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# In vivo vulnerability of dopamine neurons to inhibition of energy metabolism

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**Abstract**

In vitro studies indicate that mesencephalic dopamine neurons are more vulnerable than other neurons to impairment of energy metabolism. Such findings may have bearing on the loss of dopamine neurons in Parkinson's disease, in which mitochondrial deficiencies have been identified, but would only be relevant if the selective vulnerability were maintained in vivo. To examine this, rats were stereotaxically administered various concentrations of the succinate dehydrogenase inhibitor, malonate (0.25–4 μmol), either into the left substantia nigra or striatum. One week following injection, dopamine and γ-aminobutyric acid (GABA) levels in the mesencephalon and striatum were measured. Intranigral injection of malonate caused nigral dopamine and GABA to be comparably reduced at all doses tested. The 50% dose level for malonate vs. dopamine and GABA loss was 0.39 and 0.42 μmol, respectively. Tyrosine hydroxylase immunocytochemistry of the midbrains of rats which received an intranigral injection of malonate showed normal staining with 0.25 μmol malonate, but almost complete loss of tyrosine hydroxylase positive nigral pars compacta cells with 1 μmol malonate. Intrastriatal injection of malonate produced a loss of both tyrosine hydroxylase activity and dopamine. In contrast to what was seen in substantia nigra, there was a greater loss of dopamine than GABA in striatal regions nearest the injection site. In striatal regions most distal to the injection site, and which received the lowest concentration of malonate due to diffusion, dopamine levels were significantly reduced with all doses of malonate (0.5–4 μmol), whereas GABA levels were unaffected. Intrastriatal coinfusion of succinate along with malonate completely prevented the loss of dopamine and GABA indicating that succinate dehydrogenase inhibition was the cause of toxicity. These findings indicate that dopamine terminals in the striatum of adult rats are selectively more vulnerable than are the GABA neurons to a mild energy impairment.

*Keywords:* Succinate dehydrogenase, inhibition; Catecholamine; Toxicity; Energy metabolism; Basal ganglia

## 1. Introduction

Dopamine neurons in the substantia nigra pars compacta and their axonal projections in the striatum are the primary neurotransmitter population lost in Parkinson's disease. Recent studies from several laboratories have demonstrated mitochondrial defects in Parkinson's disease. In some studies, the defects were specific to the substantia nigra (Mizuno et al., 1989; Schapira et al., 1989, 1990; Mann et al., 1992), while in others, a more generalized impairment involving muscle and platelet mitochondria was observed (Parker et al., 1989; Shoffner et al., 1991; Bindoff et al., 1991; Benecke et al., 1993). At present, it is not known whether mitochondrial defects are a cause or an effect of Parkinson's disease. Also not known is the reason for the specific targeting of dopamine neurons should a generalized mitochondrial defect be found to be an underlying cause of the disease. Understanding how mild to moderate metabolic stress affects dopamine neurons, however, may provide insight into the loss of these neurons in Parkinson's disease.

The greater vulnerability of mesencephalic dopamine neurons to impairment of energy metabolism has been suggested by several findings. The loss of high affinity uptake of dopamine in synaptosomal preparations was demonstrated to be more sensitive to acute rotenone (mitochondrial complex I inhibitor), antimycin A (complex III inhibitor) and cyanide (complex IV inhibitor) treatment as compared with norepinephrine, γ-aminobutyric acid (GABA) and serotonin uptake suggesting that a constitutive metabolic deficiency existed in dopamine versus other

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neurotransmitter populations (Marey-Semper et al., 1993). Dopamine neurons in mouse mesencephalic cultures were three times more sensitive than mesencephalic GABA neurons or striatal GABA or cholinergic neurons to a sequential exposure to rotenone and glutamate (Marey-Semper et al., 1995), also suggesting an inherent vulnerability of dopamine neurons to energy impairment. Consistent with the findings of Marey-Semper et al. (1993, 1995), our laboratory recently reported that dopamine neurons in rat mesencephalic culture were more sensitive to a mild metabolic stress produced by inhibiting the Krebs cycle enzyme succinate dehydrogenase with the reversible, competitive inhibitor, malonate (Zeevalk et al., 1995). The purpose of the present work was to examine the relative vulnerability of dopamine neurons in vivo to energy impairment. Sensitivity to malonate was examined at the level of the cell body in the nigra and at its axonal projection sites in the striatum. In each case, the vulnerability of the dopamine population to malonate was compared with the GABA population.

## 2. Material and methods

### *2.1. Animal handling and treatment*

Male Sprague-Dawley rats (350–400 g; approximately 3–4 months; Harlan Farms, Indianapolis, IN, USA) were used in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All procedures were approved by the local Animal Care Committee. Rats were grouped 2 per cage in a room maintained at 20–22°C on a 12-h light-dark cycle with food and water available ad libitum. Animals were anesthetized with Brevital (16.7 mg/kg). Vehicle (saline, pH 7.6) or malonate (0.25–4 μmol, pH 7.6) in 1 μl, was stereotaxically injected using a 32 gauge needle into either the substantia nigra or striatum using a David Kopf model 900 stereotaxic apparatus. Needle placement corresponded to the following coordinates from the rat atlas of König and Klippel (1967): for striatal injections, AP 7.2, L 2.6, DV 0.4 and for nigral injections, AP 2.6, L 1.8, D −2.4. Injections were delivered at a rate of 0.5 μl/min. The needle was left in place for an additional 1.5 min prior to its removal. Animals were killed 1 week after treatment. To assess the persistence of dopamine loss, in one study, some animals were allowed to recover for 4 weeks and were used for determination of tyrosine hydroxylase activity and dopamine levels. Two minutes prior to death, rats received a tail vein injection of 3-mercaptopropionic acid to inhibit glutamic acid decarboxylase and prevent GABA synthesis (Korf and Venema, 1983). The brains were removed and cut coronally at the mid-hypothalamic region. The hindbrain was frozen on dry ice and stored at −80°C until the left and right mesencephalic regions were dissected, homogenized in 0.2 M perchloric acid and analyzed for dopamine or GABA. The left and right striata were dissected immediately. The nucleus accumbens was removed ventral to the anterior commissure and the remaining striatum was subdivided into 3 sections of similar mass. Each of the 3 striatal regions was homogenized in 0.2 M perchloric acid and analyzed for dopamine and GABA.

### *2.2. Detection of catecholamines*

Dopamine was measured by high-performance liquid chromatography (HPLC) with electrochemical detection as described previously (Sonsalla et al., 1991). Homogenates were centrifuged and a 15 μl aliquot of the supernatant was injected onto a HPLC (BAS, Wast Lafayette, IN, USA) equipped with a Hewlett-Packard integrator. The mobile phase was comprised of 26 ml of acetonitrile, 21 ml of tetrahydrofuran and 960 ml of 0.15 M monochloroacetate, pH 3.0, containing 50 mg/l of EDTA and 200 mg/l sodium octyl sulfate. Concentrations of dopamine and metabolites were determined by comparison to peak heights of known standards.

### *2.3. Detection of GABA*

Amino acids in the substantia nigra and striatum were extracted with 0.2 M perchloric acid. GABA in the extract was quantified by fluorescent detection of the *o*-phthalaldehyde adduct as described previously (Nicklas and Browning, 1983). Briefly, prior to derivatization, extracts were neutralized to pH 5 with $K_2CO_3$. A Beckman HPLC system Gold Model 338 fitted with a Beckman 175 fluorometer was used in the separation and detection. The derivatives were separated on a Beckman, 5 μm, $C_{18}$ Ultrasphere ODS column. Samples were reacted with an equal volume of *o*-phthalaldehyde reagent and injected at 1.25 min. The programmed gradient elution was formed from 2 buffers: A, 0.1 M sodium acetate, pH 5.9 in 10% methanol, and B, 80% methanol. Quantitation was by comparison to known standards of amino acids.

### *2.4. Tyrosine hydroxylase immunocytochemistry*

The brains were immersion fixed overnight in 4% paraformaldehyde. Brains were cryoprotected in a 20% sucrose solution, sectioned at 20 μm and mounted onto gelatin coated slides for staining. Sections from the midregion of the nigra (rostral to caudal) were processed for tyrosine hydroxylase staining using the Elite Avidin-Biotin-Peroxidase Kit (Vector Labs, Burlingame, CA, USA) with diaminobenzidine as peroxidase substrate and counterstained with cresyl violet. Anti-tyrosine hydroxylase antibody was purchased from Eugene Tech and used at a 1:1500 dilution. As a control, the primary antibody was substituted with pre-immune serum. Neuronal staining was absent in primary antibody substituted sections.

### 2.5. Statistics

Results were analyzed by analysis of variance and significance determined by the Student-Newman Keuls multiple comparisons post test.

## 3. Results

Injection of 4-month-old rats with various amounts of malonate into the left substantia nigra produced a similar loss of nigral dopamine and GABA when assayed 1 week after the injection (Fig. 1A). The 50% dose level for malonate-induced loss of dopamine and GABA was 0.39 or 0.42 μmol, respectively. Animals that received greater than 1 μmol malonate had excessive tissue destruction and cavitation in the mesencephalon and were eliminated from the study. Injection of vehicle (saline, pH 7.4) did not produce any loss of dopamine or GABA. In animals receiving 1 μl saline into the left substantia nigra, dopamine levels were $0.85 \pm 0.13$ and $0.80 \pm 0.09$ μg/g tissue ± S.E.M. in left and right mesencephalon, respectively, $n = 4$. Nigral GABA in saline injected animals was $5.78 \pm 0.36$ and $5.41 \pm 0.31$ μmol/g tissue ± S.E.M. in left and right mesencephalon, respectively, $n = 3$.

Damage to the dopamine cell bodies in the substantia nigra by intranigral injection of malonate resulted in a loss of dopamine at their axonal projection sites in the ipsilateral striatum 1 week following the injection (Fig. 1B). Striatal dopamine levels were reduced in the ipsilateral striatum by 27% with 0.5 μmol malonate and 64% with 1 μmol malonate delivered intranigrally.

Table 1
Succinate reversal of malonate-induced toxicity

| | *n* | Dorsal | Middle | Ventral |
|---|---|---|---|---|
| *Dopamine (μg / g tissue ± S.E.M.)* | | | | |
| Malonate | | | | |
| Left | 5 | 4.5 ± 0.8 [a] | 6.4 ± 0.7 [a] | 10.4 ± 0.9 [a] |
| Right | 5 | 10.6 ± 0.4 | 14.5 ± 0.3 | 14.2 ± 0.3 |
| Malonate plus succinate | | | | |
| Left | 4 | 11.7 ± 1.4 | 15.2 ± 0.8 | 14.0 ± 0.5 |
| Right | 4 | 11.2 ± 0.7 | 16.2 ± 0.5 | 15.0 ± 0.8 |
| *GABA (μmol / g tissue ± S.E.M.)* | | | | |
| Malonate | | | | |
| Left | 4 | 1.64 ± 0.30 [a] | 1.85 ± 0.27 | 2.99 ± 0.51 |
| Right | 4 | 2.50 ± 0.23 | 2.50 ± 0.66 | 2.65 ± 0.73 |
| Malonate plus succinate | | | | |
| Left | 4 | 2.00 ± 0.55 | 2.97 ± 1.11 | 3.32 ± 1.58 |
| Right | 4 | 2.07 ± 0.61 | 2.67 ± 1.12 | 2.72 ± 1.61 |

Malonate (1 μmol) was infused into the left striatum with or without succinate (3 μmol). Total volume for all injections was 1 μl. Animals were killed 1 week later and the striata rapidly removed and sectioned into 3 masses. Dopamine and GABA in each section were measured as described in Section 2. [a] Different from right side, $P < 0.05$ or better.

The hindbrains of some animals that received an intranigral infusion of malonate (0.25–1 μmol) were immersion fixed 1 week after the injection and immunostained for tyrosine hydroxylase. As shown in Fig. 2, the number of tyrosine hydroxylase positive cells in the substantia nigra pars compacta appeared normal in animals that received 0.25 μmol malonate, whereas 1 μmol malonate caused severe loss of tyrosine hydroxylase positive neurons. The steep dose response was consistent with biochemical indices. Tyrosine hydroxylase positive neurons in the ventral

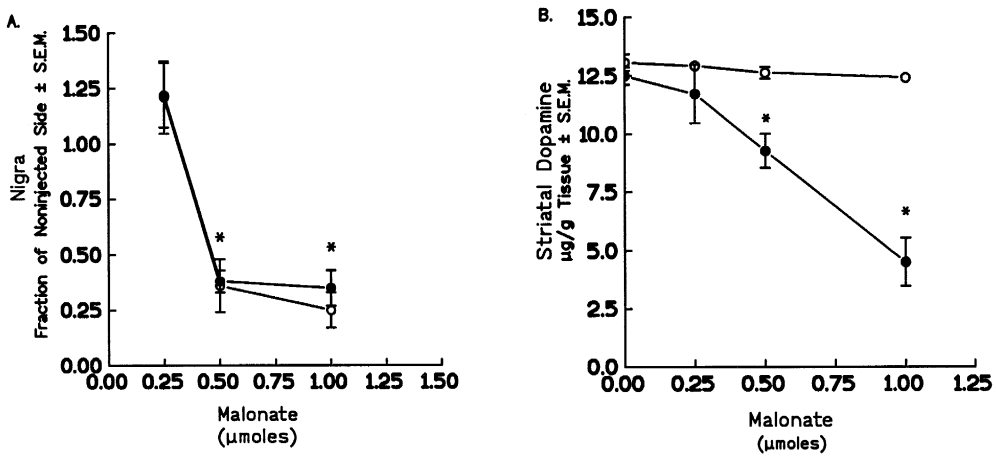



Fig. 1. (A) Rats received an intranigral injection of malonate into the left side and dopamine (DA) and GABA in the mesencephalon were measured at one week. DA and GABA levels in saline injected animals did not differ from levels on the noninjected side in either saline or malonate treated animals. Thus, the dopamine and GABA levels from the left injected sides from each animal were compared to their right, noninjected sides and expressed as a fraction of the noninjected side ± S.E.M. Malonate produced a similar dose dependent loss of DA and GABA. Closed circle, GABA; open circle, DA. (B) Striatal DA levels from rats that had received an infusion of malonate into the left nigra one week prior. Injection of malonate into the nigra resulted in anterograde loss of DA from projection terminals in the striatum. Closed circle, injected side; open circle, noninjected side. *n* is from 3–5 animals per malonate concentration. * $P < 0.05$ or better, different from the noninjected side.

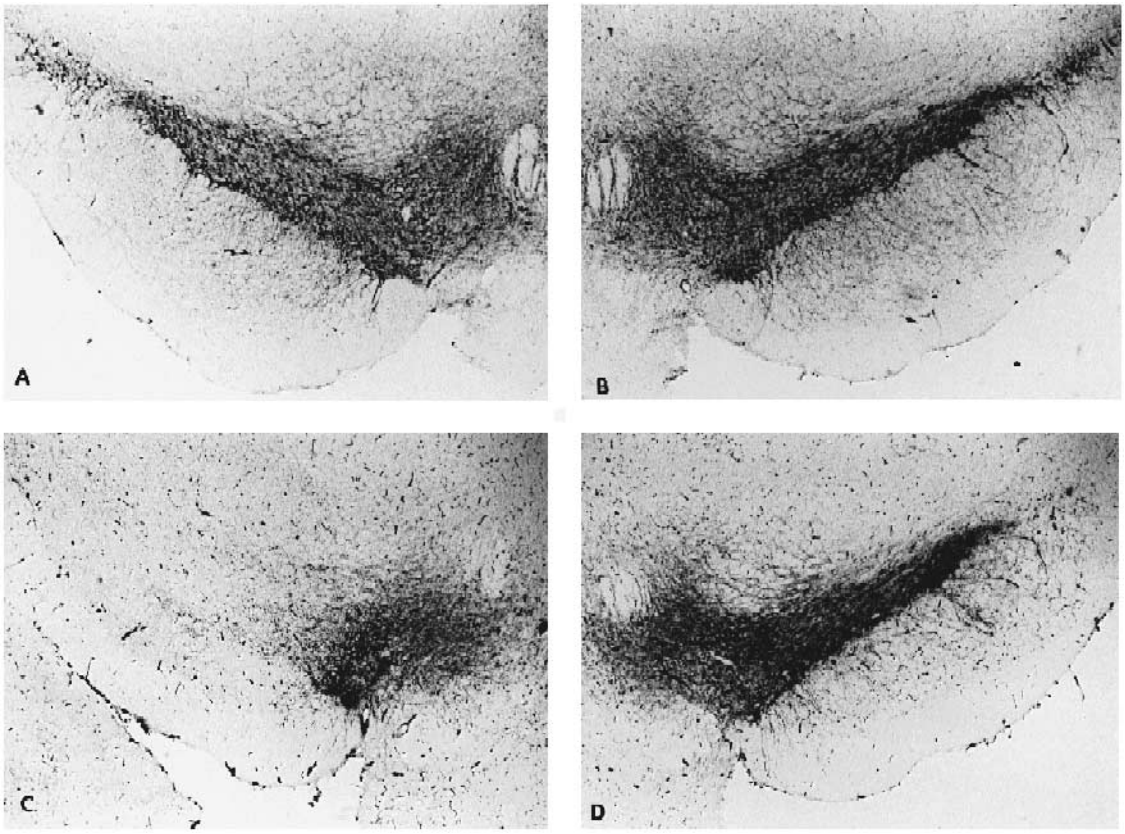
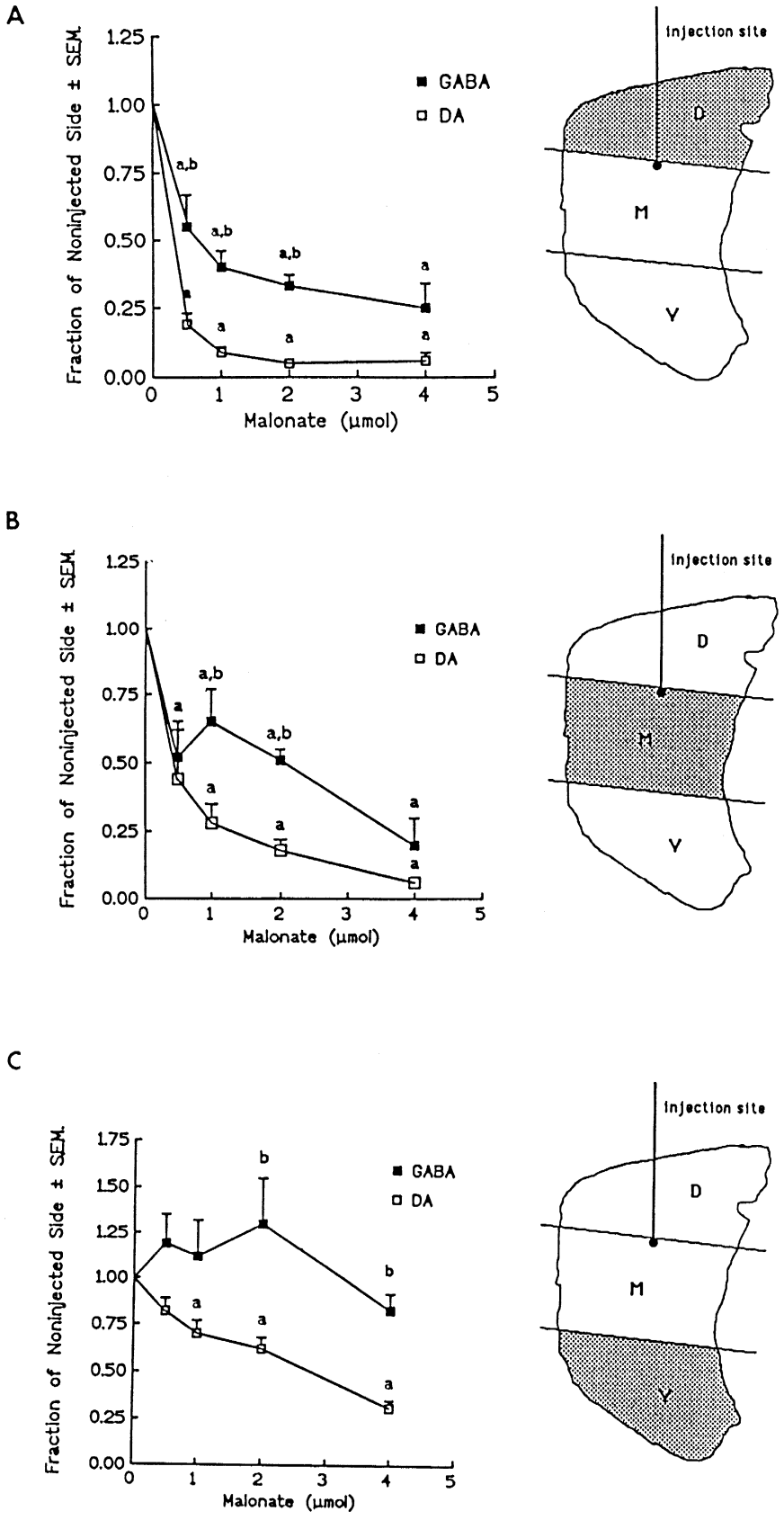



Fig. 2. Light micrographs of rat mesencephalon immunostained for tyrosine hydroxylase, 1 week following intranigral infusion of (A) 0.25 μmol malonate or (C) 1 μmol malonate into the left substantia nigra. (B) and (D) are the corresponding right sides from the same section. Consistent with biochemical data, no obvious loss of tyrosine hydroxylase immunostaining was seen with 0.25 μmol malonate, whereas 1 μmol malonate resulted in almost complete loss of tyrosine hydroxylase-like immunoreactivity in the substantia nigra.

tegmental area, however, appeared normal even at the highest dose of malonate tested (1 μmol, Fig. 2C).

To determine the sensitivity of the dopamine terminals to malonate, rats received an injection of malonate (0.5–4 μmol) into the left striatum and were killed 1 week later. Since the striatum is a large brain structure relative to the substantia nigra, and diffusion from the site of injection will produce a greater concentration gradient throughout the striatum as compared with the nigra, the striatum was subdivided into dorsal, middle and ventral thirds and the site of injection relative to each third was noted (Fig. 3). This was done to permit more meaningful comparisons between the striatum and substantia nigra with regard to sensitivity of the dopamine population to malonate-induced toxicity. The site of injection was identified as the junction between the dorsal and middle thirds of the striatum as shown graphically in Fig. 3. In these 2 regions, malonate produced a dose dependent loss of dopamine. The 50% doses of malonate for dopamine loss were 0.4 and 0.5 μmol in the dorsal and middle thirds, respectively, and were very similar to the 50% dose of malonate needed to produce dopamine loss in the nigra with an intranigral injection. Striatal GABA levels were also significantly reduced in the two striatal regions nearest the injection site (dorsal and middle) with all doses of malonate. However, GABA loss was significantly less than dopamine loss, except at the highest dose of malonate tested (4 μmol). The 50% dose of malonate for loss of GABA in the dorsal third of the striatum was approximately 0.8 μmol, and in the middle third, 2 μmol malonate. The ventral third of the striatum was the region most distal to the site of injection and thus received the lowest concentration of malonate due to diffusion. In this region, dopamine loss revealed a more shallow dose response. Significant loss was found with 1

Fig. 3. Rats received an intrastriatal injection of malonate (total amount as indicated in 1 μl) into the left side. The figure to the right denotes the site of injection and the shaded area, the region of the striatum analyzed for dopamine (DA) and GABA content. Both DA and GABA showed a dose dependent loss vs. malonate with the greatest loss occurring near the injection site (D and M) as compared with the striatal area most distal (V). All regions, however, showed a greater loss of DA as compared with GABA. [a] Different from the noninjected side, [b] different from dopamine at the same concentration of malonate, $P < 0.05$ or better. D, dorsal; M, middle; V, ventral.


A
Fraction of Noninjected Side ± SEM
1.25
1.00
0.75
0.50
0.25
0.00
0
1
2
3
4
5
Malonate (µmol)
GABA
DA
a,b
a,b
a,b
a
a
a
a
injection site
D
M
V
B
Fraction of Noninjected Side ± SEM
1.25
1.00
0.75
0.50
0.25
0.00
0
1
2
3
4
5
Malonate (µmol)
GABA
DA
a
a,b
a,b
a
a
a
a
injection site
D
M
V
C
Fraction of Noninjected Side ± SEM
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
1
2
3
4
5
Malonate (µmol)
GABA
DA
b
b
a
a
a
injection site
D
M
V

μmol malonate or greater. In contrast, GABA in the ventral third was not reduced at any dose of malonate (Fig. 3C).

Persistence of damage to dopaminergic terminals was followed in some rats that received an intrastriatal injection of 4 μmol malonate. These animals were allowed to recover for 1 month before measurements of striatal dopamine and tyrosine hydroxylase activity were performed. Tyrosine hydroxylase activity and dopamine content were reduced by 48% and 65%, respectively, as compared with the noninjected striata. Tyrosine hydroxylase activity was $679 \pm 19.9$ vs. $355 \pm 54.3^{a}$ nmol/g per h ± S.E.M. and dopamine levels $14.4 \pm 0.3$ vs. $5.1 \pm 0.8^{a}$ μg/g tissue ± S.E.M. in right and left striata, respectively ([a]different from noninjected side, $P < 0.05$ or better, $n = 11$ animals). The lower levels of striatal dopamine at 1 month indicate persistant damage; however, the loss (65% of the noninjected side) was less than that found at 1 week (85% of the noninjected side) when averaged over the 3 regions of the striatum. This suggests that either some recovery had occurred or that there was upregulation of dopamine in the remaining terminals.

In order to confirm that malonate-induced loss of dopamine and GABA was the result of inhibition of succinate dehydrogenase, some rats received a stereotaxic intrastriatal coinfusion of 1 μmol malonate plus the endogenous substrate for succinate dehydrogenase, succinate (3 μmol). Malonate alone caused a significant loss of dopamine in the dorsal, middle and ventral thirds of the striatum with the greatest loss in the region nearest the injection site (Table 1). GABA loss was significant only in the dorsal region. The competition by succinate for succinate dehydrogenase completely prevented the loss of dopamine and GABA indicating that succinate dehydrogenase inhibition by malonate was responsible for the loss of these neurotransmitter systems. Although the absolute loss of dopamine and GABA was not as great in this group of animals as compared with those used for Fig. 3, there was still a greater loss of dopamine as compared with GABA in the 3 regions of striata, consistent with what was found previously.

## 4. Discussion

In the last few years, many laboratories have reported deficiencies in energy metabolism in patients with Parkinson's disease, thus positing inefficient energy metabolism as a possible cause of the loss of nigral dopamine neurons in this disease (Beal, 1992; DiMauro, 1993). In several of these studies (Parker et al., 1989; Shoffner et al., 1991; Bindoff et al., 1991; Benecke et al., 1993), deficits have been reported in the mitochondria isolated from blood platelets and muscle from Parkinson patients, thus evoking the question of the reason for the specific targeting of dopamine neurons. In vitro studies by Marey-Semper et al. (1993) indicate that dopamine uptake into striatal synaptosomal preparations is more sensitive than other neurotransmitters (i.e., GABA, serotonin, norepinephrine) to the energy depleting effects of several mitochondrial inhibitors, findings which suggest that dopamine terminals may be more susceptible to decreased energy metabolism. Relatively selective toxicity of cultured mouse mesencephalic dopamine neurons, as compared with the mesencephalic GABA and striatal GABA and cholinergic populations, to the sequential exposure to the mitochondrial inhibitor rotenone and the excitotoxin, glutamate (Marey-Semper et al., 1995), further supports the hypothesis that dopamine neurons may be inherently more vulnerable than other neuronal populations to mild impairments of energy metabolism. Consistent with these findings, our laboratory reported that dopamine neurons in rat mesencephalic cultures were more vulnerable than GABA neurons to the toxic consequences of malonate (Zeevalk et al., 1995). Intrastriatal administration of malonate has been shown to lesion the striatum (Greene et al., 1993) and produce loss of GABA and substance P, while sparing somatostatin (Beal et al., 1993a). The effect of malonate on striatal dopamine, however, was not evaluated in these studies. The present study is the first to demonstrate that in vivo, dopamine terminals in the striatum show a greater vulnerability than the GABA population to impairment of energy metabolism by malonate. Similar to what Greene and Greenamyre (1995) first reported, the toxicity due to malonate was the result of succinate dehydrogenase inhibition since coinfusion with succinate, the endogenous substrate for succinate dehydrogenase, completely prevented neurotransmitter loss.

For studies of the effects of intrastriatal infusion of malonate, the striatum was subdivided into 3 regions of similar mass in order to better evaluate the dose dependent effects of intrastriatal malonate-induced toxicity and to provide a more meaningful comparison to intranigral administration of malonate. It was thought that the small injection volume (1 μl), the high concentration of the injected solution, and the large tissue mass of the striatum would yield a distinct concentration gradient throughout the region. The region closest to the injection site should most closely compare with the intranigral injection, whereas the region farthest from the injection site, due to diffusion, would contain the lowest concentration of malonate and would better differentiate sensitivities to energy impairment. Results support this rationale. In the ventral region, the region most distal to the site of injection, dopamine loss was dose dependent with an expected greater 50% toxic dose for malonate as compared with those regions nearest the injection site (0.4 and 0.5 μmol in the dorsal and middle regions, respectively). Part of the difference in dopamine sensitivity between the ventral region and dorsal/middle regions might also be due to inclusion in the ventral region of a small section of nucleus accumbens just dorsal to the anterior commissure. Dopamine

projections from the ventral tegmental area to the nucleus accumbens are known to be more resistant to *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity and are relatively spared in Parkinson's disease (Langston et al., 1984; Schneider et al., 1987; Yamada et al., 1990). The ventral third of the striatum used for these studies was estimated to include approximately 25% nucleus accumbens. However, it is unlikely that this would account entirely for the 6–8-fold difference in sensitivity of dopamine to malonate in the ventral versus other regions. GABA levels in the ventral region, on the other hand, were not reduced with any dose of malonate, indicating a lack of toxicity to the GABA population when concentrations of malonate are low. The difference in sensitivity of the GABA population in the ventral versus other regions also argues that a lowered concentration of malonate was responsible for the lessened effect on dopamine and GABA in this region. Striatal regions nearest the injection site (dorsal and middle) displayed a discrimination between dopamine and GABA loss at the lower doses of malonate (0.5–2 μmol); dopamine loss was significantly greater than that of GABA. However, at very high doses of malonate (4 μmol), dopamine and GABA were similarly reduced. The above findings demonstrate that when malonate concentrations are low and, therefore, inhibition of succinate dehydrogenase is less, dopamine terminals in the striatum exhibit a greater susceptibility than the GABA population. These results are consistent with the effects of intrastriatal administration of 3-nitropropionic acid, an irreversible inhibitor of succinate dehydrogenase. 3-Nitropropionic acid, injected into the striatum, produced nonspecific loss of several neurotransmitter and neuropeptide populations (Beal et al., 1993b). At low concentrations of 3-nitropropionic acid, however, only dopamine levels were significantly reduced (Beal et al., 1993b). Similar results were reported by Brouillet et al. (1993), in which dopamine, as compared with GABA, substance P-like immunoreactivity and neuropeptide Y-like immunoreactivity, showed greater sensitivity to an intrastriatal injection of 3-nitropropionic acid. Intrastriatal injection of malonate into mouse striatum was recently reported to produce a dose dependent loss of dopamine and tyrosine hydroxylase activity (Albers et al., 1996). Consistent with our findings in the rat, striatal GABA loss in the mouse decreased to a lesser extent (unpublished observations). In contrast with the dopamine loss produced by direct intrastriatal injection of 3-nitropropionic acid or malonate, systemic delivery of lower concentrations of 3-nitropropionic acid administered over 5 days caused an increase in dopamine turnover (dopamine levels unchanged and increased homovanillic acid and 3,4-dihydroxyphenylacetic acid), but significant loss of GABA (Beal et al., 1993b). Secondary effects of systemic 3-nitropropionic acid administration have been described (Hamilton and Gould, 1987), i.e., decreased blood arterial pH and blood bicarbonate levels and loss of blood-brain barrier integrity, which could confound interpretation of any differences in central vs. systemic administration of 3-nitropropionic acid.

When comparing intranigral and intrastriatal sensitivities to malonate, several questions can be raised. Does the cell body versus terminal region of dopamine neurons display similar sensitivities to malonate inhibition? The data suggest that this is the case. When the 50% dose of malonate for dopamine loss is compared in the nigra (0.39 μmol) versus striatal regions nearest the injection site (dorsal, 0.4 μmol; middle, 0.5 μmol), doses are comparable. The extremely steep dose response, however, makes subtle discrimination difficult. Examination of the sensitivity of nigral and striatal GABA is more problematic. Although the dopamine cell body versus terminal regions are well separated anatomically, such resolution is not possible with the GABA population. GABA cell bodies and terminals are contained within the striatum and nigra. Results none the less suggest that the midbrain GABA elements may be more sensitive to malonate versus striatal GABA elements. Comparison of GABA loss with 0.5 or 1 μmol malonate into the nigra versus striatum showed significantly greater loss in the nigra as compared with any of the 3 regions examined in the striatum. Even in the region nearest the injection site, the 50% dose for GABA loss was approximately twice that in the nigra (0.8 μmol in dorsal striatum versus 0.42 μmol in the nigra). Since the midbrain GABA population is made up predominantly of striatal efferent terminals and some GABAergic interneurons, whereas the striatal GABAergic population consists mainly of GABAergic cell bodies, one possible explanation for the greater sensitivity of nigral vs. striatal GABA is that the GABAergic terminals are more sensitive than their cell bodies to malonate exposure. Also of interest is the issue of the relative sensitivity of the dopamine versus GABA population in the nigra. Results from intranigral administration of malonate suggested that, at the level of the midbrain, dopamine and GABA elements are equally sensitive. However, the small tissue mass of this region and proximity to the injection site may not have produced a sufficiently mild enough metabolic stress to allow discrimination. Thus, a definite conclusion as to relative sensitivity of the dopamine and GABA population in the midbrain would be premature.

In summary, the findings from this study demonstrate that the striatal dopamine terminals in adult rats are selectively more vulnerable than the striatal GABA population to a mild inhibition of oxidative phosphorylation. Several possibilities may account for the enhanced vulnerability of the dopamine population. Excitotoxicity has been demonstrated to be a component of malonate toxicity in the striatum in vivo (Beal et al., 1993a; Greene et al., 1993) and in mesencephalic cultures in vitro (Zeevalk et al., 1995). Although dopamine terminals in the striatum are not very sensitive to excitotoxin exposure (Smith et al., 1987), retrograde involvement may occur at the level of the cell body making these neurons more vulnerable to a

metabolic challenge. Based on the findings of Marey-Semper et al. (1993, 1995) (see Section 1), it has been suggested that the nigrostriatal dopaminergic neurons may possess a constitutive metabolic deficiency relative to other neurons which renders them more vulnerable to energy stress. On the other hand, dopamine neurons and their terminals may be more sensitive to a mild deficiency in energy metabolism via enhanced production of free radicals generated by mitochondrial impairment. This free radical load in addition to the oxidative stress already imposed on dopamine neurons through autoxidation (Graham et al., 1978) and enzymatic metabolism of dopamine (Maker et al., 1981; Hastings and Zigmond, 1994; Hastings, 1995) may poise the dopamine terminal at a lower threshold for irreversible damage when energy metabolism is less than optimal. Alternatively, the heterogeneity of GABAergic neurons in the striatum may be reflected in a differential sensitivity to energy impairment so that when viewed as a single population appear less susceptible than the dopamine terminals to metabolic inhibition. Our findings do not distinguish between the above possibilities, but do support an enhanced vulnerability of the dopamine population to energy impairment.

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