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Repeated Treatment with Haloperidol and Clozapine Exerts Differential Effects on Dye Coupling between Neurons in Subregions of Striatum and Nucleus Accumbens

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The delayed onset of action of antipsychotic drugs (APDs) during the treatment of schizophrenia has been hypothesized to temporally correlate with the induction of depolarization block in rat mesencephalic dopamine (DA) cell groups. Nevertheless, it is unknown whether these drugs also exert a delayed action on the dopaminoceptive postsynaptic target cells in the striatal complex. Using *in vivo* intracellular recording and dye labeling techniques, the effects of APDs on dye coupling were examined in subregions of the striatal complex defined by double staining for calbindin immunoreactivity. Rats treated repeatedly with APDs were found to exhibit a 66–71% higher incidence of coupling that occurred in a drug- and a region-specific manner, that is, both drug treatments increased dye coupling in the limbic-associated accumbens shell region whereas only haloperidol increased dye coupling in the motor-related striatal matrix and accumbens core regions. In addition, cells located in regions in which dye coupling was altered also showed significantly higher input resistance. These changes were not observed in response to DA receptor blockade by acute drug administration or when haloperidol was administered for a period sufficient to induce DA receptor supersensitivity but not DA cell depolarization block (i.e., 2 weeks). Therefore, alteration in dye coupling appears to be correlated temporally with the induction of DA cell depolarization block. The finding that both APDs exert a common action on neurons in the accumbens shell region is consistent with its identification as the site of therapeutic drug actions, whereas the capacity of haloperidol to also affect cells in the motor-related matrix and core regions correlates with its high propensity to induce extrapyramidal side effects.

[Key words: antipsychotic therapeutic action, extrapyramidal motor side effects, dopamine cell depolarization block, electrotonic transmission, accumbens core/shell regions, striatal matrix/patch compartments]

Antipsychotic drugs (APDs) are dopamine (DA) receptor antagonists (Carlsson and Lindqvist, 1963) and their efficacy is highly correlated with their ability to block DA receptors (Creese et al., 1976; Seeman et al., 1976; Kebabian and Calne, 1979). Nonetheless, although APDs will block DA receptors within minutes following their administration (Rupniak et al., 1983; Sedvall et al., 1986), these drugs must be administered for weeks before achieving maximal therapeutic efficacy and before the Parkinsonian motor side effects are manifest (Spohn et al., 1977; Cotes et al., 1978; Davis and Garver, 1978; Johnstone et al., 1978; Cooper et al., 1990). The time-dependent effects of classic APDs in humans have been temporally correlated with the development of depolarization block of DA cell firing in rats, in which repeated haloperidol treatment causes a cessation of spontaneous spike discharge in the majority of mesencephalic DA neurons in the substantia nigra (SN, A9) and the ventral tegmental area (VTA, A10) (Bunney and Grace, 1978; Chiodo and Bunney, 1983; White and Wang, 1983; Grace and Bunney, 1986). In contrast, repeated treatment with atypical antipsychotic drugs such as clozapine, which is not associated with extrapyramidal side effects in humans (Gerlach et al., 1974; Baldessarini and Tarsy, 1980; Kane et al., 1988), only causes an inactivation of VTA DA cell firing (Chiodo and Bunney, 1983; White and Wang, 1983). Therefore, the therapeutic actions and extrapyramidal side effects associated with antipsychotic drug administration correlate with the time course and regional selectivity of these drugs in inducing depolarization block of the mesolimbic and nigrostriatal DA neurons, respectively. Evidence has further shown that depolarization block occurs via time-dependent changes in feedback to the DA cells from postsynaptic sites (Bunney and Grace, 1978; Grace and Bunney, 1986; Hollerman et al., 1992), since acute transection of these pathways reverses depolarization block and lesioning of the striatal target cells prevents its development (Bunney and Grace, 1978; Chiodo and Bunney, 1983; White and Wang, 1983). However, it is not clear whether corresponding changes in the striatum occur in concert with DA cell depolarization block.

Dopamine is known to exert multifaceted actions within the striatal complex, including direct actions on striatal cell membranes (Arbuthnott et al., 1984; Bernardi et al., 1984; Calabresi et al., 1987, 1988) as well as modulatory effects on neurotransmitter release from afferent terminals (Mitchell and Doggett, 1980; Rowlands and Roberts, 1980; Maura et al., 1988; O'Donnell and Grace, 1994). In addition, several studies have described an action of DA beyond its effects on the activity of individual striatal cells, in which this neurotransmitter was

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shown to regulate the interactions within networks of striatal cell assemblies by modulating dye- and electrical-coupling among sets of striatal neurons *in vitro* (Cepeda et al., 1989; Walsh et al., 1989; O'Donnell and Grace, 1993) and *in vivo* (Onn and Grace, 1994a). Thus, *in vivo* recordings have shown that the administration of a behaviorally activating dose of the D1/D2 agonist apomorphine can dramatically increase the degree of dye coupling between striatal cells (Onn and Grace, 1994a).

In this study, the effects of repeated treatment for 1 month with haloperidol (1.5–3 mg/kg; i.p. daily) and clozapine (20 mg/kg) were examined with respect to their influence on dye coupling between striatal cells in the dorsal striatum and the nucleus accumbens using *in vivo* intracellular recording techniques. This dosage schedule and duration of drug treatment has been shown to be sufficient to induce DA cell depolarization block (Bunney and Grace, 1978; Chiodo and Bunney, 1983; White and Wang, 1983). Furthermore, to determine whether any alterations may correlate with the induction of DA receptor supersensitivity during APD treatment, the assessments were also carried out in rats that had been treated with haloperidol for 2 weeks, which is sufficient to cause DA receptor supersensitivity in the dorsal striatum (Burt et al., 1977; Muller and Seeman, 1978; Bannon et al., 1980) without inducing DA cell depolarization block (Chiodo and Bunney, 1983; White and Wang, 1983). Two dimensions of drug action were assessed: (1) their effect on the incidence and extent of dye coupling, and 2) their selectivity for neurons in the calbindin-positive motor regions (e.g., striatal matrix and accumbens core regions) or the calbindin-negative limbic regions (i.e., striatal patch and accumbens shell regions) of the striatal complex. The patch and matrix compartments of the dorsal striatum, which can be distinguished by their differential staining for calbindin immunoreactivity, are considered to represent distinct functional subdivisions of the striatum (Graybiel and Ragsdale, 1978, 1983; Goldman-Rakic, 1982; Gerfen, 1984; Gerfen et al., 1987; Graybiel, 1990): the striosomes are associated with the limbic component of the striatum (White, 1989) based on their cortical afferent innervation (Gerfen, 1984; Donoghue and Herkenham, 1986), whereas the matrix is more closely related to the extrapyramidal function of this region (Fairley and Marshall, 1986; Whishaw and Tomie, 1987; Pisa and Schranz, 1988; White, 1989; Francois et al., 1994). The nucleus accumbens has also been subdivided into two functional regions, termed the core and shell subregions, based on a proposed preferential involvement in sensorimotor integrative versus limbic functions, respectively (Mogenson et al., 1980; Alheid and Heimer, 1988; Zahm and Heimer, 1990; Heimer et al., 1991; Berendse et al., 1992; Cools et al., 1993; Prinssen et al., 1994). These subregions also show a differential staining for calbindin immunoreactivity (Zahm and Heimer, 1988; Voorn et al., 1989; Zahm, 1991).

Portions of these results have been presented in abstract form (Onn and Grace, 1994b,c).

Materials and Methods

Animals. All procedures were carried out in accordance with the USPHS publication, *Guide for the Care and Use of Laboratory Animals,* and were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh. Male Sprague–Dawley rats (N = 73) weighing 175–250 gm at the beginning of drug treatment were used.

Drug treatment. Either haloperidol or clozapine was administered to rats for 1 month (N = 21 for each drug) or for 2 weeks (N = 5, haloperidol only) by injection (once per day, i.p.) or via the drinking water (N = 4 for each drug). Clozapine (RBI; Natick, MA) was first dissolved in 1N HCl and then titrated to pH 5.7–5.9 with 1N NaOH, with additional 0.9% saline added to achieve a final concentration of 1.4% (i.e., 0.043 M). Haloperidol (at 5 mg/ml; McNeil Pharmaceuticals, Spring House, PA) was supplied in a vehicle solution containing lactic acid to adjust the pH to 3.0–3.6. Haloperidol (1.5–3 mg/kg), clozapine (19–21 mg/kg) or saline solution (for controls, n = 8) was administered for 30–40 d (defined as 1 month treatment in the following text). This drug administration protocol should have been sufficient to induce depolarization block in the mesencephalic DA cells (Bunney and Grace, 1978; Chiodo and Bunney, 1983; White and Wang, 1983). The rate of body weight gain in the drug-treated rats was not significantly different from the vehicle-treated controls, which typically gained approximately 150–250 gm above their initial weight during the 1 month treatment period. In a separate group of rats (N = 5), haloperidol was administered using a similar treatment protocol except that the rats were injected with the drug for a 2 week period, which should have been sufficient to induce DA D2 receptor supersensitivity (which is known to reach maximal at 7–10 d in the dorsal striatum with a similar regimen of haloperidol treatment; Burt et al., 1977; Muller and Seeman, 1978), but would be inadequate for the induction of DA cell depolarization block (Chiodo and Bunney, 1983; White and Wang, 1983). Since clozapine has not been shown to induce DA receptor supersensitivity (Kobayashi et al., 1978; Rupniak et al., 1985; See et al., 1989, 1994; Wilmot and Szczepanik, 1989; Dell et al., 1990; Florijn et al., 1994; Tarazi et al., 1994), only haloperidol was tested at this time point. To test the effects of acute drug administration, either clozapine (N = 5) or haloperidol (N = 5) was administered intravenously once on the day of recording at the same dose used in the repeated treatment paradigm.

Electrophysiology. Following repeated drug treatment, a 36 hr drug wash-out period was allowed before recordings were performed. A drug washout period of 36 hr was used in order to examine the changes induced during repeated administration, rather than the change in the response to acute drug administration. The animals were anesthetized with 8% chloral hydrate (400 mg/kg, i.p.) for *in vivo* intracellular recording and dye injection as previously described (Onn and Grace, 1994a; Onn et al., 1994a–c). Intracellular recording microelectrodes that had been pulled on a Flaming/Brown P80/PC electrode puller and filled with Lucifer yellow (10% Lucifer yellow dissolved in 1 M LiCl; average input resistance = 110–150 MΩ) were lowered into the brain to record from neurons in the dorsal striatum and nucleus accumbens at the following stereotaxic coordinates: AP, 0 mm to 2 mm anterior to bregma; ML, 1.5 mm to 3.6 mm from the sagittal suture; and DV, 3 mm to 7.8 mm from the dura. Spontaneous basal activity was collected at the resting membrane potential for each striatal neuron impaled. Cell input resistance was calculated by measuring the membrane voltage deflections produced in response to 100–150 msec duration constant current pulses delivered across a bridge circuit integral to the preamplifier. The amplitude of the constant current pulses used in this calculation ranged between 1 nA in the hyperpolarizing direction and depolarizing current pulses that were subthreshold for triggering spike discharge. Action potential amplitude was measured as the difference between the resting membrane potential and the spike peak.

Intracellular labeling with Lucifer yellow. After achieving a stable impalement, striatal neurons were stained using Lucifer yellow–filled electrodes by injecting 1–3 nA constant hyperpolarizing current interrupted by short duration (5–10 msec) depolarizing pulses delivered at 4–7 Hz into the electrode (Grace and Llinas, 1985; Grace and Onn, 1989; Onn and Grace, 1994a; Onn et al., 1994c). During dye injection, the electrode was not moved and the cell membrane potential was closely monitored to maintain a stable penetration and to ensure that only a single cell was injected with dye. All cells used to evaluate the incidence of dye coupling were injected for a minimum of 3 min, with some injections lasting up to 15 min. The amount of time that elapsed between the injection of dye and perfusion of the rat ranged between 5 min and 4 hr and was noted for each cell injected. In general, postinjection survival time did not show any correlation with the presence of dye coupling in cells that were injected for at least 3 min, as reported previously (Onn and Grace, 1994a). After completion of the recordings, the deeply anesthetized rats were perfused transcardially with saline followed by 500 ml of 4% buffered paraformaldehyde (pH = 7.4). Frontal or sagittal sections (60 μm in thickness) were cut in series on a freezing microtome and collected in 0.1 M phosphate buffer (pH 7.4). Lucifer yellow–labeled cells were examined using a Leitz Orthoplan II epifluorescence microscope equipped with a Leitz I3 filter cube (excitation: band pass 355–425 nm; dichromatic mirror: RKP 580 nm; sup-

Table 1. Effects of repeated treatment with haloperidol and clozapine on the incidence of dye coupling between spiny cells in striatal matrix/patch and accumbens core/shell regions

Striatal subregions[a]	Basal levels[b]	Acute HAL (i.v.)	Repeated HAL (2 wks)	Repeated HAL (1 mon.)	Acute CLZ (i.v.)	Repeated CLZ (1 mon.)
Striatal matrix	2/12 (17%)	1/6 (14%)	1/7 (14%)	4/8 (50%) $p = 0.047$	0/5 (0%)	1/10 (10%)
Striatal patch	0/5 (0%)	N.T.	N.T.	0/3 (0%)	N.T.	0/3 (0%)
Accumbens core	2/9 (22%)	2/7 (28%)	1/5 (20%)	4/6 (67%) $p = 0.06$	1/7 (14%)	4/9 (44%)
Accumbens shell	1/10 (10%)	0/6 (0%)	1/7 (14%)	4/6 (67%) $p = 0.0001$	0/5 (0%)	5/7 (71%) $p = 0.0001$

p values determined by the Fisher exact test in comparison with control.

[a]Determined by calbindin immunoreactivity counterstain.

[b]Percentage injections resulting in coupling = number of cases of coupling/number of cells injected.

pression: low pass 580 nm). Cells were scored as dye coupled when the injection of a single cell resulted in the labeling of more than one cell. This typically consisted of the labeling of two or three cells with the somata located in one or two adjacent sections and dendritic processes spanning across the remaining three to seven sections.

Immunocytochemical double-labeling for calbindin. Lucifer yellow–labeled cells were also characterized with respect to their location within the motor- (matrix/core) or limbic- (patch/shell) related subregions of the striatal complex by double labeling for calbindin immunoreactivity. This was done using the Texas red indirect fluorescence method as described previously (Onn and Grace, 1994a; Onn et al., 1994c). Briefly, serial sections containing Lucifer yellow–labeled cells were processed with calbindin 28 Kd antisera raised in mouse (a gift of Dr. M. R. Celio; at 2000× dilution with 0.1 M phosphate buffer containing 0.4% Triton X-100 and 5% normal horse serum) followed in sequence by incubation with biotinylated horse- anti-mouse IgG (at 100× dilution) for 4 hr and by incubation with Texas red–conjugated avidin (at 100× dilution) overnight at 4°C. For dual light field examination, the avidin/biotin complex system (ABC system; Vector) was used to convert the Lucifer yellow and the calbindin fluorescent labels to dual-color peroxidase stains. The double-labeled sections were then examined using a Leitz Orthoplan II microscope configured for light field illumination (for the dual peroxidase-stained sections) or for epifluorescence illumination under I3 (Lucifer yellow) and N2 (rhodamine) filter cubes (for the double fluorescence-labeled sections; N2 cube-excitation: band pass 530–560 nm; dichromatic mirror: RKP 580 nm; suppression: low pass 580 nm).

Statistics. The incidence of dye coupling between neurons in the experimental groups was compared using a nonparametric Fisher exact test. Student's t test was used to test for differences between means of unpaired observations. Data are expressed as mean ± standard deviation.

Results

Several studies have shown that dye coupling can be a reliable indicator of the presence of gap junction connections between neurons (Andrew et al., 1981, 1982; MacVicar and Dudek, 1981, 1982; Dudek et al., 1983; Llinas, 1985; O'Beirne et al., 1987; Phelan et al., 1993) provided that sufficient caution is used when employing this technique (Gutnick and Prince, 1981; Grace and Bunney, 1983; Gutnick et al., 1985; O'Donnell and Grace, 1993; Onn and Grace, 1994a). It has been our experience that consistent dye coupling assessments require that stable penetrations be maintained throughout the dye injection period (Onn and Grace, 1994a). Therefore, to obtain consistent data and to circumvent the introduction of some potential artifacts, data were only collected from neurons that met the following criteria: (1) after penetration the cell exhibited a stable resting membrane potential of −60 mV or greater, and action potential amplitudes of 55 mV or greater; (2) cells were injected with dye for a minimum of 3 min, with the penetration remaining stable throughout the injection periods; (3) there was complete filling of the soma and dendritic processes of each cell comprising a coupled set; (4) all cells labeled by a single injection exhibited an equal intensity

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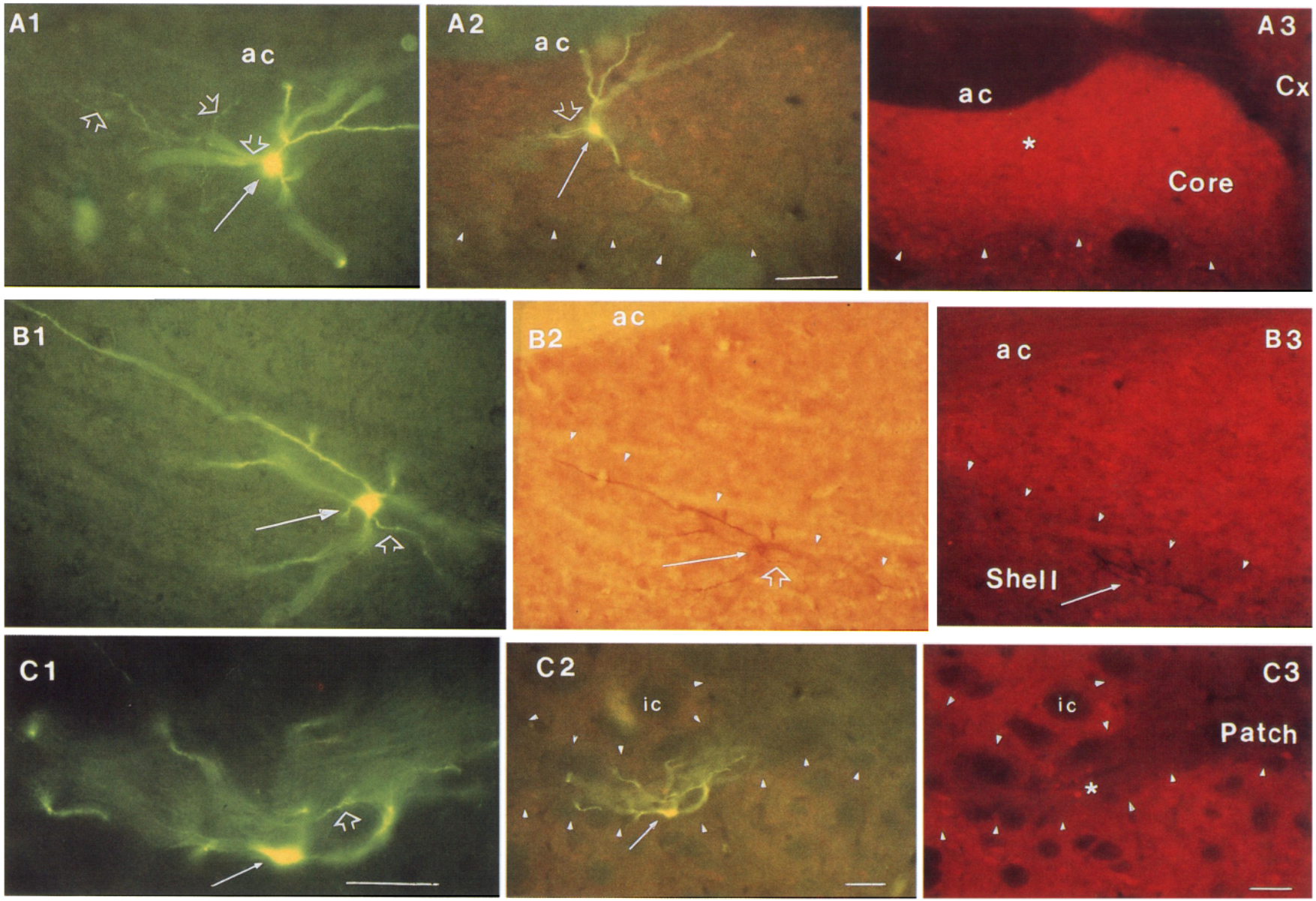
Figure 1. Photomicrographs illustrating the typical pattern of single neuronal labeling following intracellular injection of the dye Lucifer yellow into neurons in the accumbens core (*A*) and shell (*B*) regions as well as in the striatal patch region (*C*). *A*, Injection of a single cell in the accumbens core region results in the labeling of one neuron with Lucifer yellow. *A1*, A Lucifer yellow-labeled spiny neuron shown in the coronal plane had a medium-sized soma (*arrow*; 14 μm in diameter) and extensive local axonal collateralization (*open arrows*). *A2*, Subsequent staining of the same section (*A1*) for calbindin immunoreactivity reveals that the neuron is confined completely within the border (*arrowheads*) of the calbindin-positive core region. Calbindin immunoreactivity is indicated by the presence of Texas red fluorescence and the brain slice is examined using a Leitz I3 filter cube. *A3*, The location of the Lucifer yellow–labeled cell (not seen; *asterisk* indicates location of the soma) is shown in relation to the core and shell subdivisions in the same section (*A2*) illuminated using the Leitz N2 filter cube selective for Texas red fluorescence. Anterior commissure (*ac*); insular cortex (*Cx*). *B*, Injection of a single cell in the nucleus accumbens shell region results in the staining of one medium spiny neuron. *B1*, A single Lucifer yellow–labeled spiny neurons shown in the sagittal plane exhibits a 16 μm diameter soma (*arrow*) that gave rise to an axon (*open arrowhead*) and extensive axonal collaterals in adjacent sections (not shown). *B2*, subsequent conversion of the Lucifer yellow fluorescence into a peroxidase stain in the same section (*B1*) by immunocytochemical localization of Lucifer yellow. *B3*, when this same section (*B2*) was subsequently stained for calbindin immunoreactivity, the neuron (*arrow*) was found to be confined completely within the calbindin-negative shell region (*arrowheads* mark the border to the core region). *C*, Injection of a single cell with Lucifer yellow in the striatal patch region labels one medium spiny cell. *C1*, A single Lucifer yellow–labeled spiny neuron shown in the coronal plane has a 17 μm diameter soma (*arrow*) and an axon (*open arrowhead*) that originates from one of its primary dendrites; the axon (*open arrow*) also was found to give rise to extensive axonal collaterals in adjacent sections (not shown). *C2*, Subsequent staining of this section (*C1*) for calbindin immunoreactivity reveals that this neuron and its dendritic processes are confined completely within the border (*arrowheads*) of a calbindin-negative patch region (i.e., Texas red poor zone). *C3*, Examining this section (*C2*) using a Texas red filter cube (Leitz N2 filter cube) illustrates the relationship of this cell (*) to the boundary of the matrix/patch structure. *ic*, Internal capsule. Scale bars, 50 μm for all figures. *A1, B1, C1* refer to bar in *C1*; *A2* refers to bar in *A2*; *B2, C2, A3, B3, C3* refer to bar in *C3*.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of Lucifer yellow fluorescence; and (5) there was no evidence of extracellular spillage of dye in the vicinity of the stained cells.

In accordance with these restrictions, the present results were drawn from a total of 146 Lucifer yellow injections into striatal spiny cells (i.e., the Spiny I cell class): 36 injections in control or saline-treated rats and 110 injections in haloperidol- or clozapine-treated rats (acute drug = 37; 1 month drug administration = 54; 2 weeks drug administration = 19). In addition to this sample, six of the cells injected exhibited a morphology that was not of the Spiny I morphological cell class (DiFiglia et al., 1976; Preston et al., 1980; Bishop et al., 1982; Chang et al., 1982; Fisher et al., 1986; Yelnik et al., 1991; Onn et al., 1994c). Since aspiny or sparsely spined cells have not been observed to exhibit dye coupling in control rats or following acute treatment with DA agonists (Onn and Grace, 1994a), they were not included in the data used to calculate the incidence of dye coupling in these experiments.

Pattern and incidence of dye coupling in subregions of the striatal complex in vehicle-treated control rats

Out of a total of 36 striatal cells injected with Lucifer yellow in 8 vehicle-treated control rats, 5 injections resulted in the labeling of single pairs of neurons, which corresponds to a 14% incidence of dye coupling (Table 1). If the calculation is performed to include the data from all control rats studied to date ($N = 112$; $N = 36$ from present results plus $N = 76$ from Onn and Grace, 1994a), the overall incidence of coupling is 16% (i.e., 18 single pairs). For each labeled cell, Lucifer yellow was injected for a minimum of 3 min with an average time of injection of 6.3 ± 2.5 min (mean ± SD; $N = 146$; range = 3–15 min). The average length of time that dye was injected into cells that were subsequently revealed to be dye coupled (6.7 ± 2.7 min; $N = 34$) was not significantly different from those cases in which the injections only yielded single-labeled cells (6.0 ± 3.1 min; $N = 112$; $t = -0.32$; df = 144; $p > 0.7$; NS), which is consistent with our previous report (Onn and Grace, 1994a).

Cells labeled by Lucifer yellow injection were analyzed with respect to their location within striatal subregions (i.e., matrix/patch or accumbens core/shell regions) by further processing the tissues for calbindin-28 Kd immunoreactivity. In the majority of cases, injections of cells in the control striatal complex resulted in the labeling of single spiny cells which typically had their dendritic processes restricted to the same region or compartment in which the cell bodies were located (Fig. 1*A–C*). This is particularly evident for cells stained at the border of two adjacent regions; for example, between accumbens core and shell regions (Fig. 1*A,B*) or between striatal matrix and patch structures (Fig. 1*C*). Based on cells that were double labeled for calbindin immunoreactivity, approximately 58% (21/36) of the cells stained with Lucifer yellow were located in calbindin-rich regions, with 57% (12/21) of these located in the striatal matrix and 43% (9/21) in the accumbens core region. The remaining 42% (15/36) of the Lucifer yellow–stained cells were located in calbindin-negative regions [patch: 33% (5/15); shell: 66% (10/15)]. There was an approximately equal incidence of coupling observed in the striatal matrix (17%; 2/12) and the accumbens core region (22%; 2/9). In contrast, cells labeled in calbindin-negative striatal patch and accumbens shell regions exhibited comparatively low incidences of coupling (i.e., patch: 0/5; shell: 1/10; Table 1).

As reported previously (Onn and Grace, 1994a), all cases of dye coupling observed in the vehicle-treated control rats were found to occur only between cells of the same morphological class; that is, the Spiny I cell class. In almost all cases, coupling occurred only between pairs of cells; coupling involving sets of three cells was only observed in four cases across all groups, and in each case the cells were located in the ventral core region (i.e., ventral to anterior commissure) of the accumbens. In the majority of cases, the points of contact among coupled neurons occurred between the primary or between the secondary dendritic processes of the cells. Direct apposition between the somata of coupled cells was rarely observed. In all three subregions that exhibited dye coupling in controls, the somata and the dendritic processes of dye-coupled cells were confined to a single compartment, as reported previously (Onn and Grace, 1994a).

Effects of acute versus 1 month repeated administration of haloperidol on dye coupling in subregions of the striatal complex

Animals treated acutely with haloperidol (2–3 mg/kg; i.v.) did not exhibit significant differences in the incidence of dye coupling observed in the striatal matrix (1/6) or accumbens core (2/7) and shell (0/6) regions as compared to vehicle-treated controls. However, following repeated treatment with haloperidol for 1 month there was nearly a fourfold higher incidence of dye coupling in the striatal complex, with 52% (12/23) of the cells injected exhibiting coupling. Coupling was particularly high in the striatal matrix region (50%; 4/8; $p = 0.047$; Onn and Grace, 1994a) and in the accumbens core region (67%; 4/6; $p = 0.06$; Fig. 2*A,B*). The number of cells coupled per injection in the matrix compartment was identical to that found in the vehicle-treated control group (i.e., two cells coupled in every case examined). Similarly, the pattern of coupling was not altered in the accumbens core region [average 2.3 ± 0.5 for both HAL/CLZ (6 pairs, 2 triplets) vs 2.5 ± 0.7 for control (1 pair, 1 triplet), $t = 0.45$; $p > 0.7$; NS] since in both control and haloperidol-treated groups approximately 50% (control: 1/2; HAL-treated: 2/4) of the cases of coupling in the core region occurred among sets of three cells (Fig. 2*B*). In addition to exhibiting a higher incidence of dye coupling in both the matrix and core regions, rats treated with haloperidol for 1 month also exhibited a higher incidence of dye coupling in the ventrally located calbindin-poor shell region (4/6 or 67%). Although the incidence of coupling found in the shell of HAL-treated rats was similar to that observed in the matrix and core regions of these rats, it represents more than a fivefold increase in coupling when the comparatively low level of coupling found in the shell of controls is taken into account (i.e., 10%; $p < 0.0001$). Similar to that observed in the core and matrix regions, the average number of cells coupled per injection in the shell region was the same as that observed in controls [2.1 ± 0.3 vs 2.0 ± 0.0 ($N = 1$)].

As in controls, in all cases of dye coupling examined in the treated rats the cells exhibited a medium spiny morphology and the sites of direct contact between coupled neurons was limited to the dendritic regions. The somata and dendritic processes of the dye-coupled neurons did not cross the boundary between two adjacent subregions, that is, between patch and matrix structures or between accumbens core and shell subregions (Fig. 2*B,C*). Therefore, despite the higher incidence of dye coupling observed in the calbindin-rich matrix and core regions as well as in the calbindin-poor accumbens shell region following repeated HAL treatment, the pattern and the extent (i.e., the number of cells labeled per injection) of coupling was unchanged.

In summary, the incidence of dye coupling was higher in both the motor-related matrix and core regions as well as in the limbic-related shell regions in rats that had received repeated treatment with haloperidol for 1 month; no changes in the patch/matrix distribution or in the extent of coupling were observed.

Effects of acute versus 1 month repeated clozapine administration on dye coupling in subregions of the striatal complex

As found with acute haloperidol administration, the incidence or extent of dye coupling observed in rats receiving a single injection of clozapine was not significantly different from that found in either the matrix or in the core/shell region of controls (0/5 matrix; 1/7 core; 0/5 shell; $p > 0.8$). However, in contrast to the rats receiving repeated HAL, the level of dye coupling observed in the calbindin-positive matrix and core regions of rats that had been treated with clozapine for 1 month was the same as that observed in controls (i.e., calbindin- positive matrix = 10% (1/10) and core = 44% (4/9); $p > 0.5$ vs controls; NS; Table 1). In contrast, the incidence of coupling observed in the shell region of clozapine-treated rats was 71% (5/7), which was more than sevenfold higher than that observed in vehicle-treated controls ($P = 0.0001$), but not significantly different from that found following repeated HAL ($P > 0.7$; NS). Furthermore, except for a single case, the extent of coupling in clozapine-treated rats did not extend beyond two cells coupled per injection in this region. In all cases examined, each of the coupled cells exhibited a spiny morphology and did not cross the boundary between the calbindin-negative shell region and the calbindin-positive core region (Fig. 2*C*).

Effects of 2 week treatment with haloperidol on dye coupling

As outlined in Materials and Methods, a 1 month period of haloperidol treatment should have been sufficient to induce both DA cell depolarization block and DA supersensitivity in the striatum. In order to segregate the responses that may be correlated with the time course of these events, a separate group of rats was tested after 2 weeks of HAL treatment, which was shown to be sufficient to induce DA supersensitivity (Burt et al., 1977; Muller and Seeman, 1978; Bannon et al., 1980) without causing DA cell depolarization block (Chiodo and Bunney, 1983; White and Wang, 1983). In contrast to the 1 month treatment effects, 2 weeks of treatment failed to alter the incidence of dye coupling from that observed in controls (1/7 matrix; 1/5 core; 1/7 shell; $p > 0.7$ in each region, Table 1).

Effects of 1 month treatment with antipsychotic drugs on dye coupling within the striatal patch region and on dye coupling between aspiny cells

Unlike spiny cells in the striatal matrix and in the accumbens core and shell regions, none of the cells that were labeled within the patch compartment of control rats or rats treated with antipsychotic drugs for 1 month ($N = 5$ controls and $N = 3$ each drug; total = 11) were found to exhibit dye coupling. The comparatively small volume and irregular locations of the patch compartments (i.e., 5–15% of the striatal tissue volume; Groves et al., 1988; Desban et al., 1989) limited the number of dye-injected cells that could be labeled in this region; therefore, a quantitative evaluation of the basal levels of coupling and its modulation by drug treatment could not be accurately determined from these data. However, all of the single-labeled spiny cells located in the patch compartment also restricted their dendritic processes to the same compartment in which the soma was located.

Lucifer yellow labeling of aspiny cells did not result in dye coupling in the striatum of control or treated rats (i.e., 1 month repeated treatment with either APD; $N = 3$ for each) Due to the comparatively low percentage of aspiny cells in the striatum (i.e., 1–5% of striatal cells; Pasik et al., 1977, 1979; Preston et al., 1980; Graybiel and Ragsdale, 1983; Fisher et al., 1986; Kawaguchi et al., 1992), a sample size that was sufficiently large to evaluate the extent of coupling in control or treated rats could not be collected. Nonetheless, even in regions in which APD treatment caused the majority of spiny cells to exhibit dye coupling, in no instance was coupling observed between aspiny cells.

Electrophysiological properties of striatal spiny cells in rats following 1 month treatment with either haloperidol or clozapine

Spiny neurons recorded in rats that had been treated for 1 month with either antipsychotic drug had resting membrane potentials that were not significantly different from those of cells recorded in control rats (HAL: -64.4 ± 9.6 mV, $N = 23$; CLZ: -66.2 ± 13.8 mV, $N = 29$; vs Controls: -69.1 ± 7.7 mV, $N = 36$; $t = 1.38$ for HAL and $t = 0.62$ for CLZ vs controls; NS). Nevertheless, the striatal cells recorded in regions that also exhibited high levels of dye coupling in the 1 month drug-treated rats were found to have a significantly higher input resistance, which averaged 86.2% ± 5.2% above that of controls (drug-treated: HAL= 56.8 ± 19.8 MΩ, $N = 8$; CLZ = 67.7 ± 29.8 MΩ, $N = 6$; vs controls = 32.4 ± 12.6 MΩ, $N = 14$; $t = -3.56$ for HAL and $t = -3.83$ for CLZ vs controls, Fig. 3). However, within a given region the input resistance of a single cell was not correlated with the presence of dye coupling in that cell, which is consistent with that observed in comparisons between single-labeled and dye-coupled cells of control rats both in the present study and in our previous report (Onn and Grace, 1994a).

Discussion

As reviewed in the introductory section, although antipsychotic drugs will produce a maximal blockade of DA receptors within minutes of their administration, many of their clinical actions require weeks of treatment before they are fully expressed (Pickar et al., 1984). In animal studies, at least two responses to antipsychotic drugs have been shown to occur over the time course of repeated APD treatment: (1) DA receptor supersensitivity—an increase in the density of DA receptors that appears to be limited to treatment with classical APDs (Burt et al., 1977; Muller and Seeman, 1978; Rupniak et al., 1985; See et al., 1989, 1994; Wilmot and Szczepanik, 1989; O'Dell, 1990; Florijn et al., 1994) and reaches near maximal levels by 1–2 weeks after initiating treatment (Burt et al., 1977; Muller and Seeman, 1978), and (2) DA cell depolarization block—a cessation of DA cell firing in mesencephalic SN and VTA DA cells, which requires repeated administration for 3 weeks or longer to develop (Bunney and Grace, 1978; Chiodo and Bunney, 1983; White and Wang, 1983). In this study, we have found a regionally selective increase in dye coupling between striatal spiny neurons of rats that correlates with the time course of the development of DA cell depolarization block.

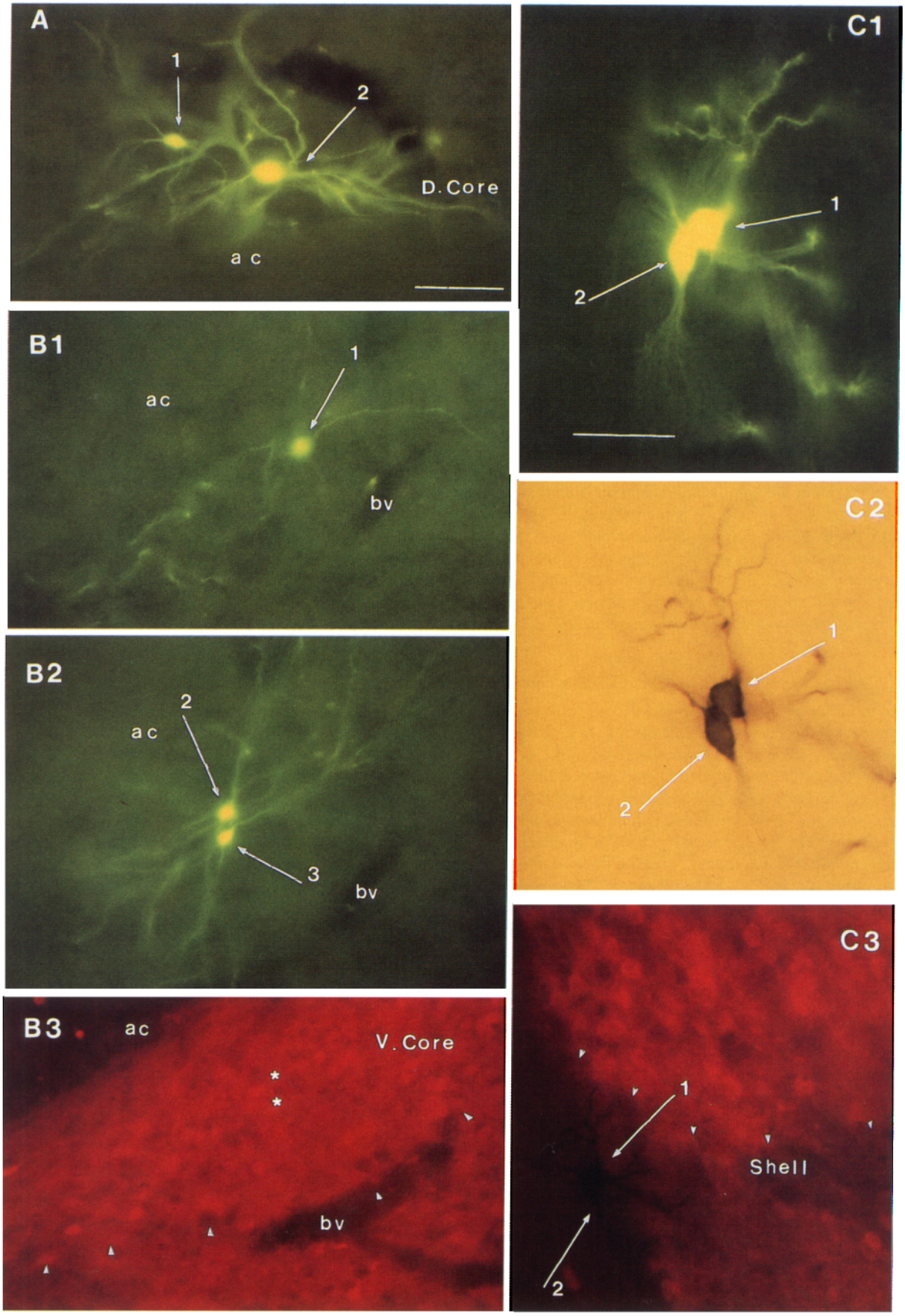

Figure 2. Photomicrographs illustrating patterns of dye-coupled cells in the accumbens core (*A*, *B*) and shell (*C*) regions following injection of single cells with Lucifer yellow in rats that had received 1 month of antipsychotic drug treatment. *A*, In haloperidol-treated rats, injection of a

Dye coupling in the adult striatal complex of vehicle-treated controls

In the adult striatal complex, intracellular injection of dye into striatal cells resulted primarily in the labeling of single spiny neurons. The dendritic arborizations of the labeled spiny cells were strictly confined to the same compartment or region in which the parent somata resided (Penny et al., 1988; Arts and Groenewegen, 1992; Onn and Grace, 1994a; Onn et al., 1994c). In the present study, dye coupling was found to occur in substantial levels (approximately 20% of the cells injected) in the calbindin-positive striatal matrix and accumbens core regions, which was similar to the levels we observed previously during *in vivo* (Onn et al., 1990; Onn and Grace, 1994a) and *in vitro* (O'Donnell and Grace, 1993) recordings. With respect to the extent of dye coupling (i.e., the number of cells coupled per injection), we have consistently found coupling to occur only between pairs of striatal cells in control rats. In contrast, in the ventral core region (i.e., the calbindin-positive region ventral to the anterior commissure), approximately one-third of the coupling was observed to occur among sets of three cells. The incidence of coupling was found to be lower in the calbindin-negative regions (i.e., striatal patch and accumbens shell regions) when compared to the calbindin-rich matrix and core regions. Although the reason for this difference is unclear, the regions (i.e., the patch or the shell regions) that exhibited a trend toward lower incidences of coupling in the present study correspond to the areas that have been shown by others to have higher levels of DA activity (Graybiel et al., 1978, 1983; Deutch and Cameron, 1992) or DA fibers (Voorn et al., 1986; Zahm, 1992; Meredith et al., 1994). Furthermore, this inverse relationship between coupling and extracellular DA levels is further supported by recent studies showing the presence of significantly higher levels of coupling in the DA depleted adult striatum (Cepeda et al., 1989; Onn and Grace, 1993) as well as in the developing striatum before the dopamine afferentation is complete (Walsh et al., 1989).

Differential modulation of dye coupling by typical versus atypical antipsychotic drugs

Acute administration of either haloperidol or clozapine did not significantly affect the level of coupling in the striatal subregions examined. In contrast, following 1 month repeated administration of haloperidol or clozapine, significantly higher levels of coupling were detected in the striatal complex in a regionally specific manner. Repeated treatment with haloperidol for 1 month increased the levels of coupling by four- to sevenfold in both motor (matrix/core)- and limbic (shell)-related striatal subregions, whereas 1 month treatment with clozapine only increased the incidence of coupling in the limbic-associated accumbens shell region. The fact that coupling was altered only in rats that had received repeated drug treatment suggests the involvement of a time-dependent alteration in the DA system that occurs beyond the initial DA receptor blockade that is present within minutes following acute drug administration (Rupniak et al., 1983; Sedvall et al., 1986).

Studies have shown that there are at least two time-dependent changes that occur in the DA system with repeated HAL administration: an increase in the number of DA receptors in the striatum (Burt et al., 1977; Muller and Seeman, 1978; Rupniak et al., 1985; Wilmot and Szczepanik, 1989; O'Dell et al., 1990; See et al., 1994; Tarazi et al., 1994) and depolarization block of mesencephalic DA cell firing (Bunney and Grace, 1978; Chiodo and Bunney, 1983; White and Wang, 1983; Grace and Bunney, 1986). Thus, DA D2 receptor supersensitivity is known to develop following approximately 1 week of treatment with haloperidol but not with clozapine (Burt et al., 1977; Kobayashi et al., 1978; Muller and Seeman, 1978) and appears to be present in a homogenous pattern throughout the extent of the dorsal striatum (Burt et al., 1977; Miller and Seeman, 1978; Rupniak et al., 1985; Wilmot and Szczepanik, 1989; O'Dell et al., 1990) but not in the nucleus accumbens (Wilmot and Szczepanik, 1989; O'Dell et al., 1990; Florijn et al., 1994; See et al., 1994; Tarazi et al., 1994). On the other hand, the development of DA cell depolarization block occurs only following repeated treatment for at least 21 d with either haloperidol or clozapine (Chiodo and Bunney, 1983; White and Wang, 1983). In order to dissociate any effects on coupling arising from D2 supersensitivity from those requiring a longer treatment period, the level of coupling was also assessed after 2 weeks of treatment with haloperidol. In contrast to the increased coupling observed with 1 month of antipsychotic drug treatment, administration of haloperidol for only 2 weeks did not result in any significant alterations in the incidence of coupling in any of the subregions examined. Although several mechanisms may contribute to this observed change, the finding that repeated treatment with haloperidol for 1 month is required for rats to exhibit a higher incidence of coupling is at least consistent with the time course for the induction of DA cell depolarization block and not DA receptor supersensitivity. Furthermore, differences in the effects produced by repeated administration of haloperidol versus clozapine on dye coupling in the motor-related striatal matrix and accumbens core regions is consistent with their differential ability to induce depolarization block in the SN nigrostriatal DA cell group (Chiodo and Bunney, 1983; White and Wang, 1983), which is known to topographically innervate both the matrix and the core regions. In a similar manner, the finding that both antipsychotic drugs increased coupling in the accumbens shell region is also consistent with their ability to induce depolarization block in the VTA mesolimbic DA cell group (Chiodo and Bunney, 1983; White and Wang, 1983), which is known to innervate

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single neuron in the dorsal core region resulted in the labeling of a pair of medium spiny neurons (*arrows 1, 2*). *B*, Only cells injected in the ventral core regions of haloperidol-treated rats resulted in dye coupling among sets of these neurons (*B1, arrow 1; B2, arrows 2* and *3, arrows* on *B1, B2* adjacent sections aligned by the *ac* and the nearby blood vessel). *B3*, After staining the section (i.e., *B2*) for calbindin immunoreactivity, the coupled cells illustrated in *B1* and *B2* are shown to be localized (*asterisks*) completely within the Texas red–labeled calbindin immunoreactive ventral core region (i.e., ventral to *ac*). *C*, A pair of medium spiny neurons are found to be dye-coupled following injection of a cell in the shell region of the accumbens in clozapine-treated rats. *C1*, A pair of neurons (*arrows 1, 2*) that is labeled following injection of a single cell with Lucifer yellow. Although not obvious at this level of magnification the somata were not in direct contact, but were separated by a small but definitive gap. *C2*, The Lucifer yellow fluorescence is converted into a dense peroxidase stain in the same pair of cells using Lucifer yellow antibodies. *C3*, Subsequent staining of this section (*C1* or *C2*) for Texas red–labeled calbindin immunoreactivity shows that these two coupled neurons (*arrows 1, 2*) are confined to the calbindin poor shell region of the accumbens (border marked by *arrowheads*). *ac*, Anterior commissure; *bv*, blood vessel. Scale bars, 50 μm. *A* and *B* sets refer to bar in *A* and *C* set refers to bar in *C1*.

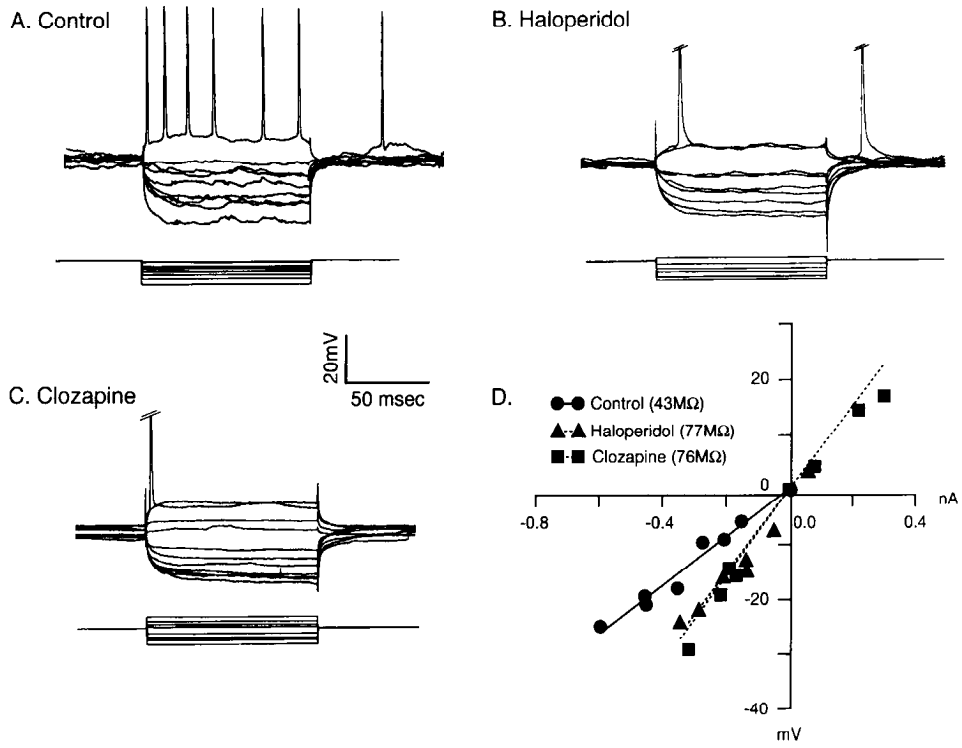

Figure 3. The input resistances of neurons recorded in antipsychotic drug-treated rats (i.e., 1 month group) were significantly higher than those of control animals. Injection of constant-current pulses was used to examine the current/voltage relationship of a neuron subsequently revealed to be dye coupled in the accumbens core region of a haloperidol-treated rat (*B*), or dye coupled in the accumbens shell region of a clozapine-treated rat (*C*), as compared with that recorded in a dye-coupled cell of a control rat (*A*). *D*, Plotting the current/voltage relationship enabled calculation of the input resistance from the slope of the regression lines for each cell. As described in Materials and Methods, only the responses to current injections that were subthreshold for evoking spikes were plotted. In these examples, cells from both haloperidol-treated (*triangles*) and the clozapine treated (*squares*) rats show higher input resistances than the cell from control rats (*circles*).

the accumbens shell region (Beckstead et al., 1979; Phillipson and Griffiths, 1985; Voorn et al., 1986; Zahm, 1991). Since these measures were made following a 36 hr drug washout, we also performed a limited number of recordings on a subset of rats treated for 1 month with haloperidol, and confirmed that the DA cells were still in a nonfiring state under these conditions (unpublished observations). Therefore, although DA cell depolarization block was not shown to be causally related to the observed changes in dye coupling, the evidence provides substantial support for this interaction. On the other hand, it is unclear why only a small nonsignificant upregulation of coupling between spiny cells in the accumbens core region was found following repeated clozapine treatment. One possibility may be related to the fact that the core neurons, in addition to receiving mesolimbic DA afferents, also are innervated by DA afferents from the SN A9 DA cell group, which is not inactivated by clozapine. As a result, the effects of APD on coupling between neurons in the core region may depend on a common action of APDs on both SN and VTA neurons, as occurs with haloperidol treatment.

Apparent absence of antipsychotic drug effect on dye coupling between striosomal spiny neurons or aspiny cells

With respect to coupling in the striosomal regions, it is not clear at present why repeated HAL did not alter dye coupling between spiny neurons in this region in a manner comparable to that observed in the matrix, given that both regions receive DA inputs from the SN mesostriatal DA cell group. However, the apparent absence of dye coupling in striosomes and its response to drugs was based on a limited number of observations, primarily due to the comparatively small and irregular volume occupied by the striosomes in the striatal complex (Graybiel and Ragsdale, 1983; Gerfen et al., 1985; Groves et al., 1988; Penny et al., 1988; Desban et al., 1989; Onn et al., 1994c). Therefore, the basal level of coupling and its modulation could not be accurately assessed in this study.

In contrast to striatal spiny cells located in the matrix, coupling has not been observed to occur between cells of the nonspiny cell classes in the striatal complex of control rats. Although a small number of aspiny cells were examined in this study, none of the stained aspiny cells exhibited coupling following treatment with antipsychotic drugs. On the other hand, gap junction-like structures have been reported to be present between at least one class of striatal nonspiny cells (i.e., the parvalbumin-immunoreactive aspiny cells; Kita et al., 1990). One possibility is that junctions between cells in this cell class may require a different type of stimulus from those examined to date in order to cause them to be in the "open" state (Onn and Grace, 1993). However, because the nonspiny cell classes comprise only approximately 1–5% of the striatal cells (Pasik et al., 1977, 1979; Preston et al., 1980; Chang and Kitai, 1982; Graybiel and Ragsdale, 1983; Fisher et al., 1986; Wilson et al., 1990; Kawaguchi, 1992), we cannot rule out the possibility that dye coupling is present in at least one subpopulation of these cells.

Changes in the electrophysiological properties of striatal cells following APD administration

In addition to the changes in coupling observed following APD administration, the striatal cells also exhibited a significant increase in input resistance following repeated HAL when compared to those recorded in control rats. This increase could not be attributed to the presence of coupling since coupled cells and noncoupled cells in the striatum of treated rats showed similar increases in input resistances. Similarly, in control rats no difference was found between the input resistance of cells that were subsequently found to be coupled and those that did not show coupling in either the present study or in our previous report (Onn and Grace, 1994a). The alteration of striatal cell input re-

sistance was not reported in striatal cells recorded *in vitro* following a similar regimen of haloperidol treatment (Calabresi et al., 1992). This discrepancy could reflect differences in several factors that are likely to exert disparate influences in each preparation, such as: (1) differences in DA cell activity such as depolarization block, which is dependent on an intact striatonigral feedback pathway (Bunney and Grace, 1978; Chiodo and Bunney, 1983; Grace and Bunney, 1983; White and Wang, 1983; Hollerman et al., 1992); (2) differences in the relative impact of other presynaptic changes present in the *in vivo* preparation, such as presynaptic D2 receptor supersensitivity (Bannon et al., 1980; Calabresi et al., 1992), changes in corticostriatal transmission (Meshul and Tan, 1993; Meshul et al., 1994), an increase in perforated synapses (Meshul et al., 1992), or a decrease in glutamate receptor binding (Creese et al., 1994).

Summary and conclusions

Repeated treatment of rats with haloperidol for 1 month or longer, but not for 2 weeks, increased the incidence of dye coupling in the matrix and core regions of the striatal complex, both of which are calbindin positive and are believed to subserve motor-related functions, as well as in the limbic-related shell region of the nucleus accumbens. In contrast, 1 month of clozapine administration increased coupling only in the shell of the accumbens. Although the specific mechanism underlying this response has not been established directly, several lines of evidence suggest that DA cell depolarization block may play a major role in this response: (1) the time course of the change in dye coupling is correlated with depolarization block but not with APD-induced DA receptor supersensitivity (Burt et al., 1977; Muller and Seeman, 1978; Chiodo and Bunney, 1983; White and Wang, 1983), and (2) the regional specificity in the actions of these classic and atypical APDs corresponds to the ability of these drugs to induce depolarization block in the DA cells projecting to these regions (Chiodo and Bunney, 1983; White and Wang, 1983). One functional impact of this drug-induced increase in coupling among sets of striatal GABAergic projection neurons would be to diminish the variability of activity among cells via the transfer of membrane polarization and second messengers through the putative gap junctions. This in turn would result in a synchronization in their efferent regulation of neurons located in the output nuclei of the basal ganglia. Separate populations of neurons (Feger and Crossman, 1984; Parent et al., 1984; Beckstead et al., 1986; Kawaguchi et al., 1990) in the same matrix compartment (Jimenez-Castellanos and Graybiel, 1989) have been shown to project to each of the three major output sites of the basal ganglia: the globus pallidus, entopeduncular nucleus, and the substantia nigra zona reticulata. Given that the vast majority of striatal spiny cells are GABAergic, electrical coupling would allow a synchronization of activity among cells that would otherwise exhibit only inhibitory interactions (Wilson et al., 1989; Bennett and Bolam, 1994). Such changes in the basic characteristics of neuronal interactions would be expected to have a potent influence on the functional properties of this structure. Consequently, the selective modulation of coupling by antipsychotic drug treatment may actually reflect the clinically relevant actions of these drugs upon administration to the human schizophrenic patient.

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