Br. J. Pharmac. (1987), **90**, 467–478

Pharmacological characterization of binding sites identified in rat brain following *in vivo* administration of [^{3}H]-spiperone.

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1 [^{3}H]-spiperone is commonly used to label dopamine receptors *in vitro* in brain tissue. However, spiperone also interacts with brain 5-hydroxytryptamine and noradrenaline receptors. *In vivo*, [^{3}H]-spiperone has been used for identifying dopamine receptors in both animals and man but the nature of the sites identified is unknown.

2 The *in vivo* administration of [^{3}H]-spiperone to rats leads to a selective accumulation of radioactivity in the olfactory lobes, tuberculum olfactorium, nucleus accumbens, striatum, substantia nigra, hippocampus, frontal cortex and hypothalamus, when compared to the cerebellum.

3 *In vivo* drug displacement studies suggest that the binding of [^{3}H]-spiperone in these areas may be to dopamine, 5-HT or noradrenaline receptors.

4 [^{3}H]-spiperone *in vivo* mainly labels dopamine receptors in striatum, tuberculum olfactorium, hypothalamus, substantia nigra and olfactory lobes. However, in the frontal cortex and nucleus accumbens specific binding involves not only dopamine receptors but also 5-HT and/or noradrenaline receptors.

5 Interpretation of *in vivo* studies in man using radioactive spiperone and its derivatives must take into account the fact that this ligand only labels dopamine receptors in some brain areas.

Introduction

Ligand binding assays employing compounds such as [^{3}H]-haloperidol, [^{3}H]-spiperone, and [^{3}H]-domperidone are used routinely for the characterization of brain dopamine receptors using *in vitro* tissue preparations. Much information has been gained on the organisation and distribution of dopamine receptor systems within the brain of rodents with such techniques (Seeman, 1980). However, the use of *in vitro* receptor binding methods does not examine the nature of dopamine receptors in the intact animal, nor does it take into account pharmacokinetic considerations of drug action. To overcome this problem, use has been made of the ability of tritiated ligands, such as [^{3}H]-spiperone, [^{3}H]-pimozide or [^{3}H]-N,*n*-propyl-norapomorphine ([^{3}H]-NPA) given intravenously to label dopamine receptors within brain tissue *in vivo* in rodents (Hollt *et al.*, 1977; Laduron *et al.*, 1978; Kuhar *et al.*, 1978; Niehoff *et al.*, 1979; Tewson *et al.*, 1980; Kohler *et al.*, 1981; Van der Werf *et al.*, 1983). Such ligands specifically accumulate in various regions of the brain from which they can be displaced by the administration of a high dose of unlabelled drug. Typically these experiments involve killing the animals and determining the radioactivity present in discrete brain regions. Recently, interest has focussed on the development of similar ligands containing ^{11}C, ^{18}F or ^{76}Br which might be used in man to establish the status of dopamine receptors as measured by positron emission topography (PET) (Tewson *et al.*, 1980; Arnett *et al.*, 1983; Maziere *et al.*, 1984; Moerlein & Stocklin, 1984).

In animals the most widely used ligand for labelling dopamine receptors both *in vitro* and *in vivo* is [^{3}H]-spiperone, which has a high affinity for dopamine receptors and shows a relatively slow dissociation from such sites (Leysen *et al.*, 1978). Some of the most promising candidates for use in man are derived from spiperone, such as [^{76}Br]-bromospiperone (Maziere *et al.*, 1984), [^{11}C]-spiperone (Arnett *et al.*, 1983). [^{18}F]-spiperone or [^{18}F]-N-methylspiperone (Arnett *et al.*,

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1985). However, none of these ligands used to identify brain dopamine receptors *in vivo* is specific for the brain dopamine receptor population. Unlabelled spiperone also interacts with both 5-hydroxytryptamine (5-HT) and noradrenaline receptors as well as dopamine receptors *in vitro* (List & Seeman, 1981).

The object of the present work was to characterize [^{3}H]-spiperone *in vivo* binding in the rat brain, and to study the ability of drugs with a range of pharmacological actions to displace the ligand from areas where it specifically accumulates.

Methods

Ligand administration, brain dissection and analysis of samples

Female Wistar rats (125–150 g; Bantin & Kingman) were used in all experiments. Animals were manually restrained and [^{3}H]-spiperone (5–45 μCi in 250 μl 0.9% saline equivalent to 1–10 μg; 21 Ci mmol^{-1}; Amersham International) was administered *via* the tail vein. Between 15 min to 8 h later the animals were anaesthetized with chloral hydrate (500 mg kg^{-1} i.p. in 0.6 ml 0.9% saline; British Drug Houses). The thoracic cavity was opened and blood was removed by cardiac puncture and placed in lithium heparin tubes. A cannula (1 mm diam.) was introduced into the aorta and the animals perfused with 0.9% saline (50 ml) to remove all blood; the jugular veins were severed to accommodate the overflow fluid. This procedure was carried out to reduce the non-specific/specific binding ratio (Niehoff *et al.*, 1979). Animals were then decapitated, the brain rapidly removed onto ice, and various brain areas dissected as described below.

The dissection of brain areas was based on the method described by Glowinski & Iversen (1966). The brain was positioned with its dorsal surface uppermost and the rhombencephalon was separated by removing the cerebellum with iris forceps and making a transverse section with a scalpel blade at a 30° angle from the dorsal surface of the medulla oblongata to the ventral surface of the brain, so as to expose the caudal surface of the substantia nigra. The brain was turned to expose the ventral surface and the substantia nigra was removed using micro-dissecting forceps. Iris forceps were used to dissect the hypothalamus, tuberculum olfactorium, olfactory lobes and nucleus accumbens. The brain was again turned to expose the dorsal surface and the two hemispheres of the forebrain were separated with iris forceps to expose the striata and hippocampus. The striatum (putamen and caudate nucleus) was removed with iris forceps. The hippocampus was then dissected out. A section of frontal cortex was removed by section with a scalpel blade.

The tissue samples were placed in Soluene 350 (1.0 ml; Packard) and dissolved. Following the addition of Instafluor II (10 ml; Packard) the samples were left in darkness for a minimum of 6 h to eliminate chemiluminescence. Subsequently radioactivity was assessed by scintillation spectrometry in a Packard 460C scintillation counter. The efficiency of counting varied between 36–39% depending on tissue weight and nature. Correction for counting efficiency was made and the radioactivity contained in each area was expressed in d.p.m. g^{-1} wet weight of tissue.

Time course and dose-related accumulation of [^{3}H]-spiperone binding in vivo

To determine the time course of [^{3}H]-spiperone accumulation in the various brain areas, animals received [^{3}H]-spiperone (25 μCi) 15 min–8 h before death. To determine the extent of dose-related accumulation of radioactivity following administration of [^{3}H]-spiperone *in vivo*, animals received [^{3}H]-spiperone (5–45 μCi) 1 h before death.

To determine the maximum degree of displacement of [^{3}H]-spiperone in each brain region, some animals received [^{3}H]-spiperone (25 μCi, i.v.) and simultaneously were given an intraperitoneal bolus of unlabelled spiperone (0.005–5.0 mg kg^{-1}) 1 h before death.

Displacement experiments

To determine the nature of binding of [^{3}H]-spiperone (25 μCi; 1 h before death) to brain tissue the following drugs were injected at the times stated prior to death and their effect on regional distribution of [^{3}H]-spiperone determined. In each case the dose of drug used and the timing of drug administration was based on previously reported studies showing behavioural or biochemical actions in brain. In other words, the dose of each drug at the time of its administration prior to [^{3}H]-spiperone were known to cause an appropriate pharmacological response at the time at which the animals were killed. The displacing agents used were (±)-sulpiride (40 mg kg^{-1}, i.p., 3 h previously; Delagrange), sultopride (40 mg kg^{-1}, i.p., 3 h previously; Delagrange), haloperidol (1.0 mg kg^{-1}, 1 h previously; Janssen Pharmaceutica), piflutixol (1 mg kg^{-1}, 2 h previously; Lundbeck), (+)- and (−)-butaclamol (5 mg kg^{-1}, 0.5 h previously; Ayerst Laboratories), cinanserin hydrochloride (5 mg kg^{-1}, 1.5 h previously; Squibb Laboratories), ketanserin (1 mg kg^{-1}, 2 h previously; Janssen Pharmaceutica), clonidine hydrochloride (0.05 mg kg^{-1}, 1.5 h previously; Boehringer), prazosin hydrochloride (1 mg kg^{-1}, 2 h previously; Pfizers Ltd.), propranolol hydrochloride (20 mg kg^{-1}, 2 h previously; ICI Ltd.), muscimol (5 mg kg^{-1}, 75 min previously; Fluka), nal-

trexone hydrochloride (1 mg kg^{-1}, 0.5 h previously; Endo Laboratories Ltd.), dexetimide (10 mg kg^{-1}, 1.5 h previously; Janssen Pharmaceutica), atropine sulphate (5 mg kg^{-1}, 0.5 h previously; Sigma), cimetidine (10 mg kg^{-1}, 1.5 h previously; SKF Ltd.) and mepyramine maleate (5 mg kg^{-1}, 1.5 h previously; May & Baker Ltd.).

In all drug displacement studies animals receiving [^{3}H]-spiperone in conjunction with a displacing agent were investigated alongside vehicle-treated animals also receiving [^{3}H]-spiperone. This procedure was used to negate variation in specific activity of the ligand, difference between batches of rats or other factors which might cause variation between experiments carried out on different occasions. Only values obtained for vehicle-treated animals on the same occasion as drug-treated animals were used to calculate the displacement caused by individual agents.

Calculation of data

In all experiments the accumulation of radioactivity in brain areas was determined by comparison to the cerebellum. The radioactivity contained in the cerebellum was chosen as a 'blank' value since there was little accumulation of radioactivity in this area and because administration of unlabelled drugs did not alter the radioactive content of the cerebellum. In addition, displacement of [^{3}H]-spiperone with a high dose of unlabelled spiperone could reduce activity to approximately cerebellar levels in each area studied. A total of 10 individual animals was used in each group. In displacement experiments the prevention of accumulation of radioactivity derived from [^{3}H]-spiperone following drug displacement was expressed as a percentage of the total binding obtained in rats treated with saline alone following subtraction of radioactivity present in the cerebellum. Total binding and drug displacement was carried out in 10 individual animals at each dosage level. All results were analysed by a Mann Whitney U test. A probability of less than 5% was considered to indicate a significant difference.

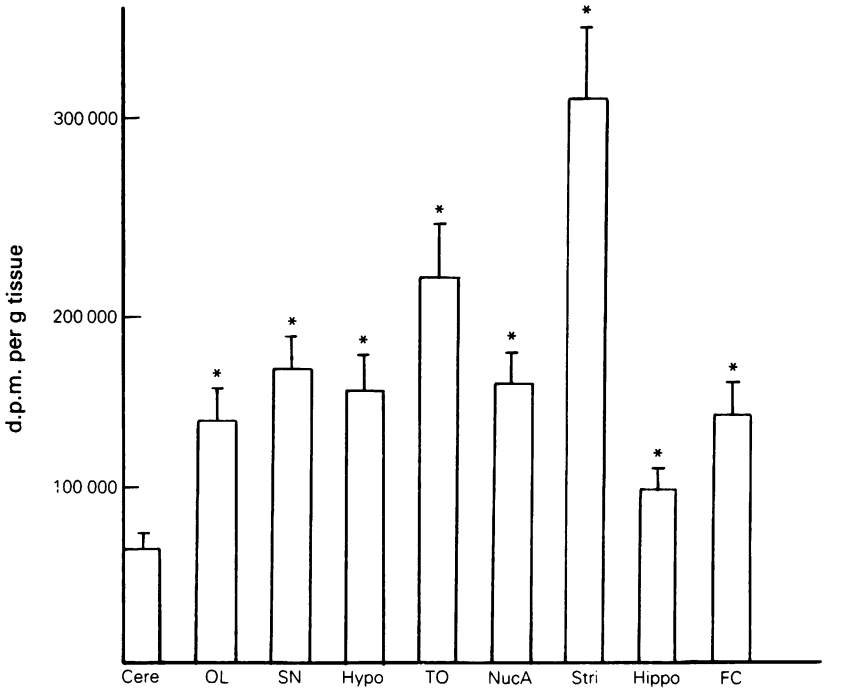

Figure 1 Accumulation of radioactivity derived from [^{3}H]-spiperone (25 μCi i.v.) in selected areas of rat brain. [^{3}H]-spiperone (25 μCi i.v.) was injected via the tail vein 1 h before death. Abbreviations: Cere – cerebellum, OL – olfactory lobes, SN – substantia nigra, Hypo – hypothalamus, FC – frontal cortex, TO – tuberculum olfactorium, NucA – nucleus acumbens, Stri – striatum, Hippo – hippocampus. Statistical analysis was carried out using a Mann Whitney U test and results are expressed as the mean for 10 animals in each treatment group with s.e.mean shown by vertical lines. *$P < 0.05$ for accumulation of radioactivity above cerebellar levels ± 1 s.e.mean.

Results

Distribution of [^{3}H]-spiperone in selected areas of rat brain (Figure 1).

The radioactivity derived from [^{3}H]-spiperone (25 μCi) administered 1 h beforehand accumulated in the olfactory lobes, substantia nigra, hypothalamus, tuberculum olfactorium, nucleus accumbens, striatum, hippocampus, and frontal cortex when compared to the cerebellum.

Dose-related accumulation of [^{3}H]-spiperone in selected regions of rat brain (Figure 2)

Administration of [^{3}H]-spiperone (5–45 μCi) 1 h before death caused a dose-related accumulation of total radioactivity in the olfactory lobes, substantia nigra, hypothalamus, tuberculum olfactorium, nucleus accumbens, striatum, hippocampus and frontal cortex. Since 25 μCi [^{3}H]-spiperone provided a reproducible and sufficiently large gate between total and non-specific binding in all areas, this dose of ligand was used for the displacement studies

Time course of accumulation of [^{3}H]-spiperone in seleted areas of rat brain (Figure 3)

Administration of [^{3}H]-spiperone (25 μCi i.v.) 15 min–8 h before death caused a time-dependent accumulation of radioactivity in the olfactory lobes, substantia nigra, hypothalamus, tuberculum olfactorium, nucleus accumbens, striatum, hippocampus, and frontal cortex when compared to the cerebellum.

In the striatum, radioactivity gradually increased following [^{3}H]-spiperone administration and reached a maximum between 1 and 4 h. By 8 h, levels of radioactivity derived from [^{3}H]-spiperone had declined only slightly. In other areas, namely olfactory lobes, hypothalamus, tuberculum olfactorium, hippocampus, and frontal cortex, total radioactivity increased up to 2 h following [^{3}H]-spiperone administration and then slowly declined. In the substantia nigra and nucleus accumbens there was a large accumulation of radioactivity 15 min following [^{3}H]-spiperone administration which then slowly declined. In the cerebellum, total radioactivity was maximal 15 min after [^{3}H]-spiperone administration and then gradually declined over the following 8 h period.

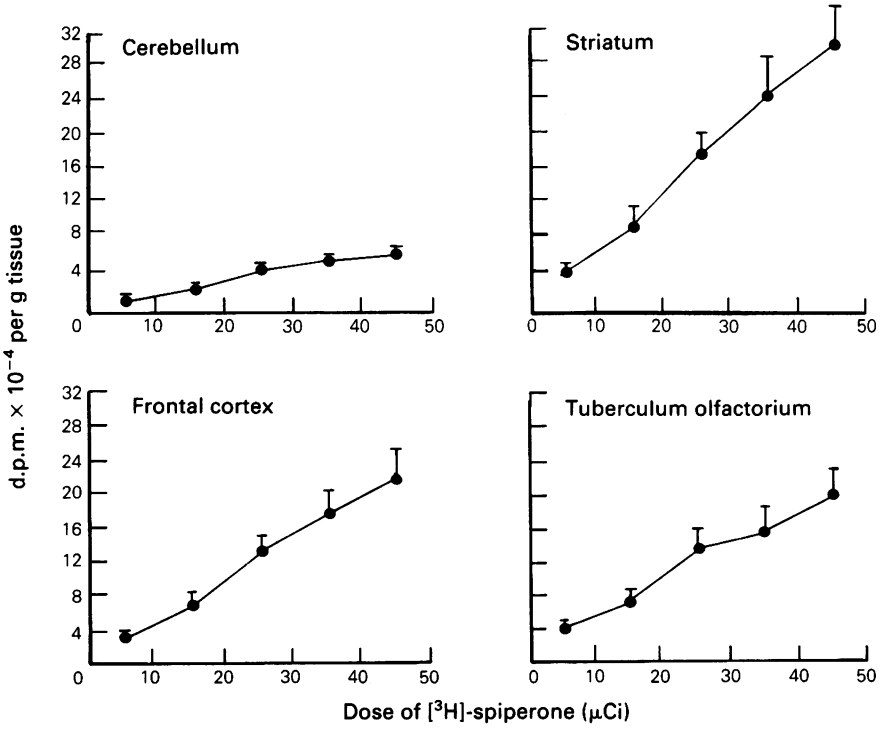

Figure 2 Accumulation of radioactivity derived from a range of doses of [^{3}H]-spiperone (5–45 μCi i.v.) in selected areas of rat brain. A range of doses of [^{3}H]-spiperone (5 μCi – 45 μCi i.v.) was administered *via* the tail vein 1 h before death. Statistical analysis was carried out using a Mann Whitney U test and results are expressed as the mean for 10 animals in each group with s.e.mean shown by vertical lines.

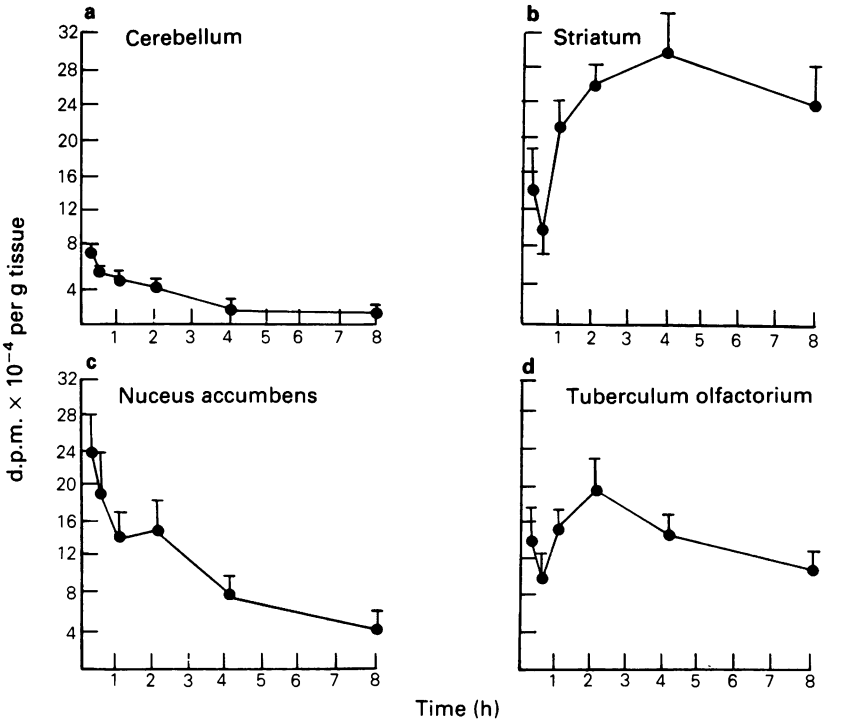

Figure 3 Time course of accumulation of radioactivity derived from [^{3}H]-spiperone (25 μCi i.v.) in selected areas of rat brain [^{3}H]-spiperone (25 μCi i.v.) was injected via the tail vein 15 min–8 h before death. All the areas studied fell into one of four distinct patterns of change: (a) cerebellum; (b) striatum; (c) substantia nigra and nucleus accumbens; (d) olfactory lobes, hypothalamus, tuberculum olfactorium, hippocampus and frontal cortex Statistical analysis was carried out using a Mann Whitney U test and results are expressed as the mean for 10 animals in each group with s.e.mean shown by vertical lines.

Based on these patterns of change in regional radioactivity illustrated in Figure 2, a time of 1 h after [^{3}H]-spiperone administration was chosen for subsequent displacement experiments. At this time there was a 6 fold difference in radioactivity in all areas compared with cerebellum, which for reasons discussed below was chosen to represent a reference area of brain in which no spiperone binding sites could be identified.

Maximal displacement of [^{3}H]-spiperone by unlabelled spiperone (Figure 4)

To determine the maximal extent to which [^{3}H]-spiperone accumulation (25 μCi) could be prevented in each brain area, animals received unlabelled spiperone (0.005–5.0 mg kg^{-1}, i.p.) at the same time as [^{3}H]-spiperone (25 μCi i.v.) 1 h before death. In every region except the cerebellum there was a dose-related decrease in the radioactivity derived from [^{3}H]-spiperone with increasing doses of unlabelled spiperone. In all regions the radioactivity present after administration of 5 mg kg^{-1} spiperone was similar to that found in the cerebellum. In the substantia nigra, tuberculum olfactorium, nucleus accumbens, striatum and frontal cortex displacement appeared to occur in a biphasic manner with the initial displacement being significant in the tuberculum olfactorium and nucleus accumbens. In these areas 30–40% of the total displaceable radioactivity was removed by 0.01 mg kg^{-1} spiperone but a ten fold increase in the dose of unlabelled spiperone was required before further radioactivity was displaced. In the presence of a high dose of unlabelled spiperone, radioactivity derived from [^{3}H]-spiperone did not accumulate over cerebellar levels in any brain region. This suggests that the cerebellum contained no specific spiperone binding sites. In other experiments, administration of (+)-butaclamol (5 mg kg^{-1} i.p., 30 min before death, a dose causing maximum inhibition of dopamine-mediated behaviour) caused no displacement of radioactivity derived from [^{3}H]-spiperone in cerebellum at any time

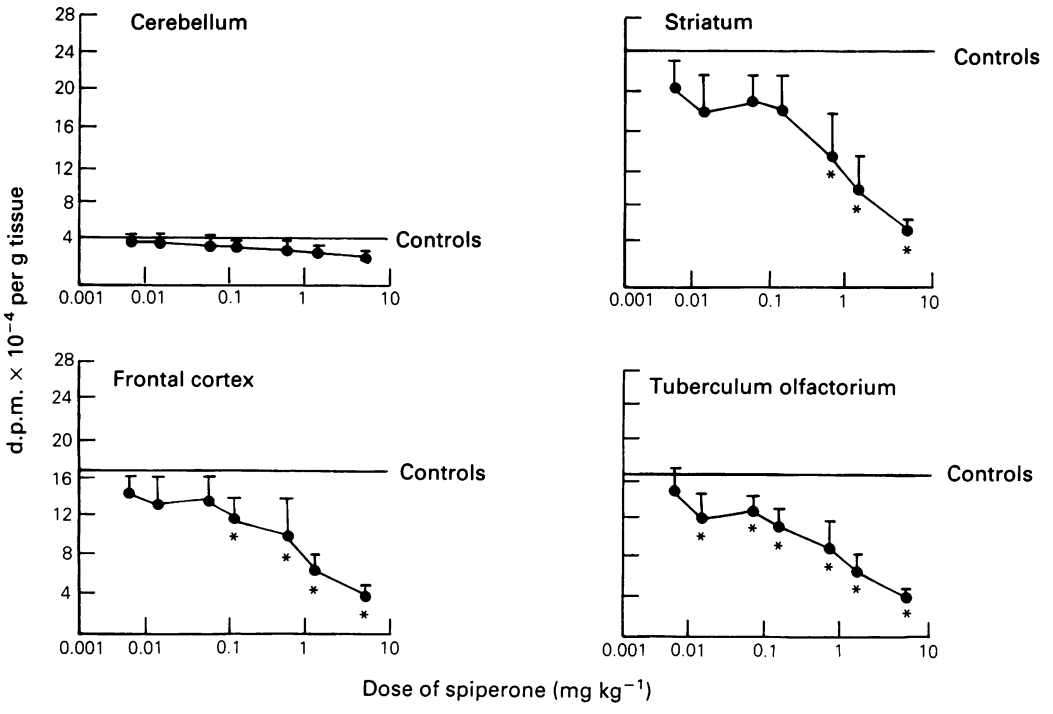

Figure 4 Displacement of radioactivity derived from [^{3}H]-spiperone (25 μCi i.v.) by a range of doses of unlabelled spiperone (0.005–5.0 mg kg^{-1}, i.p.). [^{3}H]-Spiperone (25 μCi i.v.) was injected *via* the tail vein 1 h prior to death. Some animals also received unlabelled spiperone (0.005–5.0 mg kg^{-1}i.p. 1 h before death). Each point represents the mean for 10 rats with s.e.mean shown by vertical lines.
*$P < 0.05$ for displacement of radioactivity derived from [^{3}H]-spiperone by unlabelled spiperone compared to control values, calculated using a Mann Whitney U test.

for 15 min to 8 h thereafter (data not shown). For these reasons, we chose the cerebellum as the reference brain area in subsequent experiments to express the ability of drugs to prevent [^{3}H]-spiperone accumulation from other brain areas.

Displacement experiments (Figures 5 and 6)

Neuroleptic drugs The administration of haloperidol (1 mg kg^{-1}, i.p. 1 h before death) partially prevented the accumulation of radioactivity derived from [^{3}H]-spiperone (25 μCi, 1 h before death) in striatum, tuberculum olfactorium, nucleus accumbens, hypothalamus, substantia nigra, and olfactory lobes, but not in cerebellum, hippocampus or frontal cortex. Sulpiride (40 mg kg^{-1}, 3 h before death) caused partial prevention the accumulation of [^{3}H]-spiperone in the striatum, substantia nigra, tuberculum olfactorium, hypothalamus, and olfactory lobes, but not in other brain regions examined. Sultopride (40 mg kg^{-1} i.p., 3 h beforehand) partially prevented the accumulation of radioactivity derived from [^{3}H]-spiperone in the striatum, substantia nigra, tuberculum olfactorium, hypothalamus and olfactory lobes but not in other brain regions.

Administration of (+)-butaclamol (5 mg kg^{-1} i.p., 0.5 h before death) partially prevented the accumulation of [^{3}H]-spiperone in the olfactory lobes, substantia nigra, hypothalamus, tuberculum olfactorium, nucleus accumbens, striatum, and frontal cortex, but not in the hippocampus, or cerebellum. In contrast, (−)-butaclamol (5 mg kg^{-1} i.p., 0.5 h before death) did not prevent accumulation in any brain region. Administration of piflutixol (1 mg kg^{-1}, 2 h before death) partially prevented accumulation of [^{3}H]-spiperone in olfactory lobes, substantia nigra, hypothalamus, tuberculum olfactorium, nucleus accumbens, striatum, and frontal cortex, but not in hippocampus or cerebellum.

5-Hydroxytryptamine and adrenergic drugs Cinanserin (5 mg kg^{-1} i.p., 1.5 h before death) partially prevented accumulation of [^{3}H]-spiperone in the nucleus accumbens, olfactory lobes and frontal cortex

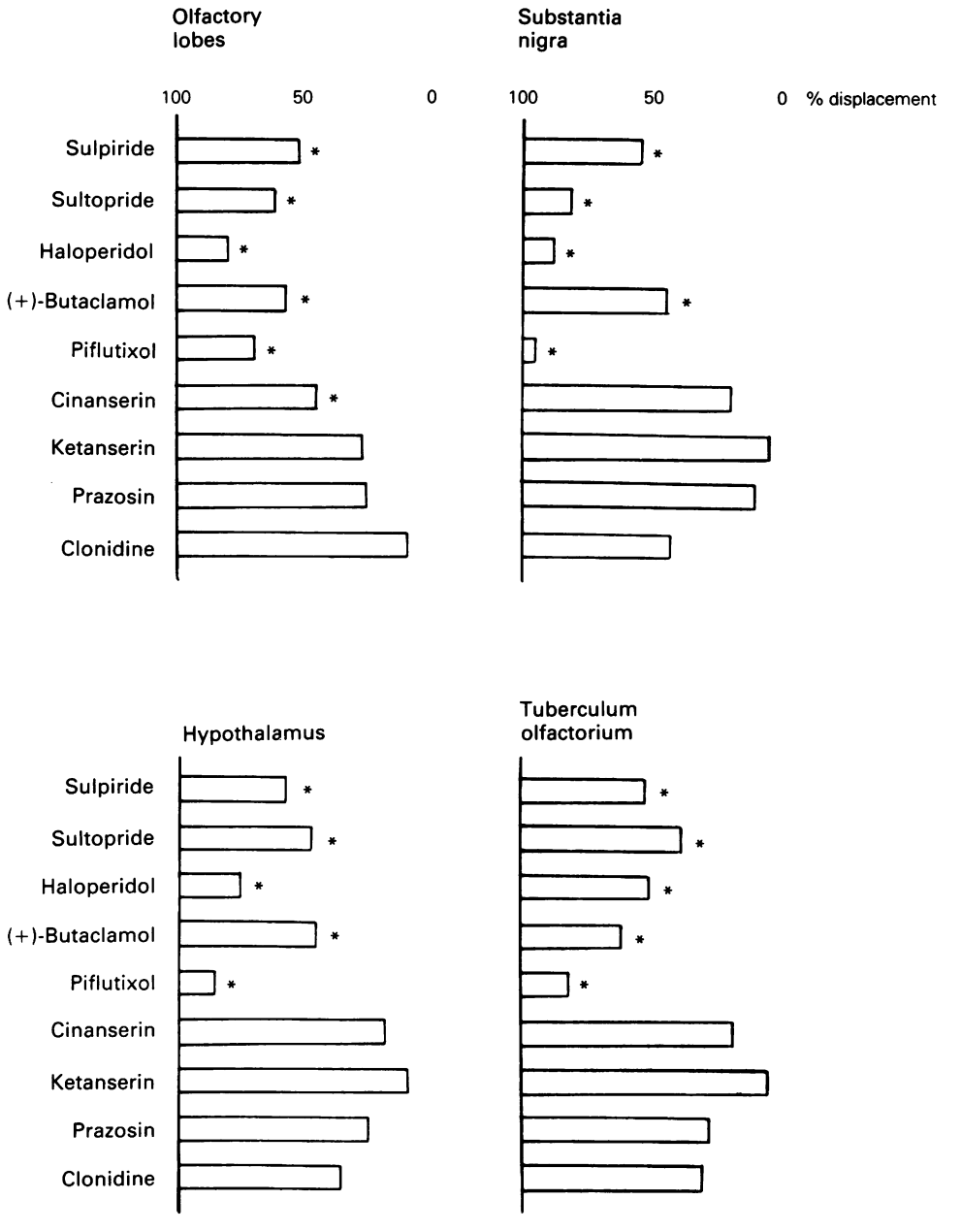

Figure 5 Ability of drugs to displace radioactivity derived from [^{3}H]-spiperone in a variety of areas of rat brain. Data presented as the percentage displacement of [^{3}H]-spiperone binding (25 μCi per rat, $n = 10$ for each drug studied) by sulpiride (40 mg kg^{-1}), sultopride (40 mg kg^{-1}), haloperidol (1 mg kg^{-1}), (+)-butaclamol (5 mg kg), piflutixol (1 mg kg^{-1}), cinanserin (5 mg kg^{-1}), ketanserin (1 mg kg^{-1}), prazosin (1 mg kg^{-1}) and clonidine (0.05 mg kg^{-1}) in the various brain areas studied. In each area the amount of binding above cerebellar levels was calculated for both the control and displaced groups. In the control group this was taken to represent total binding in that area and the amount of binding found in the presence of displacing drug was calculated as a percentage of total binding. The standard error of the mean value for binding in control and displaced groups was never greater than 15%.
*$P < 0.05$ for displacement of [^{3}H]-spiperone binding.. Results calculated using Mann Whitney U test.

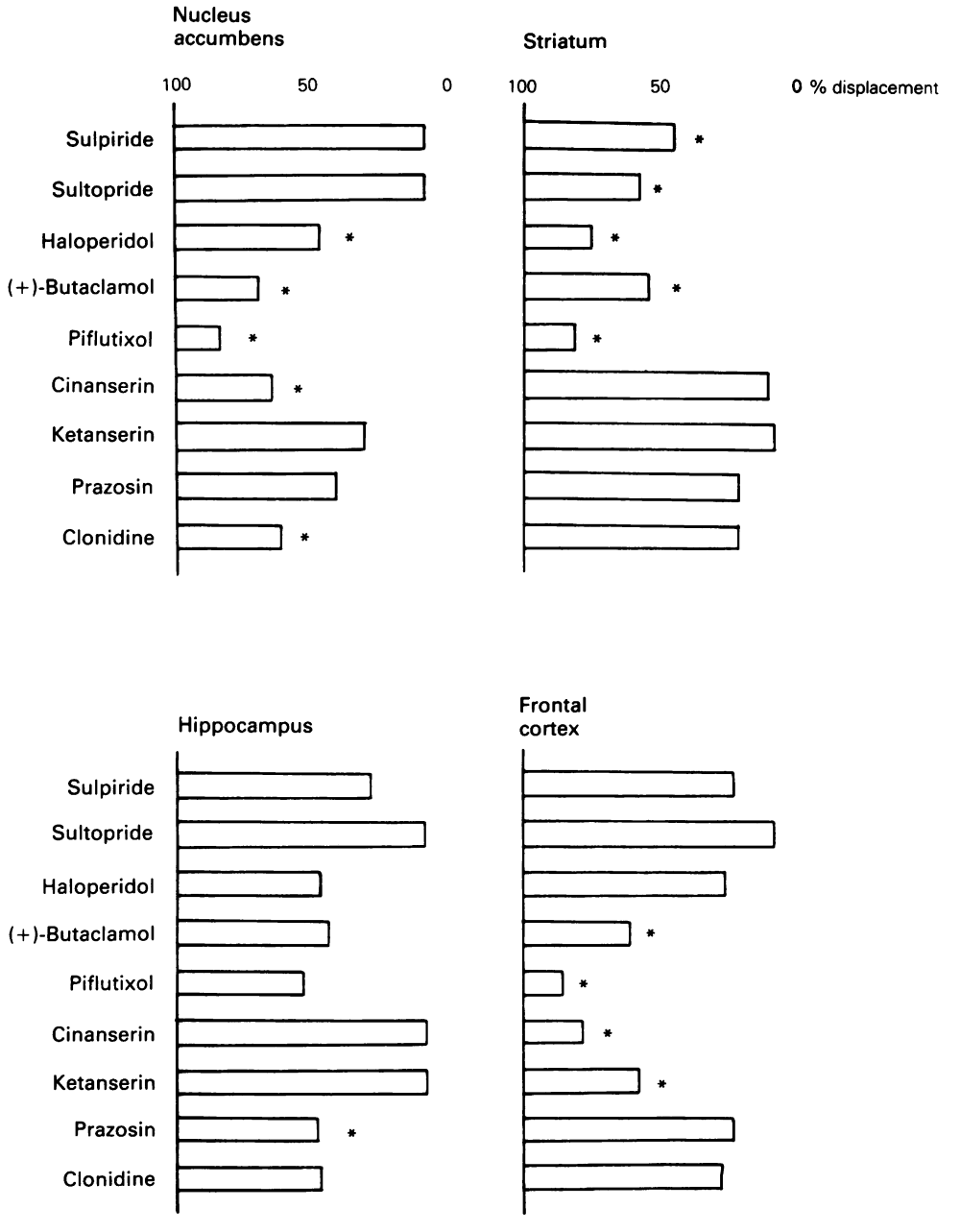

Figure 6 Ability of drugs to displace radioactivity derived from [^{3}H]-spiperone in a variety of areas of rat brain. Data presented as the percentage displacement of [^{3}H]-spiperone binding (25 μCi per rat, $n = 10$) by sulpiride (40 mg kg^{-1}) sultopride (40 mg kg^{-1}), haloperidol (1 mg kg^{-1}), (+)-butaclamol (5mg kg^{-1}), piflutixol (1 mg kg^{-1}), cinanserin (5 mg kg^{-1}) ketanserin (1 mg kg^{-1}) prazosin (1 mg kg^{-1}) clonidine (0.05 mg kg^{-1}) in the various brain areas studied. In each area the amount of binding above cerebellar levels was calculated for both the control and displaced groups. In the control group this was taken to represent total binding in that area and the amount of binding found in the presence of displacing drug was calculated as a percentage of total binding. The standard error of the mean value for binding in control and displaced groups was never greater than 15%.
*$P < 0.05$ for displacement of [^{3}H]-spiperone binding. Results calculated using Mann Whitney U test.

but not from elsewhere. Ketanserin (1 mg kg^{-1}, 2 h before death) only prevented accumulation of [^{3}H]-spiperone in the frontal cortex.

Clonidine (0.05 mg kg^{-1}, 1.5 h before death) prevented accumulation of [^{3}H]-spiperone only in the nucleus accumbens whereas prazosin (1 mg kg^{-1}, 2 h before death) prevented accumulation only in the hippocampus. In contrast, propranolol (20 mg kg^{-1} i.p., 2 h before death) did not prevent accumulation in any brain region.

Other drugs Atropine (5 mg kg^{-1} i.p., 0.5 h before death) prevented the accumulation of [^{3}H]-spiperone by 33% in the olfactory lobes and by 17% in the hypothalamus, but not elsewhere. However, in contrast, dexetimide (10 mg kg^{-1}, 1.5 h before death) did not prevent accumulation of [^{3}H]-spiperone in any brain region. Similarly, cimetidine (10 mg kg^{-1}, 1.5 h before death) was ineffective in all brain regions, although mepyramine (5 mg kg^{-1} i.p., 1.5 h before death) prevented accumulation of 33% in the tuberculum olfactorium but not elsewhere. Neither muscimol (5 mg kg^{-1}, 75 min before death) nor naltrexone (1 mg kg^{-1} 0.5 h before death) had any effect on [^{3}H]-spiperone accumulation.

Discussion

Previous studies of *in vivo* binding of [^{3}H]-spiperone (Hollt *et al.*, 1977; Laduron *et al.*, 1978; Kuhar *et al.*, 1978) generally used a non-specific agent, such as (+)-butaclamol or unlabelled spiperone itself, to define specific binding. Although [^{3}H]-spiperone was shown to accumulate in a number of brain regions only the striatum received any detailed attention. Even in such early studies doubts were raised concerning the specificity of receptors labelled by [^{3}H]-spiperone.

The *in vivo* indentification of neurotransmitter receptors involves many difficulties which are not apparent in *in vitro* ligand binding assays but which may similarly affect PET scanning studies in man. These include the far greater variation between *in vivo* ligand binding experiments in individual animals compared to tissue membrane preparations *in vitro*, and the time course of ligand accumulation in various brain areas. In addition, a single ligand concentration is employed but this may not result in a steady state situation in all brain areas. Finally, the displacing drugs used in a single dose may not penetrate equally into those brain areas in which the ligand accumulates and are usually not *specific* for one receptor population.

It is also necessary for *in vivo* binding studies to consider the way in which the data are calculated from displacement experiments. We have compared the accumulation of [^{3}H]-spiperone occurring in different brain areas relative to that occurring in the cerebellum. Since we were primarily interested in the interaction of [^{3}H]-spiperone with dopamine receptors, the cerebellum was judged to be suitable as a blank region since it is not believed to contain dopamine receptors. Accordingly, we have arbitrarily chosen the values of radioactivity in cerebellum to indicate baseline non-specific binding. The difficulty with this approach is that it is not certain whether baseline nonspecific binding would be the same in all brain regions. Using unlabelled spiperone to displace [^{3}H]-spiperone, the maximal level of displacement in all brain regions studied resulted in a level of radioactivity similar to that found in the cerebellum. This approach is consistent with that previously employed by others (Hollt *et al.*, 1977; Kuhar *et al.*, 1978).

The accumulation of radioactivity derived from [^{3}H]-spiperone administered alone over cerebellar background in the areas studied ranked in the order: striatum, tuberculum olfactorium, hypothalamus, substantia nigra, and nucleus accumbens > olfactory lobes and frontal cortex > hippocampus. The striatum > tuberculum olfactorium, substantia nigra, hypothalamus, and nucleus accumbens > olfactory alamus, substantia nigra and nucleus accumbens have 3–4 fold increases. The remaining areas of the brain show an approximately two fold increase over cerebellum. The selective accumulation of [^{3}H]-spiperone in a variety of brain regions found in these experiments is in overall agreement with that observed in previous reports (Hollt *et al.*, 1977; Kuhar *et al.*, 1978; Laduron *et al.*, 1978). The radioactivity present in the brain was not analysed to determine its composition. However, previous studies have shown that within the first 3–4 h following administration, spiperone is not metabolised to any degree (Bittiger & Bischoff, 1980), and that its metabolites are pharmacologically inactive (Soudijn *et al.*, 1967).

The time course of accumulation of total radioactivity differed in the different brain regions, as previously noted (Laduron *et al.*, 1978). In some areas, such as the cerebellum or nucleus accumbens, levels of radioactivity were maximal soon after [^{3}H]-spiperone administration and then slowly declined. In others, and in particular the striatum, radioactivity continued to accumulate for some hours following [^{3}H]-spiperone administration. This may reflect differences in regional brain blood flow, in the size of the extracellular space in the tissue components in individual brain areas, and in the density of receptors.

Pharmacological characterization of the nature of the neuronal receptors identified by [^{3}H]-spiperone *in vivo* is complex. Few drugs are available which can be regarded as selective for any one neuronal system. It is only by the use of a range of drugs affecting individual receptor systems that a comprehensive picture can be built up. Previously attempts to define the specific

Table 1 Summary of the effect of compounds studied on [^{3}H]-spiperone binding *in vivo* to rat brain in various brain regions

Drug	*OL*	*SN*	*Hypo*	*TO*	*Nuc A*	*Stri*	*Hippo*	*FC*
Sulpiride (40 mg kg^{-1})	+	+	+	+	−	+	−	−
Sultopride (40 mg kg^{-1})	+	+	+	+	−	+	−	−
Haloperidol (1 mg kg^{-1})	+	+	+	+	+	+	−	−
(+)-Butaclamol (5 mg kg^{-1})	+	+	+	+	+	+	−	+
Piflutixol (1 mg kg^{-1})	+	+	+	+	+	+	−	+
Cinanserin (5 mg kg^{-1})	+	−	−	−	+	−	−	+
Ketanserin (1 mg kg^{-1})	−	−	−	−	−	−	−	+
Prazosin (1 mg kg^{-1})	−	−	−	−	−	−	+	−
Clonidine (0.05 mg kg^{-1})	−	−	−	−	+	−	−	−

+ = significant displacement of [^{3}H]-spiperone binding *in vivo* calculated using a Mann Whitney U test.
− = no significant displacement of [^{3}H]-spiperone binding *in vivo*.
OL = olfactory lobes, SN = substantia nigra, Hypo = hypothalamus, TO = tuberculum olfactorium, NucA = nucleus accumbens, Stri = striatum, Hippo = hippocampus FC = frontal cortex.

binding of [^{3}H]-spiperone *in vivo* were limited to drugs mainly acting on dopamine receptors. However, from the present experiments using displacing drugs with a range of pharmacological action, it is possible to reach certain conclusions concerning the interaction of [^{3}H]-spiperone *in vivo* with brain neurotransmitter receptors in the rat brain (see Table 1).

In striatum the accumulation of [^{3}H]-spiperone was prevented by the various neuroleptic compounds employed (sulpiride, sultopride, haloperidol, (+)-butaclamol and piflutixol) indicating the known dopaminergic innervation of this brain region. However, haloperidol, (+)-butaclamol and piflutixol act on a variety of neuronal receptors in brain other than dopamine receptors (Enna *et al.*, 1976; Spedding & Berg, 1982). But these would not appear involved since drugs acting on 5-HT, noradrenaline, histamine, acetylcholine, opiate or γ-aminobutyric acid (GABA) receptors were without effect in striatum. The primary involvement of dopamine receptors is supported by prevention of accumulation of [^{3}H]-spiperone caused by the selective D_2-receptor antagonists, sulpiride and sultopride (Leysen *et al.*, 1980).

In three other areas receiving dopaminergic innervation namely the subtantia nigra, hypothalamus and tuberculum olfactorium, the accumulation of [^{3}H]-spiperone appears associated with brain dopamine receptors. Again, sulpiride and sultopride caused displacement of haloperidol, (+)-butaclamol and piflutixol from these regions. Drugs acting on 5-HT receptors or noradrenaline receptors were without effect on [^{3}H]-spiperone accumulation, as were compounds acting on other neuronal receptors in brain. In the olfactory lobes the accumulation of [^{3}H]-spiperone also appears largely to reflect an interaction with dopamine receptors. There was a partial displacement of [^{3}H]-spiperone in the olfactory lobes by the 5-HT antagonist cinanserin, but this was not observed with the other 5-HT antagonist used, ketanserin. So, the ability of sulpiride and sultopride to cause displacement in this region suggests identification of D_2-receptors by [^{3}H]-spiperone.

In the three remaining dopamine containing areas examined, namely the frontal cortex, nucleus accumbens and hippocampus, the nature of the receptor population identified by [^{3}H]-spiperone accumulation was less clear.

The accumulation of [^{3}H]-spiperone in the frontal cortex was not displaced by the selective D_2-receptor antagonists sulpiride or sultopride or by haloperidol suggesting a lack of involvement of dopamine receptors. However, (+)-butaclamol and piflutixol as well as the 5-HT antagonists cinanserin and ketanserin did partially prevent the accumulation of [^{3}H]-spiperone. The α-adrenoceptor compounds prazosin and clonidine were without effect as were the drugs used that

acted on histamine, acetylcholine and GABA receptors. Accordingly, much of the specific binding of [^{3}H]-spiperone to the frontal cortex *in vivo* is likely to be to 5-HT receptors as is known to occur *in vitro* (Creese & Snyder, 1978; Clements-Jewery & Robson, 1980). Presumably, the density of dopamine receptors is low in this region and is swamped by a high concentration of 5-HT receptors. Indeed, it is difficult, if not impossible, to identify cortical dopamine receptors by use of [^{3}H]-spiperone *in vitro* (List & Seeman, 1981). Recently, similar displacement experiments on the *in vivo* binding of [^{76}Br]-bromospiperone have also suggested that radioactivity measured in cortex with this ligand is associated with 5-HT receptors (Maziere *et al.*, 1984).

In the nucleus accumbens little of the accumulation of [^{3}H]-spiperone appears to be to dopamine receptors. The main evidence for lack of labelling of dopamine receptors in this area is the failure of sulpiride and sultopride to displace [^{3}H]-spiperone. In contrast, (+)-butaclamol, piflutixol, cinanserin and clonidine partially prevented [^{3}H]-spiperone accumulation, suggesting the identification of both 5-HT and noradrenaline sites in this region. But the accumulation of [^{3}H]-spiperone was not prevented by the 5-HT antagonist ketanserin or the adrenoceptor antagonist prazosin. In addition, haloperidol does cause displacement even though this drug has relatively little effect on 5-HT or noradrenaline systems *in vivo* as judged by its lack of effect in frontal cortex. So, it is probable that the interaction of [^{3}H]-spiperone with neuronal receptors in nucleus accumbens is complex involving 5-HT, noradrenaline and to some extent, dopamine receptors.

No evidence for an interaction of [^{3}H]-spiperone with dopamine receptors in the hippocampus was obtained. None of the dopamine antagonist drugs displaced [^{3}H]-spiperone from the hippocampus in contrast to a previous report (Bischoff *et al.*, 1980). Indeed, of the drugs examined only prazosin, but not clonidine, caused displacement of [^{3}H]-spiperone. Thus the nature of receptors responsible for the accumulation of [^{3}H]-spiperone in the hippocampus remains unclear. One possibility is that [^{3}H]-spiperone might identify spirodecanone sites in this brain region, since apart from prazosin, only unlabelled spiperone caused displacement of the ligand (see Howlett *et al.*, 1979).

From these experiments some general conclusions can be drawn. The use of [^{3}H]-spiperone *in vivo* does identify brain dopamine receptors, but only in certain regions among the known dopamine containing regions. Specifically, these are the substantia nigra, striatum, tuberculum olfactorium, hypothalamus and olfactory lobes. Clearly, care will be necessary when interpreting PET studies using similar ligands in man with regard to the time course of ligand accumulation in different brain areas, and the nature of the receptor population defined.

However, even in those brain regions where [^{3}H]-spiperone appears to label dopamine receptors, the situation may be more complex that it initially appears. For example, the *in vivo* displacement of [^{3}H]-spiperone by unlabelled spiperone in a number of areas, including the striatum, appeared biphasic. Again, this might reflect the interaction of [^{3}H]-spiperone with spirodecanone sites. However, other evidence suggests that *in vivo* [^{3}H]-spiperone may label more than one distinct population of dopamine receptor sites in a given brain area. Thus, Ferraro and colleagues (1983) using a range of doses of sulpiride, showed that the maximal displacement of [^{3}H]-spiperone was far less than that produced by unlabelled spiperone. Similarly, others have also noted that apomorphine was only able to displace partially specific [^{3}H]-spiperone binding *in vivo* when compared with a range of neuroleptic compounds (Hollt *et al.*, 1977; Kuhar *et al.*, 1978; Laduron *et al.*, 1978).

In the present study, different neuroleptic drugs caused differing degrees of displacement of [^{3}H]-spiperone in different brain regions. This too might initially appear to reflect involvement of different dopamine sites. However, others have found similar degrees of displacement of [^{3}H]-spiperone by neuroleptic drugs including (+)-butaclamol and haloperidol (Kohler *et al.*, 1979; 1981). The differences in effect we have observed may be due to lack of equivalence of drug dose, or to differences in drug lipophilicity which will determine the relative penetration into different brain regions. Such apparent differences reflect the limitation of this technique both in animals and in PET studies in man where only single doses of drug can be reasonably employed in displacement studies.

This study was supported by the Medical Research Council and by the Research Funds of the Royal and Maudsley Hospitals and Kings College Hospital. J.K.C. was a SERC CASE Award Student in conjunction with Chemitechna Ltd.

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(Received April 18, 1986.
Revised November 10, 1986.
Accepted November 11, 1986)