Psychopharmacology (1992) 106:45–52

Psychopharmacology
© Springer-Verlag 1992

Effect of haloperidol and clozapine on the density of "perforated" synapses in caudate, nucleus accumbens, and medial prefrontal cortex

Charles K. Meshul[1–3,6], Aaron Janowsky[2,4,5], Daniel E. Casey[2,5,7], Regina K. Stallbaumer[2], and Barry Taylor[2]

[1] Laboratory and [2] Research Services, V.A. Medical Center and Departments of [3] Medical Psychology, [4] Pharmacology, [5] Psychiatry, [6] Pathology and [7] Neurology, The Oregon Health Sciences University, 3710 S.W. Veterans Hospital Road, Portland, OR 97201, USA

Received February 26, 1991 / Final version May 1, 1991

Abstract. Perforated synapses, which contain a discontinuous density along the postsynaptic membrane, can increase or decrease in numbers following various behavioral and biochemical manipulations. We have previously established that 14-day treatment with haloperidol causes an increase in the number of perforated synapses within the caudate nucleus (dorsolateral region) but not the nucleus accumbens (Meshul and Casey 1989). This effect was reversed if the animals were withdrawn from the drug for an equivalent period of time. We have now further examined the effects of haloperidol administration, which is associated with a high incidence of extrapyramidal side effects (EPS) and tardive dyskinesia (TD), and assessed the effects of clozapine, which appears to have a lower potential for inducing EPS and TD. Administration of haloperidol for 2 weeks significantly increased the percentage of perforated synapses in the caudate, but not in the nucleus accumbens or layer VI of medial prefrontal cortex (MPCx). There was an increase in specific [^{125}I]epidepride binding to D–2 receptors in the caudate nucleus and MPCx following haloperidol. Administration of clozapine for 2 weeks did not affect the percentage of perforated synapses in any of the three dopamine (DA)-rich regions that were examined. There was an increase in specific [^{3}H]SCH 23390 binding to D–1 receptors and in specific [^{125}I]epidepride binding to D–2 receptors only within MPCx following clozapine. The absence of any change in the density of perforated synapses within the dorsolateral caudate nucleus following clozapine correlates with: 1) the lack of effect on specific DA receptor binding or down regulation of serotonin (5–HT$_2$) receptors (as reported by others), or 2) the inability in clozapine-treated animals to depolarize block substantia nigra (A9) DA neurons. These results may be related to the low incidence of EPS and TD observed with clozapine.

Key words: Haloperidol – Clozapine – Perforated synapses – Electron microscopy – Tardive dyskinesia – Extrapyramidal syndrome

Offprint requests to: C.K. Meshul, Neuroeytology Laboratory (151-Q), V.A. Medical Center, Portland, OR 97201, USA

Dopamine (DA) neurons from the midbrain substantia nigra (A9, zona compacta) innervate the basal ganglia, while neurons from the ventral tegmental area (VTA)(A10) innervate the nucleus accumbens and medial prefrontal cortex (Beckstead et al. 1979). DA released from these nerve terminals activates either D–1 DA receptors, which are linked to adenylate cyclase activation, or D–2 DA receptors, which inhibit cyclase activity (Kebabian and Calne 1979).

Classical neuroleptic drugs, like haloperidol, may exert their therapeutic effect by blocking DA D–2 receptors (Seeman and Grigoriadis 1987) and studies with animals have shown that long term blockade of DA D–2 receptors results in an increase in the number of receptors within the caudate nucleus (Duncan et al. 1987) and has been variably reported within the nucleus accumbens (Duncan et al. 1987; Wilmot and Szczepanik 1989; O'Dell et al. 1990; current study). One of the problems associated with the treatment of psychiatric patients with haloperidol is the induction of extrapyramidal side effects (EPS) and tardive dyskinesia (TD) (Baldessarini and Tarsy 1980; Casey 1987). On the other hand treatment with atypical neuroleptics, such as clozapine, is associated with a lower incidence of EPS and TD (Casey 1989a). Chiodo and Bunney (1983) have speculated that the difference in clinical effects may be due to a haloperidol-induced depolarization blockade of the A9 and A10 DA neurons. Clozapine, however, inactivates A10 but not A9 neurons (Chiodo and Bunney 1983). In radioligand binding studies, chronic administration of clozapine appears to have no effect on the number of striatal D–2 receptors, but down-regulates the number of cortical serotonin (5–HT$_2$) receptors (Wilmot and Szczepanik 1989). In addition, clozapine is one of the more potent neuroleptic antagonists at dopamine D–1 receptors (Andersen and Braestrup 1986).

As a possible correlate to the neuroleptic drug-induced changes in receptor density, behavior, and electrophysiology, electron microscopic studies of DA-rich areas have been performed in tissues following in vivo treatment with neuroleptic drugs. Benes and associates (1985a) have demonstrated that chronic haloperidol

treatment results in a small but significant increase in the neuronal area and an increase in the size of nerve terminals and dendritic diameter within the caudate nucleus. In addition, there was an increase in the number of terminals per dendrite in the substantia nigra (Benes et al. 1983). In the medial prefrontal cortex (MPCx), chronic administration of haloperidol caused a selective loss of small-calibre dendritic profiles and a loss of terminal profiles making asymmetric contact with a postsynaptic dendrite (Benes et al. 1985b). Chronic administration of clozapine, however, caused a decrease in the density of asymmetrical synapses and an increase in symmetrical synapses (Vincent et al. 1989).

In our previous study (Meshul and Casey 1989) we examined haloperidol-induced changes in the type of synaptic contact made between pre- and postsynaptic structures that could accompany the increased density of DA D–2 receptors. The vast majority of synapses contain a postsynaptic density or specialization in which the density is continuous along the entire length of contact with the nerve terminal (Fig. 1, dark arrow). We described changes in the number of synapses containing a discontinuous or "perforated" postsynaptic density (Fig. 1, open arrow). These perforated synapses are almost twice the diameter of synapses containing a continuous postsynaptic density. Administration of haloperidol to rats for 14 days caused a significant increase in the number of perforated synapses within the (dorsolateral) caudate nucleus but not the nucleus accumbens. In tissue from animals treated for 14 days and then withdrawn from the drug for 14 days, the increased number of perforated synapses was not observed. We also demonstrated that the mean diameter of all the synapses within drug-treated animals had not changed as compared to saline-treated animals, and there had not been a shift from smaller to larger diameter terminals. This was significant, since perforated synapses are larger than non-perforated synapses, and the effect of haloperidol could have been due to an increase in the number of larger terminals. We have subsequently found that the increase in striatal perforated synapses was still observed after either 6 months on haloperidol or after 6 months on and 1 month off the drug (Meshul, unpublished observation). Whether this observation could be related to TD is not known.

To further characterize the effects of neuroleptic drugs on rat brain, we examined the regional specificity of clozapine, an atypical neuroleptic, and haloperidol, a typical neuroleptic, on the appearance of perforated synapses in MPCx (layer VI) and nucleus accumbens, which both receive DA input from the VTA (Emson and Koob 1978; Lindvall et al. 1978; Beckstead et al. 1979; Van Eden et al. 1987) and in the caudate nucleus. We found that haloperidol caused an increase in the percentage of perforated synapses within the dorsolateral caudate nucleus but not the nucleus accumbens or layer VI of MPCx. In addition, haloperidol caused an increase in specific binding of [^{125}I]epidepride to D–2 receptors within the caudate and MPCx, but not the nucleus accumbens. There was no change in perforated synapses following clozapine treatment within any of the three brain areas examined, although there was an increase in specific binding of the appropriate radioligand to D–1 and D–2 receptors within the MPCx.

Materials and methods

Male Sprague-Dawley rats (180–200 g) ($n = 5$ for each group) were injected with haloperidol (0.5 mg/kg/day, SC)(McNeil Pharmaceutical), clozapine (35 mg/kg/day, SC)(Sandoz Pharmaceutical), or normal saline for 14 days. The animals were maintained on a controlled 12 h dark and 12 h light cycle, with free access to food and water. The stock solution of haloperidol (5 mg/ml) from the pharmaceutical company was diluted with normal saline. Clozapine was dissolved in a minimal amount of lactic acid, diluted with distilled water and pH balanced to 5.5. A comparable set of animals ($n = 7$) was used for the receptor binding studies as described below.

Electron microscopy. Twenty-four hours following the final injection, the animals were anesthetized with 1.0 ml/kg of a solution containing 5% ketamine, 2% xylazine and 1% acepromazine. The chest cavity was opened, and a solution consisting of 0.2 ml heparin (1000 units/ml) plus 0.2 ml 2% sodium nitrite (in normal saline) was quickly injected into the left ventricle. The left ventricle was cut and a 16 gauge blunt needle inserted through the ventricle and into the aorta. Fixative solution (300–500 ml) was perfused through the animal. The fixative consisted of 2.5% glutaraldehyde/3% freshly depolymerized paraformaldehyde in 0.1 M NaCacodylate buffer, pH 7.3, containing 0.1% $CaCl_2$. One hour after the perfusion, the brain was removed from the animal and placed in fixative overnight.

A midsaggital cut through the corpus collosum was made and then a coronal cut made at Bregma +1.7 and +2.7 mm. (Paxinos and Watson 1986). This exposes the head of the caudate and the nucleus accumbens at the first cut and the MPCx at the second cut. A 2 mm^3 piece from the dorsolateral part of the caudate, the nucleus accumbens (ventral and just medial to the anterior commissure, termed "shell of the nucleus accumbens" according to Paxinos and Watson 1986), and the medial prefrontal cortex (4 mm ventral from the skull and including all six layers of the cortex) was cut and placed into cold (4° C) Na Cacodylate buffer (pH 7.3) for 3–6 h. The tissue was then placed in cold 1% osmium tetroxide/1.5% K^+-ferricyanide for 1.5 h, en bloc stained with aqueous 0.5% uranyl acetate overnight in the cold, dehydrated in a graded series of ethanols, and embedded in Embed 812/araldite. Thick sections (0.5 μm) were cut on an RMC 6000 ultramicrotome, stained with toluidine blue, and examined with the light microscope to confirm the orientation of the tissue. Thin sections (silver-gold interface colors) were cut, placed on 200 mesh nickel grids and stained briefly (25 s) with lead citrate (0.075%)/aqueous uranyl acetate (8%) (5 min.)/lead citrate (25 s). The sections were viewed and photographed on a JEOL 1200 EX TEMSCAN electron microscope. All photographs (initial magnification: ×15000) were randomly taken throughout the section in the region of the neuropil, an area containing the greatest concentration of synapses. The negatives were all printed at a final magnification of ×42000.

A total of 25 micrographs were taken from each animal and brain region. The photographs were analyzed by an individual blind to the experimental group. The apposition of a nerve terminal and a putative postsynaptic structure was determined to be a synapse if there was an accumulation of synaptic vesicles within the nerve terminal and the pre-and postsynaptic membranes were electron dense. A synapse was determined to contain a perforated postsynaptic density if there was a gap in the electron density of the postsynaptic membrane of more than 0.05 microns (the width of a synaptic vesicle). The number of synapses with and without perforations per field of view (22 μm^2) was determined and the mean percentage of perforated synapses (±SEM) was calculated. The mean percentages between groups was statistically analyzed using Peritz' *F*-test (for multiple comparison of means) (Einot and Gabriel 1975).

Radioligand binding to dopamine receptors. The method of Neve et al. (1990) was used to determine specific binding of radioligand to D–1 and D–2 receptors. Rats were killed 24 h after the last injection by decapitation and their brains removed and placed on ice. The rostral caudate, nucleus accumbens, and MPCx were dissected out and frozen at − 70° C. Tissue was subsequently homogenized in 10 vol ice-cold TRIS buffer (50 mM TRIS–HCl, pH 7.4 at 25° C) and centrifuged at 30000 *g* for 20 min. The resulting supernatant was homogenized in 10 vol ice-cold TRIS buffer with 5 mM EDTA and incubated at 30° C for 30 min. The suspension was centrifuged again and resuspended in 600 vol (caudate and nucleus accumbens) or 30 vol (MPCx) of TRIS-isosaline for use in binding assays.

For characterization of D–2 receptors, aliquots of the membrane preparation were added to assay tubes containing (final concentrations): 50 mM TRIS–HCl, pH 7.4; 0.9% NaCl, 0.025% ascorbic acid, 100 nM idazoxan, 0.001% bovine serum albumin, [^{125}I]epidepride (2000 Ci/mmol), and appropriate drugs. No correction for radioactive decay of [^{125}I]epidepride was made because the decay product has no measurable binding activity (unpublished observations). Spiperone (2 μM) was used to define nonspecific binding. Assays were carried out in duplicate in a volume of 0.5 ml. Incubations were initiated by the addition of tissue, carried out at 30° C for 60 min, and stopped by the addition of 5 ml ice-cold wash buffer (10 mM TRIS, pH 7.4 and 0.9% NaCl) to each assay. The samples were filtered through glass-fiber filters (Whatman GF/C) and washed with an additional 2 × 5 ml wash buffer. The radioactivity retained on the filter was counted using a gamma counter (LKB Clinigamma 1272).

For characterization of DA D–1 receptors, [^{3}H]-SCH 23390 (Dupont-NEN, Boston, MA) was incubated with the tissue preparation for 60 min at 37° C. Non-specific binding was defined by the difference in binding determined in the presence or absence of SCH 23390 (1 μM). The reaction was terminated and the contents of each tube was filtered as described for the [^{125}I]epidepride binding assay (above).

Differences in specific D–1 or D–2 binding between either the haloperidol-or clozapine-treated animals and the saline control group were compared statistically using the student *t*-test. The experiment-wise statistical significance level of $P < 0.05$ was selected when comparing the two groups of data.

Results

The neuropil of the caudate nucleus is illustrated in Fig. 1. The neuropil from the nucleus accumbens and layer VI

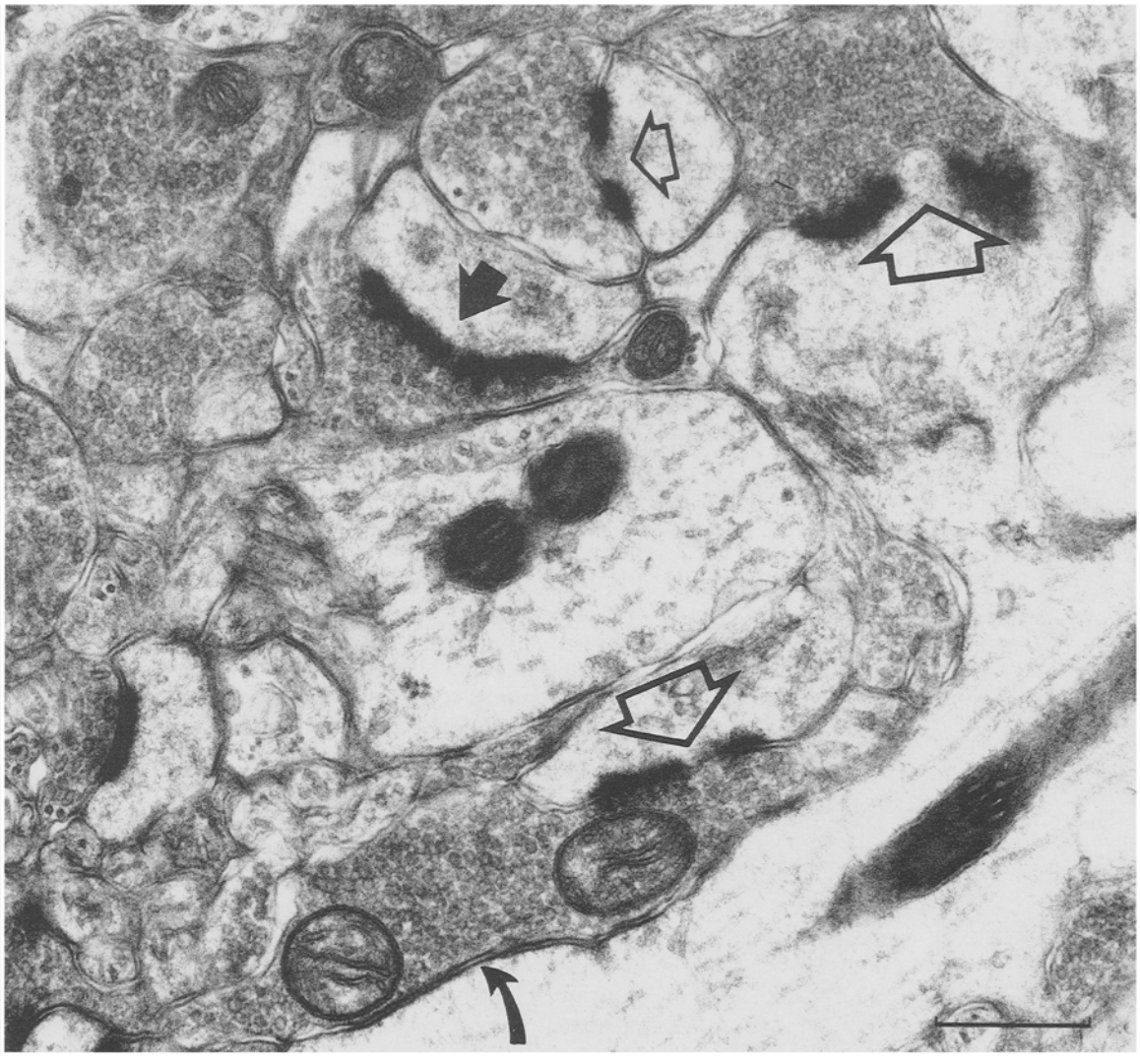

Fig. 1. Electron micrograph from the neuropil of the caudate nucleus of a rat treated with haloperidol for 14 days. A non-perforated synapse *(dark arrow)* is making an asymmetric contact with a dendritic spine, while three perforated synapses *(open arrows)* are making asymmetric contact with dendritic spines. Another perforated synapse *(curved arrow)* is shown making a symmetric contact with a dendritic shaft. Calibration bar: 0.5 μm

Table 1. Effect of clozapine and haloperidol on the number of perforated and total synapses

	Saline[a]		Haloperidol[a]		Clozapine[a]	
	Perf syn.	Total syn.	Perf syn.	Total syn.	Perf syn.	Total syn.
Caudate	1.01±0.07	11.5±0.25	1.53±0.08[b]	10.8±0.22	0.95±0.07	10.9±0.23
Nucleus accumbens	0.99±0.08	11.6±0.28	1.04±0.09	10.9±0.28	1.09±0.08	12.3±0.29
Medial prefrontal cortex	1.10±0.08	10.6±0.29	1.14±0.10	11.3±0.26	1.23±0.10	11.8±0.24

[a] Values are means±SEM per field of view
[b] Statistically significant versus saline controls or clozapine using Peritz' *F*-test ($P<0.05$)

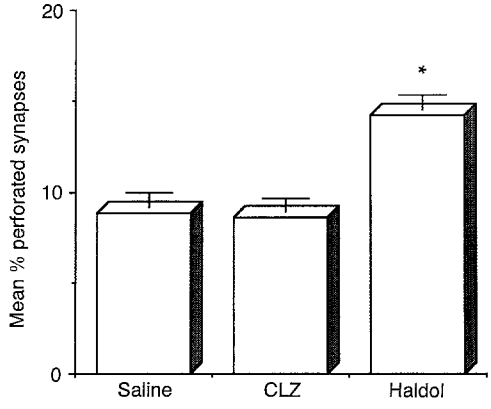

Fig. 2. Mean percentage of perforated synapses within the caudate nucleus following 14 day treatment with either saline, clozapine (35 mg/kg/day) or haloperidol (*Haldol*) (0.5 mg/kg/day). *Significantly different versus saline or clozapine ($P<0.05$), using Peritz' *F*-test

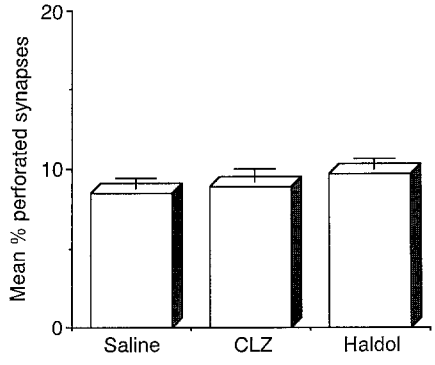

Fig. 3. Same as Fig. 2 except for the nucleus accumbens. There were no differences between any of the groups

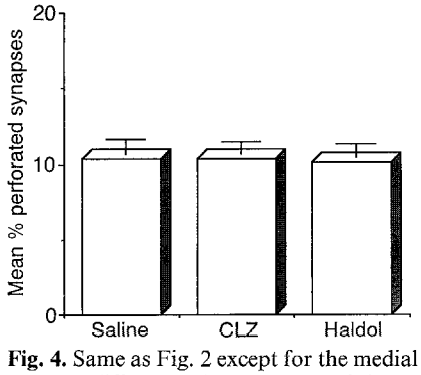

Fig. 4. Same as Fig. 2 except for the medial prefrontal cortex. There were no differences between any of the groups

of MPCx are similar in appearance. The perforated synapse contains a break in the continuity of the postsynaptic specialization such that the membrane in the area of the perforation does not contain the usual increased density. The perforation can be located anywhere along the length of the point of synaptic contact and in some instances, there can be more than one perforated region per synapse. The majority of perforated synapses (70.2%) make contact with dendritic spines, with the remainder of the contacts on dendritic shafts (29.8%). Occasional contacts are observed on the neuronal cell body. Perforated synapses were associated with asymmetrical densities 89.1% of the time, with the remainder of the perforated synapses having symmetrical contacts.

Treatment with haloperidol for 14 days resulted in a statistically significant increase in the mean percentage of perforated synapses as compared to either clozapine or saline within the neuropil of the caudate nucleus ($P<0.05$) (Fig. 2). None of the treatments lead to any change in the mean number of perforated synapses in the nucleus accumbens (Fig. 3) or layer VI of MPCx (Fig. 4). Similarly, there was no significant change in the total number of synapses in any of the brain regions following either haloperidol or clozapine treatment (Table 1). The only significant increase was in the number of perforated synapses within the caudate following haloperidol administration. Therefore, the percent increase in perforated synapses within the caudate, as shown in Fig. 2, was not due to a decrease in the total number of synapses.

Tables 2 and 3 show the specific binding for D–1 and D–2 receptors, respectively, from the caudate, nucleus accumbens, and MPCx. Haloperidol caused a significant

Table 2. Effects of haloperidol and clozapine on D–1 receptors

	Saline[a]	Haloperidol[a]	Clozapine[a]
Caudate	462 ±33	394 ±19	453 ±33
Nucleus accumbens	177 ±16	175 ±22	227 ±48
Medial prefrontal cortex	60.4± 3.2	63 ± 3.3	69.9± 2.5[b]

[a] Values are means (fmoles/mg protein)±SEM
[b] Statistically significant versus saline controls by the *t*-test ($P<0.05$)

Table 3. Effects of haloperidol and clozapine on D–2 receptors

	Saline[a]	Haloperidol[a]	Clozapine[a]
Caudate	139 ±7	172 ±11[b]	110 ±21
Nucleus accumbens	37.6±5.7	45.3± 8.2	40.7± 4.9
Medial prefrontal cortex	4.5±0.2	6.4± 0.2[b]	6.4± 0.4[b]

[a] Values are means (fmoles/mg protein) ± SEM
[b] Statistically significant versus saline controls by the *t*-test ($P<0.05$)

increase in the specific binding of D–2 receptors in the caudate nucleus and MPCx compared to the saline control ($P<0.05$). The drug did not effect D–1 receptor binding in any of the three regions examined. This was in contrast to clozapine, which caused a significant increase in D–1 and D–2 receptor binding in MPCx compared to the saline control ($P<0.05$).

Discussion

Interest in perforated synapses was stimulated by several previous studies demonstrating that these synapses can undergo changes (i.e. synaptic plasticity) in density following exposure to various environmental conditions (Greenough et al. 1978; Vrensen and Cordozo 1981; Hatton and Ellisman 1982; Geinisman et al. 1986) or lesions (Nieto-Sampedro et al. 1982). The perforated synapse is usually larger than those not perforated and at certain points along the pre- and postsynaptic density, the width of the synaptic cleft is also smaller as compared to the other synapses. Greenough and coworkers (1978) speculated that the perforated synapse is more efficient at transmitter release and this plasticity may be related to learning and memory.

Our initial study (Meshul and Casey 1989) focused on the ultrastructural effects of haloperidol within the dorsolateral caudate and the nucleus accumbens. In rat, the dorsolateral caudate receives afferents from areas associated with motor functions (Beckstead et al. 1979; McGeorge and Faull 1989). Inputs from limbic cortex to this area of caudate are sparse, suggesting a primary role in motor function. Damage to the lateral portion of the caudate results in sensorimotor impairment (Dunnett and Iversen 1982) and is consistent with the involvement of the nigrostriatal system in EPS and TD (Baldessarini and Tarsy 1980).

The nucleus accumbens mainly receives input from limbic structures (Beckstead et al. 1979; Kelly et al. 1982; Kelly and Domesick 1982). This differential innervation pattern may lend further support to the idea that the nucleus accumbens may be the site of antipsychotic action, whereas the more dorsal and lateral striatum may be involved in neuroleptic-induced EPS (Stevens 1973; Costall and Naylor 1976).

The haloperidol-induced increase in perforated synapses was observed within the dorsolateral caudate and not the nucleus accumbens. We hypothesized that the perforated synapse was not a part of the dopamine system and that its neuronal origin could be sensorimotor cortex (Meshul and Casey 1989). This hypothesis was based on the diameter of the perforated synapse, which was twice that compared to either dopamine (Pickel 1986), GABA (Ribak et al. 1981), or acetylcholine (Phelps et al. 1985) labelled terminals. The haloperidol-induced increase in perforated synapses within the dorsolateral caudate, and the importance of this area in terms of motor function, may suggest a correlation between these two observations.

Clozapine is associated with a low incidence of EPS and TD (Casey 1989a). If the motoric disorders associated with haloperidol treatment involve the dorsolateral caudate, then treatment with clozapine should not cause any changes in the density of perforated synapses in this particular area. Fig. 2 indicates that this is the case. It was also found that clozapine did not affect, ultrastructurally, the other two DA-rich areas (nucleus accumbens or layer VI of MPCx). There was no change in the total number of synapses following either drug treatment within any of the brain areas examined. This is important, since the lack of change in the percentage of perforated synapses following clozapine treatment could have been due to a significant decrease in the total number of synapses and an increase in the mean number of perforated synapses.

The lack of change in D–1 or D–2 receptor binding within the caudate following clozapine (Tables 2 and 3) correlates with the inability of this drug to effect the density of perforated synapses. The increase in D–1 and D–2 specific binding within MPCx following clozapine did *not* correlate with an increase in perforated synapses in this cortical area. This is in contrast to the increase in D–2 receptor binding within the caudate following haloperidol, where a subsequent increase in perforated synapse density was observed. On the other hand, the haloperidol-induced increase in D–2 receptors within MPCx did *not* correlate with a change in the percentage of perforated synapses within this brain region. At least within this area of cortex, differences in receptor binding by either drug was not correlated with any morphological change. This calls into question any correlation or linkage between biochemical, pharmacological, or morphological changes associated with neuroleptic drug treatment.

A number of previously reported electrophysiological and biochemical studies may help to explain the lack of ultrastructural changes within the dorsolateral caudate following clozapine. Initially, acute administration of haloperidol caused an increase in activity of the A9 DA neurons (Bunney and Grace 1978; White and Wang 1983). Chronic administration, however, resulted in a significant decrease in activity of these same neurons. When GABA or DA was applied to these silent neurons, activity could be restored, suggesting that the neurons had undergone depolarization blockade. It was later found that chronic haloperidol also caused depolarization blockade of the A10 DA neurons (Chiodo and Bunney, 1983). Chronic clozapine treatment, however,

resulted in depolarization blockade of only the A10 but not A9 DA neurons. This finding is significant, since it has been suggested that EPS associated with neuroleptic treatment may be correlated with the drug effect on A9 neurons versus the antipsychotic effect of the drug on A10 neurons (Chiodo and Bunney 1983, 1985). Blockade of A9 DA neurons by haloperidol may result in an increased density of perforated synapses within the caudate, whereas the failure of clozapine to block such activity may be responsible for the lack of change within this brain area.

Why blockade of A10 neuronal activity by both haloperidol and clozapine failed to change the density of perforated synapses within the nucleus accumbens or MPCx is not certain. What is clear is that the circuitry of the dorsolateral caudate and nucleus accumbens are distinct. Besides inputs from A9 DA neurons (Beckstead et al. 1979), and sensorimotor cortex (McGeorge and Faull 1989), the caudate also receives afferents from the parafasicular nucleus of the thalamus (Berendse and Groenewegen 1990). The nucleus accumbens receives input from the A10 DA neurons (Beckstead et al. 1979) and from widespread areas of limbic cortex, including medial prefrontal (Sesack et al. 1989), hippocampus (Kelley and Domesick 1982) and amygdala (Kelley et al. 1982). The A10 DA neurons also project to MPCx (Emson and Koob 1978; Lindvall et al. 1978; Van Eden et al. 1987). We hypothesized that a possible neuronal source of the perforated synapse was sensorimotor cortex (Meshul and Casey 1989). Injection of the tracer, WGA–HRP, into sensorimotor cortex of untreated rats labels nerve terminals associated with perforated synapses within the rostral dorsolateral caudate (Meshul et al. 1990). Injections of this tracer into the parafasicular nucleus of the thalamus labelled only small, non-perforated, nerve terminals within a similar area of the caudate (Meshul, unpublished observations).

We also suggested that activation of the corticostriatal pathway could bring about the increase in perforated synapses due to haloperidol treatment (Meshul and Casey 1989). Specifically, blockade of caudate DA receptors would prevent the inhibitory effects of DA on this area, resulting in an increase in GABA release from the caudate to the globus pallidus. This would inhibit the activity of the globus pallidus, which would cause a decrease in release of GABA from the globus pallidus to the thalamus (VA/VL). The decrease of inhibition from the globus pallidus would activate the projection from the thalamus to the motor cortex and subsequently activate the corticostriatal pathway. This suggestion is consistent with the observation that there is an increase in glucose utilization within the striatum following haloperidol (Carvey et al. 1987). Depolarization blockade of the A10 DA neurons, due to either haloperidol or clozapine treatment, could ultimately fail to activate the pathways from the various limbic cortical regions to the nucleus accumbens, thus preventing any synaptic change in this nucleus. On the other hand, the clozapine-induced increase in specific binding to D–1 and D–2 receptors within the MPCx (Tables 2 and 3) may also affect the activity of the descending cortico-accumbens pathway.

Clozapine also binds to other non-DA receptors (Anderson et al. 1985; Seeman and Grigoriadis 1987), including muscarinic (Miller and Hiley 1974; Snyder et al. 1974), alpha$_2$-noradrenergic (Bartholini et al. 1972; Burki et al. 1974; McMillen and Shore 1978; Peroutka and Snyder 1980), histaminergic (Peroutka and Snyder 1980), and serotonergic (5–HT$_2$) (Hyttel et al. 1985). In addition, chronic administration of clozapine has recently been shown to significantly decrease the density of 5–HT$_2$ receptors within the cortex (Wilmot and Szczepanik 1989; O'Dell et al. 1990), and the striatum (O'Dell et al. 1990). Chronic administration of haloperidol did not effect the density of 5–HT$_2$ receptors. The clozapine-induced decrease in 5–HT$_2$ receptors within the striatum was measured in the ventrolateral region, since binding in the dorsolateral area was near background levels. The ventrolateral striatum receives mainly limbic afferents compared to the dorsolateral striatum, which receives sensorimotor input (McGeorge and Faull 1989). This is important since the analysis of perforated synapses in the present study used tissue from the dorsolateral region of the caudate. The direct effect of clozapine in this dorsal region would, therefore, have been minimal. It is possible that blockade of cortical 5–HT$_2$ receptors could affect activity of the corticostriatal pathway, which is at least one origin of the perforated synapse (Meshul et al. 1990).

The midbrain serotonin raphe neurons also project to the substantia nigra (Steinbusch 1981), and lesions of this pathway lead to a decrease in serotonergic activity and a decrease in haloperidol-induced catalepsy (Kostowski et al. 1972). Gallager and Aghajanian (1976) reported that atypical neuroleptics inhibit the activity of neurons within the raphe, but typical neuroleptics have no effect. The atypical neuroleptics may decrease the inhibitory effect of serotonin on the substantia nigra (A9) and could play a role in preventing depolarization blockade of these midbrain neurons. Absence of depolarization blockade of the A9 DA neurons by clozapine could explain the lack of change in perforated synapses within the caudate.

The heterogeneity of the striatum, in terms of afferent connections, may be important in terms of explaining the relative lack of EPS and TD observed with clozapine versus haloperidol. Clozapine failed to change the density of perforated synapses within the motor-related dorsolateral caudate, as compared to the haloperidol-induced increase in perforations. Both drugs had no influence on the density of perforated synapses within the nucleus accumbens or MPCx, and this may be important for the antipsychotic effect of both drugs. Meltzer et al. (1989) suggested the importance of the ratio between 5–HT$_2$ and D–2 binding in terms of classifying typical and atypical antipsychotic drugs and their effects in producing EPS. Co-administration of haloperidol and a 5–HT$_2$ blocker, such as ritanserin, a drug which activates DA neurons (Ugedo et al. 1989), could prevent the increase in perforated synapses as shown with clozapine. This will be critical in determining the role of serotonergic activity in the action of clozapine, since ritanserin has been found to decrease the EPS associated with neuroleptic treatment in humans (Bersani et al. 1986, 1990). However, it has also been recently reported that ad-

ministration of a series of neuroleptic drugs with varying 5-HT_2/D-2 binding ratios produced clinically indistinguishable EPS in nonhuman primates (Casey 1989b).

In summary, we have shown that haloperidol treatment results in an increase in the mean percentage of perforated synapses only within the dorsolateral caudate and not the nucleus accumbens or MPCx. Similar treatment with clozapine failed to change the density of perforated synapses within the three brain areas examined. There was no difference in the total number of synapses following any of the treatments in any of the brain regions. Changes in specific binding to DA receptors and morphology was only correlated within the caudate following haloperidol. In contrast to the caudate nucleus, the MPCx did not show a correlation between the percentage of perforated synapses and changes in D-1 or D-2 binding induced by clozapine or haloperidol. With regard to perforated synapses, there appeared to be no direct correlation between biochemical and morphological changes following neuroleptic drug treatment.

Acknowledgements. This research was supported by the Department of Veterans Affairs Merit Review Program to C.K.M. and D.E.C. and by US Public Health Service Grant MH 42894 to A.J. and MH 36557 to D.E.C..

References

Andersen PH, Gronvald FC, Jansen JA (1985) A comparison between dopamine-stimulated adenylate cyclase and ^{3}H-SCH 23390 binding in rat striatum. Life Sci 37:1971–1983

Andersen PH, Braestrup C (1986) Evidence for different states of the dopamine D1 receptor: clozapine and fluperlapine may preferentially label an adenylate cyclase-coupled state of the D1 receptor. J Neurochem 47:1822–1831

Baldessarini RJ, Tarsy D (1980) Pathophysiologic basis of tardive dyskinesia. Adv Biochem Psychopharmacol 24:451–455

Bartholini G, Haefely W, Jalfre M, Keller HH, Pletscher A (1972) Effects of clozapine on central catecholaminergic neurone systems. Br J Pharmacol 46:736

Beckstead RM, Domesick VB, Nauta WJH (1979) Efferent connections of the substantia nigra and ventral tegmental area in the rat. Brain Res 175:191–217

Benes FM, Paskevich PA, Domesick VB (1983) Haloperidol induced plasticity of axon terminals in rat substantia nigra. Science 221:969–971

Benes FM, Paskevich PA, Davidson J, Domesick VB (1985a) The effects of haloperidol on synaptic patterns in the rat striatum. Brain Res 329:265–274

Benes FM, Paskevich PA, Davidson J, Domesick VB (1985b) Synaptic rearrangements in medial prefrontal cortex of haloperidol-treated rats. Brain Res 348:15–20

Berendse HW, Groenewegen HJ (1990) Organization of the thalamostriatal projections in the rat, with special emphasis on the ventral striatum. J Comp Neurol 299:187–228

Bersani G, Grispini A, Marini S, Pasini A, Valducci M, Ciani N (1986) Neuroleptic-induced extrapyramidal side effects: clinical perspectives with ritanserin (R 55667), a new selective 5-HT_2 receptor blocking agent. Curr Ther Res 40:492–499

Bersani G, Grispini A, Marini S, Pasini A, Valducci M, Ciani N (1990) 5-HT_2 antagonist ritanserin in neuroleptic-induced parkinsonism: a double-blind comparison with orphenadrine and placebo. Clin Neuropharmacol 13:500–506

Bunney BS, Grace AA (1978) Acute and chronic haloperidol treatment: comparison of effects of nigral dopaminergic cell activity. Life Sci 23:1715–1728

Burki HR, Ruch W, Asper H, Baggiolini M, Stille G (1974) Effect of single and repeated administration of clozapine on the metabolism of dopamine and noradrenaline in the brain of the rat. Eur J Pharmacol 27:180–190

Carvey PM, Braun AR, Kao L-C, Klawans HL (1987) Effects of dopamine antagonists and apomorphine on regional energy metabolism in rat CNS. Clin Neuropharmacol 10:247–260

Casey DE (1987) Tardive dyskinesia. In: Meltzer HY (ed) Psychopharmacology: the third generation of progress. Raven Press, New York, pp 1411–1419

Casey DE (1989a) Clozapine: neuroleptic-induced EPS and tardive dyskinesia. Psychopharmacology 99:S47–53

Casey DE (1989b) Serotonergic aspects of acute extrapyramidal syndromes in nonhuman primates. Psychopharmacol Bull 25:457–459

Chiodo LA, Bunney BS (1983) Typical and atypical neuroleptics: differential effects of chronic administration on the activity of A9 and A10 midbrain dopaminergic neurons. J Neurosci 3:1607–1619

Chiodo LA, Bunney BS (1985) Possible mechanisms by which repeated clozapine administration differentially affects the activity of two subpopulations of midbrain dopamine neurons. J Neurosci 5:2539–2544

Costall B, Naylor RJ (1976) A comparison of the abilities of typical neuroleptic agents and of thioridazine, clozapine, sulpiride and metoclopramide to antagonize the hyperactivity induced by dopamine applied intracerebrally to areas of the extrapyramidal and mesolimbic systems systems. Eur J Pharmacol 40:9–18

Duncan GE, Creswell HE, McCown JJ, Paul IA, Mueller RA, Breese GR (1987) Behavioral and neurochemical responses to haloperidol and SCH-23390 in rats treated neonatally or as adults with 6-hydroxydopamine. J Pharmacol Exp Ther 243:1027–1034

Dunnett SD, Iversen SD (1982) Sensorimotor impairments following localized kainic acid and 6-hydroxydopamine lesions of the striatum. Brain Res 248:121–127

Einot I, Gabriel KR (1975) A study of the powers of several members of multiple comparisons. J Am Statist Assoc 70:574–583

Emson PC, Koob GF (1978) The origin and distribution of dopamine-containing afferents to the rat frontal cortex. Brain Res 142:249–267

Gallager DW, Aghajanian GK (1976) Effect of antipsychotic drugs on firing of dorsal raphe cells. Eur J Pharmacol 39:341–363

Geinisman Y, de Toledo-Morrell L, Morrell F (1986) Loss of perforated synapses in the dentate gyrus: morphological substrate of memory deficit in aged rats. Proc Natl Acad Sci USA 83:3027–3031

Greenough WT, West RW, DeVoogd TJ (1978) Subsynaptic plate perforations: changes with age and experience in the rat. Science 202:1096–1098

Hatton JD, Ellisman MH (1982) A restructuring of hypothalamic synapses is associated with motherhood. J Neurosci 2:704–707

Hyttel J, Larsen J-J, Christensen AV, Arnt, J (1985) Receptor binding profiles of neuroleptics. Psychopharmacology 96[Suppl]:9–18

Kebabian JW, Calne DB (1979) Multiple receptors for dopamine. Nature 277:93–96

Kelley AE, Domesick VB, Nauta WJH (1982a) The amygdalostriatal projection in the rat-an anatomical study by anterograde and retrograde tracing methods. Neuroscience 7:615–630

Kelley AE, Domesick VB (1982b) The distribution of the projections from the hippocampal formation to the nucleus accumbens: an anterograde- and retrograde-horseradish peroxidase study. Neuroscience 7:2321–2335

Kostowski W, Gumulka W, Czlonkowski A (1972) Reduced cataleptogenic effects of some neuroleptics in rats with lesioned midbrain raphe and treated with p-chlorophenylalanine. Brain Res 48:443–446

Lindvall O, Bjorklund A, Divac I (1978) Organization of catecholamine neurons projecting to the frontal cortex in the rat. Brain Res 142:1–24

McGeorge AJ, Faull RLM (1989) The organization of the projec-

tion from the cerebral cortex to the striatum in the rat. Neuroscience 29:503–537
McMillen BA, Shore PA (1978) Comparative effects of clozapine and alpha-adrenoreceptor blocking drugs on regional noradrenaline metabolism in rat brain. Eur J Pharmacol 52:225–230
Meltzer HY, Matsubara S, Lee J-C (1989) Classification of typical and atypical antipsychotic drugs on the basis of dopamine D–1, D–2 and serotonin$_2$ pK_i values. J Pharmacol Exp Ther 251:238–246
Meshul CK, Casey DE (1989) Regional, reversible ultrastructural changes in rat brain with chronic neuroleptic treatment. Brain Res 489:338–346
Meshul CK, Janowsky A, Casey DE, Stallbaumer RK (1990) Haloperidol-induced synaptic changes in rat caudate nucleus are prevented by prior treatment with MK–801 or lesioning of the thalamus. Soc Neurosci Abstr 16:419
Miller RJ, Hiley CR (1974) Anti-muscarinic properties of neuroleptics and drug-induced parkinsonism. Nature 248:596–597
Neve KA, Henningsen A, Kinzie JM, de Paulis T, Schmidt DE, Kessler RM, Janowsky A (1990) Sodium-dependent isomerization of dopamine D–2 receptors characterized using [^{125}I]epidepride, a high affinity substituted benzamide ligand. J Pharmacol Exp Ther 252:1108–1116
Nieto-Sampedro M, Hoff SF, Cotman CW (1982) Perforated postsynaptic densities: probable intermediates in synapse turnover. Proc Natl Acad Sci USA 79:5718–5722
O'Dell SJ, LaHoste GJ, Widmark CB, Shapiro RM, Potkin SG, Marshall JF (1990) Chronic treatment with clozapine or haloperidol differentially regulates dopamine and serotonin receptors in rat brain. Synapse 6:146–153
Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2nd edn. Academic Press, San Diego
Peroutka SJ, Snyder SH (1980) Relationships of neuroleptic drug effects at brain dopamine, serotonin, alpha-adrenergic, and histamine receptors to clinical potency. Am J Psychiatry 137:1518–1522
Phelps PE, Houser CR, Vaughn JE (1985) Immunocytochemical localization of choline acetyltransferase within the rat neostriatum: a correlated light and electron microscopic study of cholinergic neurons and synapses. J Comp Neurol 238:286–307
Pickel VM (1986) Ultrastructure of central catecholaminergic neurons. In: Panula P, Paivarinta H, Soinila S (eds) Neurohistochemistry: modern methods and applications. Liss, New York, pp 397–423.
Ribak CE, Vaughn JE, Barber RP (1981) Immunocytochemical localization of GABAergic neurones at the electron microscopical level. Histochem J 13:555–582
Seeman P, Grigoriadis D (1987) Dopamine receptors in brain and periphery. Neurochem Int 10:1–25
Sesack SR, Deutch AY, Roth RH, Bunney BS (1989) Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with *Phaseolus vulgaris* leucoagglutinin. J Comp Neurol 290:213–242
Snyder SH, Banerjee SP, Yamamura HI, Greenberg D (1974) Drugs, neurotransmitters, and schizophrenia. Science 184:1243–1253
Steinbusch HWM (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat – cell bodies and terminals. Neuroscience 6:557–618
Stevens JR (1973) An anatomy of schizophrenia? Arch Gen Psychiatry 29:177–189
Ugedo L, Grenhoff J, Svensson TH (1989) Ritanserin, a 5-HT_2 receptor antagonist, activates midbrain dopamine neurons by blocking serotonergic inhibition. Psychopharmacology 98:45–50
Van Eden CG, Hoorneman EMD, Buijs RM, Matthijssen MAH, Geffard M, Uylings HBM (1987) Immunocytochemical localization of dopamine in the prefrontal cortex of the rat at the light and electron microscopical level. Neuroscience 22:849–862
Vincent SR, McSparren J, Wang RY, Benes FM (1989) Effect of chronic administration of neuroleptics on synapses of layer VI medial prefrontal cortex. Soc Neurosci Abstr 15:269
Vrensen G, Cardozo JN (1981) Changes in size and shape of synaptic connections after visual training: an ultrastructural approach of synaptic plasticity. Brain Res 218:79–97
White FJ, Wang RY (1983) Comparison of the effects of chronic haloperidol treatment on A9 and A10 dopamine neurons in the rat. Life Sci 32:983–993
Wilmot CA, Szczepanik AM (1989) Effects of acute and chronic treatments with clozapine and haloperidol on serotonin (5-HT_2) and dopamine (D_2) receptors in the rat brain. Brain Res 487:288–298