Striatal Extracellular Dopamine Levels in Rats with Haloperidol-Induced Depolarization Block of Substantia Nigra Dopamine Neurons

Holly Moore, Christopher L. Todd, and Anthony A. Grace

Departments of Neuroscience and Psychiatry, Center for Neuroscience, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

Correlations between substantia nigra (SN) dopamine (DA) cell activity and striatal extracellular DA were examined using simultaneous extracellular single-unit recordings and in vivo microdialysis performed in drug-naive rats and in rats treated repeatedly with haloperidol (HAL). Intact rats treated with HAL for 21–28 d exhibited significantly fewer active DA cells, indicating the presence of depolarization block (DB) in these cells. However, in rats that received surgical implantation of the microdialysis probe followed by a 24 hr recovery period, HAL-induced DA cell DB was reversed, as evidenced by a number of active DA neurons that was significantly higher than that in HAL-treated intact rats and similar to that of drug-naive rats. In contrast, using a modified probe implantation procedure that did not reverse SN DA neuron DB, we found striatal DA efflux to be significantly lower than in controls and significantly correlated with the reduction in DA neuron spike activity. Furthermore, although basal striatal DA efflux was independent of SN DA cell burst-firing activity in control rats, these variables were significantly correlated in rats with HAL-induced DA cell DB. Therefore, HAL-induced DB of SN DA neurons is disrupted by implantation of a microdialysis probe into the striatum using standard procedures. However, a modified microdialysis method that allowed reinstatement of DA neuron DB revealed that the HAL-induced inactivation of SN DA neurons was associated with significantly lower extracellular DA levels in the striatum. Moreover, the residual extracellular DA maintained in the presence of DB may, in part, depend on the burst-firing pattern of the noninactivated DA neurons in the SN.

Key words: microdialysis; single-unit recording; dopamine; substantia nigra; depolarization block; antipsychotic drugs; schizophrenia; haloperidol; striatum; baclofen

Antipsychotic drugs (APDs) display a range of potency and selectivity in their ability to reduce psychotic symptoms in schizophrenia (Arnt and Skarsfeldt, 1998). Of the multiple effects of APDs in animal models, their impact on the physiology of dopamine (DA) cells in the midbrain of rats has been shown to correlate with the degree of dissociation between antipsychotic efficacy and extrapyramidal side effects (Grace et al., 1997). Thus, administration of APDs for 3 weeks or more induces depolarization block (DB) in midbrain DA neurons, a condition in which these cells are depolarized to the extent that action potential generation is inactivated, resulting in a cessation of spontaneous and elicited spike activity (Bunney and Grace, 1978; Grace and Bunney, 1986; for review, see Grace et al., 1997). Although one report has suggested that anesthesia contributes to the expression of DA cell DB (Mereu et al., 1995), other studies have shown that APD-induced DA cell DB does occur in nonanesthetized animals (Bunney and Grace, 1978; Chiodo and Bunney, 1983; for review, see Grace et al., 1997).

Given the evidence of its expression in nonanesthetized animals and the predictive validity of APD-induced DA cell DB for the differential clinical effects of APDs (Grace et al., 1997), the neurochemical correlates of DA cell DB are likely to be relevant to the therapeutic actions of APDs. One expected correlate of APD-induced DB in substantia nigra (SN) DA neurons would be a decrease in extracellular DA in the striatum, depending on the extent of compensatory changes in DA synthesis and release (see Biggio et al., 1980; Zigmond et al., 1990). However, numerous studies of extracellular DA levels using in vivo microdialysis or voltammetric techniques have yielded inconsistent data regarding the impact of repeated APD treatment on extracellular DA levels in forebrain regions. For example, although several in vivo microdialysis studies have reported that chronic haloperidol (HAL) treatment decreases extracellular DA levels in forebrain terminal regions, an effect consistent with the presence of DB in the DA neurons (Hernandez and Hoebel, 1989; Ichikawa and Meltzer, 1990, 1991, 1992; See and Murray, 1992), other studies have reported a lack of a change (Invernizzi et al., 1990; See et al., 1992; Moghaddam and Bunney, 1993; See, 1993; Yamamoto and Cooperman, 1994) or even increases (Imperato et al., 1994). In addition, studies using voltammetry have reported APD-induced decreases (Lane and Blaha, 1987; Chesi et al., 1995; Feasey-Truger et al., 1995) or an absence of a change (Wiedemann et al., 1992) in stimulated DA overflow in forebrain DA terminal regions.

Although the reason for these conflicting results is not imme-
Immediately evident, one important consideration is that none of these studies provided a confirmation that the microdialysis or voltammetric procedure did not alter the APD-induced state of the DA system. This consideration may be central to the interpretation of these studies, given that it has been shown that an intact striatonigral pathway is necessary for both the induction (Bunney and Grace, 1978) and maintenance (Chiodo and Bunney, 1983) of DB in SN DA neurons. Disruption of the striatonigral pathway is likely to occur at least temporarily during the surgical implantation of a microdialysis probe (Egan et al., 1996), a manipulation that can alter the blood–brain barrier integrity and metabolism of the surrounding tissue for several hours after surgery (Benveniste, 1989; Shuaib et al., 1990; Dykstra et al., 1992; Georgieva et al., 1993; Morgan et al., 1996). Thus, short-term changes in the striatum associated with surgical probe implantation could presumably disrupt APD-induced DB of SN DA cells. Given this possibility, we examined the impact of microdialysis probe implantation on APD-induced physiological changes in midbrain DA neurons to evaluate accurately the impact of APDs on striatal DA efflux.

We first examined the effects of two striatal microdialysis probe implantation procedures on the spike activity of SN DA neurons in control and HAL-treated rats. Then using a probe implantation method that allowed repositioning of DB, we performed microdialysis in the striatum and single-unit electrophysiological recordings in the SN simultaneously within subjects to determine correlations between HAL-induced SN DA cell DB and striatal extracellular DA levels. Parts of this paper have been published previously in abstract form (Todd et al., 1996, 1997).

**Materials and Methods**

All experiments were performed in accordance with the guidelines established in *The Guide for the Care and Use of Animals in Research* (United States Public Health Service) and were approved by the University of Pittsburgh Animal Care and Use Committee.

**Drug treatment**

Male Sprague Dawley rats (Zivic Miller, Allison Park, PA) weighing ~100–125 gm at the beginning of the study were randomly assigned to a drug-naive or HAL-treated group. HAL (R. W. Johnson Pharmaceutical; 150 mg/kg/hr) was administered via a syringe pump (Razel model A-90 or Baby Bee; BioAnalytical Systems, West Lafayette, IN). Recordings were constructed using 2.0 mm o.d. glass pipettes (WPI) that were pulled on a vertical electrode puller (Narishige PE-2) and broken back under microscopic control to a diameter of ~1–2 µm before filling with 2 M NaCl containing 2% pontamine sky blue. These electrodes typically exhibited impedances of 4–12 MΩ. The electrode signal was amplified, filtered, and discriminated from noise using a combination amplification and window discrimination unit for extracellular recording (Fintronics, Orange, CT) and displayed on an oscilloscope (Tektronics, Wilsonville, OR). The data were acquired, stored, and analyzed using custom-written software (Neuroscope) running on an Intel-based personal computer interfaced with a data acquisition board (Microstar Laboratories, Bellevue, WA).

The SN was systematically sampled for the presence of spontaneously discharging DA neurons by lowering the recording electrode nine times through a rectangular area in the SN from 2.0 to 2.4 mm lateral to midline and 2.7 to 3.1 mm anterior to the lambda midpoint, with each electrode track separated by 200 µm (Bunney and Grace, 1978). Between 2 and 5 min of spike activity was recorded from spontaneously active neurons in the SN that were identified as DA neurons according to the criteria of Grace and Bunney (1983). The mean firing rate and percentage of spikes fired in bursts were calculated, with bursts defined as follows (Grace and Bunney, 1983, 1984): the beginning of a burst was defined as the occurrence of two consecutive spikes with an interspike interval of 80 msec or less, and the termination of a burst was defined as the subsequent occurrence of an interspike interval of 160 msec or more. The percentage of spikes fired in bursts during a 2–5 min recording sample was calculated by dividing the total number of spikes occurring in bursts by the total number of spikes fired in the same period of time. At the conclusion of each experiment, pontamine sky blue dye was ejected from the recording pipette with anodal current (30 µA for 30 min) to mark the location of the electrode at the completion of the final electrode track. The brain was removed and placed in 10% buffered formalin for subsequent histological verification of the site of the dialysis probe and the recording electrode tracks (see below).

**Striatal microdialysis**

At 2.5–3.5 hr before electrophysiological recording, rats in the chronic guide cannula group were lightly anesthetized with chloral hydrate, and the probe was inserted slowly (0.1–0.3 mm/min) through the guide cannula into the striatum. The probe was perfused with artificial CSF (aCSF: 145 mM NaCl, 2.7 mM KC1, 1.0 mM MgCl2, and 1.2 mM CaCl2) at a rate of 1.5–2.0 µL/min for at least 2 hr before recording. Immediately before recording, the animal was fully anesthetized with a supplemental administration of chloral hydrate, and the procedure described above was used for electrophysiological recording. Dialysis samples were collected every 15 or 20 min during simultaneous electrophysiological sampling of the SN (see above); samples were placed immediately on dry ice and then stored at −70°C until analyzed.
Quantification of DA in the dialysis samples was performed using HPLC coupled with electrochemical detection as described previously (King et al., 1997). Briefly, DA was separated from its metabolites and other monoamines with a reverse-phase C18 column (Brownlee Velosor; Applied Biosystems, Foster City, CA) and a mobile phase composed of 0.1 M sodium acetate, 0.1 mM EDTA, 7% v/v methanol, and 0.7–1.4 mM octyl sodium sulfate, pH 4.1, flowing over the column at 0.7 ml/min. The concentration of DA was quantified by comparing the peak height of DA in the sample with its height in external standards.

Experimental design and statistical analyses

The aim of experiment 1 of this study was to determine whether microdialysis probe implantation would alter HAL-induced DA neuron DB and, if necessary, to modify the probe implantation procedure to allow DB in SN DA cells to be maintained during microdialysis sampling in the striatum. In the subsequent experiment, the modified microdialysis method (chronic guide cannula implantation and extended recovery from surgery with continued HAL treatment) was used to test the effects of repeated HAL treatment on extracellular DA levels in the striatum in animals in which DA neuron DB in the SN was confirmed electrophysiologically. The treatments used in these two experiments are described in detail below.

Measurement of the effects of probe implantation on DA neuron activity (experiment 1). Drug-naive and HAL-treated rats were randomly assigned to one of three probe implantation protocol groups: intact, 24 hr-probe, or chronic guide cannula. Intact rats (i.e., either drug-naive or treated with HAL for 21–28 d) were removed from their home cages on the day of testing and subjected to electrophysiological recordings as described above. In the 24 hr probe groups, surgical implantation of the concentric microdialysis probe was performed as described above, and the animals were allowed to recover for 20–30 hr in polycarbonate tubs. To be consistent with previous studies examining extracellular DA in the striatum after chronic treatment with HAL (e.g., Ichikawa and Meltzer, 1990, 1992; Moghaddam and Bunney, 1993), we withdrew all drug-treated animals from HAL during the recovery period. Electrophysiological recordings of SN DA neurons were performed 24–30 hr after surgery as described above; in these rats, the probe was not perfused during recording. Rats in the chronic guide cannula groups underwent surgical implantation of a guide cannula as described above and then were allowed to recover for 4–7 d before the electrophysiological recording session. During the recovery period, HAL-treated rats continued to receive the same dose of HAL in their drinking water, whereas drug-naive rats continued to receive regular water. Food and water were available ad libitum until the recording session, and all rats were at or above presurgery weight at the time of electrophysiological recording. On the day of recording, the probe was inserted and perfused with aCSF, and microdialysis and electrophysiological procedures were performed simultaneously according to the methods described above. In a subset of the intact and 24 hr probe groups, recording was begun after the rat had been anesthetized for 2 hr to control for the possible effects of prolonged anesthesia on the activity of DA cells.

For each rat, the number of spontaneously active cells per electrode track (six to nine tracks per animal), the mean firing rate, and the mean percentage of spikes fired in bursts were calculated. The effects of HAL treatment and probe implantation were analyzed using a two-way ANOVA with HAL treatment (two levels) and microdialysis probe implantation procedure (three levels) as between-subjects factors. Planned comparisons testing the effect of HAL treatment in intact rats and the effects of the 24 hr probe and chronic guide cannula procedures in drug-naive or HAL-treated rats were conducted with independent t tests corrected for multiple comparisons by the layered Bonferroni technique (Darlington, 1990).

Simultaneous measurement of striatal DA efflux and electrophysiological indices of SN DA neuronal activity (experiment 2). All rats in this experiment were either drug-naive or treated with HAL in their drinking water for 21–30 d as described above. After treatment, the chronic guide cannula was surgically implanted, and rats were allowed to recover for 4–7 d with continued HAL treatment as described above for the chronic guide cannula groups. Electrophysiological recording of SN DA neurons and microdialysis sampling of extracellular DA in the striatum were performed as described above. After at least three baseline dialysis samples were taken and after sampling of DA cell activity was completed in six electrode tracks, some rats then received either baclofen (0.2 mg/kg, i.p., or 0.1 mg/kg, i.v.) or tetrodotoxin (TTX; 10 μM into the striatum via the probe). Basal striatal DA efflux and the electrophysiological measures (spontaneously active cells per electrode track, mean firing rate, and mean percentage of spikes fired in bursts) were compared between drug-naive and HAL-treated rats using independent t tests. In addition, two other measures of DA cell population activity were calculated for the rats used in experiment 2. For each rat, “population firing rate” was calculated by multiplying the number of cells per electrode track by the mean firing rate, and “population burst firing” was calculated by multiplying population firing rate by the mean percentage of spikes fired in bursts. Correlations between basal striatal DA efflux and measures of DA cell activity (spontaneously active cells per electrode track, mean firing rate, mean percentage of spikes fired in bursts, population firing rate, and population burst firing) were determined with the Pearson correlation method. Values are reported as mean ± SEM. For all statistical analyses, α = 0.05.

RESULTS

All microdialysis probe tips were confirmed to lie in the dorsal striatum between 1.7 mm anterior and 0.2 mm posterior to bregma, 1.5–4.0 mm lateral from the midline, and 3.3–5.5 mm deep from the dural surface (Fig. 1; Paxinos and Watson, 1986); all electrode tracks were confirmed to pass through the SN between 2.7 and 3.4 mm anterior to the interaural line and 1.8 and 2.3 mm lateral to the midline (Paxinos and Watson, 1986).

Effects of probe implantation on DA neuron activity

The effects of HAL treatment and probe implantation were evaluated with respect to the number of spontaneously firing DA cells per electrode track (Fig. 2, top), firing rate (Fig. 3, top), and burst firing (Fig. 3, bottom). Both HAL treatment and microdialysis probe implantation significantly affected the number of spontaneously active DA cells in the SN (the main effect of drug treatment, t(14) = 33.22; p < 0.001; the main effect of the probe implantation protocol, t(24) = 6.22; p < 0.004; Fig. 2). Similar to previous reports, the HAL-treated intact group (n = 14) exhibited significantly fewer spontaneously active DA cells in the SN than did drug-naive intact rats (n = 7; t(19) = 7.54; p < 0.001), supporting the interpretation that the repeated HAL treatment was sufficient to induce DB.

There was also a significant interaction between the effects of HAL treatment and probe implantation condition [Fig. 2, top; t(2,42) = 6.26; p < 0.01]. Planned comparisons revealed that in drug-naive rats, probe implantation had no significant effect on the number of active DA cells per track, regardless of the method of implantation [24 hr probe (n = 8) vs intact, t(13) = 0.27; p > 0.7; chronic guide cannula (n = 6) vs intact, t(13) = 1.07; p > 0.3]. On the other hand, probe implantation significantly affected the activity of the SN DA neurons in HAL-treated rats. Rats in the HAL-treated 24 hr probe group (n = 7) exhibited significantly more spontaneously firing DA cells than did HAL-treated intact rats [t(19) = 5.62; p < 0.01]. Indeed, the number of active DA cells in the HAL-treated 24 hr probe group was not significantly different from that of drug-naive rats [HAL-treated 24 hr probe vs drug-naive 24 hr probe, t(13) = 0.49; p > 0.63; HAL-treated 24 hr probe vs drug-naive intact, t(12) = 0.87; p > 0.39]. In contrast, the number of spontaneously active DA cells in HAL-treated chronic guide cannula rats (n = 6) was not significantly different from that of HAL-treated intact rats [t(18) = 0.55; p > 0.58]. As seen with the HAL-treated intact group, the number of active cells in the HAL-treated chronic guide cannula rats was significantly lower than that in the drug-naive rats [t(11) = 5.19; p < 0.001] or in the HAL-treated rats with the 24 hr probe treatment [t(11) = 3.72; p < 0.01].

To detect early effects of probe implantation surgery in the striatum on chronic HAL-induced DB of SN DA cells, we sampled cell activity in a subset of HAL-treated rats (n = 6) before
Figure 1. Microdialysis probe placements and location of recording electrode track. *Top,* Photomicrograph of a Nissl-stained sagittal section showing the recording electrode track terminating in the substantia nigra pars compacta and the guide cannula track terminating just ventral to the corpus callosum. Extending ventrally from the guide cannula track is the dorsal extent of the smaller track made by the dialysis probe. The probe terminated medial to this plane at −1.9 mm lateral to the midline and 5–6 mm ventral to the skull surface (represented in the bottom panel). The relatively intensely stained cells near the termination of the electrode track indicate that the electrode tip was in the region of the SN DA cells. *Bottom,* Schematic drawings of coronal sections (Paxinos and Watson, 1997) showing the placements of the microdialysis probes in the striatum. Vertical bars represent probe tips, with HAL-treated and drug-naive rats represented in the left and right hemispheres, respectively. Probe tips used in the 24 hr probe condition are represented by the narrower, longer bars, whereas the wider, shorter bars represent probes in the chronic guide cannula condition. Hatched bars show placements in subjects in which extracellular DA levels were measured.
were similar with respect to firing rate [4.44 ± 0.83 and 4.46 ± 0.83].

The active DA cells measured before and after probe implantation then began sampling of DA cell activity in the SN ipsilateral to the probe was lowered into the striatum ipsilateral to the final recording site. After completion of that sampling, a microdialysis probe was first sampled in the SN contralateral to the final recording site, and for up to 2 hr after probe implantation. DA neuron activity was significantly lower than that in the contralateral SN [Fig. 2, top]. In the 24 hr probe group, the number of active DA cells was significantly higher than that in the HAL-treated intact group and was not different than that in drug-naive animals (t < 0.01). In contrast, HAL-treated animals in the chronic guide cannula group showed a lower number of active DA cells in the SN compared with that in drug-naive intact rats (p < 0.001) but were not different than the HAL-treated, intact animals. Bottom, The acute effect of microdialysis probe insertion on SN DA cell activity in rats with HAL-induced DA cell DB. In a subset of the intact HAL-treated group, SN DA cell activity was measured before and after insertion of a microdialysis probe into the striatum. As shown in the top, in the intact hemisphere of HAL-treated rats, few SN DA cells were found to be spontaneously active (left bar). Within 2 hr of striatal probe implantation into the opposite hemisphere, a significantly greater number of DA cells in the SN ipsilateral to the probe were spontaneously active (right bar), relative to the control hemisphere (8p < 0.05).

and for up to 2 hr after probe implantation. DA neuron activity was first sampled in the SN contralateral to the final recording site, and after completion of that sampling, a microdialysis probe was lowered into the striatum ipsilateral to the final recording site. Sampling of DA cell activity in the SN ipsilateral to the probe then began ~20 min after the probe was lowered to its final position and continued for 1.5–2 hr. In these rats, the number of active DA cells in the SN ipsilateral to the microdialysis probe was significantly higher than that in the contralateral SN [Fig. 2, bottom; paired t(5) = 4.29; p < 0.01]. The firing characteristics of the active DA cells measured before and after probe implantation were similar with respect to firing rate [4.44 ± 0.83 and 4.46 ± 0.83].

Figure 2. Effects of microdialysis probe or guide cannula implantation on the number of spontaneously firing DA cells in drug-naive and chronic HAL-treated rats. Top, The number of spontaneously active cells per electrode track in drug-naive and HAL-treated rats in the intact (open bars), 24 hr probe (solid bars), and chronic guide cannula (hatched bars) groups. Values represent between-animal means and SEMs. HAL-treated intact rats exhibited significantly fewer active DA cells per track than did the drug-naive group (t < 0.01). In the 24 hr probe group, the number of active DA cells was significantly higher than that in the HAL-treated intact group and was not different than that in drug-naive animals (t < 0.01). In contrast, HAL-treated animals in the chronic guide cannula group showed a lower number of active DA cells in the SN contralateral to the final recording site, and after completion of that sampling, a microdialysis probe was first sampled in the SN contralateral to the final recording site. After completion of that sampling, a microdialysis probe was first sampled in the SN contralateral to the final recording site. Sampling of DA cell activity in the SN ipsilateral to the probe then began ~20 min after the probe was lowered to its final position and continued for 1.5–2 hr. In these rats, the number of active DA cells in the SN ipsilateral to the microdialysis probe was significantly higher than that in the contralateral SN [Fig. 2, bottom; paired t(5) = 4.29; p < 0.01]. The firing characteristics of the active DA cells measured before and after probe implantation were similar with respect to firing rate [4.44 ± 0.83 and 4.46 ± 0.83].

Figure 3. Effects of microdialysis probe or guide cannula implantation on the number of spontaneously firing DA cells in drug-naive and chronic HAL-treated rats. Presented are means and SEMs from drug-naive and HAL-treated rats that were in the intact (open bars), 24 hr probe (solid bars) or chronic guide cannula (open hatched bars) groups and from HAL-treated rats in which the SN was sampled during the first 2 hr after striatal probe implantation (shaded hatched bar; from Fig. 2, bottom). Top, Average firing rate of all active DA cells sampled in six to nine tracks. Neither drug treatment nor microdialysis probe implantation significantly affected the average firing rate of DA cells in the SN. Bottom, Burst firing. The percentage of spikes fired in bursts in the HAL-treated 24 hr probe group was significantly higher than that in the HAL-treated intact group ($p < 0.05$).
toward effects of drug \( F_{(1,38)} = 2.49; p = 0.12 \) and probe implantation \( F_{(2,28)} = 2.13; p = 0.13 \) and a trend for an interaction between these factors \( F_{(2,38)} = 2.52; p = 0.11 \). Planned comparisons revealed that, relative to the HAL-treated intact group, the HAL-treated 24 hr probe group displayed a significantly larger percentage of spikes fired in bursts \( t_{(16)} = 2.14; p < 0.05 \), whereas the HAL-treated chronic guide cannula group did not differ from the HAL-treated intact group \( t_{(14)} = -0.19; p > 0.85 \). On the other hand, probe implantation conditions did not significantly affect burst firing in the drug-naive rats [24 hr probe vs intact: \( t_{(13)} = 0.59; p > 0.55 \); chronic guide cannula vs intact: \( t_{(11)} = 1.76; p > 0.1 \)].

The effect of repeated HAL treatment on striatal DA efflux in animals with HAL-induced decreases in spontaneously active SN DA cells

As reported above, the method of probe insertion used in the chronic guide cannula group allowed in vivo microdialysis to be performed in the striatum, whereas DB was maintained in the DA cells of the SN. Under these conditions, administration of tetrodotoxin (10 \( \mu \)M) through the probe during dialysis suppressed striatal DA efflux [suppression, 84 ± 11% for drug-naive rats \( n = 3 \); 68 ± 19% in HAL-treated rats \( n = 2 \)], indicating that DA release was spike-dependent. As observed previously and described above, the number of spontaneously active DA cells per electrode track in HAL-treated rats \( 0.39 ± 0.23; n = 12 \) was significantly lower than that in drug-naive rats \( 0.95 ± 0.13; n = 5 \); \( t_{(15)} = 5.22; p < 0.001 \). Under these conditions, HAL-treated rats exhibited significantly lower basal striatal DA efflux than did drug-naive rats [Fig. 4, top; \( t_{(16)} = 2.52; p < 0.05 \)].

To determine whether the decrease in extracellular DA in the striatum might be related to DB in the SN, the \( \gamma \)-aminobutyric acid \( \beta \) (GABA\( \beta \)) agonist baclofen was administered at a dose shown previously to reverse SN DA cell DB \( 0.2 \) mg/kg, i.p., or \( 0.1 \) mg/kg, i.v.) (Grace and Bunney, 1980) after the collection of the last baseline dialysis sample. There was a trend for baclofen to affect striatal DA efflux differently in these two groups [independent \( t \) test on percentage change from prebaclofen baseline efflux, \( t_9 = -1.46; p = 0.09 \)], with baclofen appearing to increase striatal DA efflux only in HAL-treated rats (Fig. 4, bottom).

Correlations between striatal DA efflux and SN DA cell firing

Basal extracellular DA in the striatum was found to be positively correlated with both the number of spontaneously active DA cells per track (Pearson correlation coefficient, \( r = 0.5191; p < 0.05 \); data not shown) and the population firing rate \( r = 0.50; p < 0.05; n = 16 \). This was consistent with the fact that both DA efflux (Fig. 4) and the population firing rate (Fig. 5, top, inset; \( r = 3.91; p < 0.001 \)) were found to be lower in HAL-treated rats. This is also consistent with the observation that HAL treatment did not affect the correlation between extracellular levels of DA in the striatum and the population firing rate of DA cells in the SN, in that data from both drug-naive and HAL-treated rats were distributed along one regression line (Fig. 5, top). The relationship between striatal DA efflux and population burst firing appeared to be more complex in that it was not predicted by the group mean values of striatal DA efflux (Fig. 4, top) or population burst firing (Fig. 5, bottom, inset). Although mean population burst firing did not differ between drug-naive and HAL-treated rats \( t = 0.20; p > 0.8 \), the correlation between striatal DA efflux and population burst firing appeared to depend on the drug treatment. Thus, when data from drug-naive and HAL-treated rats were considered as one population, there was only a weak trend for a correlation between striatal DA efflux and population burst firing in SN DA cells \( r = 0.36; p = 0.087; n = 16 \). However, when the data from drug-naive and HAL-treated rats were analyzed separately, it was observed that in drug-naive rats, striatal DA did not correlate with burst firing (Fig. 5, bottom, triangles, dotted line; \( r = 0.30; p = 0.31; n = 5 \)). In contrast, the correlation between striatal DA efflux and SN DA cell burst firing in HAL-treated rats was positive and statistically significant (Fig. 5, bottom, circles, solid line; \( r = 0.64; p < 0.05; n = 11 \)).

DISCUSSION

In the present study, repeated administration of HAL resulted in ~79% fewer spontaneously active SN DA cells. Microdialysis probe implantation was found to disrupt DA cell DB, depending on the experimental conditions under which probe insertion took place. Consequently, a method of probe implantation that allowed the expression of HAL-induced DA cell DB was used to determine the changes in striatal extracellular DA that accompanied SN DA cell DB.
probe implantation surgery suggested that a reversal of DB had occurred in these cells. Moreover, in rats in which HAL-induced DA cell DB was present, the subsequent appearance of spontaneously active DA cells occurring within 2 hr after the probe implantation also suggested that a reversal of DB had occurred. On the other hand, the small number of spontaneously active SN DA cells detected in the chronic guide cannula group indicated that DA cell DB could be reinstated after this alternative probe implantation procedure. The trend for a differential effect of the GABAA receptor agonist baclofen on striatal DA efflux in HAL-treated and drug-naive rats also suggests the presence of DB in the HAL-treated group, given that this treatment has been shown to reverse DB and to increase DA cell activity in HAL-treated rats (Grace and Bunney, 1980) and yet either to decrease or not to affect striatal DA efflux in drug-naive rats (Santiago and Westerink, 1992; Santiago et al., 1993; Yoshida et al., 1994). The brief time course of baclofen-induced activation of DA cells in rats treated repeatedly with HAL, compounded with the relatively extended collection period used in microdialysis, is likely to have contributed to the variability in our measure of baclofen-induced increases in striatal DA.

The effect of probe insertion on the striatonigral pathway is likely to contribute significantly to the disruption of DB. The GABAAergic striatonigral projection (Gale et al., 1977; Tulloch et al., 1978; Bolam and Smith, 1990) regulates firing of SN DA neurons both directly and via its inputs onto the SN GABA neurons (Collingridge and Davies, 1981; Waszczak et al., 1981; Grace and Bunney, 1985). Moreover, whereas infusion of the excitatory amino acid kainic acid into the striatum has been shown to induce a temporary state of DB in SN DA neurons of untreated rats (Braszko et al., 1981), lesions of the striatonigral pathway can prevent the induction (Bunney and Grace, 1978) or disrupt the maintenance (Chiodo and Bunney, 1983) of SN DA cell DB in HAL-treated rats. Thus, changes in the activity of the striatonigral pathway are likely to significantly affect DA cell DB.

The insertion of a microdialysis probe into the striatum has several time-dependent effects that could potentially disrupt the striatonigral pathway and, thus, striatal regulation of DA neuron DB. After probe insertion, the blood–brain barrier is significantly compromised in the striatum (Dykstra et al., 1992; Morgan et al., 1996). Moreover, probe insertion also is accompanied by changes in local glucose metabolism, including increases in metabolism in the tissue proximal to the probe, with a general depression of metabolism in the surrounding tissue within the first 2 hr of dialysis membrane implantation (Benveniste et al., 1987). Decreases in metabolism also occur in nuclei that receive inputs from the site of the dialysis membrane. These decreases are smaller but still significant at 24 hr after implantation (Benveniste et al., 1987). We would predict that analogous changes would occur with striatal probe implantation, such as an acute activation of subsets of striatal cells proximal to the probe accompanied by a general decrease in synaptic activity in the striatonigral projection neurons that would persist for up to 24 hr after probe implantation surgery. Indeed, spreading depression in the striatum has been shown to alter cell activity in the SN pars reticulata in drug-naive rats (Albe-Fessard and Sanderson, 1987). This depression did not seem to change the SN DA cell firing rate, a result that is consistent with the present results in the drug-naive, 24 hr probe group. Nonetheless, given the evidence of regulation of DA cell firing by striatonigral projections and the impact of striatal manipulations on DA cell DB (see above), depression of

Impact of microdialysis probe implantation on the physiology of substantia nigra DA cells

In previous studies using in vivo microdialysis to examine the effect of repeated HAL treatment on striatal DA efflux, dialysates were typically collected in the awake or reanesthetized subject 18–24 hr after probe implantation surgery (e.g., Ichikawa and Meltzer, 1990, 1991; Moghaddam and Bunney, 1993). However, the present results indicate that HAL-induced DA cell DB in the SN is reversed under these conditions. Thus, the presence of a large number of spontaneously active DA cells with an elevated level of burst firing in HAL-treated rats observed 24 hr after
striatal activity would be expected to interact significantly with the mechanisms underlying DA cell DB.

The increase in the number of active DA cells in HAL-treated rats observed 1–2 h after striatal probe insertion may have resulted from the probe-induced changes in the striatonigral pathway (see above). However, this did not prevent the DA cells from re-entering DB because DA cell DB appeared to be reinstalled in the chronic guide cannula group. The relative importance of the modifications incorporated in the chronic guide cannula procedure (i.e., extended recovery from surgery, the reinstatement of HAL treatment, and dialysate collection conditions) is not clear. Nonetheless, the present experiments point to possible confounding variables involved in microdialysis sampling from regions that provide feedback to the cells releasing the neurotransmitter of interest.

The relationship between the physiological and neurochemical effects of repeated haloperidol treatment

Animals with HAL-induced SN DA cell DB exhibited extracellular striatal DA levels that averaged ~50% less than that in controls. In contrast, previous neurochemical studies of the effects of subchronic HAL administration showed a significant amount of variability in this measure that may have depended on the route and pattern of HAL administration (See and Murray, 1992; See and Kalia, 1996), as well as the dialysis probe implantation and sampling conditions as discussed above (see also Egan et al., 1996). However, the decrease in striatal extracellular DA observed in the present study is consistent with ex vivo studies that have reported small to moderate decreases in indices of striatal DA release in rats treated subchronically with HAL (Rastogi et al., 1982; Lappalainen et al., 1990; Essig and Kilpatrick, 1991). This decrease in striatal DA efflux in rats with HAL-induced DA cell DB is also consistent with the in vivo neurochemical studies when considered in light of the heterogeneity of the methods used. For example, in 11 of 12 of the experiments reported in these studies, the mean dorsal striatal DA efflux was lower in rats treated subchronically with HAL than in controls [Lane and Blaha, 1987; Hernandez and Hoebel, 1989; Ichikawa and Meltzer, 1990, 1991, 1992; See and Murray, 1992; Moghadam and Bunney, 1993 (anesthetized group); Osborne et al., 1994; Yamamoto and Cooperman, 1994; Klimenck et al., 1996 (two experimental groups)], although only four studies reported the decreases to be statistically significant (Lane and Blaha, 1987; Ichikawa and Meltzer, 1990, 1991, 1992). Even though DA cell DB was not confirmed in these studies, on average, the basal dorsal striatal extracellular DA levels were 26 ± 7% lower in HAL-treated rats than in controls. Indeed, the present results indicate that the magnitude and reliability of this effect would be greater had DB been maintained in previous studies. Furthermore, subchronic HAL treatment is consistently associated with a decrease in “stimulated” DA efflux or turnover, such as that evoked by electrical stimulation of the DA fibers (Wiedemann et al., 1992; Chesi et al., 1995; Feasey-Truger et al., 1995), perfusion with CSF containing supraphysiological levels of K⁺ or Ca²⁺ (Westerink et al., 1988; Ichikawa and Meltzer, 1990, 1991; Osborne et al., 1991; Moghadam and Bunney, 1993; Yamamoto and Cooperman, 1994), or acute administration of HAL [Racagni et al., 1980; Matsumoto et al., 1983; Hernandez and Hoebel, 1989; Essig and Kilpatrick, 1991; Wiedemann et al., 1992 (before withdrawal)]. This effect may reflect a condition in which HAL treatment has caused the midbrain DA system to be partially in DB or vulner-able to DB, which would limit the response of these cells to further depolarization and thereby reduce the capacity for stimulated DA release (Grace et al., 1997; see also Egan et al., 1996). Thus, it is likely that the reduced levels of basal extracellular DA and the reduced capacity for stimulated DA efflux in the striatum reflect limitations on DA release in terminal regions that result, at least in part, from DA cell DB in the midbrain.

The significant correlation between the population firing rate and basal striatal DA efflux also provided preliminary evidence that the “tonic” extracellular pool of DA in the striatum (Grace, 1991, 1992) depends in part on the number of DA cells that are active and their average firing rate. On the other hand, several studies have provided evidence that the “firing pattern” of midbrain DA neurons, specifically a change in the proportion of spikes fired in bursts, is related to the extent to which DA levels can be “increased” from this baseline level. For example, administration of γ-hydroxybutyrate at a dose that does not affect the average firing rate of DA cells but eliminates burst firing produces, at most, a 35% decrease in extracellular DA in the striatum (Nisbrandt et al., 1994). This indicates that the majority of the extracellular pool is not regulated by burst firing under normal conditions. On the other hand, increases in extracellular DA in the striatum evoked by prefrontal cortical stimulation are associated with increases in burst firing in the DA cells occurring without a change in the average firing rate (Murase et al., 1993). Taken together, this evidence supports a model of the nigrostriatal DA system in which the tonic level of extracellular DA is maintained by the average activity of the DA cell population, with additional “phasic” changes in extracellular DA levels occurring as a result of changes in DA cell burst firing. Thus, it is likely that increases in striatal DA efflux evoked by environmental events such as reward-related stimuli [Hoebel et al., 1989; Westerink et al., 1994; Mas et al., 1995; Wilson et al., 1995; Taber and Fibiger, 1997] result from increases in DA cell burst firing evoked by such stimuli [see Mirenowicz and Schultz (1994), their Fig. 1; (1996), their Fig. 1].

In the present study, although significantly fewer DA cells were active, population burst firing appeared to be maintained in HAL-treated animals. This may contribute to the apparent compensation in striatal DA efflux (efflux in HAL-treated rats was ~50% of that in controls even though only 30% of the DA neurons were spontaneously active). Because of the small number of drug-naïve animals, the effects of repeated HAL on the relationship between SN DA cell firing pattern and striatal DA efflux should be considered preliminary. Nonetheless, this correlation suggests that a significant proportion of striatal basal extracellular (i.e., tonic) DA depends on burst firing in SN DA neurons under the condition of HAL-induced DB but not under normal conditions. This has several implications for mechanisms underlying the effects of APDs. For example, because basal extracellular DA may depend to a significant extent on burst firing in the remaining active DA cells after repeated HAL treatment, we would expect the capacity of the DA system to respond to environmental events (i.e., with increases in burst firing and DA release) to be limited. This hypothesis is consistent with the reduced responsivity of striatal DA transmission (i.e., reductions in HAL- and K⁺-depolarization-stimulated DA efflux observed in animals treated chronically with APDs) and may contribute to the behavioral effects of these drugs, which include reduced instrumental responding for reward (Horvitz and Ettenberg, 1988; Asin and Wirthschafter, 1990; Hammond et al., 1991; Salamone et al., 1991; Harrow et al., 1994). With respect to the effects of APDs in
humans, this reduced responsivity of forebrain DA systems induced by subchronic HAL treatment (see also See and Kalivas, 1996), coupled with the decrease in basal DA levels, would be expected to reduce psychotic symptoms but not the cognitive or negative symptoms of schizophrenia, which are characterized by a lack of selective attention and varying degrees of sensorimotor neglect.

REFERENCES


