‘Passive stabilization’ of striatal extracellular dopamine across the lesion spectrum encompassing the presymptomatic phase of Parkinson’s disease: a voltammetric study in the 6-OHDA-lesioned rat

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Abstract

Symptoms of Parkinson’s disease do not present until the degeneration of nigrostriatal dopaminergic neurons is nearly complete. Maintenance of dopaminergic tone governing striatal efferents is postulated to preserve motor control during the presymptomatic phase, but the neuroadaptation responsible for normalization is not completely understood. In particular, the prevailing view that surviving dopaminergic neurons compensate by up-regulating release has been difficult to demonstrate directly. Here we investigate dopaminergic neurotransmission in the hemiparkinsonian rat using fast-scan cyclic voltammetry at carbon-fiber microelectrodes. Electrical stimulation was used to elicit extracellular dopamine levels mimicking the steady-state dynamics of tonic dopaminergic signaling. In agreement with microdialysis studies, evoked steady-state dopamine levels remained constant over the entire lesion spectrum (0 to ~85%) observed during the presymptomatic stage. Kinetic analysis of the voltammetric recordings demonstrated that evoked dopamine concentrations were normalized without plasticity of dopamine release and uptake, suggesting that the primary mechanisms controlling ambient levels of extracellular dopamine were not actively altered. In the present study, we formalize this neuroadaptation as ‘passive stabilization’. We further propose that passive stabilization is mediated by the simple physical principles of diffusion and steady state, is predicated on extrasynaptic transmission, and forms the basis for a new compensation model of preclinical parkinsonism.

Keywords: compensation, dopamine, Parkinson’s disease, release, uptake, voltammetry.

symptoms until severe denervation (Hornykiewicz 1966; Hornykiewicz and Kish 1987), a full understanding of this compensation remains unrealized today.

Studies using the 6-hydroxydopamine (6-OHDA)-lesioned rat model of Parkinson’s disease demonstrate that dialysate DA levels collected in the striatum are normal until the loss of dopaminergic terminals is nearly complete (Zhang et al. 1988; Abercrombie et al. 1990; Robinson et al. 1994). Striatal denervation at which ambient concentrations of extracellular DA fall thus coincides with that for the emergence of parkinsonism. Plasticity of signaling mechanisms intrinsic to the damaged dopaminergic system is proposed to underlie the normalization of dopaminergic tone. The prevailing view, pioneered by Zigmond and colleagues (Zigmond et al. 1990, 1992; Zigmond 1997) and supported by others (Zoli et al. 1998; Bezard and Gross 1998), is that increased DA synthesis and release by remaining dopaminergic neurons, and diffusion of the neurotransmitter from intact to denervated regions, combine to preserve tonic DA delivery to striatal target cells. Alterations in other mechanisms of dopaminergic neurotransmission, such as firing rate and postsynaptic receptor response, are not indicated [but see Bezard et al. (2001) for receptors]. Enhanced DA synthesis is well established (Zigmond et al. 1984; Hefi et al. 1985; Altar et al. 1987; Wolf et al. 1989), and DA diffusing into depleted areas concurs with the hypothesis that this neurotransmitter signals by volume or extrasynaptic transmission (Zoli et al. 1998; Vizi 2000). Recent studies using real-time voltammetry to probe neurotransmitter dynamics, however, have not only failed to confirm up-regulated DA release but have additionally suggested a down-regulation of DA uptake (Garris et al. 1997; Rothblat and Schneider 1999; Bezard et al. 2000; Dentresangle et al. 2001), although a clear consensus has yet to emerge.

We have previously hypothesized that extracellular DA is maintained in the moderately lesioned (50%) rat striatum without active changes in either DA release or uptake (Garris et al. 1997). This postulate, based on kinetic analysis of electrically evoked DA levels measured by voltammetry, is entirely consistent with existing compensation models of Parkinson’s disease (Zigmond et al. 1990; Bezard and Gross 1998; Zoli et al. 1998). The reason is that observed adaptations of dopaminergic terminal function, such as enhanced DA efflux, synthesis and turnover, occur only after a critical lesion threshold of ~50% is reached and increase progressively with greater denervation (Zigmond et al. 1984; Hefi et al. 1985; Altar et al. 1987; Snyder et al. 1990). The aim of the present study was to use voltammetry, electrical stimulation and kinetic analysis to investigate the relationship between extracellular DA levels and the capacity for DA release and uptake across the entire lesion spectrum encompassing preclinical parkinsonism.

Materials and methods

Drugs and chemicals

All chemicals and drugs were used as received and purchased from Sigma Chemical Company or RBI-Sigma (St Louis, MO, USA). Aqueous solutions were prepared in double-distilled, deionized water (Barnstead/Thermolyne, Dubuque, IA, USA).

Experimental design

The overall experimental design is shown graphically in Fig. 1(a). Nigrostriatal dopaminergic neurons were lesioned by injecting 6-OHDA into the lateral substantia nigra (Fig. 1a, left panel). The lateral injection site of the graded lesion procedure denervated the medial and lateral striatum to different degrees in the same animal. Dopaminergic neurotransmission was investigated in these and intact animals under anesthesia by means of electrical stimulation and voltammetry. The stimulating electrode was positioned in the medial forebrain bundle (Fig. 1a, middle panel) to evoke DA release in the striatum. One carbon-fiber microelectrode was implanted in both the medial and lateral striatum of each animal for simultaneous measurement of DA in these two regions (Fig. 1a, right panel). The voltammetric recordings were analyzed to determine indices characterizing extracellular dopaminergic signaling. Relationships between indices and denervation degree, as estimated by tissue DA content, were assessed by correlation.

Animals

Adult male Sprague–Dawley rats (250–350 g) were purchased from Harlan (Indianapolis, IN, USA). Animals were housed under standard conditions of lighting, temperature and humidity. Food and water were provided ad libitum. Care was in accordance with NIH guidelines (publication 86-23) and approved by the Institutional Animal Care and Use Committee of Illinois State University.

Lesioning procedure

Partial, graded lesions of nigrostriatal dopaminergic neurons were produced by injecting 6-OHDA into the lateral edge of the substantia nigra (Bergstrom et al. 2001). A gradient of neurotoxin develops across the dopaminergic cell body region with highest concentrations nearest the injection site, generating a variable pattern of denervation with greatest neuron loss in the lateral substantia nigra. Because of the topographic dopaminergic projection (Fallon and Moore 1978; Bjorklund and Lindvall 1984; Gerfen et al. 1987), a comparable medial to lateral loss of DA content is observed in the striatum. Animals were anesthetized with Equithesin (6 mL/kg i.p.) and immobilized in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Deltapharh Isothermal Pads (Braintree Scientific, Braintree, MA, USA) maintained core temperature throughout surgery and postoperative care. Animals were pretreated with the norepinephrine uptake blocker desipramine (25 mg/kg i.p.) and the monoamine oxidase inhibitor pargyline (40 mg/kg i.p.). After exposing the skull and drilling one hole, hypodermic tubing (30 gauge; Small Parts, Miami Lakes, FL, USA) sharpened at the tip was positioned in the lateral substantia nigra of the right brain for injecting 7 or 14 µg 6-OHDA. Stereotaxic coordinates were −5.4 anteroposterior (AP), +3.0 mediolateral (ML) and −8.2 dorsoventral (DV) (Paxinos and Watson 1986). The neurotoxin was dissolved in a 0.9% sodium chloride solution.
Tissue content is expressed as a percentage of that in the contralateral striatum. Data are mean ± SEM and represent a total of 55 determinations. In the intact or control group, 18 determinations were collected medially and laterally in nine animals representing a total of 55 determinations. In the lesioned group, 20 determinations, with content measured in both the medial and lateral regions of 20 animals. The variability of the lesion protocol produced the wide range of denervation observed in preclinical parkinsonism (0 to 85%). Unilateral lesions were generated so that tissue DA content in the lesioned striatum was lesioned to 41 and 78% respectively. The solid line under each recording demarcates the pulse train application. Background-subtracted cyclic voltammograms collected in vivo (circles) and for applied DA during postcalibration (solid line) are shown in the inset above 60-Hz traces. The x-axis is applied potential (mV) and the y-axis is normalized current (i).

**Voltammetry**

Electrically evoked levels of extracellular DA were monitored in the striatum of anesthetized rats by fast-scan cyclic voltammetry at carbon-fiber microelectrodes (Bergstrom and Garris 1999). All voltammetric recordings were collected 2–4 weeks after lesion surgery. As above, but under urethane anesthesia (1.5 g/kg i.p.), holes were drilled in the skull ipsilateral to the lesion for placement of stimulating, reference and carbon-fiber microelectrodes. The stimulating electrode was positioned just dorsal to the medial forebrain bundle (± 4.6 AP, ± 1.4 ML and − 7.0 DV) and incrementally lowered until a robust signal for DA, monitored in the striatum voltammetrically, was obtained. This depth, typically between − 8.0 and − 8.8 DV, was not changed thereafter. The chloridized silver wire reference electrode was placed in the contralateral superficial cortex. Carbon-fiber microelectrodes, angled at 6° to accommodate placement in the same anteroposterior plane, were positioned in the medial and lateral striatum (± 1.2 AP, and ± 1.0 and ± 4.0 ML, respectively). Recordings evoked by pulse trains delivered at 20 and 60 Hz were collected at three dorsoventral locations (± 4.3, − 4.5 and − 4.7 DV) and averaged to characterize each striatal region. Both 20- and 60-Hz recordings were collected at each location except that 60-Hz responses only were measured in one animal. In another experiment, carbon-fiber microelectrodes were initially lowered to − 4.3 DV and adjusted slightly to obtain a more robust signal. A frequency series, consisting of pulse trains delivered between 10 and 60 Hz, was then collected. The time interval between pulse trains varied between 2 and 10 min, with less time in between for the lower-frequency trains. This variable interval protocol decreased the overall time of data collection and negated interactions between a previous pulse train and subsequent stimuli. The position of the carbon-fiber microelectrode was not altered during these measurements.

**Electrochemistry**

Cylinder carbon-fiber (r = 2.5 µm) microelectrodes were prepared as described previously (Cahill *et al.* 1996). The fiber extended approximately 100 µm beyond the tip of the glass insulation. Electrochemistry was performed by means of an EI400 bipotentiostat (Ensmann Instruments, Bloomington, IN, USA) and was
Fig. 2 Comparison of functional characteristics for dopaminergic neurons innervating the medial and lateral striatum in the intact animal. (a) Tissue DA content. Data are expressed in absolute terms. (b) \([DA]_{\text{max}}\) evoked by electrical stimulation at 20 and 60 Hz. (c) \([DA]_p\). (d) \(V_{\text{max}}\). Data are mean ± SEM (n = 9). Medial and lateral refer to regions of the striatum.

Fig. 3 Relationship between characteristics of dopaminergic neurotransmission and extent of lesion. The x-axis in all panels is tissue DA content (i.e. innervation level), calculated as a percentage of that in the contralateral intact striatum (% Lesioned/Non-lesioned). Characteristics are presented on the y-axis: (a) \([DA]_{\text{max}}\) evoked by 20 Hz ([DA]_{20 Hz}), \(r = 0.044\) (p > 0.8). (b) \([DA]_{\text{max}}\) evoked by 60 Hz ([DA]_{60 Hz}), \(r = 0.555\) (p < 0.01). (c) \([DA]_p\), \(r = 0.597\) (p < 0.01). (d) \(V_{\text{max}}\), \(r = 0.761\) (p < 0.01). Filled circles are individual determinations in the denervated striatum. The open circle with error bars (SEM) is the value averaged for intact animals. The solid line describes the best-fit relationship calculated by linear regression.

minimization algorithm (Wu et al. 2001b). Before comparison with data, simulated curves were convoluted to take into account distortion of the observed dynamics by adsorption at the carbon-fiber microelectrode (Bath et al. 2000). For the analysis shown in Figs 2 and 3, one set of values for \([DA]_p\) and \(V_{\text{max}}\) was calculated for each set of 20- and 60-Hz recordings after fixing \(K_m\) to 0.2 µm, the value obtained previously in the intact striatum (Wu et al. 2001b). Frequency series (Fig. 4) were evaluated kinetically in a similar manner, except that \(K_m\) was determined and not fixed. Therefore, in this case, all three parameters (\([DA]_p\), \(V_{\text{max}}\) and \(K_m\)) floated during curve fitting.

Tissue DA content

Tissue DA content was determined in all animals after the voltammetric experiment (Bergstrom et al. 2001). Immediately after the electrodes had been withdrawn, the brain was removed, chilled in ice-cold saline (150 mM NaCl), and sliced while fresh into 1-mm sections using razor blades and a locally constructed block, also chilled on ice. The appropriate slice was selected, and the right striatum was dissected into the approximately 1-mm³ tissue cube immediately surrounding each recording site. Corresponding tissue was dissected from the contralateral striatum. Tissue samples were frozen at −80°C until assay, which typically was less than 4 days later. DA content was determined by high performance liquid chromatography with electrochemical detection (HPLC-EC) (BAS 200B; Bioanalytical Systems, West Lafayette, IN, USA) using a millibore, reverse-phase column (Phase-II; Bioanalytical Systems). The mobile phase, pH 2.8, contained (in 2 L water): 0.5 g EDTA,
where $r$ is correlation coefficient. The difference by the value at 100%, and then dividing by 0.5. The set was at $p<0.05$ for all comparisons.

**Statistical analysis**

Data are expressed as individual values and as the mean ± SEM. Both the number of animals ($n$) and total number of determinations (typically two per animal) are given. Statistical analysis of averaged effects was performed by SAS (Cary, NC, USA) and used $t$-test or ANOVA (Sokal and Rohlf 1995). Where appropriate, ANOVA was followed by the post hoc test of least squares with a Bonferroni correction. The significance of differences between the slope ($b$) of the regression line and zero was determined using a $t$-statistic ($t$):

$$t = \frac{b}{\sigma_b}$$

where $\sigma_b$ is the standard error of the slope regression coefficient (Sokal and Rohlf 1995). The equation of the regression line was used to determine a normalized slope, calculated by taking the difference between the $y$-value at 50 and 100% lesion, dividing this difference by the value at 100%, and then dividing by 0.5. The correlation coefficient ($r$) was also calculated. The significance level was set at $p < 0.05$ for all comparisons.

**Results**

**Lesion range**

On average, the graded lesion procedure resulted in approximately a 25 and 50% decrease of tissue DA content in the medial and lateral striatum respectively (Fig. 1b). The control group comprised pooled values from the medial and lateral striatum of intact animals based on the lack of significant difference in content between the two regions (Fig. 2a). Tissue DA content was expressed as a percentage of that in the contralateral intact striatum. Statistical analysis revealed a significant main effect (ANOVA, $F_{2,55} = 33.96, p < 0.001$), and values in both denervated regions were significantly ($p < 0.05$) different from control values and each other. As shown later in correlation plots (Fig. 3), the averaged values described in Fig. 1(b) reflect individual lesions spanning the denervation observed during the presymptomatic phase of Parkinson’s disease (0 to ~85%). Ideally, if the two averaged values fell equally spaced within this range, they would be one-third and two-thirds of 85%. They were in fact very close. Similarly, denervated animals did not exhibit behavioral deficits as assessed by apomorphine-induced rotational asymmetry and body-swing test (Bergstrom et al. 2001). Taken together, these results suggest that compensatory adaptation was occurring in the lesioned animals used in the present study.

**Evoked dynamics of extracellular DA**

Representative voltammetric recordings collected in the striatum are shown in Fig. 1(c). In both intact and lesioned animals, a frequency of 20 Hz elicited a signal that reached a plateau during the applied pulse train (Fig. 1c, top traces). Steady-state amplitude did not decrease with denervation. In contrast, a pulse train delivered at 60 Hz elicited a peak-shaped signal in intact and lesioned animals (Fig. 1c, bottom traces). The amplitude of these evoked concentration spikes decreased with denervation. In both 20- and 60-Hz recordings, denervation decreased the rate of increase of extracellular DA evoked during the pulse train and the extracellular clearance rate of released DA after the pulse train. Background-subtracted cyclic voltammograms identified evoked signals as arising from DA (Fig. 1c, inset).

**Comparing dopaminergic activity in the medial and lateral striatum of intact animals**

By enabling simultaneous measurements in differentially denervated tissue of a single animal, the graded lesion procedure increases the efficiency of the low-throughput voltammetric technique (Bergstrom et al. 2001). To take advantage of the graded lesion, data collected in the medial and lateral striatum were pooled. Pooling is only justified if the two regions are treated identically given the experimental goals. Figure 2 shows a comparison of the medial and lateral striatum in intact animals for different indices used in the present study to assess lesions. Statistical treatment used a $t$-test. Indices were obtained from the same group of animals ($n = 9$).

Figure 2(a) demonstrates that the tissue DA content (expressed in absolute terms) did not differ significantly between the medial and lateral striatum of either the right or left brain. Figure 2(b) shows that the maximal concentration

![Fig. 4 Simulated evoked responses in the denervated striatum. (a) 20 Hz. (b) 60 Hz. Both sets of simulations were calculated by reducing $[DA]_p$ and $V_{max}$ proportionally in increments of 10% until an 80% loss was achieved. $K_m$ was held constant. Initial parameters were obtained from Fig. 3. The inset for each panel shows $[DA]_{max}$ as a function of innervation level (% Intact). The solid line is the best-fit regression line.](image-url)
of extracellular DA ([DA]_{max}) elicited by either 20 or 60 Hz was not significantly different between the two striatal regions. The recordings were collected in the right brain, the side lesioned in denervated animals. Evoked signals were analyzed to determine [DA]_p and V_{max} (Figs 2c and d respectively). No significant differences were found for the release and uptake parameters between the regions. Values for tissue DA content, [DA]_{max}, [DA]_p and V_{max} were similar to our previous determinations in the mediolateral striatum of intact animals (Garris and Wightman 1994; Garris et al. 1997; Bergstrom et al. 2001). Taken together, these results support pooling of data collected in the medial and lateral striatum.

Correlating dopaminergic activity with denervation
To evaluate compensatory adaptation across the presymptomatic denervation spectrum, characteristics of dopaminergic signaling were correlated with striatal DA content (Fig. 3). Data collected in the medial and lateral striatum were pooled into either control (i.e. intact) or lesion experimental groups. Tissue DA content was expressed as a percentage of that in the contralateral intact striatum. The lack of compensation is in theory described by a linear relationship between the characteristic and innervation level with a normalized slope of 1. For example, because release and uptake sites are both located on dopaminergic terminals, 50% denervation is accompanied by an identical reduction in each characteristic if uncompensated. Adaptation, however, manifests as deviation from a slope of 1, as compensation alters the magnitude of a characteristic relative to denervation.

Consistent with the representative voltammetric recordings described in Fig. 1, [DA]_{max} evoked by 20 Hz ([DA]_{20 Hz}) showed near-complete compensation in denervated animals (Fig. 3a, filled circles; n = 14, 28 determinations). Individual measurements were scattered around the average value for intact animals (open circle with error bars; n = 9, 18 determinations). The shallow slope of the regression line was not significantly different from zero, with the normalized value of 0.06 reflecting only a 3% decrease in [DA]_{max} per 50% drop in tissue DA content. By comparison, [DA]_{max} evoked by 60 Hz ([DA]_{60 Hz}) in denervated animals showed no evidence of compensation (Fig. 3b; n = 15, 30 determinations). The linear relationship between 60-Hz measurements and content exhibited a slope that was significantly greater than zero (p < 0.01) with a normalized value of 1.1. A similar trend was observed in the representative recordings (Fig. 1). The average value for intact animals (open circle with error bars; n = 9, 18 determinations) was slightly below the regression line. No evoked signals identified as DA were obtained in a separate group of animals with near-complete lesions (≥ 95%, n = 3).

More fundamental information about extracellular dopaminergic signaling in the denervated striatum was obtained by resolving evoked signals into the underlying components of DA release and uptake. When K_m for DA uptake is fixed during kinetic analysis, voltammetric recordings of DA evoked by 20 and 60 Hz contain suitable information for determining the release parameter [DA]_p and the uptake parameter V_{max} (Wu et al. 2001b). Figure 3 shows the correlation between these indices and innervation level in the striatum. The proportional relationship between [DA]_p (Fig. 3c) or V_{max} (Fig. 3d) and tissue DA content indicated that neither release nor uptake mechanisms compensated following lesioning (n = 15, 30 determinations). In both cases, the slope was significantly greater than zero (p < 0.01) with a normalized value near 1. The regression line intersected averaged values for the parameters obtained in intact animals (open circle with error bars; n = 9, 18 determinations). A decrease in DA uptake following denervation is apparent in the individual recordings shown in Fig. 1(c) by the slower extracellular clearance rate of released DA.

Theoretical correlations
Simulations were calculated to determine whether DA release and uptake parameters obtained from the kinetic analysis of 20- and 60-Hz signals were consistent with individual voltammetric recordings shown in Fig. 1(c) and values for [DA]_{max} described in the correlation plots of Figs 3(a) and (b). The theoretical results, shown in Fig. 4, were generated for lesions simulated between 0 and 80% using uncompensated DA release ([DA]_p) and uptake (V_{max}) parameters strictly decreased according to denervation. Similar to the kinetic analysis of experimental results, K_m was also held constant at each simulated extent of lesion. The 20-Hz curves calculated for the intact condition and for most of the lesion range reached a plateau during the pulse train (Fig. 4a). As shown in the inset, [DA]_{max} calculated from 20-Hz curves was relatively constant across the range of lesions. By comparison, all simulated curves for 60 Hz were peak shaped (Fig. 4b) with [DA]_{max} decreasing in proportion to the simulated denervation (inset) and characterized by a normalized slope of 1.1 (compared with 0.12 for 20-Hz calculations). For both 20 and 60 Hz, simulated lesions decreased the rates of increase and clearance of extracellular DA.

Taken together, the simulated results support the veracity of release and uptake parameters determined by kinetic analysis of 20- and 60-Hz signals. Simulated curves based on these parameters well described important features of the experimental results. These features included frequency-dependent dynamics, denervation-induced decreases in rates of increase and clearance, and differential effects of denervation on the amplitudes of the steady-state and peak-shaped signals. The simulations also indicated that one set of parameters for DA release and uptake underlie the different responses evoked by 20 and 60 Hz in both intact and lesioned animals.

Kinetic analysis of frequency series

Dopaminergic neurotransmission in the denervated striatum was further evaluated by frequency series. Although the extended times required to collect these series precluded averaging measurements at different depths in the same animal, thereby minimizing complications arising from heterogeneous DA release and uptake rates in the striatum (Garris et al. 1994b), curves evoked by a range of stimulus frequencies between 10 and 60 Hz contain suitable neurochemical information for determining $K_m$ for DA uptake (Wu et al. 2001b). Frequency series also provide a qualitative insight into the regulation of extracellular DA levels (Garris and Wightman 1994). In agreement with previous voltammetric measurements in the intact mediolateral striatum under identical conditions (Wightman et al. 1988; Kawagoe et al. 1992; Garris and Wightman 1994), frequencies between 10 and 30 Hz elicited steady-state signals whereas frequencies between 40 and 60 Hz elicited peak-shaped signals (data not shown).

Figure 5 compares averaged values for $[DA]_{\text{max}}$ evoked by frequencies between 10 and 60 Hz in intact and denervated animals. Data were further subdivided into averaged values representing the medial and lateral striatum in each group of animals. The relationship between $[DA]_{\text{max}}$ and frequency was steep and almost identical in the two striatal regions of intact animals and similar to frequency series previously collected by us in the intact mediolateral striatum (Garris and Wightman 1994; Bergstrom et al. 2001; Wu et al. 2001b). Indicative of altered DA release and uptake rates, series became more flattened with denervation. However, lesions did not alter steady-state DA concentrations evoked by lower frequencies ($\leq 30$ Hz) but decreased the amplitude of the peak-shaped signals elicited by higher frequencies ($\geq 40$ Hz). Statistical analysis demonstrated significant main effects of group (ANOVA, $F_{3,16} = 7.89$, $p < 0.0001$) and frequency (ANOVA, $F_{3,16} = 66.07$, $p < 0.0001$), and a significant interactive term (ANOVA, $F_{3,16} = 2.30$, $p < 0.01$). The medial and lateral striatum of lesioned animals was denervated on average by 21 and 42% respectively, similar to the averaged lesions described for the entire study (Fig. 1b). There was a significant main effect of group on tissue DA content (ANOVA, $F_{3,16} = 3.34$, $p < 0.005$). Values in the medial and lateral striatum of lesioned animals were significantly lower than their corresponding intact control ($p < 0.05$). Taken together, these results further suggest that the amplitude of steady-state and peak-shaped signals respond differently to lesions.

Table 1 shows values for $K_m$ obtained in intact and denervated animals by the kinetic analysis of frequency series. This Michaelis–Menten parameter for DA uptake was not significantly affected by denervation. There was a trend for $K_m$ to decrease in the most lesioned region, the lateral striatum of denervated animals. Increasing the number of animals per group might achieve statistical significance for this decrease. However, we have previously found highly significant ($p < 0.01$–0.002) effects of competitive uptake inhibitors for a similar change in $K_m$ and sample size (Wu et al. 2001a), suggesting that other factors are involved. Values for $K_m$ in the intact animal were similar to that reported previously in the mediolateral striatum (Wu et al. 2001b). The lack of difference between $K_m$ in the medial and lateral striatum of intact animals further supported pooling data collected in these regions for the correlation analysis described in Fig. 3.

Frequency series were also simulated to determine whether the results of kinetic analysis for $K_m$ were consistent with the experimental data described in Fig. 5. As shown in the inset, mathematically decreasing $[DA]_p$ and $V_{\text{max}}$ strictly according to the observed lesion, but maintaining a constant $K_m$ produced theoretical curves that described important features of measured frequency series. For example, the overall shape

![Figure 5](image-url)

**Fig. 5** Frequency series. Frequencies series collected in the medial and lateral striatum of intact and lesioned animals. Data are mean ± SEM ($n = 5$). The inset shows simulated series for the medial intact (MI), medial lesioned (ML) and lateral lesioned (LL) striatum. One set of release and uptake parameters was used to calculate each frequency series. For simplicity, only the medial intact striatum is plotted as baseline. Labels for both axes are the same as those in the main figure.
of simulated series was similar to experimental data. The simulated denervation, more importantly, did not alter steady-state concentrations (≤30 Hz) but decreased the amplitude of peak-shaped signals (≥40 Hz).

**Correlation of DA release and uptake**

If DA release and uptake capacity both decrease proportionally with the extent of the lesion (Figs 3c and d respectively), then parameters describing these mechanisms should be linearly dependent. Figure 6 shows such a relationship. Filled circles represent individual values for [DA]₀ and Vmax obtained from the lesioned animals described in Figs 3 and 4. The accompanying solid line is the best-fit relationship calculated by linear regression. r = 0.787 (p < 0.01). The open circle with error bars (SEM) is the average for intact animals (data from Fig. 2).

**Discussion**

**Pooling voltammetric measurements of DA in the medial and lateral striatum**

The validity of pooled data from the medial and lateral striatum might be questioned on the grounds of the heterogeneous dopaminergic innervation. Dopaminergic neurons projecting to these regions originate from different loci in the ventral mesencephalon and, functionally, the lateral striatum is considered to be sensorimotor whereas the medial striatum is mixed sensorimotor and limbic (Fallon and Moore 1978; Bjorklund and Lindvall 1984; Gerfen et al. 1987). The medial striatum becomes more limbic anteriorly and ventrally. Therefore, the posterior and dorsal recording sites are located in regions with strong sensorimotor components. Functional differences are further downplayed, because presynaptic mechanisms, as opposed to postsynaptic or behavioral responses, are evaluated. Intrastriatal differences in dopaminergic terminal density, uptake and release, which also follow a dorsolateral to medioventral gradient (Marshall et al. 1990; Garris et al. 1994b; Cline et al. 1995; Bergstrom et al. 2001), are similarly minimized by recording location. Indeed, no differences were observed between the medial and lateral striatum for any index used to characterize compensation. Pooling of results obtained from voltammetric measurements in the medial and lateral striatum is thus reasonable.

**Relationship between electrically evoked DA dynamics and denervation**

Electrically evoked DA signals evaluated in this study exhibited two general types of dynamics, steady state and peak shaped. Steady-state signals were elicited by lower frequencies (≤30 Hz) whereas peak-shaped signals were elicited by higher frequencies (≥40 Hz). Differential dynamics do not result from frequency-dependent differences in either DA release and/or uptake, because one set of kinetic parameters describes both 20- and 60-Hz signals or the entire frequency series collected at one recording location in the striatum (Figs 4 and 5; Wightman et al. 1988; Kawagoe et al. 1992; Garris and Wightman 1994; Wu et al. 2001b). Rather, the determining factor is time between stimulus pulses. At lower frequencies, sufficient time allows DA uptake to oppose DA release effectively, causing the extracellular DA concentration to plateau during the pulse train when release and uptake rates become equal. In contrast, little time at higher frequencies results in release overwhelming uptake and a continual increase in DA concentration throughout the train.

Steady-state and peak-shaped dynamics evoked by electrical stimulation and measured in the striatum by voltammetry are thus mediated by different phenomena: steady-state DA concentrations reflect the balance between DA release and uptake whereas the amplitude of peak-shaped DA signals primarily reflects only DA release. More importantly, we show in this study that the type of dynamics determines the response of extracellular DA to the loss of dopaminergic terminals in the striatum. Indeed, steady-state DA levels are independent of the extent of the lesion over the range examined (0 to ~85%), but the amplitude of peak-shaped DA signals decreases in proportion to denervation.

**Electrically evoked DA dynamics and dopaminergic signaling**

Electrical stimulation used in this study was selected for its value in analyzing DA release and uptake parameters (Wu
et al. 2001b). Evoked signals may additionally provide insight into how the two modes of dopaminergic signaling, tonic and phasic, respond to denervation. During tonic signaling, slow and irregular firing of dopaminergic neurons generates a low, steady-state concentration of extracellular DA (i.e. dopaminergic tone) in projection fields (Schultz 1998; Grace 2000; Garris and Rebec 2002). In contrast, a transient DA concentration spike is produced on top of these ambient levels by synchronous burst firing of dopaminergic neurons during phasic signaling (Schultz 1998; Grace 2000; Robinson et al. 2001; Garris and Rebec 2002; Phillips et al. 2003). Strictly in terms of dynamics then, evoked steady-state signals mimic dopaminergic tone, and evoked peak-shaped signals mimic the DA concentration spikes associated with phasic signaling.

There is another important similarity between evoked steady-state signals reported in this study and dopaminergic tone: both are compensated across the presymptomatic lesion spectrum. Indeed, $[\text{DA}]_{\text{max}}$ elicited by 20 Hz (Fig. 3a) and dialysate DA (Zhang et al. 1988; Abercrombie et al. 1990; Robinson et al. 1994), an accepted measure of dopaminergic tone, are maintained at intact levels in the striatum across the denervation range between 0 and ~85%. The same compensatory mechanism may therefore normalize evoked steady-state concentrations of DA and dopaminergic tone. One caveat is that 20 Hz is higher than the basal firing rate of dopaminergic neurons, which is generally accepted as 5 Hz on average with a maximum near 10 Hz (Grace 2000; Garris and Rebec 2002). On the other hand, no effect of lesions was observed on $[\text{DA}]_{\text{max}}$ evoked by 10 Hz, a physiological frequency, and 30 Hz. The lack of denervation effect on $[\text{DA}]_{\text{max}}$ evoked by 20 Hz thus appears more related to steady-state dynamics than stimulus frequency.

How our evoked measurements of peak-shaped DA signals and phasic dopaminergic signaling compare following lesions of nigrostriatal dopaminergic neurons is more difficult to establish. One problem is that, because characterizing phasic signaling is technically demanding in the freely behaving animals (Garris and Rebec 2002), little information is currently available. Electrophysiological recordings in anesthetized animals demonstrate that firing properties of dopaminergic neurons are similar in intact rats and in rats with striatal dopaminergic denervation up to 95% (Hollerman and Grace 1990; Zigmond et al. 1990). Combined with our data showing that $[\text{DA}]_{\text{max}}$ evoked by 60 Hz decreases in proportion to striatal denervation and that $[\text{DA}]_{\text{max}}$ evoked by 40 and 50 Hz is also sensitive to lesions, this result suggests that the magnitude of DA concentration spikes associated with phasic dopaminergic signal decreases with denervation. However, although unstimulated DA concentration transients have now been recorded in freely behaving animals (Robinson et al. 2001; Phillips et al. 2003), similar measurements have not been collected after lesioning.

Another consideration is that the size (low micromolar) of our peak-shaped DA signals electrically evoked in intact animals is larger than that (few hundred nanomolar) for unstimulated DA transients (Robinson et al. 2001; Phillips et al. 2003). Recent electrophysiological recordings of dopaminergic neurons in freely behaving rats indicate that stimulation frequencies used in the present study to evoke peak-shape DA signals are within the physiological range for phasic signaling (Garris and Rebec 2002; Hyland et al. 2002). Because dopaminergic neurons burst with only a few action potentials, perhaps up to about 20 maximally (Hyland et al. 2002), the large number (80–120) of stimulus pulses applied for our voltammetric measurements may instead account for the difference in DA concentration. Whether peak-shaped DA signals evoked by electrical stimulation, and similar in magnitude to unstimulated DA transients, decrease following striatal denervation was not examined in the present study. We speculate that they should, because the amplitude of peak-shaped signals reflects DA release, which decreases with denervation. Clearly, more studies are required to establish the relationship between phasic dopaminergic signaling and denervation.

Compensatory changes in DA release and uptake

The present study found no evidence for compensatory changes in DA release or uptake across the lesion spectrum encompassing the presymptomatic phase of Parkinson's disease. The absence of up-regulated release is consistent with our previous kinetic analysis at 50% depletion (Garris et al. 1997) and semiquantitative evaluation using four-pulse, 100-Hz stimulation in the mouse lesioned to 75% by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Bezard et al. 2000). The amplitude of this 'pseudo-one pulse' measurement mirrors release for the same reason as that for 60-Hz signals. These results oppose the postulate of Zigmond et al. (1990) and Zoli et al. (1998) that surviving terminals compensate by releasing more DA following denervation. One possible explanation for this discrepancy is that earlier assessment of release used indirect indices such as the ratio of DA metabolites to DA in tissue (Hefti et al. 1985), the ratio of dialysate DA to tissue DA (Zhang et al. 1988), and the electrically evoked 'fractional efflux' of DA from striatal slices (Stachowiak et al. 1987; Snyder et al. 1990). Zoli et al. (1998) also concluded that DA release was enhanced, from anatomical data describing hypertrophy of dopaminergic terminals. An increase in dopaminergic terminal size was reported previously by Pickel et al. (1992). However, both studies examined lesions of 80% or greater, and a connection between dopaminergic terminal hypertrophy and increased DA release has not been established. Lack of adaptation in $V_{\text{max}}$ for DA uptake agrees well with our previous kinetic characterization at the single, moderate lesion (Garris et al. 1997), uptake of $[^{1}H]$DA into synaptosomes (Zigmond et al. 1984; Altar et al. 1987), and binding.
studies of the DA transporter in the denervated rat (Dentresangle et al. 2001), mouse (Bezard et al. 2000) and monkey (Bezard et al. 2001). The present and another (Zigmond et al. 1984) study also report that the affinity of the transporter for DA, as evaluated by $K_m$, is not altered by lesioning. Although extracellular DA clearance slower than that expected for a 75% denervation has been described in a voltammetric study (Bezard et al. 2000), no kinetic constants were calculated. Hence, the apparent decrease might be related to lower DA concentrations evoked after lesioning. Down-regulated uptake is observed in cats spontaneously recovered from MPTP-induced degeneration (Rothblat and Schneider 1999) and in parkinsonian patients (Uhl et al. 1994; Joyce et al. 1997), but the relevance of these findings to the preclinical phase is not clear. There is one report of up-regulated DA uptake measured by immunofluorescence and confocal laser microscopy in the striatum denervated to 80% and greater (Zoli et al. 1998). These authors proposed that the reverse transport of DA, coupled to up-regulated DA synthesis, acts as a compensatory mechanism.

New model of compensation during preclinical parkinsonism

The present results extend our hypothesis that extracellular DA is normalized in the moderately lesioned striatum without active changes in either DA release or uptake (Garris et al. 1997) across the entire denervation range observed during preclinical parkinsonism. Here we formalize this hypothesis as ‘passive stabilization’ and propose it as the basis of a new model of compensatory adaptation (Fig. 7). Passive stabilization is based on two simple physical principles, steady state and diffusion. Steady state is defined as a condition of constancy mediated by finite yet balanced input and output fluxes. In the specific case of the voltammetric recordings evoked by 20 Hz, a plateau is reached during the pulse train when rates of DA release (input flux) and uptake (output flux) equalize. A consequence of steady state is that the amplitude remains unaltered if input and output fluxes change identically. Thus, steady-state concentrations evoked by 20 Hz do not decrease with lesion size, because input (release) and output (uptake) fluxes are located on dopaminergic terminals and decrease proportionally with the lesions. We postulate in this study that a similar phenomenon is expected for dopaminergic tone under physiological conditions, where steady-state DA levels are established by tonic firing (Schultz 1998; Grace 2000).

Diffusion plays a critical compensatory role by delivering DA released from remaining neurons to target cells in denervated regions (Zigmond et al. 1990; Zoli et al. 1998). This neuroadaptation is consistent with DA signaling in the intact brain by volume transmission (Zoli et al. 1998; Vizi 2000), a hypothesis supported by rapid efflux of DA from the synaptic cleft immediately following release (Garris et al. 1994a) and extrasynaptic locations for DA transporters

Fig. 7 Passive stabilization model of compensatory adaptation during the presymptomatic phase of Parkinson’s disease. Similar to the Zigmond hypothesis (Zigmond et al. 1990), DA released from remaining neurons diffuses into denervated regions to maintain control of striatal target cells. In the passive stabilization model, however, dopaminergic tone is normalized without up-regulation of release or down-regulation of uptake when conditions of steady state are met. Rather than support up-regulated DA release, increased DA synthesis from tyrosine (Tyr) replenishes terminal stores when DA is cleared and degraded by non-dopaminergic cells in denervated areas. Uptake plays a permissive role in compensation, because transporter loss in depleted areas enhances extrasynaptic DA diffusion. Alterations in other mechanisms of dopaminergic neurotransmission, such as firing rate and postsynaptic receptor response, may not occur after partial denervation. Thicker arrows represent enhanced mechanisms.

(Nirenberg et al. 1996) and receptors (Sesack et al. 1994). Because uptake limits DA diffusion in the striatum, the loss of DA transporters on lesioned neurons passively potentiates volume transmission (Doucet et al. 1986; Zigmond et al. 1990; van Horne et al. 1992; Schneider et al. 1994). In contrast to Zigmond et al. (1990) and Zoli et al. (1998), our compensation model does not require the active component of up-regulated release to drive enhanced volume transmission in order to supply normal levels of DA to denervated regions.

We propose additionally that the capability of DA for extensive extrasynaptic diffusion is essential for compensation. Rapid mixing of DA released from different sources quickly establishes steady-state levels of the neurotransmitter in brain extracellular fluid. Thus, concomitant losses of release and uptake capacity on lesioned neurons precisely offset and do not alter DA concentration when the condition of steady state is met. It is interesting to speculate that the failure of steady state to develop and the subsequent breakdown of passive stabilization is responsible for the decrease in dopaminergic tone in severe lesions (> 85%). The large distance DA must diffuse between now widely spaced dopaminergic terminals might result in extracellular concentration gradients, with high DA levels in the immediate proximity of surviving terminals but more distally in denervated regions, and inadequate DA delivery to target
cells. In support of this possibility, lesion degree slowed time to reach the plateau for 20-Hz recordings.

The role of enhanced DA synthesis, a well established adaptation after creation of moderate to severe lesions (Zigmond et al. 1984; Hefti et al. 1985; Altar et al. 1987; Wolf et al. 1989), and proposed to support up-regulated release (Zigmond et al. 1990; Zoli et al. 1998), may need re-evaluation. One possibility is that, in denervated regions, DA is more likely to be cleared and degraded by other monoaminergic neurons or glia rather than up-taken by dopaminergic terminals for re-release. In this scenario, synthesis is increased to replenish diminished terminal stores of DA (Garris et al. 1997). In support of this idea, transgenic animals with deletion of the DA transporter gene exhibit high DA synthesis rates (Jones et al. 1998).

The present results also give rise to the predication that the same capacity for DA release and uptake in the partially denervated striatum maintains tonic but not phasic dopaminergic signaling. Nigrostriatal dopaminergic neurons may not, in fact, be able to normalize both signaling modes. For example, adaptation to restore phasic DA transients by increasing DA release or decreasing DA should concomitantly increase dopaminergic tone, resulting in a hyperdopaminergic basal state. Interestingly, stress elicits parkinsonian symptoms in subclinical animals and worsens symptoms in patients with mild impairment (Zigmond 1997). It might be that stress activates phasic firing of dopaminergic neurons and that this signaling mode is not fully supported by compensation. It is also worthwhile speculating that subtle behavioral deficits recently identified in moderately denervated rats (Kirik et al. 1998; Schallert et al. 2000), monkeys (Annett et al. 2000) and humans (Mazzoni and Ford 1999) result from improper phasic dopaminergic signaling.

Conclusions

We propose that passive stabilization normalizes extracellular neurotransmitter levels following partial denervation without plasticity of release or clearance mechanisms. This mode of compensation is efficacious for DA, a well established extrasynaptic messenger, in an animal model of Parkinson’s disease. Given that signaling for most neurotransmitters involves an extrasynaptic component (Zoli et al. 1998; Vizi 2000), passive stabilization may generalize to non-dopaminergic systems and other neurodegenerative disorders, in particular Huntington’s and Alzheimer’s diseases, which, like parkinsonism, are progressive and emerge later in life. Thus, the brain exercises another option to counter cell loss in addition to sprouting, presynaptic and postsynaptic hypertrophy, receptor sensitization and neurogenesis (Hornykiewicz and Kish 1987; Pierce and Lewin 1994; Gage 2000).

It should be emphasized that passive stabilization only addresses the control of dopaminergic tone. Other compensatory mechanisms preserving normal behavior are thought to occur in Parkinson’s disease. For example, studies using a progressive denervation procedure with the MPTP-treated monkey demonstrate hyperactivity of the subthalamic nucleus and globus pallidus pars interna, and overexpression of striatal preproenkephalin-A mRNA levels at the late preclinical stage before classical symptoms appear (Bezard et al. 2003). These results suggest that non-dopaminergic systems in the basal ganglia and outside participate in the overall adaptive response.

Another important point to consider is whether results obtained in the 6-OHDA-lesioned rat have relevance to Parkinson’s disease given the differences in etiology, time course and progression of the pathology (Gerlach and Riederer 1996; Schwarting and Huston 1996). Although comparisons should be made cautiously, passive stabilization treats the brain as a physical system, obeying the laws of diffusion and steady state. Additionally, because basic characteristics of dopaminergic neurotransmission, such as DA receptors (Hooks et al. 1994; McCauley et al. 1995; Ridd et al. 1998) and transporters (Eshleman et al. 2001), and dopaminergic terminal density (Fernandez et al. 1996; Bergstrom et al. 2001), are similar in rat and human, passive stabilization may well apply to aspects of Parkinson’s disease.

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