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Response of the Ventral Pallidal/Mediodorsal Thalamic System to Antipsychotic Drug Administration: Involvement of the Prefrontal Cortex

Antonieta Lavin, Ph.D., and Anthony A. Grace, Ph.D.

Intracellular recordings were obtained from rat ventral pallidal (VP) and mediodorsal thalamic (MD) cells in vivo *and the effects of antipsychotic drugs on their basal and evoked electrophysiological characteristics were assessed. Administration of either haloperidol or clozapine caused a significant decrease in the average firing rate, accompanied by a hyperpolarization of the membrane potential in the VP cells recorded. However, neither drug induced a substantial change in the other basic membrane properties of the MD cells or VP cells tested. In addition, in 50% of the MD cells tested, both antipsychotic drugs caused a change in spike discharge from an oscillatory pattern to a tonic discharge mode. In rats that had received ibotenic acid lesions of the prefrontal cortex (PFCtx) 4–8 weeks prior to recording, cells in the VP exhibited similar changes in firing frequency in response to haloperidol administration as those in the intact rats. However, in contrast to the intact rats, MD cells recorded from rats with PFCtx lesions exhibited a significant increase in firing rate after haloperidol administration. The results from this study suggest that the prefrontal cortex plays a role in modulating the response of the thalamus to antipsychotic drugs.*
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KEY WORDS: *Mediodorsal thalamic nucleus; Ventral Pallidum; Haloperidol; Clozapine; Schizophrenia; Prefrontal cortical lesions*

The ventral pallidum (VP) is a ventral extension of the globus pallidus, and together with its projection to the mediodorsal thalamic nucleus (MD) are integral parts of the limbic system within the basal ganglia. The VP is the major synaptic target of nucleus accumbens neurons, and in return influences higher cortical structures via its projections to the MD (Cornwall and Phillipson 1988; Gröenewegen 1988; Gröenewegen et al. 1993; Haber et al. 1985; Lavin and Grace 1994; Young et al. 1984; Zahm and Heimer 1987) and the reticular thalamic nucleus (RTN) (Cornwall et al. 1990; Jones 1985; Lavin and Grace 1994), thus forming a loop that encompasses the prefrontal cortex–nucleus accumbens–VP–thalamus–cortex. The MD thalamic nucleus has attracted a great deal of interest due to its proposed role in the integration of information flow between the limbic system and centers of higher cognitive function. The MD nucleus has also been an area of interest due to its potential involvement in schizophrenia, both in its role as a major cortical relay of the limbic system and because of its interconnections with the prefrontal cortex (Divac et al. 1978; Goldman-Rakic et al. 1984; Goldman-Rakic and Porrino 1985; Markowitsch 1982; O'Donnell et al. 1997).

Models of cortical function suggest that the thalamocortical system, in conjunction with the RTN, serves as a gate for cortical input or cortico-cortical integration. Re-

From the Departments of Neuroscience and Psychiatry, Center for Neuroscience, University of Pittsburgh, Pittsburgh, Pennsylvania.

Address correspondence to: Dr. Antonieta Lavin, Department of Neuroscience, 446 Crawford Hall, University of Pittsburgh, Pittsburgh, PA 15260.

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sults of anatomical (Cornwall et al. 1990; Jones 1985; O'Donnell et al. 1997) and electrophysiological (Lavin and Grace 1994) studies suggest that the limbic system output to the thalamus is positioned to exert a strong influence on prefrontal cortical activity via its projection to the MD and the RTN. Therefore, information arising from cortical and limbic sites is integrated within the ventral striatum under the modulatory influence of dopamine, which then exerts regulatory control over MD and RTN activity via the ventral striatal projection to the VP and the medial segment of the globus pallidus (Gröenewegen 1988; Young et al. 1984). Like the MD, the VP itself is thought to play a significant role in the cognitive and motor skills necessary to perform reward-mediated behaviors (Grace 1995; Mogenson and Yang 1991; Richardson and DeLong 1991; Wilson and Rolls 1990) and represents the main output of the limbic system.

Because of this potential relationship between cortical-subcortical interactions and schizophrenia, many studies have examined the mode of action of antipsychotic drugs by testing their effects on basal ganglia neurons. However, comparatively less effort has been expended to examine how classical and atypical antipsychotic drug treatment affects the output sites of the limbic system and their subsequent impact on thalamocortical activity. For example, Deutch et al. (1995) found that clozapine administration increases the number of cells expressing Fos-like immunoreactivity and the amount of Fos protein in the paraventricular thalamic nucleus, but D_2-like or D_1-like antagonists did not induce Fos in this nucleus. Furthermore, extracellular recordings of VP neuron activity revealed that systemic administration of haloperidol produced a suppression in their firing rate (Napier et al. 1991a). In this article, we examined the spontaneous and evoked activity of neurons that comprise the output sites of the limbic system (i.e., the VP and the MD thalamic nucleus) in response to acute administration of two drugs with distinct pharmacological profiles that are known to have potent therapeutic actions in schizophrenia: the classical antipsychotic drug haloperidol and the atypical antipsychotic drug clozapine (Chouinard and Annable 1976; Gerlach et al. 1974, 1975; Racagni et al. 1980). The impact of lesions of the prefrontal cortex on these responses was also examined as a way of evaluating whether the hypofrontality that is reported to be present in schizophrenics (Ingvar and Franzen 1974; Pettegrew et al. 1989, 1991; Williamson et al. 1991; Weinberger et al. 1986) may alter the effects of antipsychotic drugs when compared to the intact system.

METHODS

All animals were handled in accordance with the procedures outlined in the *Guide for the Care and Use of Laboratory Animals* published by the USPHS, and the specific protocols used were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Most of the methods used have been described previously (Lavin and Grace 1994).

In Vivo Intracellular Recording and Drug Administration

Sprague-Dawley albino male rats were anesthetized with chloral hydrate (400 mg/kg IP) and fixed in a stereotaxic apparatus (Narishige model SN-2). In experiments using animals with cortical lesions, the recordings were performed 4–6 weeks after the lesion. Supplemental doses of chloral hydrate and test drugs were administered IV via a lateral tail vein cannula. The scalp was exposed and holes drilled in the skull overlying the VP (coordinates: AP = −0.4mm; L = −1.9 mm; V = 7.5–8.5 mm), the MD thalamic nucleus (coordinates: AP = −3.4 mm; L = −0.5 mm; V = 5.2–5.6 mm), and the prefrontal cortex (coordinates: AP = 2.7 mm; L = −1.6 mm; V = 2.5 mm, according to Paxinos and Watson 1986). Body temperature was maintained at 37 ± 0.5°C by a thermostatically controlled heating pad. Intracellular recordings were performed using electrodes pulled from 1.0 mm O.D. Omegadot glass tubing (WPI) using a Flaming-Brown P-80/PC electrode puller and filled with either: (1) 3 M K + acetate (resistance = 60–120 MΩ), or (2) 10% Lucifer yellow in 0.1 M LiCl (resistance = 100–250 MΩ).

All electrophysiological recordings were digitized using a NeuroData Neurocorder and stored on VHS videocassettes for subsequent off-line analysis with a custom-designed data analysis program (Neuroscope). Input resistance, time constant, firing pattern, and evoked responses from VP or MD stimulation were examined for each cell recorded. Electrical stimulation of the VP or MD nuclei was performed using bipolar concentric electrodes (Kopf, model SNE-10). Single pulses (0.3 ms duration, 0.1 to 1.5 mA) were delivered at approximately 1 Hz. Spontaneous and evoked responses were sampled from single cells before and after drug administration. The input resistance was determined by injecting a series of hyperpolarizing current pulses of increasing amplitude into the neuron and measuring the resultant changes in membrane potential. The drugs used were: haloperidol (0.5 mg/kg) (McNeil Pharmaceutical), or clozapine (20 mg/kg, dissolved in 1N HCl, pH = 4.5) (RBI). The final volume was adjusted using isotonic saline. Only haloperidol was tested in the animals with prefrontal cortical lesions. In the cases in which the effects of drugs were examined, only one cell per animal was recorded.

Prefrontal Cortical Lesions

Lesions of neurons intrinsic to the PFCtx were produced in Sprague-Dawley (Zivic-Miller) albino male rats weigh-

ing 200–250 g. The rats were anesthetized with Equithesin (0.3 ml/0.1 kg) followed by local bilateral injection of the excitotoxin ibotenic acid (5 μg/0.5 μl/side) into the prefrontal cortex (coordinates: AP +3.2; L ±0.7; V 3.2 mm, according to Paxinos and Watson 1986) using a glass micropipette (75–100 μm tip diameter). After the micropipette had remained in position for at least 5 min, ibotenic acid was introduced by pressure ejection over a 2.5-min period. The micropipette was then left in place for an additional 5 min before withdrawal. Following the lesions, animals were allowed to recover for 4–6 weeks before recording, with food and water available ad libitum. Rats were housed 2 per cage on a 12-h light-dark cycle. The animals did not exhibit any substantial behavioral abnormalities following recovery from the lesion and were observed to consume food and water normally. The lesions did not encompass the entire PFCtx, but instead were limited to a region of the cortex that included the medial prefrontal cortex, dorsal anterior cingulate cortex and prelimbic cortex, as illustrated in Figure 1.

Histology

In a subset of experiments, the location of the cells was verified by staining the cells after recording via intracellular injection of the fluorescent dye Lucifer yellow (Stewart 1978). Upon stable penetration of VP or MD neurons, the dye was injected by applying 1–2 nA continuous hyperpolarizing current through the electrode, which was interrupted by 12-ms, 2-nA depolarizing current pulses delivered at 7 Hz to prevent blocking of the electrode (Grace and Llinás 1985). At the end of the experiment, the animal was killed by administering an overdose of chloral hydrate and was perfused transcardially with saline followed by 10% formalin. The brain was removed and placed overnight in 15% sucrose solution at 4°C. Slices of 75–120 μm thickness were cut in the

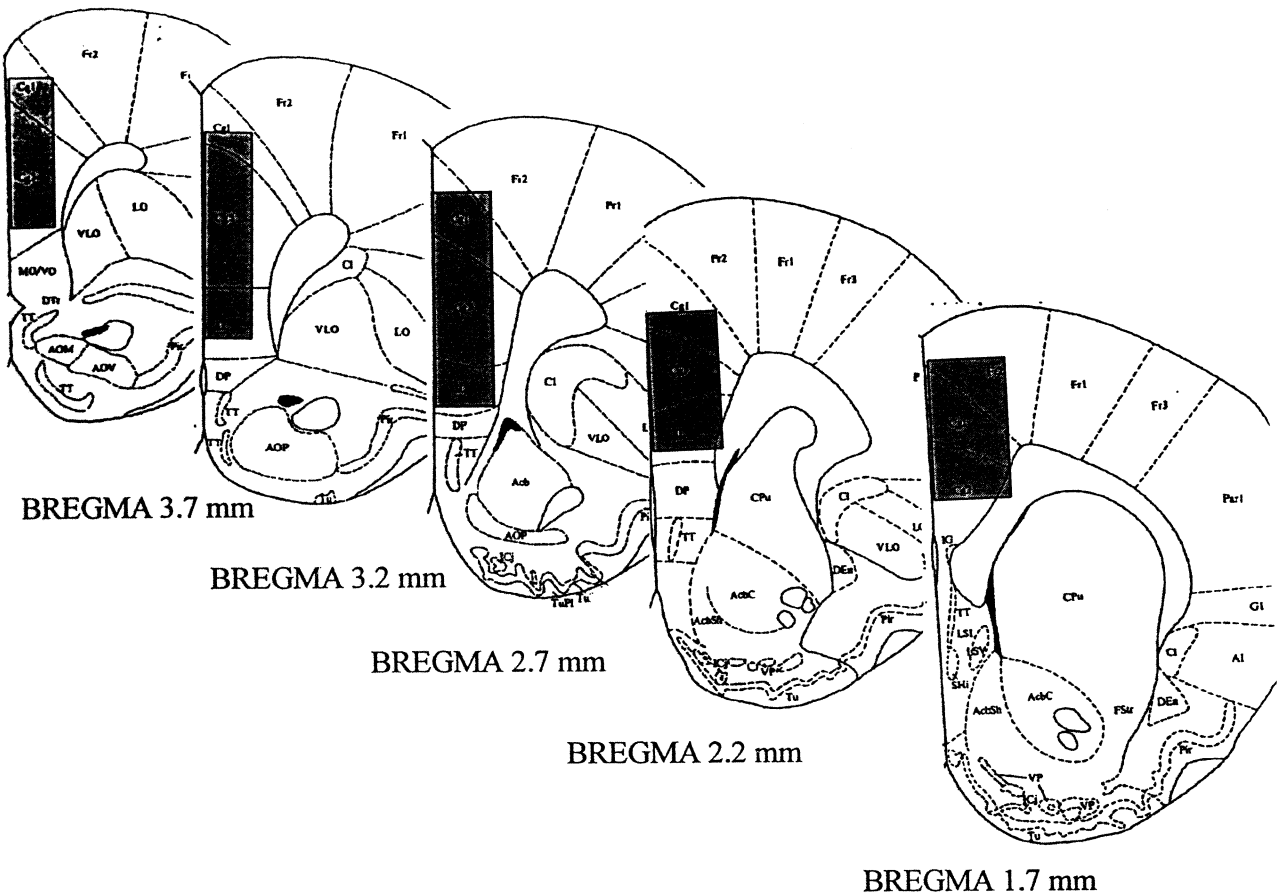

Figure 1. Extent of lesion induced by ibotenic acid injection into the prefrontal cortex. Diagrams modified from Paxinos and Watson atlas (1986) show the extent of the lesion. Ibotenic acid was infused bilaterally by pressure ejection into the prefrontal cortex of rats 4–8 weeks before recording. The area of the lesion encompassed the cingulate cortex areas 1 and 3 and the infralimbic cortex (*hatched blocks*). The AP coordinate listed in each diagram reflects the distance in mm anterior to Bregma.

coronal plane using a freezing microtome and collected in phosphate buffer (1.0 M, pH 7.4). The slices were then washed with 100% DMSO for 2 × 20 min. The Lucifer yellow–labeled cells exhibiting fluorescence were observed using a Leitz fluorescence microscope fitted with a custom-made filter cube (Omega Optical; excitation bandpass = 370–490 nm, low pass filter = 510 nm, dichromatic mirror = 500 nm, 45°). In the cases in which the cells recorded were not stained with Lucifer yellow, slices cut in an identical manner were transferred to phosphate buffer and then stained with cresyl violet to aid in the localization of the electrode tracks. To facilitate localization of the electrode tracks in stained tissue, the electrode was "jiggled" after completion of the recording to increase the diameter of the track sufficient for visual confirmation of the recording site.

Data Analysis

A two-way ANOVA with mixed design was used with "lesion" as the between-subjects factor and "drug" as the within subject (repeated) factor. Planned comparisons of the effects of lesion or drug were made with independent or paired *t*-tests, respectively. The effects of drugs on cell firing rate are expressed as percent of control to compensate for the variability in baseline firing rate among individual neurons. Therefore, in experiments using repeated measures of drug effects on single cells, changes of >35% were considered to be biologically significant. All data are presented as mean ± SEM. Only data collected from cells that exhibited stable intracellular penetrations were used, with stability defined as exhibiting a membrane potential of at least −50 mV, action potentials with amplitudes of 50 mV or greater, and cell impalements maintained for at least 15 min to allow sufficient time for data collection. The membrane potential histograms were generated using a minimum of 260 samples for each histogram. This number of samples represents a minimum of 5 min sampling during control and during drug conditions.

RESULTS

Stable intracellular recordings were obtained in vivo from a total of 79 neurons. Of these neurons, 45 cells were recorded in 20 intact animals and 34 cells were recorded in 21 rats with bilateral prefrontal cortical lesions (PFCtx lesion). The cells were divided as follows: $n = 28$ VP cells in intact animals (haloperidol = 10, clozapine = 12); $n = 17$ MD cells in intact animals (haloperidol = 7, clozapine = 6); $n = 16$ VP cells in animals with PFCtx lesions (haloperidol = 9); $n = 18$ MD cells in animals with PFCtx lesion (haloperidol $n = 5$). The effect of clozapine was not assessed in PFCtx-lesioned animals. The responses to drug administration were examined on one cell per animal in a repeated measures design with one dose of a single drug tested for each cell. In addition, eight of the cells were stained by intracellular injection of Lucifer yellow to confirm their location as follows: in animals with PFCtx lesions, two of the cells were located in the VP, and three cells were located in the MD nucleus; in intact animals three cells were localized to the MD nucleus. All of the MD cells stained with Lucifer yellow exhibited six to 10 primary dendrites and a spindle or stellate shape, with the somata ranging between 16 and 26 μm in diameter. The cells stained and recorded in rats with lesions did not differ in gross morphological characteristics from the cells stained in intact animals. The MD neurons could be reliably identified by their stereotaxic location and the short-latency ipsps (1.3 ± 0.6 ms) that were evoked in response to VP stimulation; this served as identification criteria for subsequent recordings using KAc-filled electrodes. As we described previously (Lavin and Grace 1996), neurons in the VP can be classified as Type A, B, or C, according to the action potential waveform and firing pattern. Briefly, Type A cells are characterized by short duration action potentials (1–1.5 ms) that do not exhibit a substantial afterhyperpolarization (AHP), the Type B cells exhibit longer duration action potentials (2–3 ms) and a pronounced AHP, and cells classified as Type C fire spikes grouped in couplets or in bursts. In the VP of lesioned rats, two cells were stained and exhibited the Type A spike pattern. These cells had small polygonal-shaped somata measuring 23.6 ± 11.8 and 28.3 ± 9.1 μm across their short and long axes, respectively, and exhibited four thick primary dendrites that extended from their somata without branching. Overall, 15 cells were classified as Type A, nine were Type B, and four were Type C. The anatomical location of the recording sites was also assessed by reconstruction of the electrode tracks in cresyl violet–stained tissue slices.

Ventral Pallidal Neurons

Electrophysiological Properties of VP Neurons. The basic properties of the VP cells recorded in intact and lesioned rats are listed in Table 1. The VP cells recorded from animals with PFCtx lesions ($n = 16$) did not display significant differences in their basic physiology when compared with the VP cells recorded in intact rats (Table 1). Possibly due to the small number of VP cells recorded in lesioned rats and the variability in baseline rates, the firing rate of VP cells recorded in PFCtx-lesioned rats was not significantly different from controls, although there was a trend towards lower firing frequencies (ANOVA $p < .11$). In the intact animals, 17.8% of the cells (5/28) were quiescent, whereas in the PFCtx-lesioned animals 50% (8/16) of the cells recorded were found to be in the silent state.

Table 1. Comparison of the Basic Physiological Characteristics of VP and MD Cells Recorded in Intact and PFCtx-Lesioned Animals

	VP Intact	VP PFCtx Lesion	% Change	MD Intact	MD PFCtx Lesion	% Change
Membrane Potential (mV)	63.8 ± 1.7 (n = 28)	66.9 ± 0.9 (n = 16)	−3.7 ± 1.4	63.4 ± 2.3 (n = 17)	62.7 ± 1.5 (n = 18)	1.1 ± 1.9
Input resistance (MΩ)	48.4 ± 22.8 (n = 20)	55.9 ± 29.3 (n = 13)	+15.4 ± 26	34.1 ± 22.1 (n = 14)	59.2 ± 12.2 (n = 5)	+73.6 ± 17.1
Firing rate (Hz)	5.3 ± 1.0 (n = 18)	3.9 ± 1.8 (n = 8)	−26.4 ± 1.4	7.2 ± 0.5 (n = 7)	10.5 ± 5.3 (n = 10)	+45.8 ± 2.9
Type A (%)	53.5 (15/28)	62.5 (10/16)	−12.4			
Type B (%)	32.1 (9/28)	12.5 (2/16)	−70.7			
Type C (%)	14.2 (4/28)	18.7 (3/16)	−1.5			
% of cells with slow oscillations (<1 Hz)	21.4 (6/28)	18.7 (3/16)	−34.3			

Effects of Haloperidol Administration. The primary response of VP cells recorded in intact animals to acute administration of haloperidol (0.5 mg/kg, IV) was a statistically significant decrease in the firing rate ($p < .05$, $t = 2.21$) and a significant hyperpolarization of the membrane potential ($p < .009$, $t = -3.0$) (Table 2). The response to acute haloperidol administration was also examined in nine cells in the VP following PFCtx lesions. Haloperidol administration again produced a statistically significant decrease in firing rate (by 64%; $p < .05$, $t = 2.2$), accompanied by a trend toward more hyperpolarized membrane potentials (by 4.8 ± 3.0 mV; $p < .06$, $t = 1.4$). The cells also exhibited an increase in average input resistance by 48%, although the magnitude of the changes in input resistance did not reach statistical significance (Table 2). Approximately 28% of the VP cells recorded in intact animals also exhibited a slow (<1Hz) oscillatory pattern of spike discharge. In these cells, spikes were found to ride on a wave of depolarized membrane potential (4.8 ± 1.5 mV amplitude, 58.0 ± 151.2 ms duration). This pattern was not disrupted by antipsychotic administration. However, after the antipsychotic application there was a shift in the distribution of membrane potentials, with the hyperpolarized phase reaching more negative membrane potential values (Figure 2). Thus, the membrane potential histograms revealed that, after antipsychotic drug administration these cells spent more time in the hyperpolarized state. No changes in latency or amplitude of the MD-evoked epsps were found after drugs or PFCtx lesions.

Effects of Clozapine Administration. The response of VP cells to clozapine administration was examined in intact animals. The primary response to this drug was a significant decrease in the mean firing rate ($p < .05$, $t = 2.21$) of the cells recorded ($n = 12$), which shifted from a control rate of 1.3 ± 0.8 Hz to an average of 0.3 ± 0.2 Hz after clozapine administration. It should be noted that this decrease was significant despite the fact that, due to the variability in basal firing rates, baseline pre-drug firing rate of the cells tested in this group were lower than those recorded during the same period in the haloperidol group (Table 2).

Mediodorsal Thalamic Neurons

Electrophysiological Properties of MD Thalamic Neurons. In the MD of intact animals 10/17 cells (58.8%) were found to be in a quiescent state; on the other hand, in animals with PFCtx lesions 8/18 (44.4%) were found to be nonfiring. In animals that had received PFCtx lesions, the average firing frequency of neurons in the MD thalamic nucleus was significantly higher than those recorded in intact rats ($p < .05$, $t = -1.1$ Table 1). With respect to oscillatory activity, neither the frequency of oscillations (5.1 ± 0.2 Hz in lesion animals vs. 5.4 ± 0.3 Hz in intact animals) nor the proportion of MD cells that were found in the oscillatory state (40% in lesion animals vs. 38.0% in intact animals) were different in the lesioned rats.

Effect of Haloperidol Administration. All of the MD neurons tested responded to VP stimulation with evoked ipsps. Seven MD cells in intact rats were tested for their response to haloperidol administration. Haloperidol administration caused a change in the firing pattern of 3/5 MD cells, with neurons changing from an oscillatory discharge pattern to a tonic firing mode, accompanied by a trend toward higher firing frequencies (increase by 22%; Table 3, Figure 3).

The effect of haloperidol was studied on five MD cells recorded in rats with PFCtx lesions. As in the control

Table 2. Effects of Antipsychotic Drug Administration on VP Cells Recorded in Intact Animals, and in Animals with PFCtx Lesions

	Intact						PFCtx Lesion		
	CTRL	HAL (0.5 mg/kg)	% Change	CTRL	CLOZ (20 mg/kg)	% Change	CTRL	HAL (0.5 mg/kg)	% Change
Membrane potential (mV)	63.2 ± 2.2 (n = 10)	**70.7 ± 2.9**[a] (n = 10)	−11.6 ± 2.5	69.7 ± 5.1 (n = 12)	71.8 ± 7.2 (n = 12)	−3.0 ± 6.1	65.7 ± 1.0 (n = 9)	72.0 ± 0.5 (n = 9)	−7.9 ± 0.7
Input resistance (MΩ)	71.9 ± 4.5 (n = 10)	70.8 ± 12.2 (n = 10)	−1.5 ± 8.3	70.7 ± 8.6 (n = 10)	70.9 ± 8.3 (n = 10)	+0.28 ± 8.5	77.9 ± 2.9 (n = 5)	**115.2 ± 11.5** (n = 5)	**+47.8 ± 7.2**
Firing frequency (Hz)	5.9 ± 0.5 (n = 10)	**1.0 ± 0.1**[b] (n = 10)	**−83 ± 0.3**	1.3 ± 0.8 (n = 8)	**0.3 ± 0.2**[b] (n = 8)	**−76.9 ± 0.5**	4.2 ± 2.1 (n = 7)	**1.5 ± 0.1**[b] (n = 7)	**−64.2 ± 1.1**

CTRL = Control; HAL = Haloperidol; CLOZ = Clozapine.
[a] $p < .009$, $t = -3.07$.
[b] $p < .05$, $t = 2.21$.
Bold = statistical significant difference $p < .05$ or >35% change in response to drug administration (repeated measures design).

sample, haloperidol did not elicit significant changes in the input resistance, membrane potential or in the proportion of MD cells that exhibited oscillations following haloperidol. Furthermore, unlike that observed in intact animals, haloperidol caused a change in the activity state of 2/5 cells, which shifted from a non-oscillatory mode to an oscillatory pattern (Table 3). Neither haloperidol nor PFCtx lesions produced changes in the am-

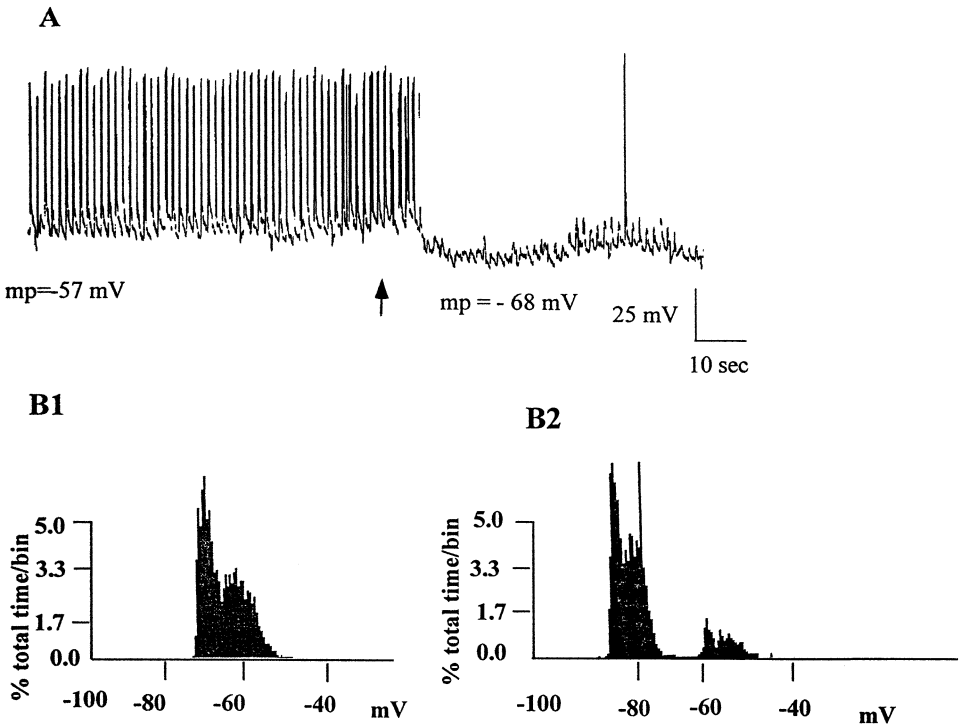

Figure 2. Representative example of the response of a VP neuron recorded in an intact rat to haloperidol administration. **(A)** Prior to haloperidol administration, this cell was tonically discharging action potentials in a regular pattern. Acute injection of haloperidol (0.5 mg/kg IV, *arrow*) caused an 11 mV hyperpolarization of the membrane and an attenuation of spontaneous spike discharge. **(B1)** Membrane potential histograms of the same cell shown in **(A)** reveal the bimodal distribution of membrane potentials characteristic of cells exhibiting spontaneous oscillatory activity. **(B2)** The membrane potential histogram constructed from the same VP neuron shown in **(B1)**, 2 min following haloperidol administration shows a shift toward more hyperpolarized values and also an increase in the proportion of time that the cell membrane is in the hyperpolarized state. The abscissa represents the membrane potential in mV, the ordinate represents the % of total time/bin, calculated by taking the number of events/bin × bin width (0.1 ms) divided by the total time of sampling.

Table 3. Effects of Antipsychotic Drug Administration on MD Cells Recorded in Intact Animals, and in Animals with Prefrontal Cortical Lesions

	Intact					
	CTRL	HAL (0.5 mg/kg)	% Change	CTRL	CLOZ (20 mg/kg)	% Change
Membrane potential (mV)	63.6 ± 2.5 (n = 7)	60.9 ± 4.7 (n = 7)	+3.7 ± 3.6	65.1 ± 5.3 (n = 6)	63.6 ± 3.3 (n = 6)	−2.3 ± 4.3
Input resistance (MΩ)	47.1 ± 9.6 (n = 6)	40.2 ± 7.2 (n = 6)	−14.6 ± 8.4	42.1 ± 2.4 (n = 3)	43.3 ± 1.0 (n = 3)	+2.8 ± 1.7
Firing frequency (Hz)	6.7 ± 0.9 (n = 7)	8.2 ± 7.0 (n = 7)	+22.3 ± 3.9	8.6 ± 6.0 (n = 3)	15.9 ± 12.4 (n = 3)	**+84.8 ± 9.2**
Change in state		OSC→ TONIC 3/5			OSC→ TONIC 3/7	
VP-evoked IPSP amplitude (mV)	7.3 ± 0.7	4.9 ± 0.8	−32.8 ± 7.1	5.9 ± 2.2	4.3 ± 0.65	−27.1 ± 35.2

	PFCtx Lesions		
	CTRL	HAL (0.5 mg/kg)	% Change
Membrane potential (mV)	63.8 ± 3.6 (n = 5)	65.3 ± 4.9 (n = 5)	−2.3 ± 4.2
Input resistance (MΩ)	62.2 ± 6.2 (n = 5)	58.0 ± 8.3 (n = 5)	−6.7 ± 7.2
Firing frequency (Hz)	5.3 ± 2.0 (n = 5)	**10.3 ± 3.6**[a] (n = 5)	**+94.34 ± 8.4**
Change in state		Non-OSC →OSC 2/5	
VP-evoked IPSP amplitude (mV)	7.5 ± 1.3	6.7 ± 0.85	−10.6 ± 17.3

[a] $p < .05$.
Bold = statistically significant difference $p < .05$ or >35% in response to drug administration (repeated measures design).

plitude of VP stimulation-evoked ipsps recorded in MD cells in lesioned animals (pre-haloperidol: 7.5 ± 1.3 mV, haloperidol: 6.7 ± 0.85 mV).

Effect of Clozapine. The response to the acute administration of clozapine was examined in six MD cells recorded in control animals. Clozapine produced an increase in firing frequency (85%) in the MD cells tested (Table 3 and Figure 4). In three out of seven cases the drug changed the mode of discharge from an oscillatory pattern to one of tonic firing. No changes in input resistance or resting membrane potential were observed after clozapine; however, the amplitude of the ipsps evoked by VP stimulation exhibited a trend toward a decrease.

DISCUSSION

Antipsychotic drugs exert a broad array of actions on neurons within the basal ganglia; as a result, it has been difficult to ascertain which of the actions are related to the therapeutic response of schizophrenic patients. In an initial approach to this complex problem, we have focused on an examination of antipsychotic drug action on the primary common output pathway of the limbic system: the ventral pallidal-mediodorsal thalamic pathway.

Impact of Prefrontal Cortical Lesions on VP-MD Cell Physiology

Studies into the potential sites of pathology in the schizophrenic brain have failed to yield consistent results, with alterations described with respect to sulcal size, ventricular enlargement, and neuronal disarray in the hippocampus and prefrontal cortex (Benes et al. 1991; Bogerts et al. 1985; Conrad et al. 1991; Roberts and Bruton 1990; Shelton et al. 1988). However, one characteristic that has been described repeatedly in the schizophrenic is the presence of hypofrontality—i.e., a decrease in either the activity or in the task-specific activation of the prefrontal cortex in patients with

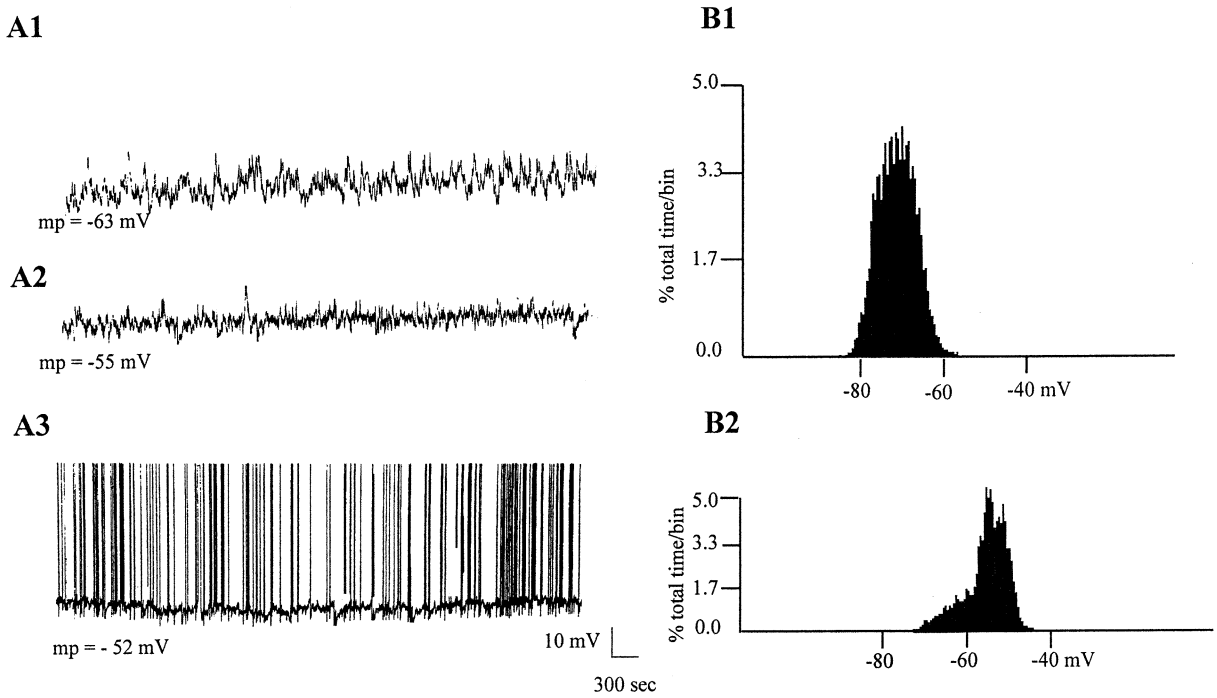

Figure 3. Typical example of the activation of a nonfiring MD thalamic cell by systemic administration of halperidol in an intact animal. **(A1)** This MD thalamic cell was quiescent prior to drug administration. **(A2)** Two and a half minutes following haloperidol administration (0.5 mg/kg, IV) the cell exhibited a small depolarization of the membrane potential, and **(A3)** by 4.5 min the cell displayed a tonic spike discharge pattern and a change to a non-oscillatory membrane potential activity state. Overall, this cell exhibited an 11 mV depolarization of the membrane from pre-drug levels. **(B)** Membrane potential histograms showing the changes in firing states produced by the systemic administration of haloperidol (0.5 mg/kg, IV) to a MD cell **(B1** = control period, **B2** = after haloperidol**)**.

schizophrenia (Ingvar and Franzen 1974; Pettegrew et al. 1989; 1991; Weinberger 1988; Williamson et al. 1991). Therefore, in an attempt to assess the relevance of prefrontal cortical function to the response to antipsychotic drugs, we performed cell-specific lesions of the prefrontal cortex in one set of rats approximately 1 month prior to recording and evaluation of drug action.

Following prefrontal cortical lesions, only minor changes in the basal activity of limbic system neurons were noted. Neurons in the VP showed a tendency to fire more slowly (26% decrease) with even smaller changes in their other physiological properties such as input resistance or resting membrane potential. This effect could be due to the loss of direct excitatory PFCtx input to the VP (Sesack et al. 1989) or via an effect in the PFCtx–nucleus accumbens–VP circuit, since at least a portion of the n. accumbens input to the VP is also excitatory (Chrobak and Napier 1993; Lavin and Grace 1996; Napier et al. 1995). Neurons in the MD of prefrontally lesioned rats showed a more marked (46%) increase in baseline firing rate, however this change did not reach statistical significance. It is not clear why removal of excitatory PFCtx drive to MD cells (Groenewegen 1988) did not result in a decrease in MD cell activity. One possibility is that, as a result of decreased PFCtx input to the limbic system, compensatory processes acting via a decrease in VP-mediated MD inhibition were introduced. This would be consistent with the increase in firing rate combined with an increase in the average input resistance in the MD occurring through a removal of inhibitory inputs secondary to decreased VP afferent activity.

Response of VP and MD Cells to Administration of Antipsychotic Drugs

In this study, we examined the actions of both the classical neuroleptic drug haloperidol and the atypical antipsychotic drug clozapine. Although these classes of drugs vary in several dimensions with regard to their receptor binding specificity and propensity for inducing extrapyramidal side effects, the one feature that these drugs have in common is their ability to elicit a therapeutic response in schizophrenic patients. Consistent with this common therapeutic action, cells in the VP exhibited a significant decrease in firing rate in response to both drugs; in both cases drug administration caused a hyperpolarization sufficient to bring the cells

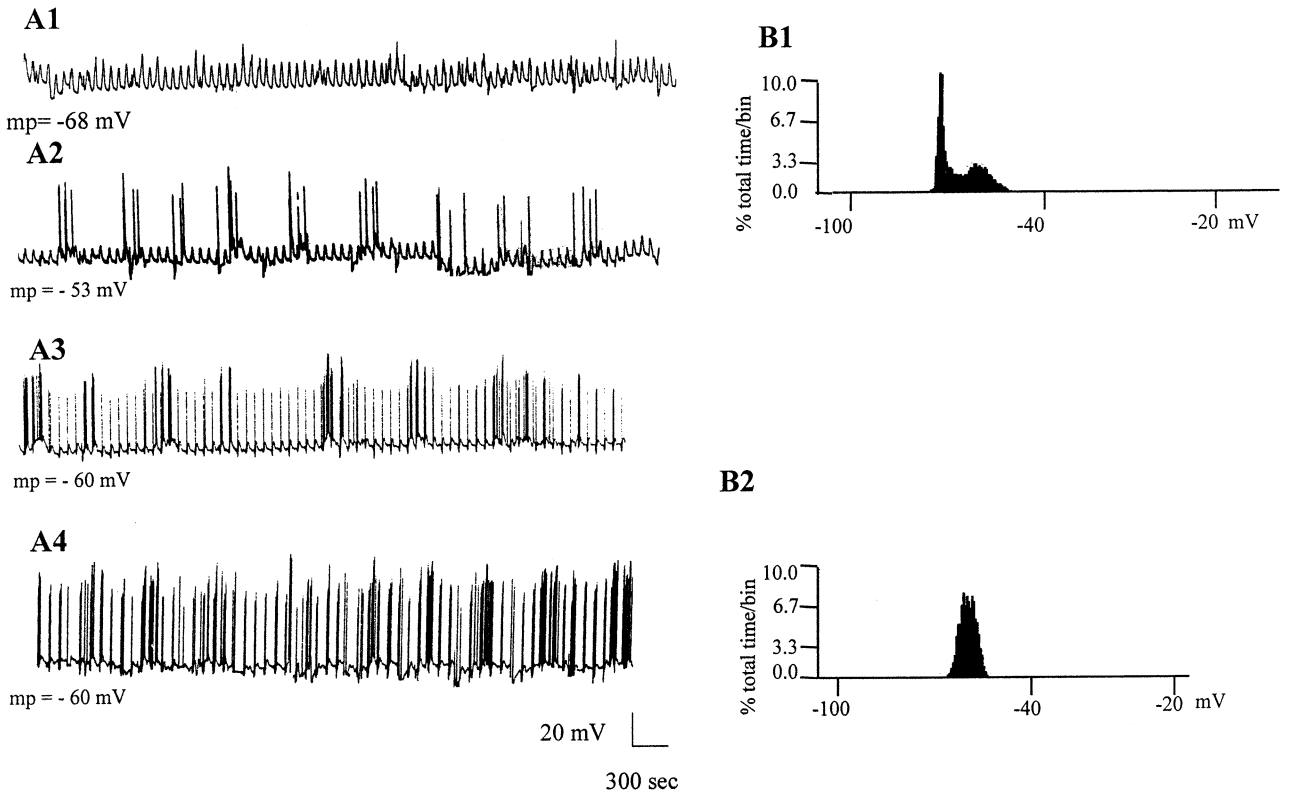

Figure 4. Response of an MD thalamic cell in an intact rat to systemic administration of clozapine (20 mg/kg). **(A1)** Prior to drug administration, the neuron exhibited oscillations (5 Hz) that elicited action potentials sporadically. **(A2)** Forty-five seconds following clozapine the cell began to exhibit an increase in the firing rate and a small depolarization of the membrane. **(A3)** At 1.5 min following clozapine administration the increase in firing rate is more evident, and **(A4)** at 4 min following drug application, the cell exhibits a higher firing frequency and the oscillatory activity has changed to a primary tonic activity state. **(B)** Membrane potential histogram showing the changes in the distribution of membrane potential induced by an acute clozapine injection (20 mg/kg IV).

to approximately the same resting membrane potential (i.e., −71 to −72 mV). In the case of cells that exhibited slow oscillations, the drugs also produced a shift toward more hyperpolarized membrane potential levels and also increased the time during which the cells were in the more hyperpolarized state. It is possible that some of the acute actions of antipsychotic drugs in the VP occur secondary to the blockade of DA afferents to this region and/or an increase in GABA release (Drew et al. 1990; Moghaddam and Bunney 1990). Thus, Napier et al. (1991a) reported that haloperidol administered iontophoretically to VP cells attenuated spontaneous spike firing by blocking a tonic dopaminergic tone in the VP. VP cells have been shown to be responsive to DA agonists (Napier et al. 1991a,b) and the VP is known to receive substantial DA afferent input from the ventral tegmental area and the substantia nigra pars compacta (Klitenick et al. 1992; Grove 1988; Jones and Cuello 1989). The balance between DA blockade in the accumbens leading to increased n. accumbens–VP inhibitory tone versus a direct blockade of DA actions on VP neurons in mediating the responses observed here is yet to be determined. In the intact rats, haloperidol caused a significant hyperpolarization of the membrane potential without causing observable changes in input resistance. However, in rats with PFCtx lesions, the hyperpolarization was accompanied by a significant increase in input resistance. Whereas the explanation for such a finding is not clear, it does suggest that the lesion is removing some offsetting influence with respect to haloperidol-induced conductance changes in VP neurons that may be obscured in the intact rat, as discussed below.

In contrast to their different action in VP, both the classical neuroleptic haloperidol and the atypical drug clozapine caused an excitation of MD thalamic cells; however, clozapine appeared to produce a greater increase in firing rate (85%) when compared with haloperidol (22%). Given that the VP projection to the MD is inhibitory in nature (Gröenewegen et al. 1990; Haber et al. 1985; Lavin and Grace 1994), the data obtained after haloperidol administration are consistent with a disinhibition of MD cell firing secondary to inhibition of VP neu-

ronal afferent discharge. However, it was not clear why haloperidol and clozapine exhibited equivalent levels of inhibition of VP neurons, whereas clozapine caused a much greater elevation in the firing rate of MD neurons. Although the reason for this is not apparent, one possibility is that it is related to the broad pharmacological profile of clozapine and its actions at other receptor sites, such as serotoninergic, muscarinic, and adrenergic receptors. In this context, it is interesting to note that PFCtx lesions can augment the responses of MD cells to haloperidol to an extent in which it is equivalent to clozapine without affecting the response of VP neurons. It should be noted, however, that since these recordings were obtained in anesthetized animals, and chloral hydrate is known to attenuate some of the responses of neurons to antipsychotic drugs (Bunney et al. 1973), the effects of these drugs may be more robust in awake preparations.

Another effect observed in MD cells in response to both haloperidol and clozapine administration was a shift in activity from an oscillatory to a tonically discharging state. Studies have shown that the reticular nucleus of the thalamus has a central role in regulating primary thalamic cell activity patterns (Steriade and Deschênes 1984). Furthermore, our work has shown that projections from the VP innervates both the MD and the reticular thalamic nucleus (RTN) (O'Donnell et al 1997) and that stimulation of the VP both inhibits the MD directly as well as disinhibits dorsal thalamic nuclei via an action in the RTN (Lavin and Grace 1994). Therefore, it is possible that inhibition of neurons in the VP by antipsychotic drugs may affect MD cell firing directly as well as modulating its activity pattern via the RTN.

Conclusions and Functional Extensions

The augmented response of MD cells to haloperidol administration in rats with lesions of the PFCtx may reflect differences in the acute response to these drugs as observed in normal versus schizophrenic patients. Several studies have shown that schizophrenics exhibit hypofrontality, or a decrease in basal activity and/or task-specific activation of the PFCtx when compared with controls (Chabrol et al. 1986; Farkas et al. 1984; Ingvar and Franzen 1974; Pettegrew et al. 1989; 1991; Weinberger 1987). Drawing from our observations, it is possible that the augmented effect produced by haloperidol administration in rats with PFCtx lesions is a consequence of the diminished activity in the prefrontal cortical efferent pathways. Since this does not occur in concert with an augmented effect on the VP, one possibility is that it involves an alteration in the PFCtx–MD loop. For example, if one proposes that the MD–PFCtx loop functions to provide a stabilizing influence in this system, one possible explanation is that the intact PFCtx–MD connection partially offsets the influence of haloperidol acting via the nucleus accumbens–VP–MD circuit. This would be consistent with models of cortical/subcortical DA interactions, in which PFCtx DA and subcortical DA are regulated in opposite directions. In this context, one could suggest that the potent effects of clozapine in the MD as well as in the schizophrenic patients are at least partially due to its ability to circumvent this stabilizing influence. Furthermore, the finding that the responses of MD cells to haloperidol in the lesioned rats are similar in magnitude to the effects of clozapine in intact animals may relate to the greater sedative actions of haloperidol in a system that has not been compromised—i.e., the non-schizophrenic individual (Berger and Waldhorn 1995; Kornetsky and Mirsky 1966; King and Henry 1992).

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