (mean $\pm$ SEM) of the various areas on the side contralateral to the lesion are given in picomoles per milligram of tissue. B_{max} values of corresponding structures ipsilateral to the lesion are expressed as ratios of those on the control side.

Region	Control Side B_{max}	Lesioned Side (% Control)	n	p^a
Striatum	0.38 ± 0.03	83 ± 4	7	<0.016
Globus pallidus	0.54 ± 0.04	118 ± 4	12	<0.038
Substantia nigra pars reticulata	1.28 ± 0.14	138 ± 8	12	<0.006
Entopeduncular nucleus	0.28 ± 0.02	122 ± 7	12	<0.002
Ventroanterior lateral thalamus	0.85 ± 0.06	92 ± 2	11	<0.012
Cortex, layer IV	1.25 ± 0.09	91 ± 4	6	NS
Stratum moleculare	1.01 ± 0.07	105 ± 2	6	NS
Superior colliculus, superior layer	1.01 ± 0.17	100 ± 2	6	NS
Central gray	0.94 ± 0.16	98 ± 3	6	NS

^a Two-tailed sign test, NS, not significant.

netic, and competitive studies were carried out to establish the characteristics of the binding of the GABA receptor agonist, muscimol. Previous studies had found binding to be a saturable process with widely varying B_{max} but relatively constant K_D (Penney et al., 1981b).

Figure 6. Autoradiograms of [^3H]QNB, [^3H]naloxone, or [^3H]spiroperidol (with 100 M serotonin displacement) binding in SN 7 days post-kainate lesion. Binding conditions and procedures were as specified in the text. No differences in binding were found between left and right SN_r for [^3H]QNB (A) or [^3H]naloxone (B). A 12% increase in [^3H]spiroperidol binding was noted in SN_r (C). Densitometric readings of these autoradiograms are tabulated in Table III. The experiment was repeated four times.

TABLE IV

Binding of other ligands in SN_r 1 week postlesion

Binding in SN_r contralateral to the lesion is expressed in picomoles of ligand bound per milligram of tissue (mean $\pm$ SEM). Binding on the lesioned side is given as percentages of that on the control side. The values represent the mean $\pm$ SEM of four experiments for each ligand.

Ligand		Control Side	Lesioned Side (% Control)
$[^3\text{H}]\text{QNB}$	SN _r	0.200 $\pm$ 0.002	99 $\pm$ 3
$[^3\text{H}]\text{Spiroperidol}$	SN _r	0.75 $\pm$ 0.006	112 $\pm$ 8 ^a
$[^3\text{H}]\text{Naloxone}$	SN _r	0.45 $\pm$ 0.002	95 $\pm$ 6

^a $p < 0.1$ by two-tailed paired t test.

Binding was nearly complete in 20 min and k_1 was $15 \times 10^6 \text{ M}^{-1} \text{ min}^{-1}$ and k_{-1} was 0.46 min^{-1} (Penney et al., 1981b). Scatchard analysis of the binding curves over the ligand range of 3 to 150 nM indicates a homogeneous population of receptors. The plot gives a K_D of 56 nM and a B_{max} of 1.00 pmol/mg of tissue in layer IV of the cerebral cortex. The K_D is slightly higher than that suggested by homogenate studies using tritonized or extensively washed and freeze-thawed membranes (Beaumont et al., 1978; Williams and Risley, 1979; DeFeudis, 1980) or by binding studies using thin sections from lightly fixed tissue (Palacios et al., 1980). It is consistent, however, with the K_D observed in lightly washed, nontritonized tissues (Beaumont et al., 1978; Leach and Wilson, 1978; Snodgrass, 1978). The K_D obtained from equilibrium saturation data is consistent with the K_D ($45 \text{ nM} \pm 2$ (SEM)) obtained from kinetic data of $[^3\text{H}]\text{muscimol}$ binding reported previously (Penney et al., 1981b). The discrepancy in K_D values between previous homogenate studies and the present study presumably reflects differences in tissue preparation and fixation. It is unlikely that the prewashing procedure used in this study removes all endogenous GABA, and thus it is possible that a higher affinity site has not been unmasked. Nevertheless, no high affinity site was seen at ligand concentrations in the low nanomolar range. Also, no consistent differences in K_D were seen between binding in intact striatal projection areas and in deafferented ones after unilateral striatal kainate lesions. Striatal lesions are known to produce large decreases in endogenous GABA concentrations in striatal projection areas, and, if endogenous GABA was not adequately washed out, one might expect to see K_D differences between the intact and lesioned areas.

Some of the B_{max} values obtained in the present study are higher than those reported in the literature. In the homogenate studies, $[^3\text{H}]\text{muscimol}$ binding represents an average of binding in the area of interest and neighboring regions with varying receptor concentrations due to limitations in dissection. Using the autoradiographic method, specific structures of interest can be studied without contamination from neighboring regions containing little $[^3\text{H}]\text{muscimol}$ binding.

The pharmacology of $[^3\text{H}]\text{muscimol}$ binding is comparable to that described previously (Beaumont et al., 1978; Snodgrass, 1978). The present method yields a complete displacement of $[^3\text{H}]\text{muscimol}$ by 100 μM non-radioactive GABA. At 100 μM , the GABA antagonist, (+)bicuculline, which competes with binding to the GABA receptor site, caused 75% displacement. Given

that the inhibitory constant, K_i , and the amount of displacement are related by $K_i = \text{IC}_{50} / (1 + L/K_D)$ and that the concentrations of $[^3\text{H}]\text{muscimol}$ and bicuculline used were 150 nM and 100 μM , respectively, 75% displacement seen with 100 μM bicuculline corresponds to a K_i for bicuculline of 10 to 15 μM , similar to that seen in other studies (DeFeudis, 1980). Picrotoxin (100 μM), an antagonist which does not bind to the GABA binding site, was not effective in the displacement of muscimol, as found by other investigators (Enna et al., 1977; Greenlee et al., 1978; Williams and Risley, 1979). Strychnine (100 μM) displaced 45% of $[^3\text{H}]\text{muscimol}$ binding. Although strychnine is known to be a specific glycine antagonist (Curtis et al., 1971), at high concentrations it has been shown to partially block the action of GABA (Scholfield, 1982) and to inhibit $[^3\text{H}]\text{GABA}$ binding with an IC_{50} of 33 to 50 μM (Zukin et al., 1974; Lloyd et al., 1977). Other amino acids and the uptake blocker, 2,4-diaminobutyric acid produced little displacement. This evidence suggests that $[^3\text{H}]\text{muscimol}$ binding in the present study represents selective binding to synaptic GABA receptors.

We investigated changes in GABA receptor numbers and affinities after striatal lesions. Unilateral microelectrophoretic application of kainic acid to the striatum of rats was used to produce lesions with maximal local destruction of striatal neurons and with minimal distant effects (Stephenson et al., 1981). Many striatal output cells are thought to be inhibitory and GABAergic (Fonnum et al., 1978; Nagy et al., 1978). These cells may send collaterals to GP, EPN, and SN_r (Yoshida et al., 1972; Bishop et al., 1982). Electrophysiological data suggest that EPN and SN_r are one brain structure separated by the internal capsule (DeLong and Georgopoulos, 1979). Lesions of EPN and SN_r may, therefore, result in similar receptor changes in their respective projection areas. Recent evidence also implicates the pallidothalamic, nigrothalamic, and nigrocollicular pathways as inhibitory and GABAergic (DiChiara et al., 1979; Kilpatrick et al., 1980; MacLeod et al., 1980; Penney and Young, 1981). If striatal output disinhibits thalamus, then striatal lesions could result in increased activity of entopedunculothalamic and nigrothalamic GABAergic projections. The effect of striatal lesions might, therefore, be an increase in GABA receptors in primary striatal projection areas (GP, EPN, and SN_r) and a decrease in GABA receptors in the secondary projection areas (AVL/VM). Others have seen increases in GABA receptors in SN_r after lesions of striatonigral pathways (Waddington and Cross, 1978, 1980; Guidotti et al., 1979). In the studies reported here, increases in receptor densities were observed in GP, EPN, and SN_r ipsilateral to the lesion, whereas a decrease in $[^3\text{H}]\text{muscimol}$ binding was found in the ipsilateral AVL/VM. Equilibrium saturation data indicated that the changes in receptor densities resulted from a change in B_{max} whereas K_D remained essentially constant. Layer IV of cortex, central grey, stratum moleculare, and substantia nigra pars compacta, areas which are not known to receive GABAergic striatal input, showed no significant changes in $[^3\text{H}]\text{muscimol}$ binding on the two sides of the brain.

The regions of brain which sometimes showed distant necrosis (amygdala, hippocampus, and pyriform cortex) have no known direct connections with globus pallidus, entopeduncular nucleus, substantia nigra, or ventral thal-

amus. Furthermore, the distant lesions were symmetric on the two sides of the brain. Thus, these distant lesions would be an unlikely source of the unilateral receptor changes observed in this study.

To examine the possibility that the observed changes in [^3H]muscimol binding were due to shrinkage after deafferentation, the binding of [^3H]QNB (a cholinergic muscarinic antagonist), [^3H]spiroperidol (a dopamine antagonist), and [^3H]naloxone (an opiate antagonist) were additionally studied. These ligands were chosen for study because they have not been found to be coupled to GABA receptors and yet are present in striatal projection areas. Binding of [^3H]QNB and [^3H]naloxone was symmetric in SN_r of lesioned rats. A 12% increase in [^3H]spiroperidol binding in SN_r was observed. This change may represent a trans-synaptic effect of striatal lesions on nigral dopamine autoreceptors. Alternatively, the change in the amount of spiroperidol bound may indicate the increase in the density of cells receiving striatal input due to the loss of presynaptic terminals. Taking this base level shrinkage phenomenon into consideration, GABA receptors are still increased in SN_r ipsilateral to the lesion. This potential concentrative phenomenon would not affect AVL/VM because it receives secondary projections from the striatum. Therefore, we conclude that the changes observed in [^3H]muscimol binding represent a true increase in the number of GABA receptors, not an artifactual change due to tissue shrinkage.

Changes in GABA receptor density could not be demonstrated in PF, LH, or SC. There is evidence that SC receives GABAergic input from SN_r (Vincent et al., 1978). PF receives input from collaterals of the EPN cells which project to AVL/VM while LH gets input from other EPN cells (van der Kooy and Carter 1981). SC and PF are thus analogous to AVL/VM as receiving areas for second-order striatal output. The apparent lack of GABA receptor changes in LH and PF may be related to the low concentration of muscimol binding sites in these regions which precludes accurate quantitation.

Substance P (Brownstein et al., 1977; Jessel et al., 1978), enkephalin (Cuello and Paxinos, 1978; Sar et al., 1978), angiotensin (Sirett et al., 1981), and CCK (Emson et al., 1980) are also contained in striatal efferents. Perhaps loss of one or more of these peptidergic systems is responsible for the GABA receptor changes found. Further studies must be carried out to establish what the effects of these peptides are on neurons in striatal projection areas.

The striatal kainate model has been proposed as a neurochemical model of Huntington's disease (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976). It is of interest that changes in GABA receptors similar to those found in this study have been found also in the brains of patients dying of Huntington's disease (Enna et al., 1976; Lloyd et al., 1977; Iversen et al., 1978; Waddington and Cross, 1980; Penney and Young, 1982).

On a more general level, quantitative autoradiography provides a sensitive assay for receptor changes in immediate and trans-synaptic projection areas of a lesioned site. It also makes possible biochemical investigation of receptors in many discrete areas which previously required pooling of tissues.

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