ing: differential attentional demands may have confounded the results. In the two experiments reported here we controlled both for attentional and for motor requirements; no differences in alpha activity resulted, in terms of cognitive and emotional processes. However, EEG alpha activity is important in its ability to reflect attentional processes. In addition, even with the motor and attentional controls, we report beta differences reflecting both cognitive and emotional dimensions (*25*), suggesting that EEG beta may be a useful measure of appropriate cognitive and emotional processes.

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26. Supported in part by BRSG grant RR07082-14 (NIH) and the Faculty Research Fund (Liberal Arts) of Pennsylvania State University.

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Widespread Distribution of Brain Dopamine Receptors Evidenced with [^{125}I]Iodosulpride, a Highly Selective Ligand

Abstract. *The new benzamide derivative [^{125}I]iodosulpride is a highly sensitive and selective ligand for D-2 dopamine receptors and displays a very low nonspecific binding to membrane or autoradiographic sections. On autoradiographic images, D-2 receptors are present not only in well-established dopaminergic areas but also, in a discrete manner, in a number of catecholaminergic regions in which the dopaminergic innervation is still unknown, imprecise, or controversial, as in the sensorimotor cerebral cortex or cerebellum. This widespread distribution suggests larger physiological and pathophysiological roles for cerebral dopamine receptors than was previously thought.*

More than 20 radioactive ligands have been used to label dopamine receptors, and these ligands have already provided much information about the pharmacology, biochemistry, and localization of the receptors, as well as information about the mode of action of antipsychotic drugs (*1*). Nevertheless, the ligands available until now either have lacked selectivity or have a relatively high nonspecific binding, so that a detailed autoradiographic mapping of cerebral dopamine receptors, particularly in regions of low density, has not been obtained despite several attempts (*2*). Ligands labeled with ^{125}I, because their specific radioactivity is 50 to 100 times higher than that of corresponding ^{3}H-labeled ligands, have been successfully used in sensitive assays of various receptors (*3*) but so far have not been proposed for dopamine receptors. We have now shown that [^{125}I]iodosulpride—that is, *N*-[(1-cyclopropylmethyl-2-pyrrolidinyl) methyl]-2methoxy-4-iodo-5-ethylsulfonylbenzamide (specific activity, 2000 Ci/mmol)—is a highly selective dopamine ligand that allows the demonstration of dopamine receptors in cerebral areas in which they were only suspected or were not known to occur.

[^{125}I]Iodosulpride was prepared (*4*), and its properties were first assessed in standard filtration assays. With 0.2 n*M* [^{125}I]iodosulpride, binding was linear with up to 100 μg of tissue protein in striatum and up to at least 150 μg of tissue protein in substantia nigra (Fig. 1A). The sensitivity of the receptor assay is such that 5 μg of striatal or 40 μg of nigral protein per incubation was sufficient to ensure a total binding value twice as high as the nonspecific binding plus the filter blank. Scatchard and computer-assisted (*5*) analyses of the saturation curve at equilibrium (reached after 15 to 20 minutes at 30°C) revealed an apparently homogeneous population of striatal sites (Fig. 1B) with a dissociation constant (Kd) of 1.6 ± 0.3 n*M*, a Hill coefficient of 0.99 ± 0.12, and a maximum number of binding sites (B_{max}) of 449 ± 25 fmol per milligram of protein, the latter value being closely similar to the B_{max} of [^{3}H]domperidone in the same preparation. The pharmacology of striatal [^{125}I]iodosulpride recognition sites (Fig. 1C) was also similar to that of sites labeled with [^{3}H]domperidone, which is generally recognized (*5, 6*) as the most selective ligand yet available for D-2 receptors—that is non–D-1 receptors, according to the nomenclature of Kebabian and Calne (*7*). More recently, there has been a proposal to distinguish two subclasses of [^{3}H]domperidone sites in striatum (termed D-2 and D-4, respectively, among which only D-2 sites are present in the pituitary) on the basis of discrimination by a few benzamide derivatives like sulpiride (but not metoclopramide) (*5*). Initial studies indicate that

iodosulpride does not discriminate these two putative subclasses of the D-2 receptor, as shown by the value of its Hill coefficient close to unity. Hence the number and pharmacology of [^{125}I]iodosulpride binding sites, both similar to those of [^{3}H]domperidone (*8*), together with the much lower nonspecific binding, suggest that [^{125}I]iodosulpride is, by far, the most sensitive and selective D-2 receptor ligand now available.

[^{125}I]Iodosulpride should prove useful in a number of studies of these receptors (pharmacology in regions of low density, purification, and so forth). However, the potential usefulness of [^{125}I]iodosulpride is best illustrated in autoradiographic studies performed by the method developed by Kuhar and his colleagues (*9*). Well-contrasted images of regions known to receive major dopaminergic inputs and to contain high densities of receptors (for example, the striatum, nucleus accumbens, or olfactory tubercles) were obtained after a few hours of exposure (not shown). With a 5-day exposure, additional binding sites with a widespread distribution were present (except in fiber regions like the corpus callosum) (Fig. 2), and their occurrence was almost completely prevented when incubations took place in the presence of apomorphine in moderate (10 μM) concentration (compare a and b in Fig. 2 or d and e in Fig. 2). With a slightly higher apomorphine concentration (25 μM) or with 5 μM (−)-sulpiride the nonspecific binding was almost nullified in most areas, including the cerebellum (not shown).

Many labeled areas corresponded to well-established dopaminergic areas containing either the cells of origin in mesencephalon (substantia nigra or ventral tegmental area) or the projection fields of the mesostriatal (striatum, globus pallidus, and nucleus accumbens), mesocortical (olfactory tubercles, septum, and entorhinal or frontal cortex), or mesodiencephalic systems (lateral habenula and subthalamic nucleus). In these areas, the distribution and marked differences in density generally paralleled those of dopaminergic innervation (*10–12*). For instance, [^{125}I]iodosulpride sites of moderate density were localized in a discrete manner in the deepest layers of frontal cortex and in more superficial layers of the anterior cingulate cortex, apparently matching exactly the distribution of cortical dopaminergic innervation (*13*).

A dopaminergic innervation of the hippocampus, probably emanating from the ventral tegmental area, has been recently proposed but is still controversial, and its precise projection has not been established (*14*). The high density of sites restricted to the stratum lacunosum moleculare of the dorsal hippocampus (Fig. 2, a and d), which decreases slightly from CA_1 to CA_3, strongly suggests that it projects mainly to this layer.

Specific [^{125}I]iodosulpride sites were also present in areas in which dopaminergic innervation is not yet unequivocally established. Thus in all studied regions of the cerebral neocortex—for example, in the sensorimotor parietal areas (Fig. 2, a and d) or in the visual posterior areas (not shown)—large continuous bands of sites were observed mainly at the level of the deepest layers (approximately layers

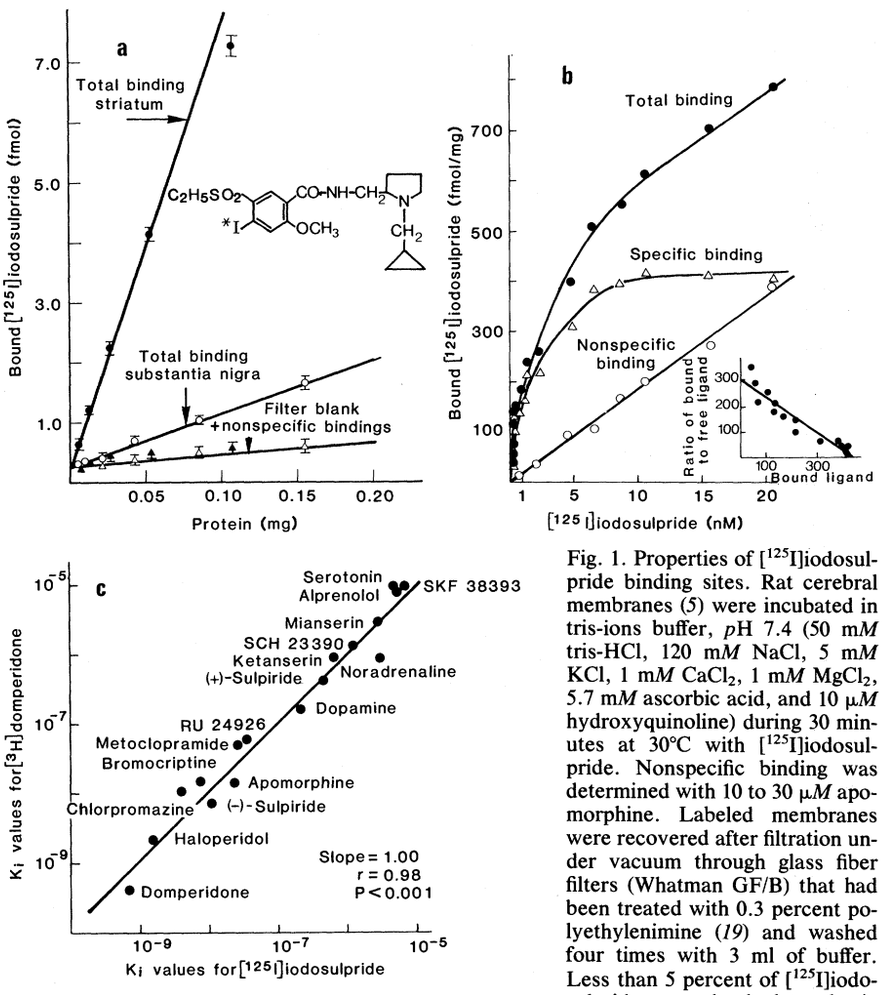

Fig. 1. Properties of [^{125}I]iodosulpride binding sites. Rat cerebral membranes (*5*) were incubated in tris-ions buffer, *p*H 7.4 (50 m*M* tris-HCl, 120 m*M* NaCl, 5 m*M* KCl, 1 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 5.7 m*M* ascorbic acid, and 10 μM hydroxyquinoline) during 30 minutes at 30°C with [^{125}I]iodosulpride. Nonspecific binding was determined with 10 to 30 μM apomorphine. Labeled membranes were recovered after filtration under vacuum through glass fiber filters (Whatman GF/B) that had been treated with 0.3 percent polyethylenimine (*19*) and washed four times with 3 ml of buffer. Less than 5 percent of [^{125}I]iodosulpride was adsorbed to plastic tubes, and binding to filters corresponded to 0.2 to 0.4 percent of total radioactivity. (a) Influence of tissue concentration. Striatal or nigral membranes in various amounts were incubated with 0.2 n*M* [^{125}I]iodosulpride in the absence (circles) or presence (triangles) of 25 μM apomorphine (incubation volume, 400 μl). Values were not corrected for binding of [^{125}I]iodosulpride to the filters. Values are given as means ± S.E.M. of triplicate determinations. The slopes were 75.3, 9.0, and 1.9 fmol per milligram of protein for the total binding in the striatum and the substantia nigra, and for the nonspecific bindings, respectively. Inset: structure of [^{125}I]iodosulpride. (b) Saturation of [^{125}I]iodosulpride binding to striatal membranes. Nonspecific binding was measured in the presence of 25 μM apomorphine. For up to 0.6 n*M* [^{125}I]iodosulpride, the total incubation volume was 1.6 ml, whereas for higher concentrations the incubation volume was 0.4 ml, and [^{125}I]iodosulpride was diluted with nonradioactive iodosulpride, so that bound [^{125}I]iodosulpride never exceeded 4 percent of the total. All values were corrected for filter blanks. Means of two experiments with triplicates. Inset: Scatchard representation of the specific binding. The ratio of bound to free ligand is given as femtomoles per milligram of protein per nanomole per liter and bound ligand is given as femtomoles per milligram of protein. (c) Compared potencies of various agents as displacers of [^{125}I]iodosulpride and [^{3}H]domperidone bindings on striatal membranes. [^{125}I]Iodosulpride (0.1 n*M*) was incubated in the presence of the indicated compounds in 6 to 10 concentrations. The IC_{50} values were determined from pooled data obtained in two independent experiments with the use of a nonlinear regression computer program for a one-site model (*5*). K_i values (moles per liter) were derived according to the relation $K_i = IC_{50}/(1 + L/K_d)$ where K_d = 1.5 n*M*. K_i values for [^{3}H]domperidone were obtained from (*5*). For clonidine, scopolamine, etorphine, and histamine, IC_{50} values were higher than 50 μM.

V and VI). Although dopaminergic projections were so far not described in these large neocortical regions, their presence would be consistent with indirect neuroanatomical data (*15*).

In the cerebellum, in which the presence of dopamine-containing afferents is highly controversial (*16*), a moderate binding largely displaceable by apomorphine occurred in the gray matter. The presence of dopamine-related sites pharmacologically similar to those characterized in striatum was confirmed in studies with membranes from the cerebellum and parietal cortex (*17*).

Labeling inhibited by 25 μM apomorphine or 5 μM (−)-sulpiride also occurred in the inferior and superior colliculi (particularly the superior gray layer), the spinal nucleus of the trigeminal nerve and the mammillary bodies, but the identity of these sites as D-2 receptors remains to be confirmed by detailed pharmacological analysis similar to those performed in striatum, parietal cortex, and cerebellum. In all of these areas a catecholamine innervation occurs but so far has been considered exclusively noradrenergic (*10, 11, 18*).

Although in well-established dopaminergic areas, the density of [^{125}I]iodosulpride sites seems to strictly parallel that of dopaminergic projections, the possibility remains that in the other areas these sites do not correspond to a dopaminergic innervation. Because of the limitations of available histochemical methods, it is still difficult to identify dopaminergic projections in areas where they are sparse relative to the noradrenergic projections (*10, 11*). However, novel methodologies (retrograde tracing and histochemistry after selective destruction of noradrenergic neurons) have provided evidence for several new dopaminergic projections (*11, 12, 16, 18*). Hence, our data suggest that dopaminergic projections within mammalian brain may be more widely distributed than was previously thought and therefore that new brain areas have to be considered in the pathophysiology of conditions in which dopaminergic systems seem to participate, as well as in the mode and loci of action of drugs such as the antipsychotics.

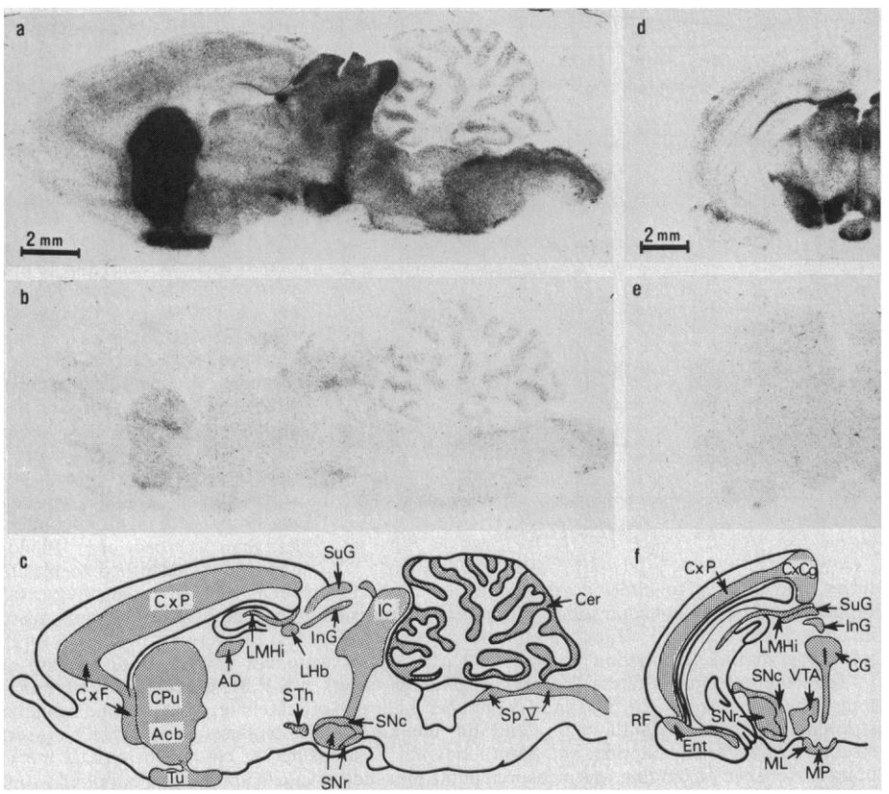

Fig. 2. Autoradiograms of rat brain sections generated with [^{125}I]iodosulpride. Cryostat sections (15 μm) were prepared and incubated for 30 minutes at 25°C in tris-ions buffer containing 0.3 nM [^{125}I]iodosulpride in the absence (a and d) or the presence (b and e) of 10 μM apomorphine to determine nonspecific binding. After two 2-minute rinses in fresh buffer, sections were dried and apposed on ^{3}H-Ultrofilm (LKB) during 5-day periods. Structures in the sagittal (a, b, c; lateral 1.9 mm) or frontal sections (d, e, f; interaural 3.7 mm) were identified with a brain atlas (*20*). Abbreviations: Acb, nucleus accumbens; AD, anterodorsal thalamus nucleus; CG, central gray matter; Cer, cerebellum; CPu, caudate putamen; CxCg, cingulate cortex; CxF, frontal cortex; CxP, parietal cortex; Ent, entorhinal cortex; IC, inferior colliculus; InG, intermediate gray layer of superior colliculus; LHb, lateral habenular nucleus; LMHi, lacunosum moleculare layer of CA_1-CA_2 hippocampus; ML, median mammillary nucleus, pars lateral; MP, median mammillary nucleus, pars posterior; RF, rhinal fissure; SNc, substantia nigra, pars compacta; SNr, substantia nigra, pars reticulata; SpV, nucleus spinal of trigeminal nerve; STh, subthalamic nucleus; SuG, superficial gray layer of superior colliculus; Tu, olfactory tubercle; and VTA, ventral tegmental area.

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17. A highly reproducible specific binding of 0.15 n*M* [^{125}I]iodosulpride representing 1.1 ± 0.1 and 0.6 ± 0.1 fmol per milligram of protein was detected in parietal cortex and cerebellum, respectively (corresponding values being 54.0 ± 2.5 in striatum and 2.0 ± 0.3 in frontal cortex) over a nonspecific binding defined with 25 μ*M* apomorphine corresponding to less than 20 percent of the total (filter blank deduced). In both regions, the pharmacology of these sites was studied with the dopaminergic and nondopaminergic compounds depicted in Fig. 1C for striatal sites. No significant difference in the three regions was found for the values of the median inhibition constant (IC_{50}) of the various agents (for example, the IC_{50} value of (−)-sulpiride was 11.8 ± 2.0, 12.0 ± 1.8, and 12.3 ± 2.3 n*M* and of (+)-sulpiride 495 ± 123, 423 ± 56, and 553 ± 188 n*M*, in the striatum, parietal cortex, and cerebellum, respectively). Moreover, the extremely high correlation between the three sets of IC_{50} values indicates that the sites labeled by [^{125}I]iodosulpride in the parietal cortex and cerebellum have a dopaminergic nature (slope, 0.998; $r = 0.989$, $P < 0.001$; slope, 1.036, $r = 0.996$, $P < 0.001$ between striatum and parietal cortex and between striatum and cerebellum, respectively) (M.-P. Martres, M.-L. Bouthenet, N. Salés, P. Sokoloff, J.-C. Schwartz, in preparation).
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Detection of Serum Antibodies to Borna Disease Virus in Patients with Psychiatric Disorders

Abstract. *Borna disease virus causes a rare meningoencephalitis in horses and sheep and has been shown to produce behavioral effects in some species. The possibility that the Borna virus is associated with mental disorders in humans was evaluated by examining serum samples from 979 psychiatric patients and 200 normal volunteers for the presence of Borna virus-specific antibodies. Antibodies were detected by the indirect immunofluorescence focus assay. Antibodies to the virus were demonstrated in 16 of the patients but none of the normal volunteers. The patients with the positive serum samples were characterized by having histories of affective disorders, particularly of a cyclic nature. Further studies are needed to define the possible involvement of Borna virus in human psychiatric disturbances.*

Borna disease virus causes a rare meningoencephalitis in horses and sheep in certain areas of Germany and Switzerland, where it has been endemic for over 150 years. The virus has not been classified, but because it may lead to persistent infections it is often considered to be a member of the slow virus group. The incubation period varies between a few weeks and several months. Characteristic symptoms of the disease are excitability or apathy, spasms, and partial paralysis. The disease is usually fatal (*1*).

The Borna virus has not been characterized biochemically. It replicates in a variety of cell lines after cocultivation with brain cells from infected animals. The virus persists in these cell lines and is noncytopathic. Intranuclear viral antigen can be demonstrated by immunohistology (*2*). Filtrates of brain homogenates from infected animals can be used to transmit the virus to a broad spectrum of animals ranging from chicken to chimpanzee, but the incubation periods and the clinical manifestations vary considerably. Whereas the course of the disease in some experimentally infected animals is similar to that observed in the natural disease of horses and sheep, in other species the disease remains subclinical or is evidenced only by behavior abnormalities (*1, 3, 4*). Behavioral changes resulting from Borna virus infection have been described in detail in the tree shrew *Tupaia glis* (*5*). The changes are manifested as a disinhibition toward the environment or, more specifically, as a reduction in cognitive ability. Infected tree shrews show a slight drowsiness and a disturbance in sexual behavior. Morphological studies implicate the limbic system in these alterations (*5*). Similar behavioral disorders occur in Borna virus–infected rats (*6, 7*), in which a virus-specific cellular immune response can be demonstrated (*7, 8*). Virus-specific antibodies can be demonstrated in the serum of infected animals by an immunofluorescence binding assay (*4*).

In view of the prominent central nervous system and behavioral effects produced by the Borna virus in experimentally infected animals, we wondered whether mental disorders in humans might, in some cases, be accompanied by the appearance of Borna virus–specific antibodies. To explore this possibility we obtained serum samples from 979 patients with emotional and depressive disorders from psychiatric clinics in the United States (Philadelphia) and in different areas of Germany (Giessen and Würzburg), and screened them for the presence of Borna virus–specific antibodies.

The patients in Philadelphia were attending the Depression Research Unit or the Lithium Clinic of the Hospital of the University of Pennsylvania. All of them were evaluated in a semistructured interview format, and diagnoses were assigned according to Research Diagnostic Criteria (*9*). Normal control subjects were obtained primarily from the hospital and university communities. They were evaluated in a similar semistructured interview format, and only those who were found to be free of significant medical illnesses, psychiatric disorders, or family histories of psychiatric illnesses were included in the study. Blood samples were obtained from a total of 285 patients with unipolar and bipolar depression and 105 normal healthy volunteers. The samples were centrifuged at 2500 rev/min for 15 minutes. The sera were then immediately frozen in coded tubes, in randomized order with respect to patients and healthy controls, and were shipped on dry ice to Giessen for analysis.

In addition, 686 psychiatric patients from Würzburg and eight patients from Giessen were evaluated, along with 95 control subjects. The patients were randomly selected from a heterogeneous population of hospitalized patients and represented a variety of psychiatric disturbances.

Antibodies were detected by the indirect immunofluorescence focus assay (*2, 4*). Sera were diluted 1:10 in swine serum, absorbed with swine liver powder (100 mg/ml) to eliminate nonspecific background staining, and added in twofold dilutions to acetone-fixed Madin Darby canine kidney (MDCK) cells persistently infected with Borna virus strain He/80, originally isolated from a horse (*4*). Cells were incubated for 30 minutes at 37°C, washed in phosphate-buffered saline, and reacted with fluorescein isothiocyanate (FITC)-conjugated goat antiserum to human immunoglobulin G