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Classification of Neuroleptic Drugs According to Their Ability to Inhibit Apomorphine-Induced Locomotion and Gnawing: Evidence for Two Different Mechanisms of Action

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Abstract. Apomorphine 5 mg/kg given s.c. induces two different behaviours that can be separately measured in a special test box: one characterized by increased locomotion and one characterized by strong compulsive gnawing. Six different neuroleptic drugs with different clinical profiles (metaclopramide, haloperidol, chlorpromazine, thioridazine, clozapine, and sulpiride) were tested for their ability to antagonize either of these two different behaviours. We found that the neuroleptic drugs causing high incidences of extrapyramidal side effects (metoclopramide and haloperidol) predominantly antagonized the apomorphine-induced compulsive gnawing, while the 'atypical' neuroleptic drugs causing low incidences of extrapyramidal side effects (thioridazine, clozapine, sulpiride) instead antagonized the apomorphine-induced locomotion. When the drugs were rank-ordered according to their relative potencies in antagonizing gnawing as compared to locomotion, the rank order paralleled clinical data concerning the incidence of extrapyramidal side effects. The findings are tentatively explained by the existence of two different dopamine receptors. The test may be useful for the screening of new neuroleptic drugs because it seems possible to distinguish drugs producing extrapyramidal side effects from drugs that do not.

Key words: Automatic registration of behaviour – Apomorphine – Neuroleptics – Stereotyped behaviour – Open-field activity – Locomotor activity

Clinically used antipsychotic drugs are thought to be effective because of their ability to block dopamine (DA) receptors in the brain (see Matthysse, 1973; Iversen, 1975; Creese et al., 1976). In animal models the selection of new possible antipsychotic drugs has therefore often been based on their ability to block the stereotyped behaviour induced by dopaminergic drugs (e.g., apomorphine and amphetamine). But such screening models are somewhat misleading because such drugs as thioridazine, clozapine, and sulpiride are antipsychotically potent but inefficient blockers of apomorphine-induced stereotyped behaviour (Janssen et al., 1965; Stille et al., 1971; Laville, 1972). In the clinic these drugs have also been found less potent in inducing extrapyramidal side effects (see Klein and Davies, 1969; Angst et al., 1971; De Maio, 1972; Carranza and Toro, 1974; Ishimaru et al., 1974) and it therefore seems possible that the ability to antagonize apomorphine-induced stereotypy may, in fact, predict the incidence of extrapyramidal side effects rather than the antischizophrenic potency (Janssen et al., 1960).

In a recent study (Ljungberg and Ungerstedt, 1977) we identified and quantified two different apomorphine-induced behaviours, one characterized by compulsive gnawing and another characterized by increased locomotion accompanied by sniffing and repetitive head and limb movements. Because of the characteristics of these two behaviours, we concluded that they were not expressions of different apomorphine doses (intensity of the stimulation) but were elicited by different synaptic mechanisms in the brain (Ljungberg and Ungerstedt, 1977).

In view of our results it seemed highly interesting to test whether different types of neuroleptic drugs with different clinical profiles may differ in their ability to antagonize the two different apomorphine-induced behaviours, and whether a difference in their specificity would relate to their ability to induce extrapyramidal side effects. In this paper we now report that neuroleptic drugs differ widely in their ability to antagonize the two different apomorphine-induced behaviours and that their relative potencies in antagonizing these two behaviours relates to clinical data concerning the incidence of extrapyramidal side effects.

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MATERIALS AND METHODS

General. The experiments were performed on 159 male Sprague Dawley rats (Anticimex, Stockholm) weighing 160–210 g. The animals arrived at least 3 days before the experiments and were kept 5/cage under constant temperature and humidity conditions on a 12 h light/dark schedule (6 a.m.–6 p.m.). One hour before the start of the experiment the animal was weighed, isolated in a clean cage, and moved to the weakly illuminated experimental room. All animals were used only once.

Apparatus. The behaviour of the animal was recorded in a test box designed for the automatic recording of eight components of behaviour. It consisted of a modified open-field area (69 × 69 cm, with 25 cm high walls), where the animal could move around freely in the periphery but was unable to cross the middle part of the area (see Fig. 1). The movements of the animal was detected by interruptions of ten photobeams, using visible light, symmetrically covering the open-field area. *Activity*[1] was defined as the number of interruptions of these ten photobeams. By feeding the photobeam interruptions into a digital logic, the locomotion of the animal in the open-field area could also be recorded and separated from repetitive interruptions of one photobeam. *Total locomotion* was defined as the number of times the animal walked a fixed distance, which was slightly less than the length of the side of the box and defined by the arrangement of the photocells. *Forward locomotion* was defined as the number of times the animal walked the same distance but continued from one arm into the next arm (see Fig. 1). Thirty-two holes (2.5 cm in diameter) were symmetrically distributed over the entire floor. Six photobeams, one for each row of holes, were mounted under the floor in such a way that a beam was interrupted as soon as the nose of the animal was lowered deeper than 0.5 cm into a hole as measured from the surface of the floor. *Hole count* was defined as the number of interruptions of these photobeams and *hole time* was the accumulated time these photobeams were interrupted. One vertically directed photobeam was positioned in each corner. *Corner count* was defined as the number of interruptions of these photobeams and *corner time* was the accumulated time these photobeams were interrupted. The box was built out of black 1 cm thick PVC plastic and supported on rubber pads. The gnawing of the animal, which was almost always performed on the edges of the holes, caused a characteristic sound/vibration that was detected, amplified, triggered, and converted to digital pulses and counted. The detection was adjusted so that one 'gnaw' produced one digital pulse. The test box itself was placed in an outer sound-protecting box (1 × 1 × 1 m) with a small Plexiglass observation window at the top. The floor of the test box was positioned 40 cm above the bottom of the outer box. A rat looking down through a hole in the test box could thus see the bottom of the outer box 40 cm underneath. The bottom of the outer box was dark blue and weakly illuminated. The floor of the test box also received weak illumination from four 2.5-W lamps positioned just underneath the observation window. An electric fan ventilated the box at the same time as it provided a constant background noise. The assembly was placed in a weakly illuminated room.

All data from the test box were fed on-line into a WANG 2200 S minicomputer for plotting of the individual experiment and for statistical analyses and group comparisons. The data were accumulated in 15-min periods and were analyzed and presented either in counts/15 min or in total counts for the whole apomorphine activation (0–90 min after the injection).

Concomitant with the automatic recordings, more than half the animals were also observed 1–2 times during the habituation period and at the apomorphine *locomotion* and *gnawing* peak effects, and notes with a description of the behaviour were taken.

For further description of this technique see Ljungberg and Ungerstedt (1978a).

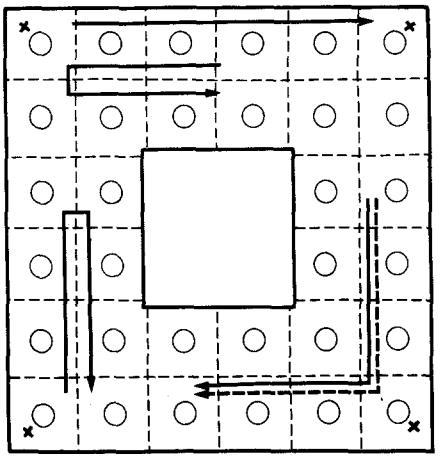

Fig. 1. The test box consists of a modified open-field symmetrically covered by ten photobeams (*thin dotted lines*). *Activity* is number of interruptions of these photobeams. A *locomotion* count is obtained every time animal walks a fixed distance defined by programming of photobeams. A *total locomotion* count corresponds to distance walked by animal (*solid lines*) and a *forward locomotion* count corresponds to distance walked by animal (*thick dotted line*). The 'X' in the corners shows position of vertical photobeam detecting entries of animal into corner. *Corner count* is number of interruptions of these photobeams and *corner time* is accumulated time of interruption. Thirty-two holes are distributed over floor. Six photobeams, one for each row of holes, are mounted under floor to detect when animal lowers its head into hole. *Hole count* is number of interruptions of these photobeams and *hole time* is accumulated time of interruption. Compulsive gnawing, which is nearly always performed on edges of the holes, causes characteristic sound/vibration, which is detected and converted to digital pulses and counted. Detection is adjusted so that one 'gnaw' gives one count

Drug Treatments. Apomorphine was obtained in commercially available 1 ml ampoules (Injectabile apomorphini 5 mg/ml, Apoteksbolaget, Sweden, with an injection vehicle containing 1 g $NaHSO_3$, 0.9 g 1 M HCl, 8.33 g NaCl, 2 g methylparaoxybenzoate, aq. dest. add 1000 ml), and was injected s.c. in a volume of 1 ml/kg body weight.

Metoclopramide (Primperan, Lundbeck), haloperidol (Haldol, Leo), chlorpromazine (Hibernal, Leo), and sulpiride (Equilid, Lepetit) were also obtained as injection ampoules. They were diluted with isotonic saline to reach the volume of 5 ml/kg body weight. The doses refer to the above-mentioned form. Thioridazine-HCl (Sandoz) was dissolved in isotonic saline to reach the volume of 5 ml/kg body weight (dose refers to the base). Clozapine (Sandoz) was dissolved in a minimal quantity of 1 M HCl and made up to volume (5 ml/kg body weight) with isotonic saline. The drugs were injected i.p.

All doses, number of animals used, and pretreatment times are summarized in Table 1.

Statistics. All data are presented as the medians. The Mann-Whitney *U*-test was used to calculate the degree of significance and the Spearman Rank correlation was used to calculate the degree of correlation between the ED_{50} values (Siegel, 1956). The ED_{50} was defined as the dose of drug needed to produce a 50% inhibition

[1] In order to differentiate between the actual behaviour of the animal and the automatically recorded behavioural components all recorded components are written in italics

Table 1. The different drugs and doses tested, pretreatment times and number of animals used are shown

Drug	Dose	Minutes	*N*	Act.	Tot. loc.	For. loc.	Gnawing	Hole c.	Hole t.	Corner c.	Corner t.
NaCl			8	353	23	11	1	102	121	160	4135
Apomorphine	5		13	2478*	201*	162*	2248*	125	56	487*	1527*
Metoclopramide	2.5	(30)	5	3421	438	293	1072	465	218*	559	781*
(before apo 5)	5	(30)	5	2332	208	181	851	336	217*	395	860
	10	(60)	5	1846	210	189	18*	200	160*	457	1446
	20	(60)	4	1009*	58*	28*	5*	171	158	135*	285*
	40	(60)	4	112*	1*	0*	0*	109	397*	11*	448
Haloperidol	0.1	(30)	4	3027	159	90	1642	280	178*	254*	827*
(before apo 5)	0.2	(30)	6	2286	236	192	549*	611	333*	382	1033
	0.4	(60)	6	1684*	146	116	11*	138	66	176*	446
	0.7	(60)	4	522*	24*	11*	1*	211	152	77*	235*
	1.0	(60)	6	295*	6*	4*	1*	101	145	7*	17*
Chlorpromazine	0.5	(30)	4	3637	226	165	2102	523	194	439	998
(before apo 5)	2	(30)	6	2759	110*	66*	1773	162	106	240*	713
	5	(30)	6	1557*	90*	62*	1975	200	236*	238*	635*
	20	(60)	4	1882	136	100*	10*	181	2148*	152*	456*
	50	(60)	4	32*	0*	0*	1*	338	2613*	20*	1687
Thioridazine	0.5	(30)	4	2709	135	101*	1531	170	73	424	1376
(before apo 5)	2	(30)	6	1651	74*	48*	1942	201	127	310	841
	5	(30)	4	1977	63*	47*	1552	145	161	214*	1133
	20	(60)	5	425*	14*	9*	1095	725	1173*	73*	154*
	50	(60)	4	304*	8*	7*	895	255	1576*	59*	603
Clozapine	2	(30)	4	3557	153	107	2065	164	94	398	1024
(before apo 5)	5	(30)	8	2487	91*	58*	2457	200	116	421	1686
	20	(30)	7	662*	20*	10*	1994	393	762*	238*	1013
	50	(60)	5	333*	2*	1*	95*	40*	4738*	39*	243
Sulpiride	20	(30)	6	2488	200	117	2302	478	244	421	1795
(before apo 5)	50	(60)	6	1280*	98	86	2076	249	179	468	1601
	200	(60)	6	374*	2*	2*	2559	289	128	248	850

Apomorphine 5 mg/kg was injected s.c. 90 min after animals were placed in test box and data are presented as median total counts for period 0–90 min after apomorphine injection. Only apomorphine-injected animals are compared with saline-injected controls, while neuroleptic-pretreated animals are compared with apomorphine-injected animals

* $P < 0.05$

of the apomorphine-(5 mg/kg) induced activation and was calculated graphically.

RESULTS

General Results

In all experiments the animal was left for 90 min in the test box before the injection of apomorphine. The behaviour of normal animals and other characteristics of this habituation period have been described in great detail elsewhere (Ljungberg and Ungerstedt, 1978a). The duration of the apomorphine 5 mg/kg activation was 90 min, and the results shown in Tables 1 and 2 are presented as total counts obtained during this period. All the experiments were continued for at least another 30 min in order to detect any increase in the apomorphine duration, but no such increases were found. In all reported experiments the data were also analyzed and statistically tested against the apomorphine controls for each 15-min period. This way of analyzing the data was somewhat more sensitive, but the results were principally the same as when comparing the total counts. Some illustrative results are shown in this way (see Figs. 2 and 3).

The principal results obtained for *total* and *forward locomotion* were the same, and the results are therefore described in the text as only *locomotion*. Because *locomotion* and *gnawing* occurred at different times after the apomorphine injection (locomotion peak 0–30 min and gnawing peak 30–60 min, see Fig. 2 and below), a specific effect on one of these behaviours may be due to the time at which the neuroleptic drug was injected. In order to test this, the pretreatment times were varied over a range of 0–90 min in several

Table 2

	Activity	Locomotion	Gnawing	Corner c.	Act./loc.	Act./corner c.	Loc./gnaw.
Metoclopramide	17	15	2.5	16	1.1	1.1	6
Haloperidol	0.5	0.5	0.14	0.3	1.0	1.7	3.6
Chlorpromazine	28	6	9	3	4.7	9.3	0.7
Thioridazine	10	1	20	4	10.0	2.5	< 0.1
Clozapine	13	3	32	20	4.3	0.7	< 0.1
Sulpiride	50	50	> 200	200	1.0	0.25	< 0.4

The ED_{50} values were defined as dose of drug needed to produce 50% inhibition of apomorphine-induced activation and were calculated graphically. Drugs are ranked according to quote ED_{50} *locomotion*/ED_{50} *gnawing*, which parallels published clinical data concerning incidences of extrapyramidal side effects. (For further evaluation of the ED_{50} values see Discussion)

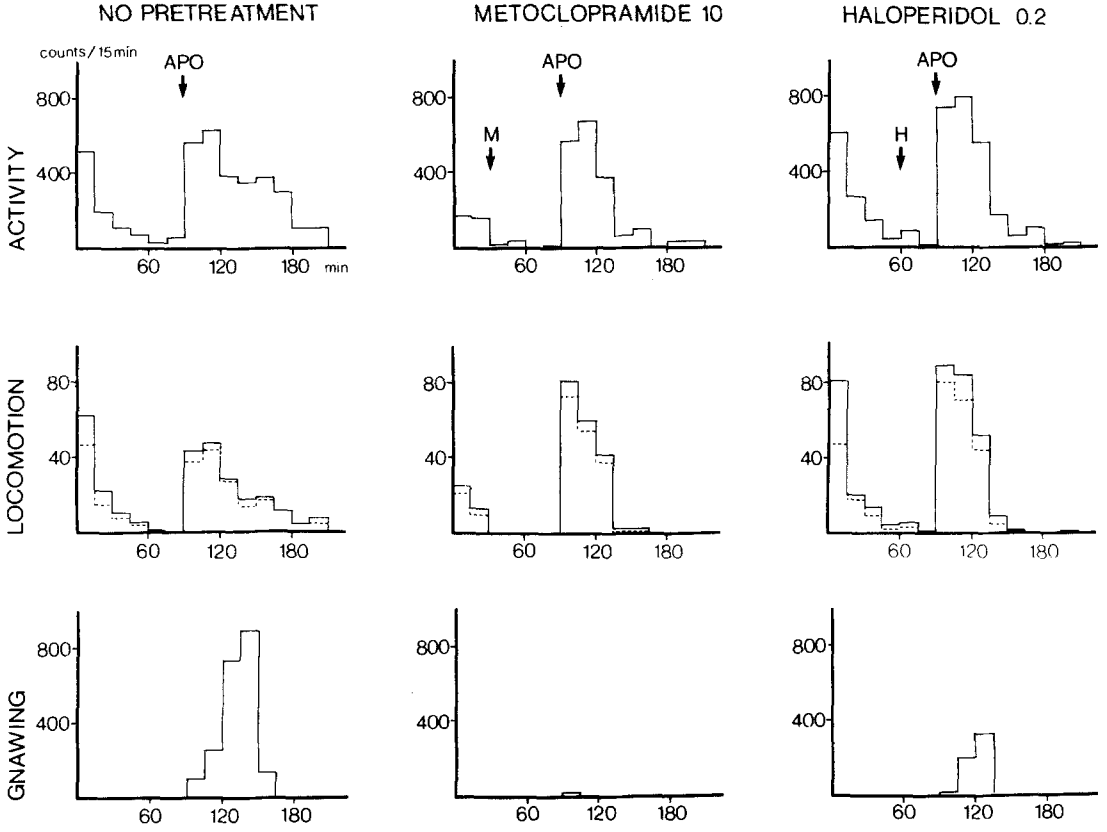

Fig. 2. Apomorphine 5 mg/kg was injected s.c. 90 min after start of recordings. Behavioural response to apomorphine was characterized 0–30 min after injection by intense *locomotion* (*solid line = total locomotion, dotted line = forward locomotion*) with only little compulsive *gnawing*. At 30–60 min after injection this pattern changed toward pattern characterized by relatively lower *locomotion* but instead intense compulsive *gnawing*. Both metoclopramide 10 mg/kg given 60 min before apomorphine and haloperidol 0.2 mg/kg given 30 min before apomorphine predominantly antagonized apomorphine-induced compulsive *gnawing* with metoclopramide being more specific

cases where specific effects were observed. In no case could the specificity be explained on the basis of this suspicion (Ljungberg and Ungerstedt, unpublished observation).

Apomorphine

Apomorphine 5 mg/kg induced a strong activation of the animals. At short time intervals after the injection (0–30 min), the activity consisted of high locomotion on somewhat straight legs with a hunched back posture and the tail bent upward. The locomotion was accompanied by sniffing, repetitive head and forelimb movements, and fast biting movements. Only a few occasions of intense compulsive gnawing was observed. After about 30 min the animals started to show less locomotion and less repetitive head and forelimb movements, but also showed an increase of

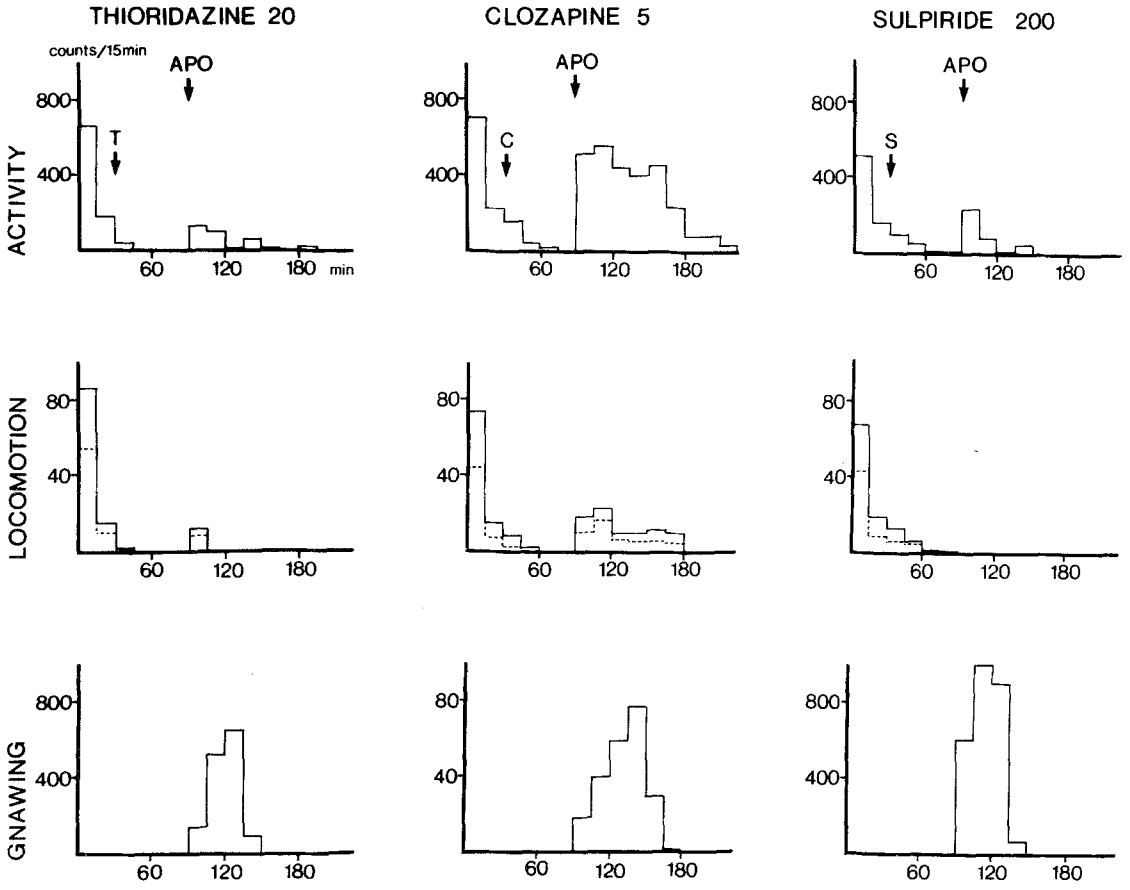

Fig. 3. Apomorphine 5 mg/kg was injected s.c. 90 min after start of recordings. Thioridazine 20 mg/kg given 60 min before apomorphine, clozapine 20 mg/kg (60 min), and sulpiride 200 mg/kg (60 min) all antagonized apomorphine-induced *locomotion* without reducing compulsive *gnawing*

strong compulsive gnawing, which was almost always performed on the edges of the holes (see Fig. 2). The apomorphine injection did not cause any specific increase in the *hole counts*. The *hole counts* recorded were recording errors due to interruption of the photobeams when a rat fell down with a leg in a hole or gnawed deeply at the edge of a hole (a decrease in recorded *hole time*). The animals were mainly active in the middle of the open-field area, but made many short entries into the corners.

Antagonistic Effect of Neuroleptic Drugs

Metoclopramide. Metoclopramide 2.5 mg/kg caused a further increase in the apomorphine-induced *activity* and *locomotion*, but caused a reduction of the *gnawing*. With increasing doses (5 and 10 mg/kg) the *activity* and *locomotion* remained in the range of the controls, while *gnawing* was completely blocked. The locomotion was quite similar to the locomotion at short time intervals after the apomorphine injection. The effect of 10 mg/kg over time is shown in Fig. 2. Higher doses (20 and 40 mg/kg) caused a dose-related decrease of apomorphine-induced *activity*, *locomotion*, and *corner count*. At 40 mg/kg the apomorphine activation was completely abolished.

Haloperidol. Haloperidol in low doses (0.1 and 0.2 mg/kg) caused a specific reduction of *gnawing* without reducing *locomotion* (see Fig. 2). The locomotion was quite similar to the locomotion at short time intervals after the apomorphine injection. Compared with metoclopramide, haloperidol did not cause as strong an increase of *locomotion* (see Table 1) and was not as potent in reducing *gnawing* (see Fig. 2). Higher doses (0.4 and 0.7 mg/kg) caused a dose-related reduction of apomorphine-induced *activity*, *locomotion*, and *corner count*. Haloperidol 1.0 mg/kg completely blocked all apomorphine activation.

Chlorpromazine. Low doses (2 and 5 mg/kg) caused a partial reduction of the apomorphine-induced *locomotion*. The remaining locomotion was somewhat

less varied and more automatized as compared with the controls. There was only a slight decrease in *gnawing* and *activity*. An increase of the dose to 20 mg/kg completely blocked *gnawing* and slightly increased *locomotion*. This locomotion was highly automatized and badly coordinated. Fifty mg/kg caused a complete blockade of the apomorphine-induced activation. *Corner count* showed a dose-dependent reduction on all doses.

Thioridazine. Thioridazine showed a very flat dose-response curve with a dose-related decrease in *activity*, *locomotion*, and *corner count*. There was no specific antagonism of *gnawing*, and the decrease observed on high doses probably reflects nonspecific effects (see below). After thioridazine 20 mg/kg (see Fig. 3) there was only a slight induction of badly coordinated locomotion, while the typical strong gnawing was still present. At 50 mg/kg a peculiar behaviour was observed: the animals were dipping their heads (and often also their forelimbs and the front part of their bodies) very deep into the holes and were trying to gnaw on the underside of the box.

Clozapine. Clozapine showed a dose-related reduction of apomorphine-induced *locomotion*, *activity*, and *corner count*, which was most potent in the reduction of *locomotion*. There was no specific reduction of *gnawing*. At 50 mg/kg the same peculiar behaviour as that after thioridazine 50 mg/kg was observed: The animals were hanging in the holes trying to gnaw on the underside of the box. The difficulties of gnawing in this position probably caused the decrease in the recorded *gnawing*.

Sulpiride. Sulpiride caused a clear dose-related reduction of apomorphine-induced *activity* and *locomotion*. The antagonism of the apomorphine-induced increase in *corner count* was much less potent, and in the dose range used there was no reduction in *gnawing* (Fig. 3).

ED_{50} Values

Because of the dose-response characteristics of the apomorphine effect (see Ljungberg and Ungerstedt, 1977b), ED_{50} values were calculated only for *activity*, *locomotion*, *gnawing*, and *corner count* (where the calculated ED_{50} values for *total locomotion* and *forward locomotion* did not differ from each other (see Tables 1 and 2).

The gnawing ED_{50} for thioridazine and clozapine have to be interpreted with some care since the decrease might have been caused by 'nonspecific effects,' as described above. In the dose range used there was no antagonism of the apomorphine-induced *gnawing* with sulpiride, and so the ED_{50} value is expressed as > 200. Clorpromazine gave a 50% reduction of *locomotion* in a wide dose range (see Results and Table 1). The middle dose in this range was taken as the ED_{50}. The ED_{50} values for *activity* and *locomotion* showed some correlation ($r_s = 0.88$, $P < 0.02$) while the other combinations did not ($P > 0.1$).

The ED_{50} quote *locomotion/gnawing* for a particular drug shows its relative potency to antagonize apomorphine *locomotion* as compared with the potency to antagonize apomorphine *gnawing*, and correlates with clinical data regarding incidences of extrapyramidal side effects (Table 2). The ED_{50} quote *activity/locomotion* subdivides the neuroleptics tested into two groups, one with equal potency to antagonize *activity* and *locomotion* and another group with a higher potency to antagonize *locomotion*. Within this group the different neuroleptics are separated by both the quote *locomotion/gnawing* and the relative potency to antagonize *corner count*. This group of neuroleptics, at high doses, also caused a strong increase in *hole time*.

During the recording period (90 – 180 min) saline-treated controls spent most of the time lying inactive in the corners, while apomorphine controls were very active in the open-field area (see also Ljungberg and Ungerstedt, 1977b). The neuroleptic-pretreated animals were, even when they were completely inactive after the apomorphine injection, not lying in the corners as controls, but in the open-field areas where they were placed after the apomorphine injection. This shows that the effect was not a 'normalization' of the behaviour.

DISCUSSION

The neuroleptic drugs tested in this study were selected to include drugs known to have different clinical profiles as regards antipsychotic properties in relation to incidences of extrapyramidal side effects (EPS): metoclopramide, which is not ordinarily used as an antipsychotic drug but which is known to elicit certain EPS (Borenstein and Bles, 1965), haloperidol, which is used as an antipsychotic drug and is known to induce EPS (see Klein and Davies, 1969), chlorpromazine, which is considered to give rise to less EPS than haloperidol (see Klein and Davies, 1969), and, finally, three 'atypical' neuroleptics, i.e., thioridazine, clozapine, and sulpiride, which do not possess typical neuroleptic properties in animal models (Janssen et al., 1965; Stille et al., 1971; Laville, 1972) and give few EPS in the clinic (see Klein and Davies, 1969; Angst et al., 1971; De Maio, 1972; Carranza and Toro, 1974; Ishimaru, 1974).

The neuroleptic drugs tested differed greatly in their ability to inhibit apomorphine-induced *gnawing*

and *locomotion* (Table 1). Metoclopramide blocked *gnawing* at a dose that did not reduce the locomotion. The action of haloperidol was similar but less specific, i.e., after a dose that blocked *gnawing* completely there was also a reduction in the apomorphine-induced *locomotion*. Chlorpromazine was more complicated since lower doses reduced both *locomotion* and *gnawing* while *locomotion* was slightly increased at a higher dose when *gnawing* was completely blocked. This locomotion was, however, different from the locomotion seen after apomorphine alone because it was highly 'automatic' and badly coordinated. Metoclopramide, haloperidol, and chlorpromazine were similar in that a sufficiently high dose was able to block completely *locomotion* as well as *gnawing* (cf. Buus Lassen, 1977). Thioridazine, clozapine, and sulpiride were qualitatively different from the first-mentioned neuroleptic drugs in that the apomorphine-induced *locomotion* was blocked at doses that did not reduce the apomorphine-induced *gnawing* (Table 1). After high doses of thioridazine and clozapine the animals developed a peculiar behaviour, hanging with the front part of their body into the holes and trying to gnaw on the underside of the box, which was reflected in the recordings as a strong increase in *hole time* (Table 1). The same type of behaviour has been observed after a combined treatment with haloperidol and scopolamine before apomorphine, and we have attributed this behaviour to a blockade of muscarinic receptors (Ljungberg and Ungerstedt, 1978b). This behaviour did not occur after sulpiride.

By forming the quote ED_{50} (for inhibition of locomotion)/ED_{50} (for inhibition of gnawing) for each neuroleptic drug, it seems possible to achieve a characterization of its pharmacological profile in our test. When the neuroleptic drugs are ranked according to this 'index' (Table 2), metoclopramide and haloperidol are ranked on one end of the scale, while thioridazine, clozapine, and sulpiride are ranked on the other side of the scale. When comparing this result with the clinical data on the incidence of EPS it seems that the neuroleptic drugs that cause a high incidence of EPS have a high index value, while those neuroleptic drugs that show antipsychotic potency with low incidence of EPS have a low index value.

The results in the present study on the antagonisms of the apomorphine-induced *gnawing* agrees with previous studies since thioridazine, clozapine, and sulpiride have been shown not to antagonize apomorphine-induced gnawing (Janssen et al., 1965; Stille et al., 1971; Laville, 1972), while chlorpromazine, haloperidol, and metoclopramide have been shown to antagonize gnawing in relative ED_{50} doses directly correlated with ours (Janssen et al., 1965, 1967; Puech, 1976). Our automatically recorded *locomotion*, however, probably does not correspond completely to the behaviour referred to as 'hypermotility' or 'hyperactivity' in other publications, because the apomorphine-induced 'hypermotility' has been effectively antagonized by haloperidol (Buus Lassen, 1976) and because Puech (1976) found that the ED_{50} values for the inhibition of apomorphine-induced 'stereotyped behaviour' and 'hyperactivity' were highly correlated for several neuroleptic drugs. Puech (1976) therefore concluded that the 'stereotyped behaviour' and 'hyperactivity' were mediated by the same mechanism. These differences in results are probably partly explained by the fact that the apomorphine-induced behaviours are highly dependent on the way of dissolving and injecting the drug and that only some ways of administrating the apomorphine produces the type of locomotion we have recorded in the present study (see Ljungberg and Ungerstedt, 1977). It is unclear whether this locomotion has been induced and studied by the other investigators. The other main difference is the definition and principle of recording locomotor behaviour. In the present study *locomotion* was defined as an actual distance covered by the animal while the 'hyperactivity' defined and measured in ordinary activity boxes is a nondescriptive 'mixed' recording that consists of several components of behaviour, locomotion being only one of them. The other components, like repetitive movements, rearing, and sniffing, might have other pharmacological properties than the actual locomotion, and the net result might therefore become quite different from ours. However, the observation that clozapine and thioridazine can antagonize the apomorphine induced 'hyperactivity' agrees well with our results (Maj et al., 1974; Buus Lassen, 1976, 1977).

Local injection of DA or drugs interfering with DA neurotransmission indicate that locomotion may be related to limbic DA neurotransmission (Pijnenburg and van Rossum, 1973). These findings, together with the suggested involvement of limbic DA neurotransmission in psychotic behaviour (see review by Matthysse, 1973), led Costall and Naylor (1976) to investigate the ability of various neuroleptic drugs to inhibit the locomotor behaviour induced by injecting DA into limbic areas, i.e., the nucleus accumbens septi. They were able to distinguish metoclopramide from a number of such other neuroleptics as clozapine, sulpiride, thioridazine, and haloperidol because metoclopramide in the dose range tested failed to reduce the DA-induced 'hyperactivity'. The fact that metoclopramide stood out in their test agrees well with our results; they were not, however, able to distinguish the other neuroleptic drugs (e.g., sulpiride from haloperidol) that have widely different clinical profiles

and that were clearly different in our test system when their potency to antagonize apomorphine-induced *gnawing* or *locomotion* was considered.

The fact that different neuroleptic drugs are markedly different in their ability to block apomorphine-induced *gnawing* and *locomotion* indicates that they differ in their mechanism of action. The experiments with local injections of DA into the brain suggests that the locomotion effect of apomorphine may be elicited from the limbic system (see above), while the gnawing effect may arise in the striatum (Ernst and Smelick, 1966). The neuroleptic drugs may thus differ in their ability to inhibit, for example, striatal and limbic DA neurotransmission. If this were the prime cause of their specificity in our test system, one would expect that they showed equally distinct effects in their ability to affect DA neurotransmission in these areas as reflected by, for example, regional levels of the DA metabolite homovanillic acid (HVA). But the literature describing regional HVA levels after various neuroleptic drugs is rather conflicting. Andén and Stock (1973) found that clozapine, which is quite specific in antagonizing *locomotion*, was more effective in raising HVA in limbic structures than in the striatum. This finding was confirmed by Stawarz et al. (1975), Westerink and Korf (1976), and Bartholini (1976) while, on the contrary, clozapine was found more effective in raising HVA in the striatum by Wiesel and Sedvall (1975) and Wilk et al. (1975). Finally, Bartholini et al. (1975), Westerink and Korf (1975), and Wilk and Glick (1976) failed to see any difference in the ability of clozapine to increase HVA levels in striatal and limbic brain areas.

Another possible explanation of our findings may be that the neuroleptic drugs affect neurotransmitters other than dopamine. The ability to block muscarinic receptors, for example, has in fact been suggested as an explanation of low incidence of EPS after certain drugs (Miller and Hiley, 1974; Snyder et al., 1974). In a separate study we tried to mimic the effect of clozapine and thioridazine by combining scopolamine and haloperidol, but it has not been possible to create a clozapine effect on locomotion in this way while still preserving the apomorphine-induced *gnawing* (Ljungberg and Ungerstedt, 1978 b). This agrees with the suggestion of Bürki et al. (1975) that the anti-muscarinic action of clozapine cannot explain its pharmacological difference from 'classical neuroleptics' like haloperidol. Finally, the fact that sulpiride, which in contrast to clozapine and thioridazine blocks neither muscarinic nor noradrenergic α-receptors (Laville, 1972; Tagliamonte, 1975), is able to block apomorphine-induced *locomotion* while not reducing the *gnawing* shows that the ability to interfere with acetylcholine or noradrenaline neurotransmission is not sufficient to explain the specific blockade of the apomorphine-induced *locomotion*.

Recently, much interest has centered around the possibility that more than one type of DA receptor may exist (for review see Cools and van Rossum, 1976). Costall and Naylor (1975) found that hyperactivity induced by intrastriatal administration of DA to guinea pigs was inhibited by many neuroleptic agents, while dyskinetic movements were inhibited only by pimozide and oxiperomide, and they postulated that this may be due to the existence of two DA receptors. Puech et al. (1976) found that sulpiride antagonized hypothermia and climbing behaviour induced by apomorphine in mice, in contrast to its inability to antagonize stereotyped behaviour induced by apomorphine in rats, and suggested that this may be due to the presence of two DA receptors. Buus Lassen (1976) also suggested that two DA receptors may exist, because he found that clozapine and thioridazine inhibited apomorphine 'hypermotility' in contrast to its known inability to inhibit apomorphine-induced gnawing. Our results in this study seem compatible with the idea that the various neuroleptic drugs block different DA 'receptors' related to the mediation of apomorphine-induced *locomotion* and *gnawing*. But when considering the data on HVA levels (see above), these 'receptors' seem not to be strictly localized in distinct anatomical regions.

In summary, it has been possible to characterize different neuroleptic drugs according to their ability to inhibit apomorphine-induced *gnawing* or *locomotion*. Neuroleptic drugs causing high incidence of extrapyramidal side effects predominantly antagonized the apomorphine-induced *gnawing*, while the 'atypical' neuroleptic drugs, showing antipsychotic potency together with low incidence of extrapyramidal side effects, instead antagonized the apomorphine-induced *locomotion*. The results may tentatively be explained by the existence of two different DA 'receptors'.

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