Age-Related Changes in the Capacity, Rate, and Modulation of Dopamine Uptake within the Striatum and Nucleus Accumbens of Fischer 344 Rats: An In Vivo Electrochemical Study\textsuperscript{1}

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ABSTRACT

Age-related changes in the capacity, rate, and modulation of dopamine (DA) uptake within the striatum and the nucleus accumbens core of Fischer 344 rats were investigated using in vivo electrochemical recordings coupled with local drug application techniques. Equimolar amounts of DA were pressure ejected into the striatum and the nucleus accumbens of 6-, 12-, 18-, and 24-month-old rats. The DA ejections produced larger DA signal amplitudes in the older rats, suggesting age-related differences in the capacity to clear extracellular DA. Within the striatum, the capacity and rate of DA uptake were reduced by 50% in the aged groups (18 and 24 months) compared with the younger rats (6 and 12 months). In the nucleus accumbens, significant reductions in DA uptake capacity and rate were observed in the 24-month group. In both brain regions and in all age groups studied, the rate of DA uptake was found to be concentration-dependent until a maximal rate was reached. The maximum rate of DA transport was significantly reduced in both the striatum and the nucleus accumbens of aged rats (18 and 24 months versus 6 and 12 months). The ability of nomifensine, an inhibitor of the DA transporter, to modulate DA signal amplitudes in the striatum and the nucleus accumbens was also decreased with age (24 months versus 6 months). Taken together, these findings demonstrate substantial age-related deficits in DA uptake processes within the striatum and the nucleus accumbens, consistent with the hypothesis that DA uptake may be slowed in aged animals to compensate for reductions in DA release.

The progressive development of motor and cognitive dysfunction during senescence may be associated with alterations in neurotransmitter function in the central nervous system. In particular, age-related diminutions in dopamine (DA) neurotransmission within the basal ganglia of humans and animals are believed to contribute to changes in the execution and coordination of voluntary movements (Finch et al., 1981). Age-related alterations in both pre- and postsynaptic indices involved in dopaminergic (DAergic) neurotransmission have been well documented (Joseph et al., 1978; Goldman-Rakic and Brown, 1981; Rose et al., 1986; Friedemann and Gerhardt, 1992; Hebert and Gerhardt, 1998). The major regulator of DAergic neurotransmission is the uptake of released DA via the dopamine transporter (DAT) (Giros et al., 1996). This high-affinity uptake process is sodium-, chloride-, and temperature-dependent; it is also saturable and has an affinity for monoamine substrates of approximately 0.1 to 2 \( \mu \)M (Meiergerd and Schenk, 1994; Lenhard et al., 1998). Not surprisingly, this important regulatory mechanism has also been shown to be altered with age. Several studies have shown a progressive age-related decline in the number of DA transporters (Zelnik et al., 1986) and a sharp decline in DAT mRNA in both rats and humans (Bannon and Whitty, 1997). As the status of this integral membrane protein during aging may have important implications for the proper functioning of the DAergic system, the actual dynamic performance of the DA uptake mechanism is of vital importance, because it regulates the amount of DA available for neurotransmission. It has been shown that the affinity of ligand binding to the rat and human DA transporter decreases with age (Shimizu and Prasad, 1991; Volkow et al., 1994). In addition, previous studies from our laboratory involving measures of stimulus-evoked release of DA in aged animals have yielded data implicating age-related changes in DAT function (Friedemann and Gerhardt, 1992; Hebert and

ABBREVIATIONS: DA; dopamine; DAergic, dopaminergic; DAT, dopamine transporter; F344, Fischer 344; NET, norepinephrine transporter; SERT, serotonin transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; \( T_c \), clearance rate; \( T_{50} \), time for signal amplitude to decay by 50%; ANOVA, analysis of variance.
Gerhardt, 1998). The purpose of this study was to systematically study changes in DAT function that may occur with age.

The in vivo activity of the DA transporter can be assessed in various brain regions by monitoring the disappearance of locally ejected DA with high-speed electrochemical recordings (Cass et al. 1993; Gerhardt et al. 1996). This technique allows the precise measurement of DA concentration (nanomolar) within a discrete brain region at a fast sampling rate (5 Hz). The peak response conveys information regarding a brain region's ability (capacity) to clear the exogenous DA (Cass and Gerhardt, 1995). The duration of the electrochemical signal indicates the rate at which the removal is occurring. Direct electrochemical measurements of the presence and successive uptake of exogenously applied DA eliminates contributions from the release of endogenous DA and confines the processes being examined to those responsible for clearing DA from the extracellular space (Cass et al. 1993). The electrochemical recordings are reproducible and the resulting kinetic parameters permit quantitative comparison of extracellular DA regulation between age groups and brain regions. This technique also provides in vivo assessment of the efficacy of drugs that modulate the DA uptake process (Cass et al., 1993).

Our investigations have focused on DA uptake within the striatum and the core of the nucleus accumbens because of their distinct role in motor activity (Beninger, 1983) and because each system appears to be differentially affected by the aging process (Friedemann, 1992; Friedemann and Gerhardt, 1992). More important, reports have suggested differences in the status and function of high-affinity DA uptake systems within these two regions. The differences observed include regional variation in the density of DATs (Marshall et al., 1990), differences in the molecular features of the DAT in the two areas (Amara and Kuhar, 1993), and a reduced capacity of DA neurons in the nucleus accumbens to clear DA from the extracellular space as compared with those in the striatum (Stamford et al. 1988). Additionally, reports indicate that DA uptake inhibitors produce differential effects in the two regions, both in vitro (Izenwasser and Cox, 1990) and in vivo (Cass and Gerhardt, 1995).

To determine age-related and region-specific differences in the capacity to clear DA, we compared the amplitudes of electrochemical signals generated by applying a range of amounts of exogenous DA in young (6-month), middle-aged (12-month), and aged (18- and 24-month) Fischer 344 (F344) rats. Next, we compared the decay portion of equivalent amplitude signals to assess age-related and region-specific differences in the rate of DA uptake. Finally, we measured the abilities of nomifensine, a combined DAT and norepinephrine transporter (NET) inhibitor, desipramine, a selective NET inhibitor, and citalopram, a selective inhibitor of the serotonin transporter (SERT), to alter the capacity of DA uptake in the striatum and the nucleus accumbens of young (6-month) versus aged (24-month) rats. These uptake inhibitors have been used to investigate age-related differences in the stimulation of motor behavior (Hebert and Gerhardt, 1998) and their ability to inhibit monoamine uptake in vivo has been demonstrated with in vivo electrochemistry (Cass and Gerhardt, 1995; Hoffman and Gerhardt, 1998).

### In Vivo Electrochemistry

Rats were anesthetized with urethane (1.0–1.5 g/kg i.p.) and placed in a stereotaxic frame. Body temperature was maintained at 37°C with a heating pad coupled to a rectal thermometer. The skull and dura overlying the striatal recording sites were removed bilaterally. Electrochemical reference electrodes (Ag/AgCl) were implanted into brain regions remote from recording sites and were cemented into place with dental acrylic.

ELECTRODE/MICROPIPETTE ASSEMBLIES

The electrode/micropipette assemblies used for the in vivo recordings consisted of a working electrode and a single-barrel (1 mm o.d., 0.58 mm i.d. glass, A-M Systems, Inc., Everett, WA) or a triple-barrel micropipette (1.2 mm o.d., 0.68 mm i.d. glass, World Precision Instruments, Inc., Sarasota, FL). The tips of the micropipettes had outer diameters of 10 to 15 μm and were positioned 300 ± 10 μm from the tips of the electrodes. Single-barrel micropipettes contained 200 to 400 μM DA in 0.9% NaCl with 100 μM ascorbic acid added as an antioxidant. Triple-barrel pipettes contained 200 to 400 μM DA solution in one or two barrels and drug solutions (800 μM nomifensine maleate, 800 μM desipramine, and/or 800 μM citalopram) in the other barrel(s). All locally applied drugs were prepared in saline and adjusted to pH 7.4.

High-temperature Nafion-coated (5% solution, 4–7 coats at 200°C, Aldrich Chemical Co., Milwaukee, WI) single carbon-fiber electrodes (fiber diameter 30 μm; exposed length 100–150 μm) were used as recording electrodes (Hebert et al., 1996). The sensitivities and linearity of all recording electrodes were determined by generating calibration curves for each recording electrode in stock solutions of DA in 0.1 M phosphate-buffered saline at 22°C. All electrodes showed linearities for increments of DA ranging from 2 to 14 μM (r² ≥ 0.997) and selectivities greater than 500:1 for DA versus 3,4-dihydroxyphenylacetic acid (DOPAC) and ascorbic acid. Extracellular changes in DA were expressed quantitatively in terms of the DA calibration curves (Gerhardt, 1995). Electrodes were used to record from one animal and then discarded.

High-speed chronamperometric electrochemical measurements were continuously made and averaged to 1 Hz with an IVEC-10 system (Medical Systems Corp., Greenvale, NY). Square-wave pulses of 0.00 to +0.55 V, with respect to a Ag/AgCl reference electrode, were applied to the working electrode for 0.10 s and repeated 5 times/s (Gerhardt, 1995). The resulting oxidation and subsequent reduction currents were digitally integrated during the last 80 ms of the 100-ms pulses. The detection limits of the electrodes averaged 25 ± 5 nM (n = 107), with a signal-to-noise ratio of 3.0.

All coordinates used for recordings were based on the rat atlas of Paxinos and Watson (Paxinos et al., 1985; Paxinos and Watson, 1986). With the incisor bar positioned at −2.3 mm, electrode assemblies were positioned in the striatum (1.0–1.7 mm anterior to bregma, 2.0–2.2 mm lateral from midline, 3.5–5.0 mm below the surface of the brain) or in the core of the nucleus accumbens (1.5–1.7 mm anterior to bregma, 2.0–2.2 mm lateral from midline, 6.5–8.0 mm below the surface of the brain). Once the electrode/micropipette recording assembly was positioned at each recording site, a baseline signal was recorded for approximately 5 to 10 min before DA was ejected. Baseline signals obtained from each recording position were considered to be the theoretical zero response. DA (2–100 pM) was applied by pressure ejection (10–40 psi for 0.5–6 s) with a Picospritzer II pressure ejection system (General Valve Corp., Fairfield, NJ). The volume applied was determined and controlled with a

### Materials and Methods

**Animals.** Young adult (4–6 months, n = 28), middle-aged (10–12 months, n = 24), and aged (18–20 months, n = 30; 24–26 months, n = 32) male F344 rats obtained from the National Institute on Aging (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were used for all experiments. Protocols for animal use were approved by the Institutional Animal Care and Use Committee. Animals were housed according to approved guidelines under a 12 h light/dark cycle with food and water available ad libitum. All recordings were conducted in naive rats during the light cycle.
stereomicroscope fitted with a reticule in one eyepiece to measure the movement of the meniscus in the micropipette (Friedemann and Gerhardt, 1992).

For experiments designed to compare DA uptake in the striatum and the nucleus accumbens of different ages of animals, various volumes of DA solution (10–250 nl containing 2–100 pmol DA) were applied at each individual site before lowering to a new site. Characteristics of the resulting signals were used to quantitate DA uptake capacity and rate (see Data Analysis). Specific volumes of DA were pressure ejected to determine the capacity of DAergic terminals to clear exogenous DA and the rate at which DA uptake occurs in these regions. After completing recordings resulting from various concentrations of DA, the electrode assembly was lowered 250 to 500 μm and allowed to equilibrate at the new site for 5 to 10 min.

For experiments involving the local application of uptake inhibitors, a specific amount of DA was applied by pressure ejection at 5-min intervals to obtain reproducible responses (usually two to three applications). Once the DA signals were stable (≤ 10% variation), the drug solution (nomifensine, desipramine or citalopram) was applied slowly over 10 to 20 s, approximately 30 s before the next application of DA. The volume of drug solution applied was two to three times the volume of DA solution applied to “saturate” the uptake sites. Because the concentration of drug was twice the concentration of the DA solution, the amount (in picomoles) of drug applied was four to six times the amount (in picomoles) of DA applied. After the effects of locally applied drugs were recorded, the electrode assembly was lowered 250 to 500 μm and allowed to equilibrate at the new site for 5 to 10 min. At the end of the experiments, animals were euthanized and their brains were removed for histological confirmation of electrode placements. Only recordings in which the electrodes were clearly positioned in the striatum or the core of the nucleus accumbens were included in Results.

Data Analysis. For each individual electrochemical signal, two parameters were analyzed: 1) the peak amplitude resulting from DA pressure ejection; and 2) the clearance rate (Tc in μM/s), defined by the change in DA concentration between the T30 and T60 time points (e.g., the slope of a pseudolinear portion of the decay curve). The peak amplitude is a measure of extracellular DA. The decay slope of the signal indicates the rate at which the DA is removed from the extracellular space. For purposes of analysis, peak amplitude and clearance values for each signal were grouped and averaged according to concentration (for capacity analysis) or signal amplitude (for rate analysis). The relative capacity of each brain region to clear DA was determined by correlating the amplitude of the resulting signal with the moles of DA applied by pressure ejection. The rate of DA uptake was determined by plotting the amplitude of each signal against the Tc for that signal. A total of 128 to 357 signals recorded in the striatum and 52 to 92 responses in the core of the nucleus accumbens for each age group were used to analyze properties of DA uptake. Linear regression analyses were performed with Prism v2.01 (GraphPad Software, Inc., San Diego, CA). The capacity and rate data were analyzed by a repeated-measures analysis of variance (ANOVA) followed by Tukey-Kramer post hoc comparisons. For experiments involving the local application of uptake inhibitors, baseline parameters were defined at each recording site by averaging the reproducible signals (two to three) preceding drug application; the first DA signal after the drug ejection was taken as the modulated response. Changes in amplitude and Tc after the application of uptake inhibitors were analyzed by repeated-measures ANOVA followed by Tukey-Kramer post hoc comparisons. For statistical comparisons of the electrochemical responses between the striatum and the nucleus accumbens in each age group, a two-way ANOVA (age × region) was performed. In all tests, p < .05 was defined as statistically significant. Statistical analyses were performed with Instat 2.04 and StatMate (GraphPad Software).

Materials. Nomifensine maleate and desipramine were purchased from Research Biochemicals International (Natick, MA). DA, DOPAC, ascorbic acid, and urethane were purchased from Sigma Chemical Co. (St. Louis, MO). Citalopram was generously provided by Dr. Alan Frazer (University of Texas Health Sciences Center, San Antonio). All other reagents were research grade.

Results

Age-Related Changes in Signal Amplitudes: DA Uptake Capacity. To investigate age-related changes in DA uptake, equal amounts of exogenous DA were locally applied in rats of different ages and the resulting signal amplitudes were compared. Application of exogenous DA into the striatum and the nucleus accumbens in all age groups of F344 rats produced reproducible electrochemical signals. Typical electrochemical signals detected after application of 30 pmol of DA within the striatum and the nucleus accumbens of young (6-month), middle-aged (12-month) and aged (18- and 24-month) rats are presented in Fig. 1. Using this amount of locally applied DA, the recorded responses within the striatum were significantly (F(3,75) = 59, all p values < .001) larger as a function of age, indicating reduced DA uptake. Significant age-dependent deficits in the ability to clear exogenous DA within the striatum were measured as early as 12 months and became quite pronounced (2–3 fold greater) at 18 and 24 months. Within the nucleus accumbens, the amplitude of the electrochemical signals remained unchanged until 24 months, at which age modest, but significantly increased, amplitudes were measured (F(3,61) = 11, p < .001).

To examine age-related differences in the capacity to clear DA over a range of picomoles, varying amounts of DA (2–100 pmol) were pressure ejected in the striatum and the nucleus accumbens of rats in each age group and the resulting amplitudes were recorded. As shown in Figs. 2A and 3A, the...
signal amplitudes ranged between 0.1 and 18 \mu M and were linear with respect to the amount of DA applied. Each point represents an average (± S.E.M.) of 8 to 45 signals recorded after pressure ejection of that particular amount of DA. The data from 9 to 12 rats per age group were obtained at several sites within the dorsal striatum. The responses were linear for all groups (r^2 > 0.98), indicating that the change in amplitude per picomole of DA applied was constant within age groups. No significant difference in signal amplitudes from the different age groups was observed when less than 8.0 pmol of DA was ejected. B, bar graph indicates the average nanomolar increase in signal amplitude per picomole of DA applied in each age group (± S.E.M.); significant differences versus the 6-month rats are represented (***p < .001).

Age-Related Changes in Tc Values: DA Uptake Rates.

Because DAergic neurotransmission is not only regulated by the amount but also the duration of extracellular DA, we investigated possible age-dependent alterations in the rate of DA clearance from the extracellular space. The amplitude comparisons illustrated in Fig. 1 suggest significant age- and region-related differences in the relative numbers of functional DA transporters, which in all likelihood affect the DA uptake rate. Specific information regarding the rate of DA uptake was obtained from the decay portion of the electrochemical current traces. The clearance rate, Tc, defined as the micromolar change in DA concentration per second, is calculated from a pseudolinear portion of the decay curve (slope of line between T20 and T60). As seen in Fig. 4, comparison of equivalent amplitude signals recorded from the striatum of young (6-month) and aged (24-month) rats indicated significant differences (unpaired, two-tailed Student’s t test, p < .001) in the Tcs, which suggested age-related alterations in the rate of DA uptake between the young and aged rats.

To compare the apparent rates of DA uptake across age groups, the Tcs of signals over a wide amplitude range (0.1–18 \mu M) were examined in each brain region (128–357 signals in the striatum and 52–92 in the nucleus accumbens per age group). The signals were divided into amplitude ranges spanning approximately 0.5 \mu M, beginning at 0.0 \mu M. The amplitudes (\mu M DA) and respective Tcs of the electrochemical responses were averaged within each set and plotted against each other. The resulting graphs are depicted in Figs. 5 and 6; the rate of uptake in both the striatum and the nucleus accumbens of all age groups studied was found to...
uptake rate was reached. The maximal DA uptake rate (Tmax) on the extracellular concentration of DA and a maximum certain height, the rate of transport was no longer dependent until a maximal rate of DA transport was reached. The slopes of the concentration-dependent portion of the c plots (DA uptake rate per micromolar signal amplitude) for each age group are compared in Table 1. The slope was nearly 50% reduced (p < .001) in the striatum of aged rats (18 and 24 months) when compared with that of younger animals (6- and 12-month) rats. The rate of uptake remained constant (as indicated by the dashed lines) and was not dependent on signal amplitude.

increase in a concentration-dependent manner (r² < 0.97), until a maximal rate of DA transport was reached. The slopes of the concentration-dependent portion of the Tc plots (DA uptake rate per micromolar signal amplitude) for each age group are compared in Table 1. The slope was nearly 50% reduced (p < .001) in the striatum of aged rats (18 and 24 months) when compared with that of younger animals (6- and 12-month) rats. The rate of uptake remained constant (as indicated by the dashed lines) and was not dependent on signal amplitude.
The purpose of this study was to compare the regulation of extracellular DA levels in the striatum and the nucleus accumbens core of four age groups of F344 rats. We have found marked age-related differences in DA uptake processes in both brain regions. First, we observed age-related reductions in the capacity to clear exogenous DA over a wide range of concentrations in both regions. Second, the rate of DA uptake was found to be significantly decreased with age. Finally, we observed that modulation of DA uptake resulting from addition of the DAT inhibitor nomifensine was significantly reduced in aged rats.

Signal amplitudes resulting from the application of exogenous DA provide information regarding the capacity of the surrounding region to clear DA and are thought to relate to the number of functional DA transporters in that region (Cass et al. 1993; Cass and Gerhardt, 1995; Hoffman and Gerhardt, 1998). The relative number of functional DA transporter sites in that region (Cass et al. 1993; Cass and Gerhardt, 1995; Hoffman and Gerhardt, 1998) can be thought of as the in vivo “B_{\text{max}}” in that region for that age group. A comparison of the DA uptake capacity across age groups demonstrated that the ability to remove exogenous DA was significantly reduced in older rats, indicating possible age-related reductions in DAT B_{\text{max}}. Within the striatum, signal amplitudes recorded in aged (18- and 24-month) rats were almost twice those recorded in the younger (6- and 12-month) rats. Data are mean ( +/- S.E.M.) values representing the percentage of change from baseline; the baseline signal represents the recording immediately preceding drug application and the “modulated” signal is the electrochemical response directly after drug ejection. In the 6-month-old rats, nomifensine significantly (***p < .001) increased the signal amplitude in both the striatum (n = 98) and the nucleus accumbens (n = 61). In aged rats (24 months), nomifensine exhibited a significant (*p < .05) but reduced effect on signal amplitudes within the striatum (n = 68) and the nucleus accumbens (n = 43), compared with its effect in the young group. Neither desipramine (6-month striatum, n = 26; nucleus accumbens, n = 12; 24-month striatum, n = 19; nucleus accumbens, n = 10) nor citalopram (6-month striatum, n = 14; nucleus accumbens, n = 7; 24-month striatum, n = 16; nucleus accumbens, n = 7) affected signal amplitudes.

### Discussion

Age-related differences in the slope of the DA uptake rate/μM signal amplitude (concentration-dependent portion of uptake curve) were found in the striatum of the 18- and 24-month groups and in the nucleus accumbens of 24-month rats (as compared to the 6-month group, ***p < .001). Maximal uptake rates were determined by averaging the clearance rate values for signal amplitudes at which the uptake rate was no longer concentration dependent (dotted lines in Figs. 5 and 6). The maximal DA uptake rates within the striatum and the nucleus accumbens were significantly reduced with age (18- and 24-month group versus 6-month, p < .001).

### Comparison of uptake parameters for signals recorded in the striatum and the nucleus accumbens of 6-, 12-, 18-, and 24-month-old rats.

<table>
<thead>
<tr>
<th>Region</th>
<th>DA Uptake Rate/μM Signal Amplitude</th>
<th>Maximal Uptake Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Striatum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-month</td>
<td>0.028 ± 0.001 (n = 302)</td>
<td>0.40 ± 0.02 (n = 55)</td>
</tr>
<tr>
<td>12-month</td>
<td>0.028 ± 0.001 (n = 106)</td>
<td>0.35 ± 0.02 (n = 22)</td>
</tr>
<tr>
<td>18-month</td>
<td>0.017 ± 0.001*** (n = 158)</td>
<td>0.18 ± 0.02*** (n = 84)</td>
</tr>
<tr>
<td>24-month</td>
<td>0.017 ± 0.001*** (n = 103)</td>
<td>0.11 ± 0.02*** (n = 121)</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-month</td>
<td>0.017 ± 0.001 (n = 54)</td>
<td>0.16 ± 0.01 (n = 38)</td>
</tr>
<tr>
<td>12-month</td>
<td>0.016 ± 0.002 (n = 34)</td>
<td>0.21 ± 0.03 (n = 18)</td>
</tr>
<tr>
<td>18-month</td>
<td>0.015 ± 0.002 (n = 52)</td>
<td>0.12 ± 0.01*** (n = 52)</td>
</tr>
<tr>
<td>24-month</td>
<td>0.010 ± 0.001*** (n = 28)</td>
<td>0.10 ± 0.01*** (n = 28)</td>
</tr>
</tbody>
</table>

Comparison of DA uptake rate in the striatum and nucleus accumbens of 6-, 12-, 18-, and 24-month-old rats. Data represent the mean ± S.E.M.

**Fig. 7.** Effect of locally applied nomifensine on electrochemical signals recorded from the striatum. DA was pressure ejected at 5 min intervals. Nomifensine (6 times the concentration of DA) was applied 30 s before an application of DA. As shown, nomifensine pretreatment altered the amplitude and the time course of the DA signal.

**Fig. 8.** Modulation of DA uptake by nomifensine, desipramine, and citalopram in the striatum (A) and in the nucleus accumbens (B) of young (6-month) and aged (24-month) rats. Data are mean ( +/- S.E.M.) values representing the percentage of change from baseline; the baseline signal represents the recording immediately preceding drug application and the “modulated” signal is the electrochemical response directly after drug ejection. In the 6-month-old rats, nomifensine significantly (***p < .001) increased the signal amplitude in both the striatum (n = 98) and the nucleus accumbens (n = 61). In aged rats (24 months), nomifensine exhibited a significant (*p < .05) but reduced effect on signal amplitudes within the striatum (n = 68) and the nucleus accumbens (n = 43), compared with its effect in the young group. Neither desipramine (6-month striatum, n = 26; nucleus accumbens, n = 12; 24-month striatum, n = 19; nucleus accumbens, n = 10) nor citalopram (6-month striatum, n = 14; nucleus accumbens, n = 7; 24-month striatum, n = 16; nucleus accumbens, n = 7) affected signal amplitudes.
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Differences in DAT \( B_{\text{max}} \) were also noted when comparing the uptake capacity of the striatum to that of the nucleus accumbens, with the nucleus accumbens being less efficient in clearing extracellular DA. The power of in vivo electrochemistry lies with its ability to measure functional events in a dynamic manner. This aspect, combined with the short exposure times of exogenous DA to the DATs within a certain brain region, limits direct comparison of our in vivo \( B_{\text{max}} \) (DA uptake capacity) indices with previously reported in vitro brain region, limits direct comparison of our in vivo exposure times of exogenous DA to the DATs within a certain region values. However, we suggest that relative in vivo uptake capacity) indicies with previously reported in vitro brain region, limits direct comparison of our in vivo exposure times of exogenous DA to the DATs within a certain region.

In addition to conferring information regarding the capacity and rate of DA uptake, the reproducibility of electrochemical recordings permits investigation of pharmacological modulation of DA uptake. Although DAT is the primary mechanism by which DA is removed from the extracellular space, DA uptake has been reported to occur in situ via other monoamine transporters such as the NET and SERT (Izenwasser et al., 1990). Whereas the relative abundance of serotonergic and noradrenergic content within the striatum and the nucleus accumbens is less than 10% (Schenk et al., 1993), it was important to determine the relative contribution of DAT, NET, and SERT in the DA uptake process across age groups and regions. This was accomplished by pretreating DAergic terminals with uptake inhibitors selective for specific transporters. Only application of nomifensine was found to increase the concentration and duration of DA within the extracellular space, supporting the hypothesis that the DA uptake measures are recording the removal of DA by DA transporters and not by SERTs or NETs.

Nomifensine inhibited DA uptake in both the striatum and the nucleus accumbens to a larger extent in the young (6-month) rats compared with the aged (24-month) rats. These results support earlier findings that the effects of high-affinity uptake inhibitors, such as nomifensine, are diminished in the striatum of aged rats (24-month) as compared with young animals (6-month) (Friedemann, 1992; Friedemann and Gerhardt, 1992). The inability of nomifensine to alter uptake in the aged rats confirms data from a recent study investigating behavioral stimulation by various monoaminergic uptake inhibitors (Hebert and Gerhardt, 1978) and suggests age-related changes in the pharmacological properties of the DAT.

It should be noted that metabolism and diffusion, in addition to DA uptake processes, contribute to the shape of the recorded electrochemical signals. Although, extrasynaptic catabolism of DA to homovanillic acid or DOPAC reduces the concentration of DA measured in the extracellular space, we know that homovanillic acid and DOPAC do not contribute to the oxidation current measurements and metabolism appears to play a minor role (Cass et al., 1993). Diffusion of DA in the extracellular space has been measured and modeled previously by our laboratory (Gerhardt and Palmer, 1987) and has been considered to play a minor role in the measured uptake processes. In this study, we examined the clearance of exogenously applied DA in the frontal cortex, a region with little DA uptake capacity and where diffusion is considered to be the primary mechanism of DA removal (Cass and Gerhardt, 1995; Sesack et al. 1998). The average \( T_c \) values for signals within the frontal cortex of 6-month rats, measured at or near \( V_{\text{max}} \) concentrations, averaged 0.014 ± 0.001 \( \mu M/\sec \) (\( n = 4 \)) and those in the 24-month rats averaged 0.017 ± 0.002 \( \mu M/\sec \) (\( n = 5 \); data not shown). Consequently, these data support the hypothesis that diffusion plays a minor role in the measured DA uptake rates, it is consistent across age groups, and contributes less than 10% to the \( T_c \) values indicated in this paper.
In addition to age-related reductions in the density of DAT, deficits in in vivo DA uptake may also involve modifications of the DAT protein and/or compensatory regulation of the uptake process. Possible age-related alterations of DAT include phosphorylation or glycosylation of transporter protein, changes in the membrane potential or fluidity state of the membrane, or modification of DA transporter sulphydryl groups, all of which have been documented and may affect transporter affinity and, in turn, the rate of uptake (Kuhar et al., 1990; Meiergerd and Schenk, 1994; Patel et al., 1994). Further studies are needed to determine whether age-related modifications of the DAT may be responsible for altered DA uptake, or if aging results in decreased levels of functional DA transporter protein.

Age-related reductions in DA uptake in the striatum and the nucleus accumbens do not appear to be due to a reduction in the number of DA nerve terminals in these areas. Previous studies in the same strain and age groups of rats have indicated that DA whole tissue levels within the nigrostrial and mesolimbic cell body and terminal regions are not altered with age (Hebert and Gerhardt, 1998). However, the age-related deficits in DA uptake do occur along a similar time course as deficits in DA release, as well as declines in locomotor activity (Hebert and Gerhardt, 1998). The functional implication of the prolonged presence of DA within the aged rat striatum and/or nucleus accumbens is that DAergic receptor-mediated transmission can remain effective (as in young animals) in the presence of decreased DA release, thereby serving as a compensatory mechanism (Rose et al., 1986; Friedemann and Gerhardt, 1992; Hebert and Gerhardt, 1998). Compelling evidence has demonstrated the remarkable plasticity of the DAergic system (Zigmond et al., 1984; Altar et al., 1987; van Horne et al., 1992). The nigrostrial system has been suggested to adapt its capacity to maintain function despite extensive neuronal loss found in parkinsonian humans and animals (Cass et al., 1995). In Parkinson’s disease, not only is the number of DAT-binding sites reduced as one would suspect after the loss of DA neurons, but what is possibly more significant is that the number of DATs per neuron seems to also be reduced compared with normal aging (Uhl et al., 1994). This would suggest that DA neurons may undergo adaptive changes by reducing the number of DATs on their terminals, thus reducing uptake of DA in an attempt to increase the amount of extracellular DA. Likewise, a down-regulation of functional DA uptake by reducing the number of DATs available or modifying the functional capacity of the ones present may help maintain DAergic transmission in aged animals. Correspondingly, compensatory modulation of DAT may also render the DA-containing terminals of aged rats less susceptible to the degenerative influences of neurotoxins that are incorporated by the high-affinity DA uptake processes (Gainetdinov et al. 1997). Thus, changes in DAT that occur in aged animals may represent a form of compensation to maintain DA neurotransmission in senescence.

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References


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