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"Classical" and "Atypical" Antipsychotic Drugs: Differential Antagonism of Amphetamine- and Apomorphine-Induced Alterations of Spontaneous Neuronal Activity in the Neostriatum and Nucleus Accumbens

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REBEC, G. V., T. R. BASHORE, K. S. ZIMMERMAN AND K. D. ALLOWAY. *"Classical" and "atypical" antipsychotic drugs: differential antagonism of amphetamine- and apomorphine-induced alterations of spontaneous neuronal activity in the neostriatum and nucleus accumbens.* PHARMAC. BIOCHEM. BEHAV. 11(5) 529–538, 1979.—The ability of clozapine and haloperidol to antagonize the depression of firing rate produced by d-amphetamine and apomorphine in the neostriatum and nucleus accumbens was tested in immobilized, locally anesthetized rats. In the neostriatum, an intraperitoneal injection of 2.5 mg/kg d-amphetamine or 1.0 mg/kg apomorphine produced a prolonged inhibition of neuronal activity that was reversed by a subsequent injection of either 20 mg/kg clozapine or 2.0 mg/kg haloperidol. An analysis of the onset and magnitude of the blockade revealed that clozapine was more effective than haloperidol in reversing the amphetamine response but that both antipsychotic drugs produced a comparable blockade of the apomorphine-induced depression. Similar results were obtained in the nucleus accumbens. The data indicate that although clozapine acts equieffectively in the neostriatum and nucleus accumbens, this atypical antipsychotic drug, aside from blocking postsynaptic dopamine receptors, may exert at least some of its effects by preventing dopamine release.

d-Amphetamine Apomorphine Clozapine Haloperidol Neostriatum Nucleus Accumbens Unit activity

SUBSTANTIAL evidence has implicated the nigro-neostriatal and mesolimbic dopamine (DA) systems in different components of the behavioral response to d-amphetamine. Kelly and Iversen [24], for example, reported that the focused stereotyped behavior produced by d-amphetamine in rats is abolished following a large-scale depletion of DA from nerve terminals in the neostriatum but not in the nucleus accumbens, a major projection area of the mesolimbic DA system. In contrast, amphetamine-induced locomotion survives destruction of DA afferents in the neostriatum but is blocked by similar damage in the nucleus accumbens [9, 24, 50]. Furthermore, a direct infusion of amphetamine into the neostriatum of intact animals elicits stereotypy, whereas a local injection of this drug into the nucleus accumbens produces locomotor activity [23,40].

Current speculation regarding the mechanisms of action of the antipsychotic drugs has upheld the behavioral distinction between the nigro-neostriatal and mesolimbic DA systems. Thus, although both the classical and the atypical antipsychotic drugs are thought to exert their clinical efficacy in humans by blocking DA receptors in the mesolimbic system, only the classical antipsychotics produce a series of parkinsonian-like extrapyramidal side effects presumably because these drugs also block DA transmission in the neostriatum [7,36]. This hypothesis, which supports the alleged involvement of the neostriatum in some major motor dysfunctions [20], is consistent with evidence that in rats the cataleptic behavior produced by the classical antipsychotics is intensified following a depletion of DA in the neostriatum but not in the nucleus accumbens [19]. The atypical antipsychotics, on the other hand, appear to act selectively in the mesolimbic system and consequently do not produce catalepsy [1, 2, 6, 19]. Additional support for a differentiation of function between the nigro-neostriatal and mesolimbic DA systems is derived from evidence that clozapine, an atypical antipsychotic drug, selectively abolishes ampheta-

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mine-induced locomotion without impairing focused stereotypy [22].

Low doses of d-amphetamine (0.5–2.5 mg/kg) typically produce a prolonged depression of neuronal activity in the neostriatum and nucleus accumbens that is sometimes preceded by a brief, initial excitation [3, 43, 44, 55]. The inhibition of firing rate, which may represent an important correlate of the amphetamine behavioral response [16], has been attributed to a facilitation of DA transmission since this effect can be blocked by the subsequent injection of 2.0 mg/kg haloperidol, a classical antipsychotic drug, or by a depletion of forebrain DA [3, 17, 18]. To test the hypothesis that clozapine acts selectively on the mesolimbic DA system, we compared the ability of clozapine and haloperidol to antagonize the neuronal response to d-amphetamine in the neostriatum and nucleus accumbens. Clozapine was administered at a dose (20 mg/kg) previously shown to selectively abolish amphetamine-induced locomotion [22] and to match the effects of 2.0 mg/kg haloperidol on spontaneous neuronal activity in the neostriatum and nucleus accumbens [41]. Since there is evidence that this and lower doses of clozapine, apart from blocking postsynaptic DA receptors, may prevent DA release [13,21], we also examined the effectiveness of these antipsychotic drugs in blocking the neuronal response to apomorphine, a direct acting DA receptor stimulant. Our results indicate, contrary to the popular view, that clozapine is equieffective in the neostriatum and nucleus accumbens and that this drug may act, at least in part, by blocking DA release.

METHOD

Experiments were performed on male, Sprague-Dawley rats (Murphy Breeding Laboratories, Plainfield, Indiana), weighing from 350–450 g, as described elsewhere [18,43]. Briefly, after the animals were anesthetized by ether inhalation, they were mounted in a stereotaxic instrument equipped with atraumatic ear bars (Kopf Instruments), and the skull was exposed. All points of surgical and stereotaxic contact were thoroughly treated with topical application of xylocaine (Astra) and with subcutaneous injections of procaine (Abbott). Supplemental applications of local anesthetic were made periodically throughout the experiment, and commercial eyedrops (Visine) were applied intermittently to prevent corneal drying. Bilateral holes were drilled in the skull overlying the neostriatum (7.5 mm anterior and 2.5 mm lateral to stereotaxic zero) or the nucleus accumbens (9.0 mm anterior and 1.0 mm lateral to stereotaxic zero) according to the coordinates of König and Klippel [28]. In some animals, a stainless steel electrode was placed over an exposed portion of frontal cortex to record electrocorticographic (ECoG) activity.

Following surgery, ether anesthesia was discontinued and the animals were immobilized with 2.0 mg/kg tubocurarine chloride (Lilly). Tracheal intubation was not necessary since positive pressure artificial respiration was provided by a Harvard Instruments Rodent Respirator that was attached to a rubber cone fitted snugly over the snout. Respiration rate and volume were adjusted to maintain a carbon dioxide concentration in the expired air of 3.5 to 4.5 percent as measured by a Beckman Instruments LB2 Medical Gas Analyzer. Heartbeat, displayed continuously on the face of an oscilloscope, served as another indication of the state of the preparation. Body temperature, measured with the aid of an anal thermistor, was maintained between 36.5° and 37.5°C throughout the experiment by means of a thermostatically controlled heating pad placed under the animal. ECoG activity was dominated by large, slow waves indicating effective local anesthesia.

Tungsten microelectrodes (Frederick Haer), having impedences of from 1.5 to 5.0 MΩ, were lowered into the target area on both sides of the brain. This procedure permitted simultaneous recording of two different neurons from each animal. Spontaneously active single unit discharges, isolated to a signal-to-noise ratio of 3:1 or more, were amplified and displayed on the face of an oscilloscope. On-line firing rates were counted on a minute-by-minute basis by means of a neuronal spike analyzer (Mentor N-750) in conjunction with a high speed printer-counter (Digitec 6120). Neuronal activity was also stored on magnetic tape for subsequent off-line analysis. Spontaneous activity was recorded for at least 30 min prior to drug injection to insure a stable baseline firing rate.

The mean spontaneous firing rate/min was calculated for the 10-min period immediately preceding the drug injection and was defined, in each case, as 100%. Drug-induced changes in firing rate were expressed in terms of the pre-injection baseline rate for each neuron sampled. This procedure allowed comparison of group data despite individual differences with respect to pre-injection firing rate. For purposes of statistical comparison, a drug-induced increase or decrease in firing rate was defined as a change of at least 40% from the baseline rate for a period of 5 min or longer (spontaneous predrug fluctuations in activity never exceeded this value for more than two consecutive min). Unit activity that failed to maintain a constant signal-to-noise ratio and that did not return to at least 60% of the predrug baseline rate was not included in the results.

All animals received an injection (free base) of either 2.5 mg/kg d-amphetamine sulfate (Smith, Kline and French) or 1.0 mg/kg apomorphine hydrochloride (Merck) via an indwelling intraperitoneal (IP) catheter. Separate groups of animals also received an IP injection of 20 mg/kg clozapine (Sandoz) or 2.0 mg/kg haloperidol (McNeil) 15–30 min later. To verify the accuracy of the IP injections, methylene blue dye was passed through the catheter at the completion of each experiment and the peritoneal cavity was subsequently inspected. Data obtained from animals in which dye was found outside the peritoneal cavity were discarded since the absorption rate of drugs from different adjacent organs could alter the results. Each animal received only one injection of either d-amphetamine or apomorphine to avoid residual drug effects [43].

At the end of each experiment, the animal received an IP anesthetic dose of pentobarbital (Abbott). To identify the site of the recording electrodes, current was passed through each electrode to produce a small lesion (Grass DCLM5). Following perfusion with normal saline and 10% Formalin, brains were frozen, sectioned and stained with cresyl-violet for histological analysis.

RESULTS

Unit Responses in the Neostriatum

Neurons in the neostriatum (n=5) responded to an IP injection of 2.5 mg/kg d-amphetamine with a prolonged depression of firing rate that persisted for a mean duration of 85.0 min with a standard error of the mean (SEM) of 19.3 min. Unit activity was suppressed to a mean maximum value

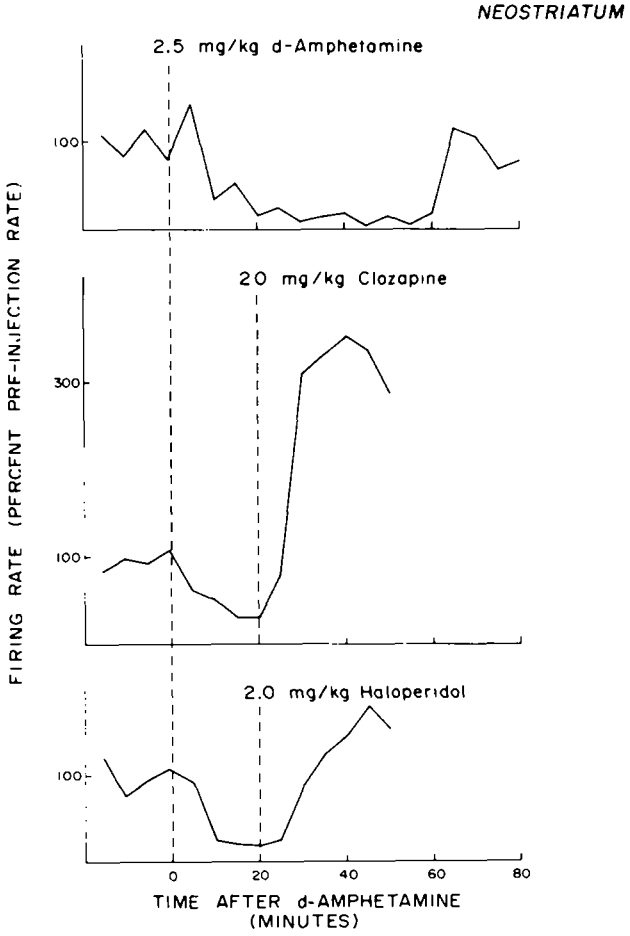

FIG. 1. Representative examples of the response of individual neurons in the neostriatum to an IP injection of d-amphetamine and some antipsychotic drugs. The top graph illustrates the time-course of the changes in the activity of a single neuron produced by 2.5 mg/kg d-amphetamine injected at Time 0. The other two graphs show the effects of injecting 20 mg/kg clozapine or 2.0 mg/kg haloperidol at 20 min after d-amphetamine. The pre-injection firing rate/minute was calculated for the 10-min period immediately before the amphetamine injection and was given a value of 100%. Spontaneous firing rate, based on the 100% value, is plotted for 20 min prior to amphetamine and for the entire postamphetamine period until unit activity returned to at least 60% of the pre-injection rate.

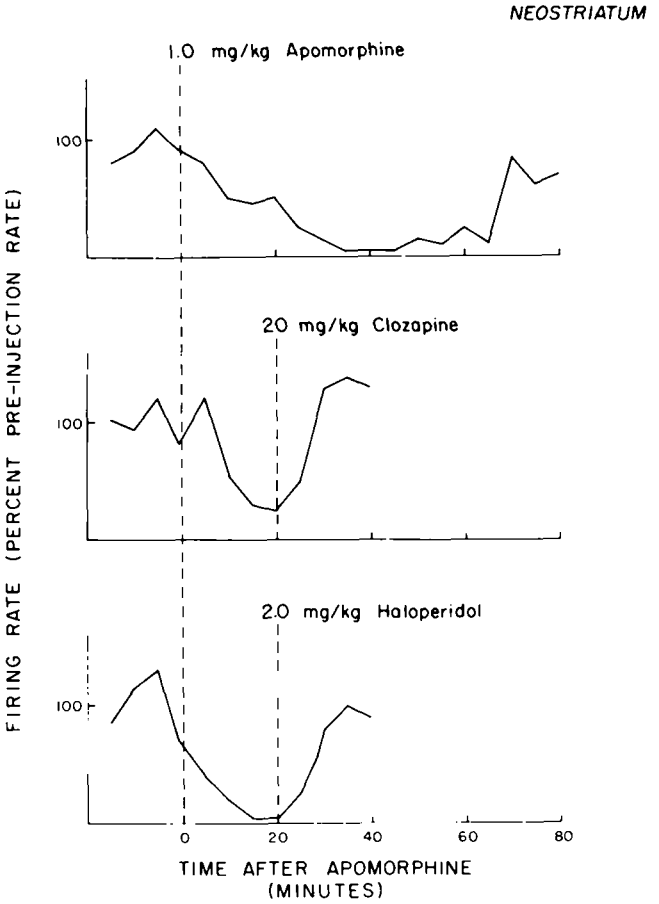

FIG. 2. Representative examples of the response of individual neurons in the neostriatum to apomorphine and some antipsychotic drugs. The top graph illustrates the time course of the neuronal response to 1.0 mg/kg apomorphine injected at Time 0. The lower graphs show the effects of administering 20 mg/kg clozapine or 2.0 mg/kg haloperidol after the apomorphine injection. Firing rate is expressed as percent of the pre-injection rate as in Fig. 1.

of 17 (SEM=10.6) percent of the pre-injection rate. Figure 1 illustrates the entire time-course of the amphetamine response for a representative neostriatal neuron. In this and all subsequent graphs, minute-by-minute counts of neuronal activity were averaged over 5-min intervals for the duration of the drug response. Note that in this case the depression of activity, which lasted for 65 min, was preceded by a brief, initial excitation.

In a separate series of animals, we examined the ability of 20 mg/kg clozapine (n=4) or 2.0 mg/kg haloperidol (n=4) to block the amphetamine-induced inhibition. The antipsychotic drugs were injected between 15 and 30 min after d-amphetamine at a time when unit activity was depressed to below 60% of the pre-amphetamine baseline rate for at least 5 min. Although both clozapine and haloperidol reversed the amphetamine response, these antipsychotics differed in their relative effectiveness (see Fig. 1). Thus, an analysis of the time required for unit activity to return to at least 60% of the baseline rate revealed that clozapine acted significantly faster than haloperidol, $F(1,7)=25.00$, $p<0.01$. Furthermore, clozapine produced a significantly greater increase in firing rate than haloperidol during the first 10 min after injection, $F(1,7)=5.99$, $p<0.05$. These differential effects of the antipsychotic drugs in blocking the response of neostriatal neurons to d-amphetamine are summarized in Table 1. Note also that in both antipsychotic drug groups, amphetamine produced a comparable depression of firing rate prior to clozapine or haloperidol administration.

Like d-amphetamine, apomorphine inhibited neuronal activity in the neostriatum (n=5). An IP injection of 1.0 mg/kg apomorphine produced a depression of firing rate that persisted for 58 (SEM=8.2) min and that reached a mean maximum value of 29 (SEM=8.2) percent of the pre-injection rate. The time-course of the apomorphine response is shown in Fig. 2. In this representative example, activity was suppressed to 5% of the baseline rate and recovery to the 60% criterion level occurred at 70 min after apomorphine administration.

TABLE 1

BLOCKADE OF THE RESPONSE TO DOPAMINE AGONISTS BY ANTIPSYCHOTIC DRUGS

Recording Site	Treatment		Mean Onset of Blockade in Minutes	Mean Firing Rate 10 Minutes Before Antipsychotic	Mean Firing Rate 10 Minutes After Antipsychotic
Neostriatum	d-Amphetamine	Clozapine (n=4)	5.0 (0.7)	21.3 (9.6)	180.5 (62.4)
		Haloperidol (n=4)	10.0 (0.9)†	30.0 (8.5)	48.2 (2.3)*
	Apomorphine	Clozapine (n=6)	10.8 (4.3)	36.5 (9.2)	99.3 (33.5)
		Haloperidol (n=5)	11.0 (2.1)	28.8 (10.6)	64.4 (6.8)
Nucleus Accumbens	d-Amphetamine	Clozapine (n=6)	5.3 (0.4)	34.8 (9.4)	139.3 (31.9)
		Haloperidol (n=7)	16.4 (2.9)†	29.3 (4.6)	45.3 (5.4)†
	Apomorphine	Clozapine (n=5)	6.1 (1.1)	42.8 (9.3)	150.8 (59.0)
		Haloperidol (n=6)	8.3 (2.7)	41.8 (5.1)	97.7 (28.0)

Following an IP injection of either 2.5 mg/kg d-amphetamine or 1.0 mg/kg apomorphine, clozapine (20 mg/kg) or haloperidol (2.0 mg/kg) was injected (IP) within 15–30 minutes to block the DA agonist-induced depression of firing rate (n=number of neurons in each group). The mean onset of blockade indicates the time after the clozapine or haloperidol injection that unit activity returned to at least 60 percent of the predrug baseline rate. Mean firing rate was calculated for the 10-minute periods immediately prior to and after the antipsychotic drug injection and was expressed in each case as percent of the spontaneous baseline rate. Numbers in parentheses refer to the standard error of the mean. Statistical differences between the clozapine and haloperidol groups are indicated by $^*p<0.05$ and $^\dagger p<0.01$.

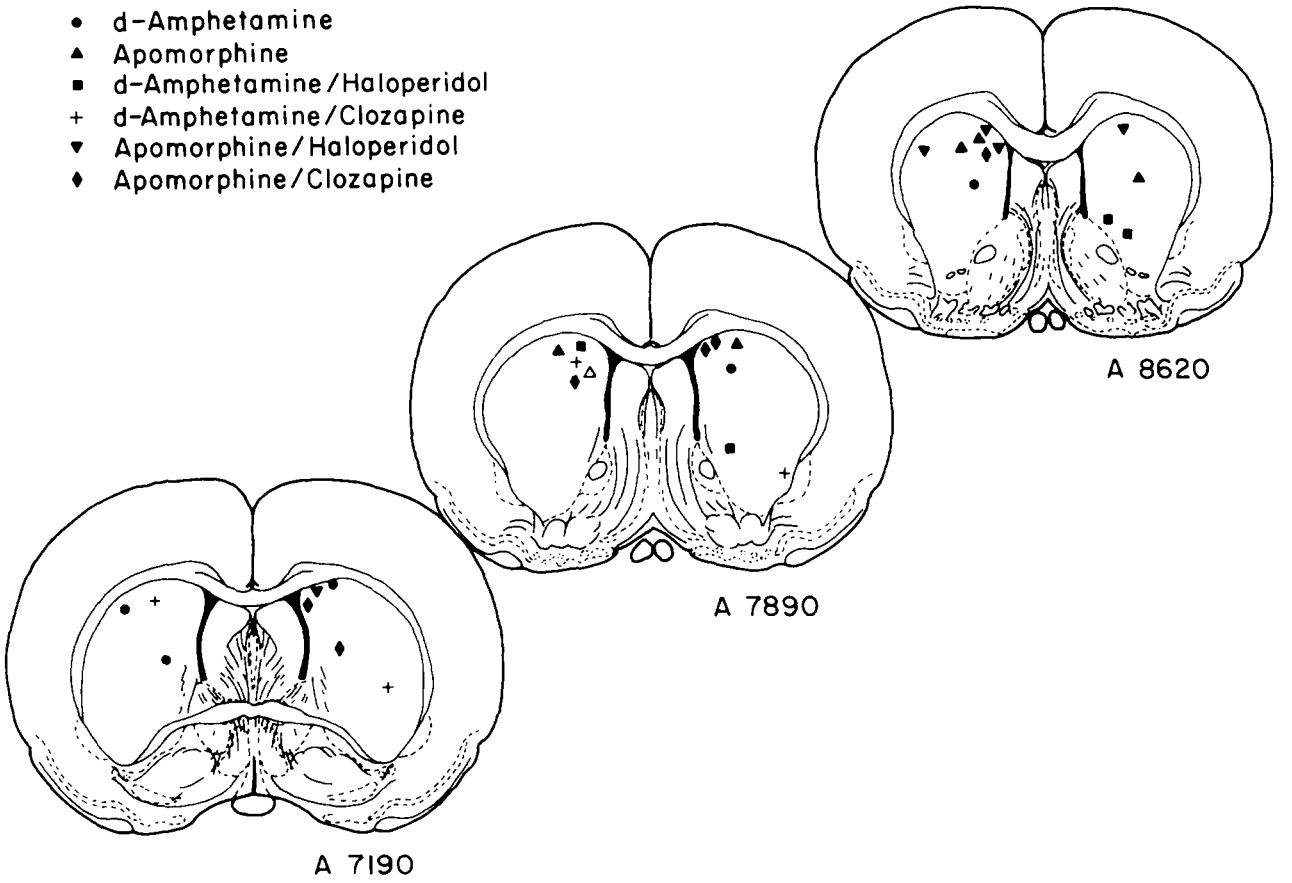

FIG. 3. Location of electrode tip placements for all neurons recorded in the neostriatum. The various symbols indicate the distribution of neurons in each drug group as described in the legend. The location of the aberrant neuron described in the text is indicated by an open triangle. Histological drawings are after König and Klippel [28].

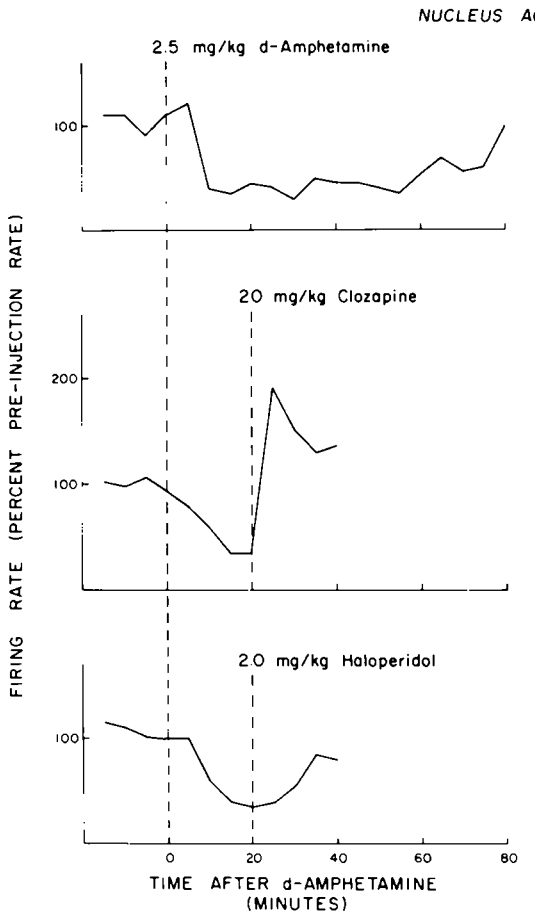

FIG. 4. Representative examples of the response of individual neurons in the nucleus accumbens to d-amphetamine and some antipsychotic drugs. The top graph shows the time course of the changes in firing rate produced by 2.5 mg/kg d-amphetamine injected at Time 0. The other graphs depict the subsequent response to an injection of either 20 mg/kg clozapine or 2.0 mg/kg haloperidol. Firing rate is expressed as percent of the pre-injection rate as in Fig. 1.

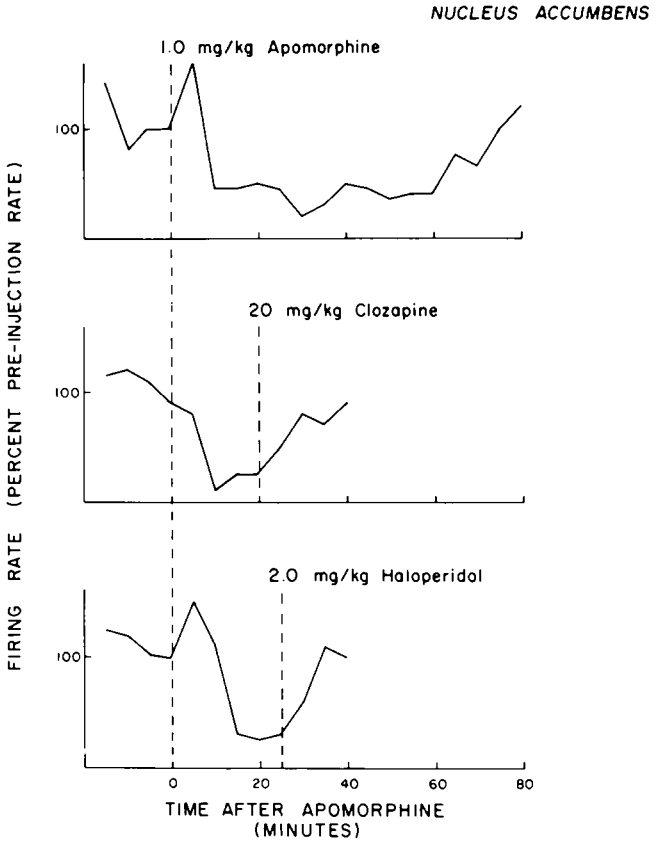

FIG. 5. Representative examples of the response of individual neurons in the nucleus accumbens to apomorphine and some antipsychotic drugs. The top graph illustrates the time course of the changes in activity produced by 1.0 mg/kg apomorphine injected at Time 0. The lower graphs depict the ability of 20 mg/kg clozapine and 2.0 mg/kg haloperidol to reverse the apomorphine response. Firing rate is expressed as percent of the pre-injection rate as in Fig. 1.

In contrast to their differential blockade of the amphetamine response, clozapine (n=6) and haloperidol (n=5) were equieffective in blocking the apomorphine-induced depression of firing rate in the neostriatum (see Fig. 2). Despite some individual unit variability, administration of 20 mg/kg clozapine or 2.0 mg/kg haloperidol within 15–30 min after apomorphine produced a blockade of the depression that was comparable in onset time and magnitude (see Table 1).

Following the drug-induced blockade of either the amphetamine or apomorphine response, continued recording in the neostriatum revealed that both antipsychotic drugs typically increased activity above the spontaneous baseline rate. Thus, in every case the maximum increase in unit activity produced by clozapine exceeded the pre-drug baseline rate, ranging in magnitude from 130 to 975%. Haloperidol, in all but 3 neurons, produced a similar, though less dramatic, increase, ranging for individual units from 120 to 425%.

For one neostriatal neuron in the apomorphine/clozapine condition, no drug response was observed. In this unusual case, an injection of 1.0 mg/kg apomorphine produced no consistent change (±40%) in unit activity from the 100% predrug baseline rate; an injection of 20 mg/kg clozapine 20 min later also failed to alter neuronal activity even when firing rate was monitored for another 60 min.

The spontaneous activity of all neurons in the neostriatum (n=30) was relatively slow having a mean predrug firing rate of 31.6 (SEM=6.0) discharges/min. Figure 3 illustrates the location of the electrode tip placements in the neostriatum.

Unit Responses in the Nucleus Accumbens

The response of neurons in the nucleus accumbens (n=5) to 2.5 mg/kg d-amphetamine was comparable to that of neostriatal neurons. Thus, the amphetamine-induced depression of firing rate lasted for a mean duration of 92.5 (SEM=17.3) min and reached a mean maximum value of 23.8 (SEM=9.2) percent of the pre-amphetamine baseline rate. A typical example of the entire time-course of the amphetamine response is depicted in Fig. 4. In this case, unit activity was depressed below 60% of the pre-injection rate until 65 min after amphetamine administration.

Although a subsequent injection of either 20 mg/kg clozapine (n=6) or 2.0 mg/kg haloperidol (n=7) in different groups of animals reversed the depression of firing rate produced by d-amphetamine, the antipsychotic drugs did not produce comparable effects, as shown in Fig. 4. Mimicking

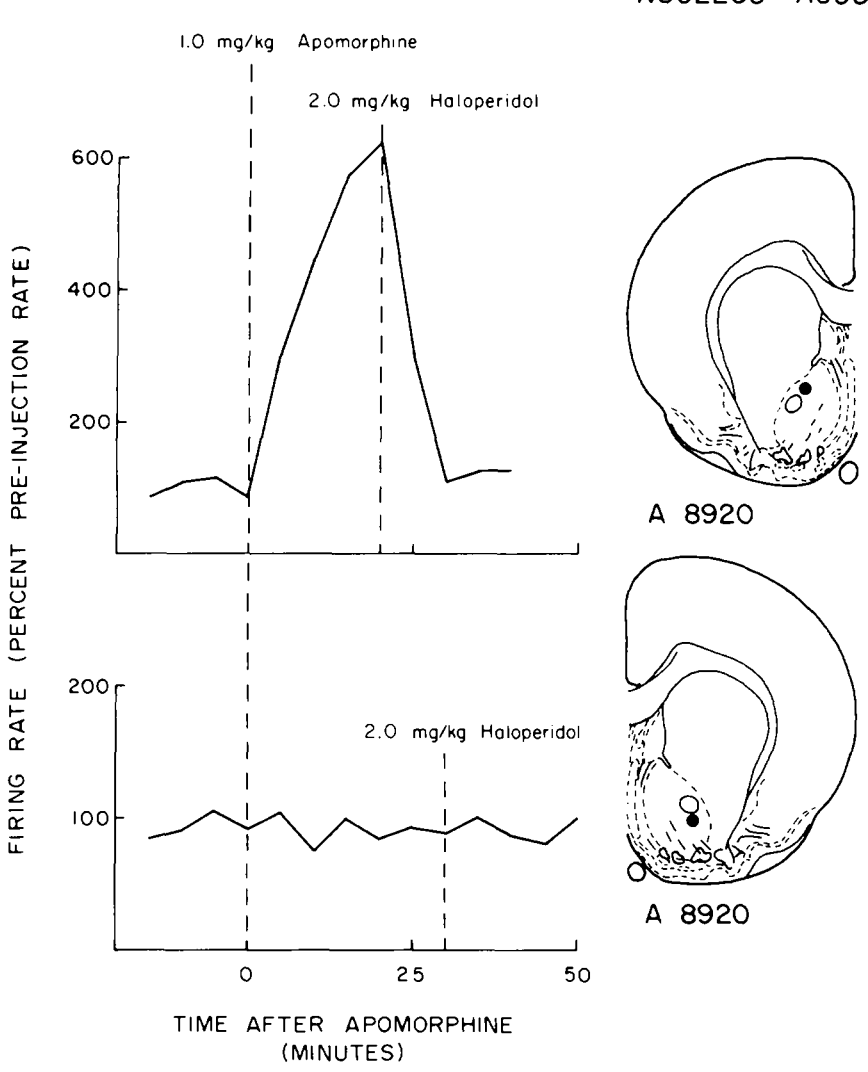

FIG. 6. Two different neurons in the nucleus accumbens that displayed an aberrant response to apomorphine and the antipsychotic drugs. In the top graph, 1.0 mg/kg apomorphine increased unit activity and a subsequent injection of 2.0 mg/kg haloperidol blocked this response. In the other exceptional case (bottom graph), neither 1.0 mg/kg apomorphine nor 2.0 mg/kg haloperidol altered the spontaneous firing rate. Unit activity is expressed as percent of the pre-injection rate as in Fig. 1. The location of the electrode tip placements for these aberrant neurons are shown to the right of each graph.

its action in the neostriatum, clozapine not only blocked the amphetamine-induced depression of activity significantly faster than haloperidol, $F(1,12)=15.15$, $p<0.01$, but also produced a significantly greater increase in firing rate during the first 10 min after injection, $F(1,12)=11.92$, $p<0.01$. A summary of these differential effects in the nucleus accumbens is presented in Table 1. Statistical comparisons of these data with those obtained for clozapine and haloperidol in the neostriatum revealed no significant regional differences.

An IP injection of 1.0 mg/kg apomorphine depressed firing rate in the nucleus accumbens (n=5) for a mean period of 52.0 (SEM=6.5) min with a mean maximum inhibition of 34 (SEM=8.9) percent of the pre-injection rate. A representative example of the apomorphine response in the nucleus accumbens is illustrated in Fig. 5. Note that following a brief, initial excitation, apomorphine, in this case, slowed unit activity to below 60% of the baseline rate for more than 60 min. In other animals, a subsequent injection of 20 mg/kg clozapine (n=5) or 2.0 mg/kg haloperidol (n=6) produced a comparable blockade of the apomorphine response in the nucleus accumbens (see Fig. 5). There were no significant differences in either the onset of the apomorphine blockade or the initial magnitude of the subsequent increase in firing rate (see Table 1). Furthermore, these measures of the apomorphine blockade were not significantly different from those obtained for clozapine or haloperidol in the neostriatum.

When we continued to monitor neuronal activity in the nucleus accumbens after the amphetamine or apomorphine response was blocked, we found that both antipsychotics

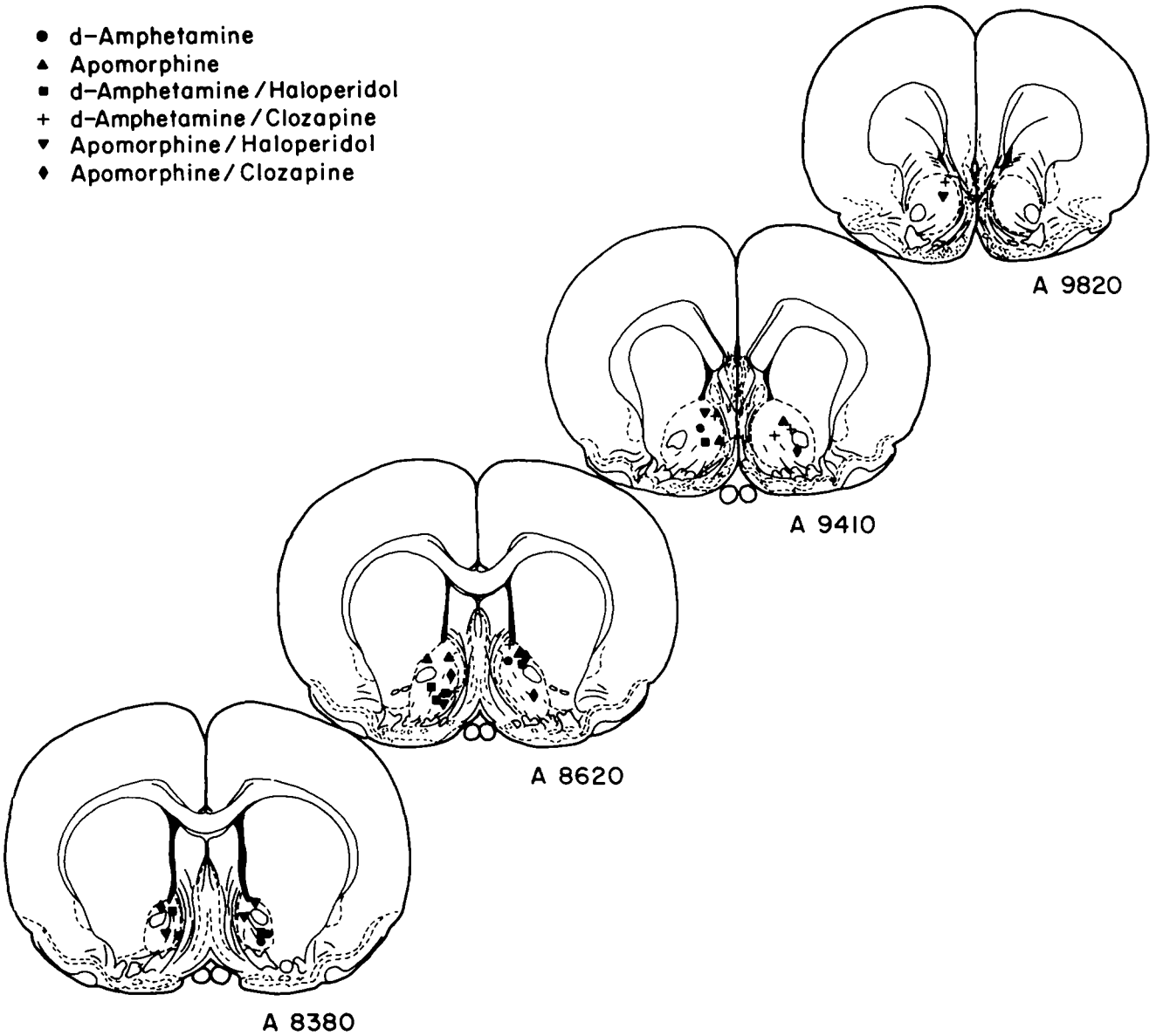

FIG. 7. Location of electrode tip placements for neurons in the nucleus accumbens. The symbols in the legend indicate the recording site for each neuron in each drug condition. Histological drawings are after König and Klippel [28].

produced marked increases in firing rate. With only one exception, clozapine increased activity above the baseline rate with a range for individual neurons of from 125 to 1,015%. The haloperidol-induced excitation, which exceeded the spontaneous rate in all but 4 cases, was less variable than the clozapine response ranging from 120 to 380%.

We encountered two additional neurons in the nucleus accumbens whose drug-induced changes in firing rate were not consistent with the remainder of our sample. In one exceptional case, 1.0 mg/kg apomorphine produced a prolonged increase in firing rate that was subsequently reversed by an injection of 2.0 mg/kg haloperidol. Another neuron in the nucleus accumbens did not respond to apomorphine administration nor was there a response to haloperidol injected 30 min later. Data obtained from both aberrant neurons are shown in Fig. 6.

All neurons in the nucleus accumbens (n=36) discharged at a slow rate having a mean spontaneous activity of 24.1 (SEM=2.0) spikes/min. The electrode tip placements in the nucleus accumbens are illustrated in Fig. 7.

DISCUSSION

Several lines of evidence have cast doubt on the hypothesis that clozapine exerts a preferential blockade of DA receptors in the mesolimbic system. For one, clozapine has been reported to produce a comparable increase in DA turnover and tyrosine hydroxylase activity in the neostriatum and nucleus accumbens [5, 48, 53, 54]. In fact, apart from a potency difference of approximately 10:1, clozapine mimics the effects of haloperidol on these measures of DA receptor blockade [49]. Furthermore, clozapine abolishes turning be-

havior in rats whether this response is produced by administration of DA agonists following unilateral lesions of the nigro-neostriatal pathway or by unilateral stimulation of DA afferents to the neostriatum [39,45]. In addition, clozapine produced qualitatively similar effects on spontaneous neuronal activity in the neostriatum and nucleus accumbens [41]. This growing body of evidence, which argues against regional selectivity in the action of clozapine on DA neurons, is consistent with the results of the present study since we found this drug to be equieffective in antagonizing the changes in firing rate produced by DA agonists in the neostriatum and nucleus accumbens. Clozapine, however, may exert at least some of its effects presynaptically, perhaps by blocking DA release, since this drug was more effective than haloperidol in reversing the inhibition of activity produced by d-amphetamine, an indirectly acting DA agonist, but not in blocking the neuronal response to apomorphine, a DA receptor stimulant.

The depression of firing rate produced by DA agonists can be attributed to an inhibitory action of DA in the forebrain [29,37]. It is possible, however, that DA may act on some neurons as an excitatory transmitter [4,27] and, in fact, we encountered one neuron in the nucleus accumbens that increased its firing rate to apomorphine. We have previously reported that in the neostriatum low doses of d-amphetamine occasionally produce a prolonged excitation and this response occurs more often as the dose is increased [44]. Different populations of inhibitory and excitatory DA receptors may account for these effects [51] although other interpretations cannot be ruled out. The lack of response of two other neurons to either apomorphine or the antipsychotics suggests that not all neurons in the neostriatum and nucleus accumbens are senstitive to these drugs. It is unlikely that any changes in firing rate produced by d-amphetamine or apomorphine are secondary to the effects of these drugs on heart rate, blood pressure or other peripheral variables since we have previously shown that mephentermine, a potent sympathomimetic with only weak central nervous system effects, produces no consistent change in neostriatal neuronal activity even at doses that exert dramatic peripheral effects [18,42]. Furthermore, the changes in firing rate that we observed were not correlated with fluctuations in heart rate, body temperature, or end-tidal carbon dioxide. It is also unlikely that our results are secondary to changes in muscle activity or respiration since the animals were immobilized and artificially respired.

The haloperidol-induced reversal of the neuronal response to d-amphetamine or apomorphine, which is consistent with previous studies [3,18], supports current speculation regarding the mechanism of action of this antipsychotic drug. Thus, haloperidol, as a DA receptor blocker, has been shown to displace the specific binding of labelled spiperone in the neostriatum and to inhibit DA-sensitive adenylate cyclase activity in the nigro-neostriatal and mesolimbic systems [8, 30, 46]. Moreover, haloperidol antagonizes both the locomotor activity and the stereotypy produced by DA agonists [15].

Although clozapine does not differ from haloperidol in blocking the neuronal response to apomorphine, this atypical antipsychotic drug is more effective than haloperidol in antagonizing the amphetamine-induced depression of firing rate. One explanation for this difference may involve a selective interference by clozapine in the uptake and distribution of amphetamine by cerebral tissue. This argument is unlikely, however, since clozapine was injected well after the time required for systemically administered amphetamine to reach the brain [52]. Furthermore, unit activity following clozapine administration does not simply return to the pre-amphetamine baseline rate, as might be expected if this antipsychotic is interfering with the access of amphetamine to its target site, but rather firing rate routinely increases above the pre-amphetamine level for several minutes. In addition, the fact that the depression produced by both DA agonists was comparable prior to administration of either antipsychotic drug rules out the possibility that the relative efficacy of clozapine in reversing the amphetamine response is related to the degree of baseline depression. Since we have previously reported that high doses of d-amphetamine (5.0–7.5 mg/kg) can actually increase unit activity in the neostriatum [44], it is conceivable that clozapine may be potentiating the amphetamine effect instead of blocking it. This explanation is also unlikely, however, since preliminary findings indicate that clozapine blocks the prolonged increase in neostriatal unit activity produced by 7.5 mg/kg d-amphetamine (Rebec and Zimmerman, in preparation). A more likely explanation is that clozapine, at least at the dose used in our study, may be acting, in part, by blocking DA release. This notion is consistent with reports that whereas low doses of clozapine (5–20 mg/kg) increase DA levels in the neostriatum and nucleus accumbens, only higher doses resemble the classical antipsychotics by increasing DA turnover without changing DA concentration [13,21]. Thus, clozapine may be more effective than haloperidol in antagonizing the amphetamine response because, aside from any blockade of postsynaptic DA receptors, clozapine may prevent DA release. This presynaptic action of clozapine should not affect the apomorphine response and, accordingly, clozapine does not differ from haloperidol in blocking the apomorphine-induced depression of activity, suggesting that both antipsychotics act similarly at postsynaptic sites.

If this is the case, however, it is difficult to explain why clozapine, unlike haloperidol, fails to block the stereotyped behavior produced by d-amphetamine and apomorphine [22,34]. Because of the large body of evidence implicating the neostriatum in this response, one might expect clozapine to exert little, if any, effect in this region relative to its effect in the nucleus accumbens. However, the sharp distinction that has traditionally been made between the nigro-neostriatal and mesolimbic DA systems may not be entirely justified. For example, such behaviors as drug-induced rotation and catalepsy, which were once thought to be mediated exclusively by an imbalance in DA transmission in the neostriatum, now appear to be regulated, in part, by an important mesolimbic component [10,26]. Similarly, the mesolimbic system may not play an exclusive role in the locomotor response to DA agonists [11]. Furthermore, recent neuroanatomical evidence that both these systems innervate, in a topographically ordered manner, a number of common forebrain structures, including the basal ganglia [32,33,37], argues against the traditional anatomical separation between the nigro-neostriatal and mesolimbic DA systems and supports, instead, the notion of integrative rather than differential mediation of drug effects [35]. In fact, because of the many similarities between the nigro-neostriatal and mesolimbic DA systems, the unique behavioral effects of clozapine may be mediated, in large measure, by an action on non-dopaminergic systems. Thus, clozapine, in comparison with haloperidol, is a strong antagonist of cholinergic and noradrenergic neurotransmission [12, 31, 47], and this action

by itself can actually potentiate the behavioral response to DA agonists [14,38]. It is tempting to speculate, therefore, that any clozapine-induced interference with DA transmission in the neostriatum and nucleus accumbens is masked by a simultaneous disruption of transmission in neuronal pathways that normally exert an antagonistic influence on dopaminergic systems.

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REFERENCES

1. Anden, N. E. and G. Stock. Effect of clozapine on the turnover of dopamine in the corpus striatum and in the limbic system. *J. Pharm. Pharmac.* **25:** 346–348, 1973.
2. Bartholini, G. Differential effect of neuroleptic drugs on dopamine turnover in the extrapyramidal and limbic system. *J. Pharm. Pharmac.* **28:** 429–433, 1976.
3. Bashore, T. R., G. V. Rebec and P. M. Groves. Alterations of spontaneous neuronal activity in the caudate-putamen, nucleus accumbens and amygdaloid complex of rats produced by d-amphetamine. *Pharmac. Biochem. Behav.* **8:** 467–474, 1978.
4. Bevan, P., C. M. Bradshaw and E. Szabadi. Effects of desipramine on neuronal responses to dopamine, noradrenaline, 5-hydroxytryptamine and acetylcholine in the caudate nucleus of the rat. *Br. J. Pharmac.* **54:** 285–293, 1975.
5. Bowers, M. B. and A. Rozitis. Brain homovanillic acid: regional changes over time with antipsychotic drugs. *Eur. J. Pharmac.* **39:** 109–115, 1976.
6. Burki, H. R., E. Eichenberger, A. C. Sayers and T. G. White. Clozapine and the dopamine hypothesis of schizophrenia, a critical appraisal. *Pharmakopsychiatrie* **8:** 115–121, 1975.
7. Carlsson, A. Antipsychotic drugs and catecholamine synapses. *J. Psychiat. Res.* **11:** 57–64, 1974.
8. Clement-Cormier, Y. C. and G. A. Robison. Adenylate cyclase from various dopaminergic areas of the brain and the action of antipsychotic drugs. *Biochem. Pharmac.* **26:** 1719–1722, 1977.
9. Costall, B., C. D. Marsden, R. J. Naylor and C. J. Pycock. Stereotyped behavior patterns and hyperactivity induced by amphetamine and apomorphine after discrete 6-hydroxydopamine lesions of extrapyramidal and mesolimbic nuclei. *Brain Res.* **123:** 89–111, 1977.
10. Costall, B. and R. J. Naylor. The nucleus amygdaloideus centralis and neuroleptic activity in the rat. *Eur. J. Pharmac.* **25:** 138–146, 1974.
11. Costall, B., R. J. Naylor and V. Nohria. Hyperactivity response to apomorphine and amphetamine in the mouse: The importance of the nucleus accumbens and caudate-putamen. *J. Pharm. Pharmac.* **31:** 259–261, 1979.
12. Dorris, R. L. and P. A. Shore. On the mechanism of action of clozapine on the adrenergic neuron. *Br. J. Pharmac.* **56:** 279–283, 1976.
13. Gianutsos, G. and K. E. Moore. Possible significance of clozapine-induced increase in brain dopamine. *Res. commun. chem. pathol. Pharmac.* **17:** 29–39, 1977.
14. Grabowska-Anden, M. Modifications of the amphetamine-induced stereotypy in rats following inhibition of the noradrenaline release by FLA 136. *J. Pharm. Pharmac.* **29:** 566–567, 1977.
15. Groves, P. M. and G. V. Rebec. Biochemistry and behavior: Some central actions of amphetamine and antipsychotic drugs. *Ann. Rev. Psychol.* **27:** 91–127, 1976.
16. Groves, P. M. and G. V. Rebec. Changes in neuronal activity in the neostriatum and reticular formation following acute or long-term amphetamine administration. In: *Cocaine and Other Stimulants*, edited by E. H. Ellinwood and M. M. Kilbey. New York: Plenum Press, 1977, pp. 269–301.
17. Groves, P. M., G. V. Rebec and J. A. Harvey. Alteration of the effects of (+)-amphetamine on neuronal activity in the striatum following lesions of the nigrostriatal bundle. *Neuropharmacology* **14:** 369–376, 1975.
18. Groves, P. M., G. V. Rebec and D. S. Segal. The action of D-amphetamine on spontaneous activity in the caudate nucleus and reticular formation of the rat. *Behav. Biol.* **11:** 33–47, 1974.
19. Honma, T. and H. Fukushima. Effects of bilateral lesions in the striatum or nucleus accumbens on the cataleptogenic activity of neuroleptics in rats. *Jap. J. Pharmac.* **28:** 231–238, 1978.
20. Hornykiewicz, O. Psychopharmacological implications of dopamine and dopamine antagonists: a critical evaluation of current evidence. *Ann. Rev. Pharmac. tox.* **17:** 545–559, 1977.
21. Hyttel, J. Effects of a single administration of clozapine on mouse brain catecholamines. *Acta pharmac. tox.* **38:** 358–365, 1976.
22. Iversen, S. D. and G. F. Koob. Behavioral implications of dopaminergic neurons in the mesolimbic system. In: *Nonstriatal Dopaminergic Neurons: Advances in Biochemical Psychopharmacology*, edited by E. Costa and G. L. Gessa. New York: Raven Press, 1977, pp. 209–214.
23. Jackson, D. M., N. E. Anden and A. Dahlstrom. A functional effect of dopamine in the nucleus accumbens and in some other dopamine-rich parts of the rat brain. *Psychopharmacologia* **45:** 139–149, 1975.
24. Kelly, P. H. and S. D. Iversen. Selective 6-OHDA-induced destruction of mesolimbic dopamine neurons: Abolition of psychostimulant-induced locomotor activity in rats. *Eur. J. Pharmac.* **40:** 45–56, 1976.
25. Kelly, P. H. and K. E. Moore. Mesolimbic dopamine neurons: Effects of 6-hydroxydopamine-induced destruction and receptor blockade on drug-induced rotation of rats. *Psychopharmacology* **55:** 35–41, 1977.
26. Kelly, P. H. and K. E. Moore. Mesolimbic dopaminergic neurons in the rotational model of nigrostriatal function. *Nature* **263:** 695–696, 1976.
27. Kitai, S. R., M. Sugimori and J. D. Kocsis. Excitatory nature of dopamine in the nigro-caudate pathway. *Expl Brain Res* **21:** 351–362, 1976.
28. Konig, J. F. R. and R. A. Klippel. *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem.* Baltimore: Williams and Wilkins, 1963.
29. Krnjevic, K. Electrophysiology of dopamine receptors. In: *Advances in Neurology, Vol. 9, Dopaminergic Mechanisms*, edited by D. B. Calne, T. N. Chase and A. Barbeau. New York: Raven Press, 1975, pp. 13–24.
30. Lauduron, P. M., P. F. M. Janssen and J. E. Leysen. Spiperone: a ligand of choice for neuroleptic receptors. 2. Regional distribution and in vivo displacement of neuroleptic drugs. *Biochem. Pharmac.* **27:** 317–321, 1978.
31. Lauduron, P. M. and J. E. Leysen. Is the low incidence of extrapyramidal side-effects of antipsychotics associated with antimuscarinic properties? *J. Pharm. Pharmac.* **30:** 120–122, 1978.
32. Lindvall, O. and A. Bjorklund. The organization of the ascending catecholamine neurons systems in the rat brain as revealed by the glyoxylic acid fluorescence method. *Acta physiol. scand.*, suppl. **412:** 1–48, 1974.
33. Lindvall, O., A. Bjorklund and I. Divac. Organization of catecholamine neurons projecting to the frontal cortex in the rat. *Brain Res.* **142:** 1–24, 1978.
34. Ljungberg, T. and U. Ungerstedt. Classification of neuroleptic drugs according to their ability to inhibit apomorphine-induced locomotion and gnawing: Evidence for two different mechanisms of action. *Psychopharmacology* **56:** 239–247, 1978.

35. Lyon, M. and T. Robbins. The action of central nervous system stimulating drugs: A general theory concerning amphetamine effects. In: *Current Developments in Psychopharmacology*. New York: Spectrum, 1975, pp. 81–163.
36. Matthysse, S. Schizophrenia: relationships to dopamine transmission motor control and feature extraction. In: *The Neurosciences: Third Study Program*, edited by F. O. Schmitt and F. G. Worden. Cambridge: MIT Press, 1974, pp. 733–737.
37. Moore, R. Y. and F. E. Bloom. Central catecholamine neuron systems: Anatomy and physiology of the dopamine systems. *Ann. Rev. Neurosci.* **1:** 129–169, 1978.
38. Mogilnicka, E. and C. Braestrup. Noradrenergic influence on the stereotyped behavior induced by amphetamine, phenethylamine and apormorphine. *J. Pharm. Pharmac.* **28:** 253–255, 1976.
39. Nakamura, S., J. Engel and M. Goldstein. Blockade of lergotrile or apomorphine induced turning behavior by haloperidol and clozapine. *Comm. Psychopharmac.* **2:** 185–190, 1978.
40. Pijnenburg, A. J. J., W. M. M. Honig, J. A. M. Van Der Heyden and J. M. van Rossum. Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. *Eur. J. Pharmac.* **35:** 45–58, 1976.
41. Rebec, G. V., T. R. Bashore, K. S. Zimmerman and K. D. Alloway. Neostriatal and mesolimbic neurons: Dose-dependent effects of clozapine. *Neuropharmacology*, in press.
42. Rebec, G. V. and P. M. Groves. Differential effects of the optical isomers of amphetamine on neuronal activity in the reticular formation and caudate nucleus of the rat. *Brain Res.* **83:** 301–318, 1975.
43. Rebec, G. V. and P. M. Groves. Enhancement of effects of dopaminergic agonists on neuronal activity in the caudate-putamen of the rat following long-term d-amphetamine administration. *Pharmac. Biochem. Behav.* **5:** 349–357, 1976.
44. Rebec, G. V. and D. S. Segal. Dose-dependent biphasic alterations in the spontaneous activity of neurons in the rat neostriatum produced by D-amphetamine and methylphenidate. *Brain Res.* **150:** 353–366, 1978.
45. Roffman, M., P. S. Bernard, K. M. Dawson, R. E. Sobiski and J. K. Saelens. The effects of haloperidol and clozapine on circling induced by electrical stimulation of the substantia nigra and the ventromedial tegmentum. *Neuropharmacology* **17:** 943–946, 1978.
46. Roufogalis, B. D., M. Thornton and D. N. Wade. Specificity of the dopamine sensitive adenylate cyclase for antipsychotic antagonists. *Life Sci.* **19:** 927–934, 1976.
47. Snyder, S. H., D. Greenberg and H. I. Yamamura. Antischizophrenic drugs and brain cholinergic receptors. *Archs gen. Psychiat. Chicago* **31:** 58–61, 1974.
48. Stanley, M. and S. Wilk. The effect of antipsychotic drugs and their clinically inactive analogs on dopamine metabolism. *Eur. J. Pharmac.* **44:** 293–302, 1977.
49. Stawarz, R. J., H. Hill, S. E. Robinson, P. Settler, J. V. Dingell and F. Sulser. On the significance of the increase in homovanillic acid (HVA) caused by antipsychotic drugs in corpus striatum and limbic forebrain. *Psychopharmacologia* **43:** 125–130, 1975.
50. Stinus, L., O. Gaffori, H. Simon and M. LeMoal. Small doses of apomorphine and chronic administration of d-amphetamine reduce locomotor hyperactivity produced by radiofrequency lesions of dopaminergic A10 neurons area. *Biol. Psychiat.* **12:** 719–732, 1977.
51. Szabadi, E. A model of two functionally antagonistic receptor populations activated by the same agonist. *J. theor. Biol.* **69:** 101–112, 1977.
52. Vree, T. B. and J. M. van Rossum. Kinetics of metabolism and excretion of amphetamines in man. In: *Amphetamines and Related Compounds*, edited by E. Costa and S. Garattini. New York: Raven Press, 1970, pp. 165–190.
53. Westerink, B. H. C. and J. Korf. Influence of drugs on striatal and limbic homovanillic acid concentration in the rat brain. *Eur. J. Pharmac.* **33:** 31–40, 1975.
54. Wiesel, F. A. and G. Sedvall. Effect of antipsychotic drugs on homovanillic acid levels in striatum and olfactory tubercle of the rat. *Eur. J. Pharmac.* **30:** 364–367, 1975.
55. Wilson, C. J., J. M. Juraska and P. M. Groves. Alteration of the neuronal response to amphetamine in the neostriatum by pretreatment with a centrally acting anticholinergic. *Neuropharmacology* **16:** 455–461, 1977.