Neurochemical and Histologic Characterization of Striatal Excitotoxic Lesions Produced by the Mitochondrial Toxin 3-Nitropropionic Acid

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An impairment of energy metabolism may underlie slow excitotoxic neuronal death in neurodegenerative diseases. We therefore examined the effects of intrastriatal, subacute systemic, or chronic systemic administration of the mitochondrial toxin 3-nitropropionic acid (3-NP) in rats. Following intrastriatal injection 3-NP produced dose-dependent striatal lesions. Neurochemical and histologic evaluation showed that markers of both spiny projection neurons (GABA, substance P, calbindin) and aspiny interneurons (somatostatin, neuropeptide Y, NADPH-diaphorase) were equally affected. Subacute systemic administration of 3-NP produced age-dependent bilateral striatal lesions with a similar neurochemical profile. However, in contrast to the intrastriatal injections, striatal dopaminergic afferent projections were spared. Both freeze-clamp measurements and chemical shift magnetic resonance spectroscopy showed that 3-NP impairs energy metabolism in the striatum *in vivo*. Microdialysis showed no increase in extracellular glutamate concentrations after systemic administration of 3-NP. The lesions produced by intrastriatal injection or systemic administration of 3-NP were blocked by prior decortication. However, the NMDA antagonist MK-801 did not block the effects of intrastriatal 3-NP, consistent with a non-NMDA excitotoxic mechanism. In contrast to subacute systemic administration of 3-NP, chronic (1 month) administration produced lesions confined to the striatum in which there was relative sparing of NADPH-diaphorase interneurons, consistent with an NMDA excitotoxic process. Chronic administration showed growth-related proliferative changes in dendrites of spiny neurons similar to changes in Huntington's disease (HD). These results are consistent with *in vitro* studies showing that mild metabolic compromise can selectively activate NMDA receptors while more severe compromise activates both NMDA and non-NMDA receptors. Chronic administration of 3-NP over 1 month produces selective striatal lesions that replicate many of the characteristic histologic and neurochemical features of HD.

[Key words: mitochondria, excitotoxicity, Huntington's disease, striatum, magnetic resonance spectroscopy]

A large number of studies now suggest that excitatory amino acids play a role in the mechanism of neuronal death in several diseases. Experimental studies in both ischemia and hypoglycemia have shown that there are dramatic increases in extracellular glutamate, and that neuronal death can be blocked by excitatory amino acid antagonists (Choi, 1988). The role of excitotoxic mechanisms in neurodegenerative diseases is more speculative. In these illnesses one has to account for gradually evolving relentless cell death that can progress over 10 or more years. These illnesses, such as Alzheimer's disease, Parkinson's disease, and Huntington's disease (HD), are characterized by circumscribed and selective neuronal death.

One possibility is that a progressive impairment of energy metabolism may secondarily result in slow excitotoxic neuronal death (Albin and Greenamyre, 1992; Beal, 1992). Disruption of ATP synthesis may lead to partial neuronal depolarization with activation of voltage-dependent NMDA receptors and secondary excitotoxic neuronal damage (Novelli et al., 1988). We have been particularly intrigued by the possibility that this mechanism might underlie neuronal degeneration in HD. Several studies have reported decreased glucose metabolism and abnormalities in mitochondrial electron transport enzymes in HD (reviewed in Beal, 1992). We have recently obtained the first *in vivo* evidence of impaired oxidative energy metabolism in HD by demonstrating increased concentrations of lactate in cerebral cortex using nuclear magnetic resonance spectroscopy (Koroshetz et al., 1992). In addition, studies in both rodents and primates show striking similarities between striatal lesions induced by NMDA agonists and the neurochemical and histologic features of HD (Beal, 1992; Ferrante et al., 1993). Further evidence in support of an NMDA excitotoxic mechanism in HD is the finding of a preferential loss of striatal NMDA receptors (Young et al., 1988), although a latter study showed depletion of both NMDA and non-NMDA receptors (Dure et al., 1991).

We therefore examined the mechanism of neuronal degeneration produced by the known mitochondrial toxin 3-nitropropionic acid (3-NP). 3-NP is an irreversible inhibitor of succinate dehydrogenase that inhibits both the Krebs cycle and complex II of the mitochondrial electron transport chain (Alston et al., 1977; Coles et al., 1979). It is of particular interest since 3-NP produces selective basal ganglia lesions and delayed dystonia when accidently ingested in human (Ludolph et al., 1991). In the present study we examined the neurochemical and histologic effects of both intrastriatal and systemic administration of 3-NP. We also determined its effects on energy metab-

olism using both *in vivo* water-suppressed proton chemical shift magnetic resonance imaging techniques, and direct measurements of ATP and lactate using the freeze-clamp technique. To determine whether lesions were caused by an excitotoxic mechanism, we examined the effects of prior decortication, which removes the corticostriatal glutamatergic input, as well as the effects of treatment with the noncompetitive NMDA antagonist MK-801. Last we examined whether chronic administration over 1 month could produce slow progressive neuronal degeneration showing the neuronal selectivity and neurochemical features of HD.

Materials and Methods

Male Sprague–Dawley rats (Charles River, Cambridge, MA) weighing 175–200 gm were anesthetized with pentobarbital (50 mg/kg, i.p.). 3-NP (Aldrich, Milwaukee, WI) was dissolved in water and the pH adjusted to 7.4 with NaOH. Intrastriatal injections of varying doses were made in the left striatum in a volume of 1 μl using a blunt-tipped 30 gauge Hamilton syringe (Beal et al., 1989, 1991). All injections were made over 1 min and the needle was left in place for 1 min before being slowly withdrawn. Nine to 10 animals were examined in each group. Prior decortication was performed by removing frontoparietal cortex by gentle suction 1 week before the intrastriatal injections. Hemostasis was achieved with Gelfoam. To examine the effects of MK-801 on 3-NP lesions, animals were administered MK-801 (5 mg/kg, i.p.) or saline 30 min before injection and then for every 4 hr for 24 hr.

Subacute systemic administration of 3-NP was made at a dose of 20 mg/kg subcutaneously by Alzet pump for 5 d. Both 1- and 4-month-old animals were examined. 3-NP was made up in water at 20 mg/kg/ml and adjusted to pH 7.4. For chronic administration 20 rats aged 12 months were treated with 3-NP for 1 month at a dose of 12 mg/kg/d by subcutaneous Alzet pump (Alzet Corp., Palo Alto, CA). Six controls received normal saline. In the case of the MK-801 experiment, animals were killed at 24 hr. Following intrastriatal administration of 3-NP animals were killed at 1 week. In the experiments in which animals received subacute 3-NP by Alzet pump, the pump was removed when animals were acutely ill (5–7 d), and the animals were then killed. The 12-month-old animals treated with 12 mg/kg/d of 3-NP for 1 month by Alzet pump were killed 1 week following the 3-NP administration. Only half the animals treated at this dosage survived 1 month. The remaining animals became acutely ill after 1–2 weeks and had to be killed at this time point. These animals showed large lesions.

For neurochemical analysis the left and right striata were rapidly dissected from a 2-mm-thick slice on a chilled glass plate as previously described (Beal et al., 1989, 1991). Tissue samples were sonicated, extracted, and assayed for somatostatin-like immunoreactivity (SLI), neuropeptide Y–like immunoreactivity (NPYLI), and substance P–like immunoreactivity (SPLI) as reported previously (Arnold et al., 1982; Beal et al., 1986b; Beal and Mazurek, 1987). GABA was measured by HPLC with electrochemical detection (Ellison et al., 1987). Monoamines and their metabolites were quantified by HPLC with 16-electrode electrochemical detection (Beal et al., 1990). Protein was measured with a fluorometric assay (Bohlen et al., 1973).

The effects of intrastriatal injections of 3-NP on ATP and lactate concentrations were examined using the freeze-clamp procedure and focused microwave irradiation for 0.6 sec at 5.4 kW. Six 1–2-month-old animals in each group were examined at 3 hr after intrastriatal injection of saline or 1000 nmol of 3-NP. In addition, the effects of systemic administration of 30 mg/kg of 3-NP intraperitoneally to 4-month-old animals were examined at 4 hr. ATP was measured by the luciferin-luciferase assay (Lust et al., 1981) and lactate was measured with an enzymatic assay (Schon, 1965).

The effects of systemic administration of 3-NP on striatal extracellular concentrations of glutamate were examined using the microdialysis technique. Male Sprague–Dawley rats (250–350 gm) were anesthetized with pentobarbital (50 mg/kg, i.p.) on the day of surgery and placed in a Kopf stereotaxic frame. Stainless steel guide shafts (21 gauge tubing) were implanted in the striatum and secured with dental cement. Animals were allowed to recover for 24 hr before probe insertion. Probes of concentric design (4 mm exposed membrane; 6000 MW cutoff) were perfused with modified Ringer's solution containing 2.7 mM K$^+$, 145 mM Na$^+$, 1.2 mM Ca^{2+}, 1.0 mM Mg^{2+}, and 2 mM phosphate, pH 7.40,

at a flow rate of 2.0 μl/min using a CMA/100 microinjection pump; samples were collected at 20 min intervals. Three animals in each group were treated with either 3-NP at a dose of 30 mg/kg intraperitoneally or normal saline. Samples were collected for 5 hr. Glutamate concentrations in the dialysates were measured by HPLC with electrochemical detection (Ellison et al., 1987).

The ability of both local and systemic administration of 3-NP to induce impairment of oxidative metabolism was evaluated in real time with water-suppressed proton chemical shift magnetic resonance imaging (Jenkins et al., 1991). 3-NP was administered at a dose of 40 mg/kg for 2 d to 1-month-old rats. Rats were imaged at 4.7 T using a GE Omega CSI imager with a 30 mm bird cage coil using a double-phase encode technique with a binomial refocusing pulse. Slice thickness was 8 mm. Phase encoding was performed in two dimensions to give a three-dimensional data set. The entire echo was collected using a TR of 2.15 sec and TE of 272 msec with an inversion pulse for lipid suppression (TI = 210 msec), eight averages, and the field of view was 50 × 50 mm with 16 × 16 phase-encoding steps. A two-dimensional image of lactate was obtained. The parameters for the water images were TR/TE = 3200/80 msec, with a slice thickness of 2.5 mm.

For histologic evaluation animals were deeply anesthetized 1 week after lesioning and perfused with ice-cold saline, followed by phosphate-buffered 4% paraformaldehyde (Beal et al., 1989, 1991). Brains were sectioned at 50 μM intervals on a freezing microtome. Sections were stained for Nissl substance using cresyl violet or examined using NADPH-diaphorase histochemistry. Combined staining for Nissl substance and NADPH-diaphorase was performed on select sections. Some sections were also stained immunohistochemically for calcium-binding protein (CaBP), somatostatin, or glial fibrillary acidic protein (GFAP), and neuronal cell adhesion molecule (NCAM) using a previously described methodology (Beal et al., 1989, 1991). Golgi studies were carried out as previously described (Ferrante et al., 1991). Quantitation of striatal neurons in combined Nissl/NADPH-diaphorase–stained sections from both 3-NP– and saline-treated animals was carried out in 350 × 500 μm fields centered around the lesions at a magnification of 400×, using an eyepiece graticule. Six to eight counts were made in each animal, and were averaged. Counts were expressed as the ratio of NADPH-diaphorase positive neurons to total Nissl-stained neurons. The results are expressed as the mean ± SEM.

Neurochemical measurements on the lesioned side were compared with those on the unlesioned (control) side and expressed as percentages of control values. The unlesioned sides did not differ from saline-injected controls (Beal et al., 1989, 1991). The results are expressed as mean ± SEM values. Both neurochemical and histologic comparisons were made using Student's *t* test (two tailed) or one-way analysis of variance.

Results

The effects of increasing doses of intrastriatal injections of 3-NP on striatal neurochemical markers are shown in Figure 1. There were dose-dependent decreases in GABA, SPLI, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA). Dopamine was depleted to a greater extent than the other neurochemical markers. SLI and NPYLI were also significantly ($P < 0.01$) depleted to 61.5 ± 13.6% and 63.7 ± 11.9% of control following a dose of 500 nmol of 3-NP. Prior decortication significantly protected against decreases in GABA, SPLI, dopamine, and DOPAC; however, the effects on dopamine and DOPAC were only partial. The effects of MK-801 on lesions induced by intrastriatal injections of 500 nmol of 3-NP at 24 hr are shown in Figure 2. There was no significant protective effect.

The neurochemical effects of subacute systemic administration of 3-NP at a dose of 20 mg/kg/d by Alzet pump in 1- and 4-month-old animals are shown in Figure 3. Controls received normal saline and measurements are expressed as percentage of control. There were no significant effects in 1-month-old animals. The 4-month-old animals, however, showed significant 25% depletions of GABA, substance P, somatostatin, and neuropeptide Y. Dopamine and 5-HT concentrations were spared
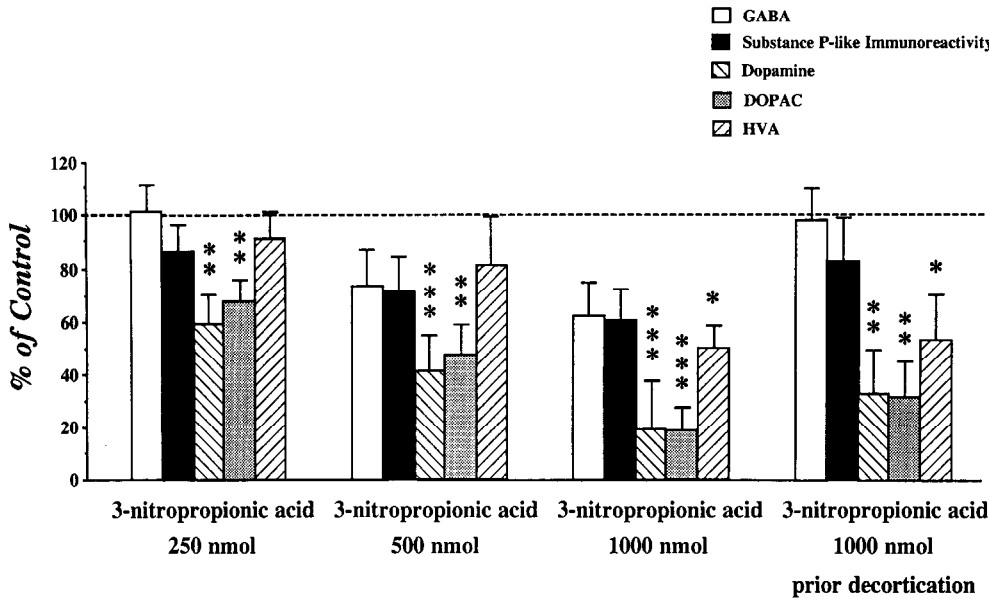
Figure 1. Neurochemical effects of intrastriatal injection of increasing doses of 3-NP, and the effect of prior decortication. Neurochemical measurements were compared with those of the unlesioned (control) contralateral striatum and expressed as percentage of control ± SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

but there were significant increases in DOPAC and HVA, consistent with increased dopamine turnover.

The effects of local injection of 1000 nmol of 3-NP on ATP and lactate concentrations at 3 hr were examined using the freeze-clamp technique. There was a significant ($P < 0.01$) decrease in ATP to 45.9 ± 14.2% of control and a significant ($P < 0.001$) threefold increase in lactate to 300 ± 13% of control. ATP concentrations in controls were 14.2 ± 0.4 nmol/mg protein and lactate concentrations were 0.016 ± 0.002 μmol/mg of protein. Focused microwave irradiation confirmed a significant ($P < 0.01$) increase in lactate to 289 ± 14% of control at 3 hr. Four hours after systemic administration of 3-NP (30 mg/kg, i.p.), there were significant ($P < 0.05$) increases in lactate concentrations in the striatum as compared with saline controls (203 ± 19%), but there were no significant changes in the cerebral cortex, hippocampus, and cerebellum.

Two-dimensional lactate imaging using water-suppressed chemical shift magnetic resonance imaging confirmed a focal increase in lactate at the injection site 3 hr after injection of

3-NP. T_2-weighted imaging showed increased signal in the striatum following local administration of 3-NP. Following systemic administration of 3-NP at a dose of 40 mg/kg/d intraperitoneally in 1-month-old animals, focal increases in lactate concentrations were seen over both striata at 2 d (Fig. 4). Lactate concentration estimates in four different animals were 1.59 ± 0.25 mM in the left striatum and 1.62 ± 0.28 mM (mean ± SD) in the right striatum. These values are two- to threefold above baseline levels. In most cases lactate signal intensity preceded the development of lesions on T_2-weighted scans in animals treated with systemic injections. Similar results were obtained with a dose of 20 mg/kg/d in 4-month-old animals.

Microdialysis studies were carried out to determine if 3-NP results in striatal glutamate release. Samples were collected for 20 min intervals following intraperitoneal administration of 30 mg/kg of 3-NP. Administration of this dose of 3-NP resulted in no significant alterations in striatal glutamate concentrations as compared with controls who received normal saline (Fig. 5). Systemic administration of the same dose of 3-NP (30 mg/kg,
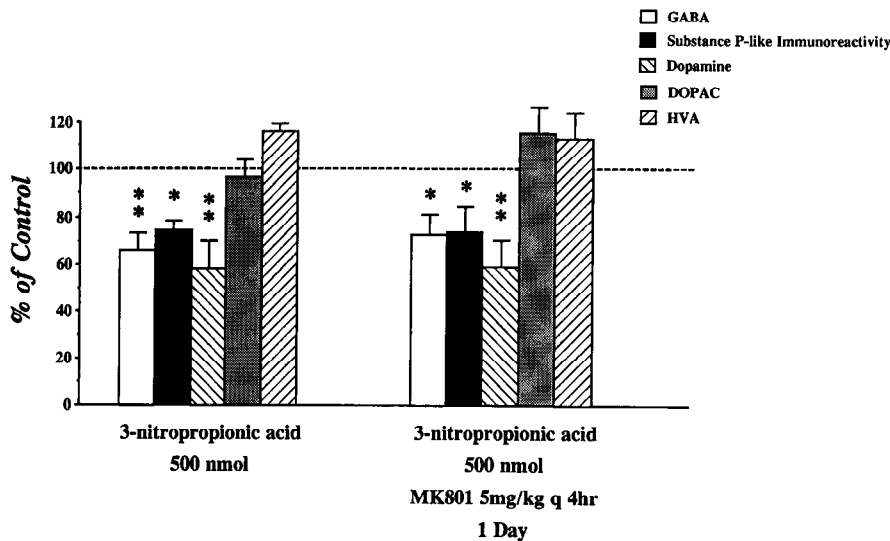

Figure 2. Effects of MK-801 treatment on GABA, substance P, and dopamine and its metabolites at 24 hr. MK-801 showed no protective effects against 3-NP lesions at 24 hr. *, $P < 0.05$; **, $P < 0.01$; as compared with the control side.
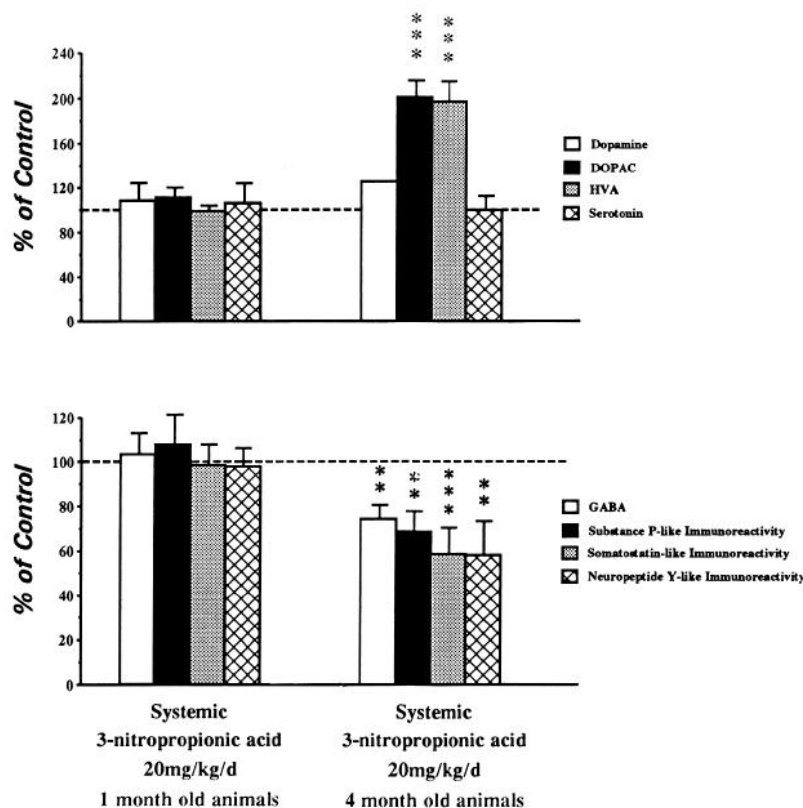
Figure 3. Neurochemical effects of systemic administration of 3-NP, at 20 mg/kg/d, or saline to 1- and 4-month-old rats for 5 d by subcutaneous Alzet pump. Neurochemical measurements are expressed as percentage of control ± SEM. Comparisons were made by nonpaired Student's *t* test in each group. As compared with saline controls there were no significant effects in 1-month-old animals. In 4-month-old animals there were significant depletions in markers of striatal neurons and significant increases in dopamine metabolites, but no significant effect on dopamine and 5-HT. **, $P < 0.01$; ***, $P < 0.001$.

i.p.) to 4-month-old rats resulted in a significant twofold increase in lactate at 4 hr in the striatum using the freeze-clamp method, but no change in cerebral cortex, hippocampus, or cerebellum (data not shown). The striatal lactate concentrations in the 3-NP–treated animals were 0.042 ± 0.013 μmol/mg of protein versus 0.017 ± 0.004 μmol/mg of protein in controls ($P < 0.05$, $n = 6$).

Histologic examination of 4-month-old rats injected with 500 nmol of 3-NP ($n = 4$) showed extensive neuronal loss and gliosis, which was confirmed by GFAP staining. There was sparing of some large neurons at the lesion site, but NADPH-diaphorase staining and CaBP showed a comparable neuronal loss throughout the lesioned area. Examination of rats that received subacute systemic administration of 3-NP at a dose of 20 mg/kg/d in-
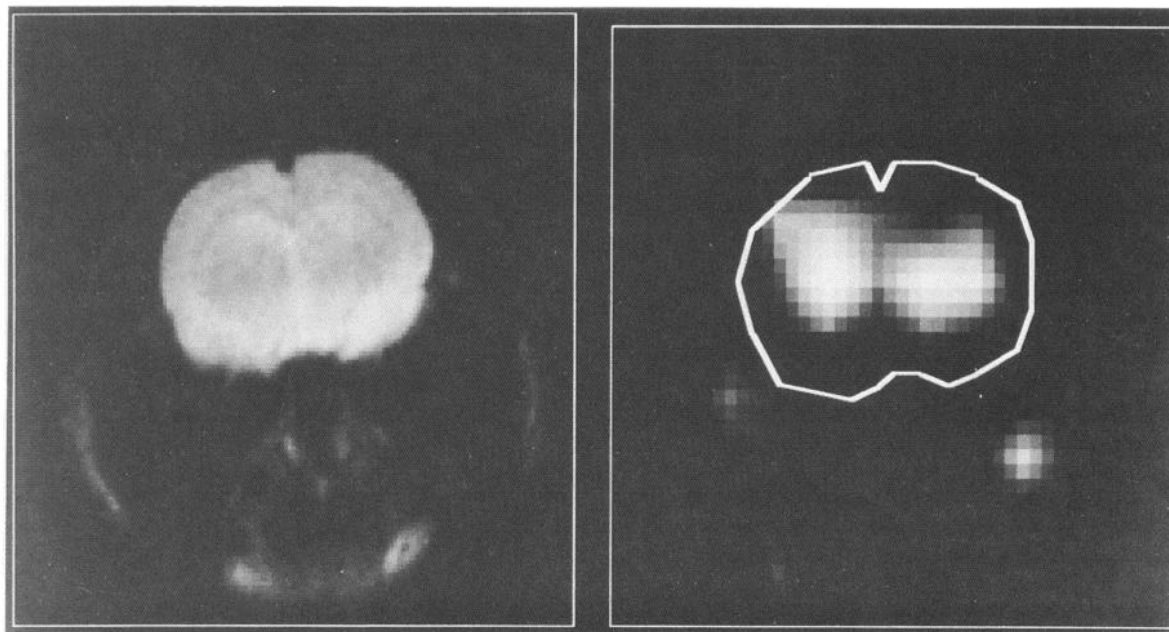

Figure 4. Water-suppressed chemical shift magnetic resonance imaging (TE/TR of 272/2100 msec) following systemic administration of 3-NP to a 1-month-old rat for 3 d at a dose of 40 mg/kg. There is essentially no increase in T_2-weighted water signal in the striata (*left*) at a time in which there are focal lactate accumulations in the striata (*right*).
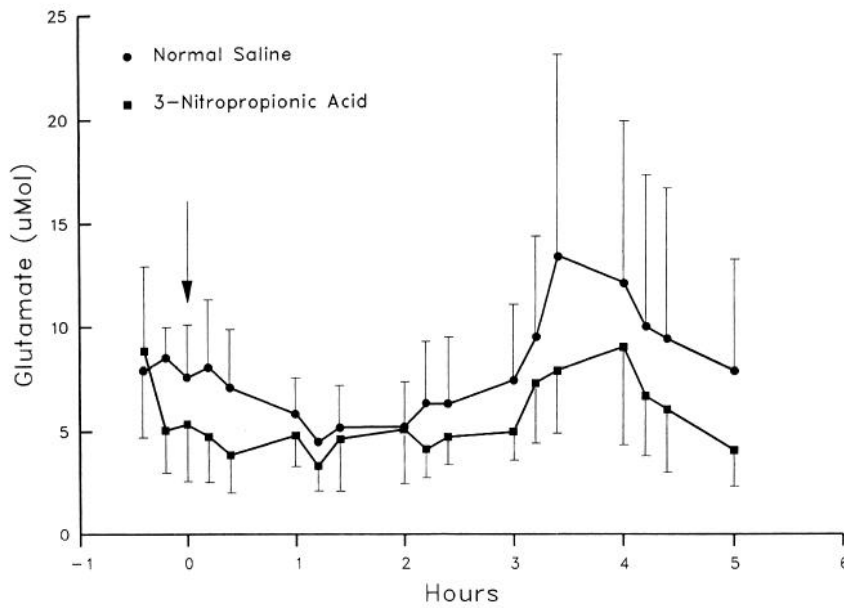
Figure 5. Glutamate concentrations in striatal dialysate after intraperitoneal injection of 30 mg/kg of 3-NP or normal saline. As compared with normal saline, there were no significant increases in glutamate concentrations at any time point over 5 hr.

traperitoneally for five d ($n = 5$) showed bilateral striatal lesions. Nissl stains showed loss of neurons and gliosis that was confirmed by GFAP staining (Fig. 6). There was a depletion of both somatostatin-immunoreactive and NADPH-diaphorase neurons within the lesioned striata, with no selective sparing relative to the loss of CaBP neurons. In some animals that received subacute administration of 3-NP, there was gliosis and neuronal loss in area CA1 of the hippocampus in addition to striatal lesions (Fig. 7).

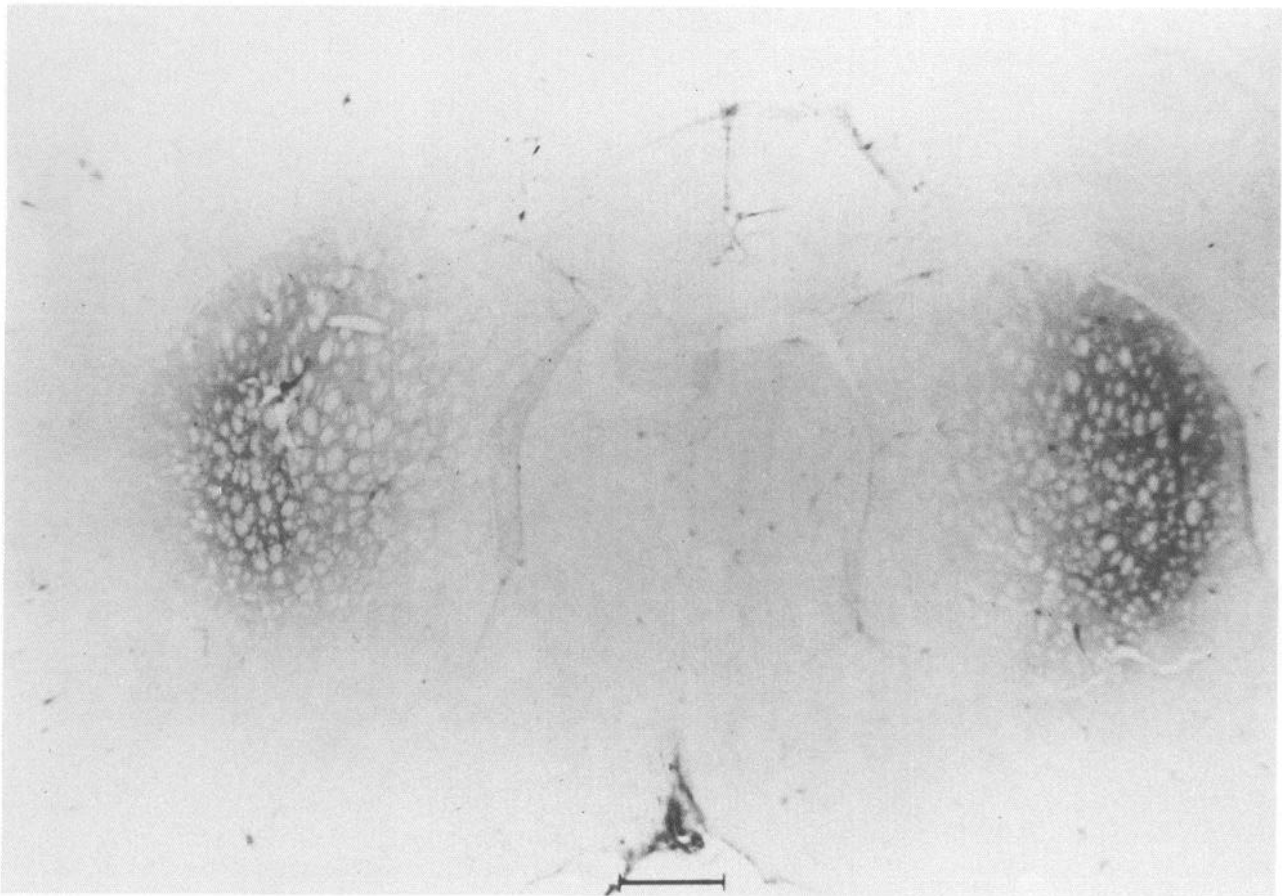Following subacute administration of 3-NP, animals devel-

Figure 6. GFAP-stained section at the level of the anterior striatum following subacute systemic administration of 3-NP. Focal increases in GFAP staining are seen in the dorsolateral striatum bilaterally.

Figure 7. GFAP-stained section of the hippocampus following subacute systemic administration of 3-NP. There is a focal increase in GFAP staining in the hippocampus that is particularly marked in the CA1 field.

oped dystonic posturing in the posterior extremities. This was characterized by slowing of movements that, when severe, resulted in animals being unable to move the posterior extremities, and dragging themselves forward using the forelimbs. In its most

Figure 8. Effects of prior decortication on lesions induced by subacute systemic administration of 3-NP. On Nissl-stained sections large bilateral lesions are seen as pale staining in the striatum (*top*), which is significantly attenuated on the side with prior decortication (*bottom*).

extreme form animals became "recumbent," lying on their sides. If they were fed and allowed to drink in this state they would subsequently improve and in some cases recover the ability to ambulate independently. However, survival was not greater than 50–60%. The dystonic posturing and rigidity appears to be related to the basal ganglia lesions since examination of the brainstem and spinal cord in three animals showing the movement disorder showed no neuropathologic abnormalities (data not shown).

Histologic evaluation following administration of MK-801 at a dose of 1 mg/kg/hr for 24 hr by Alzet pump following a loading intraperitoneal dose of 5 mg/kg produced no neuroprotective effects as compared with saline following local striatal injection of 500 nmol of 3-NP ($n = 3$ in each group). The effects of prior decortication were examined in 1-month-old rats that received intraperitoneal 3-NP at 30 mg/kg/d for 5 d ($n = 3$) or 7 d ($n = 3$). Both Nissl and GFAP stains showed that the lesion on the side with the prior decortication was markedly attenuated. At the site of the maximal lesion, the area of the Nissl-stained lesion on the side with prior decortication was 0.1 ± 0.1 mm^2, which was significantly ($P < 0.05$) less than that on the side with intact cortex, 1.7 ± 0.6 mm^2 ($n = 3$) (Fig. 8).

Chronic administration of 3-NP at a dose of 12 mg/kg/d for 1 month in 12-month-old rats produced selective bilateral lesions in the rostrolateral aspect of the caudate putamen in 11 animals. Histopathology was not observed elsewhere in the brains of these animals. The lesions were small (0.5–2.0 mm in diameter) and were variable between animals (Fig. 9*A*). In contrast to intrastriatal and subacute administration of 3-NP, the chronic administration of 3-NP resulted in histopathologic striatal sequelae characterized by a selective sparing of aspiny NADPH-diaphorase neurons concomitant with marked neuronal loss and astrogliosis (Fig. 9*B,C*). Neuronal counts within the striatum showed a statistically significant reduction in the density of Nissl-stained neurons (Table 1). The density of NADPH-diaphorase neurons was increased in the chronically 3-NP–treated animals, although the difference did not show significance. The ratio of
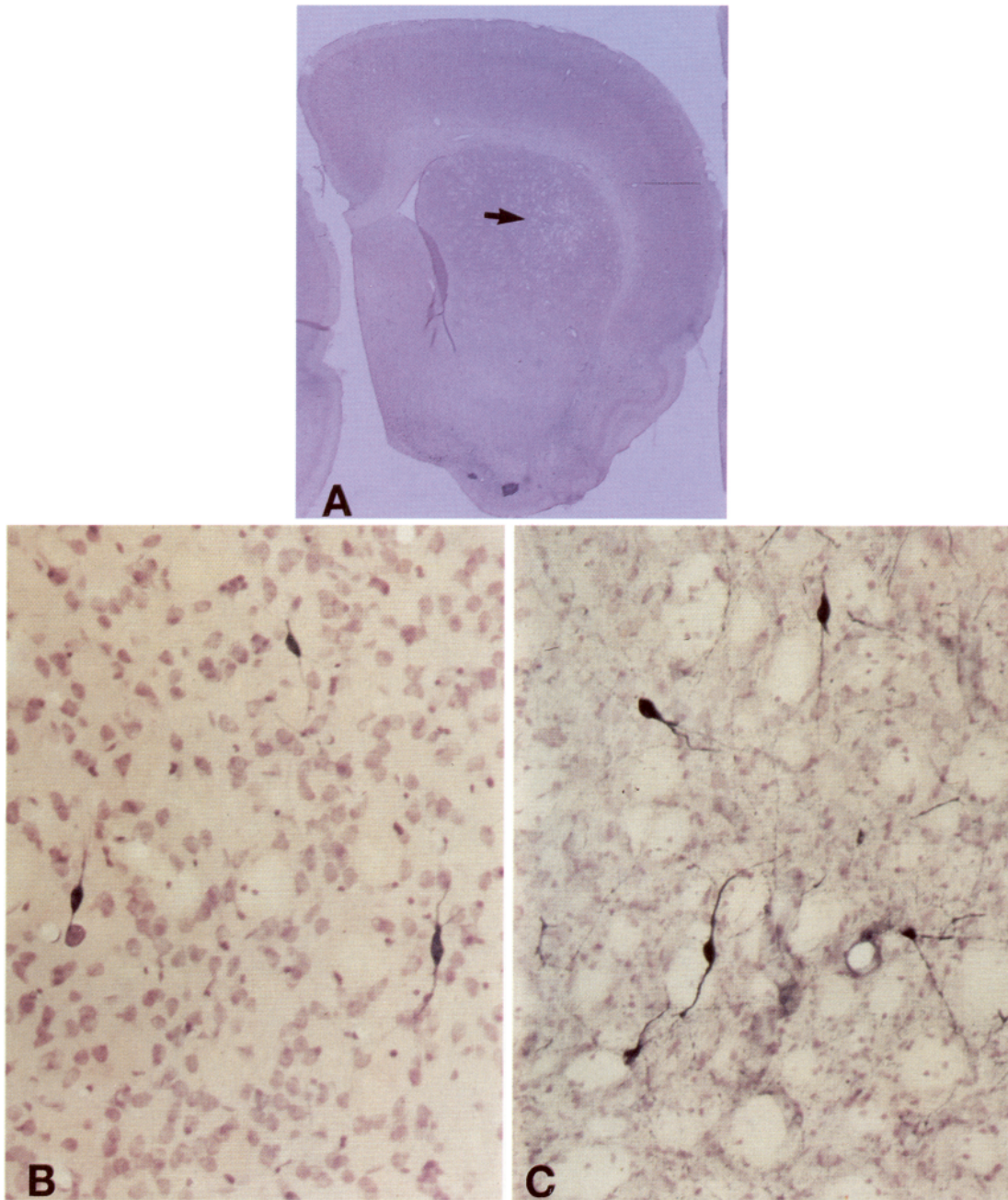
Figure 9. Chronic administration of 3-NP at a dose of 12 mg/kg/d produced selective striatal lesions within the rat caudate putamen. A small lesion in the dorsolateral caudate putamen can be identified in *A*, where there is an increased density of white matter bundles (*arrow*). These lesions were characterized by marked neuronal loss and gliosis with selective preservation of NADPH-diaphorase neurons in *C* as compared to a saline-treated control in *B*.

NADPH-diaphorase to Nissl neurons, however, was significantly increased, demonstrating a relative sparing of NADPH-diaphorase neurons. Two of the 11 animals did not show any grossly discernable lesion. Quantitative analysis of striatal neurons in these two animals, however, identified a statistically significant loss of Nissl-stained neurons with a sparing of NADPH-diaphorase aspiny neurons (NADPH-diaphorase neurons, 5.7 ± 0.8/mm²; Nissl neurons, 145 ± 7.4; density ratio, 3.9; $P < 0.05$) (Table 1) as compared with controls. Both of these animals were included in the overall statistical analysis.

In addition, growth-related dysmorphic alterations observed

Table 1. Striatal NADPH-diaphorase and Nissl neuronal counts in chronic 3-NP-treated rats

	NADPH-diaphorase	Nissl	Density ratio
3-NP lesion	5.8 ± 0.8	77.3 ± 14.2**	9.3%**
Control	5.6 ± 1.1	196.4 ± 9.6	2.8%

Eleven animals were examined with 3-NP and six saline-treated controls. Data are means ± SEM.
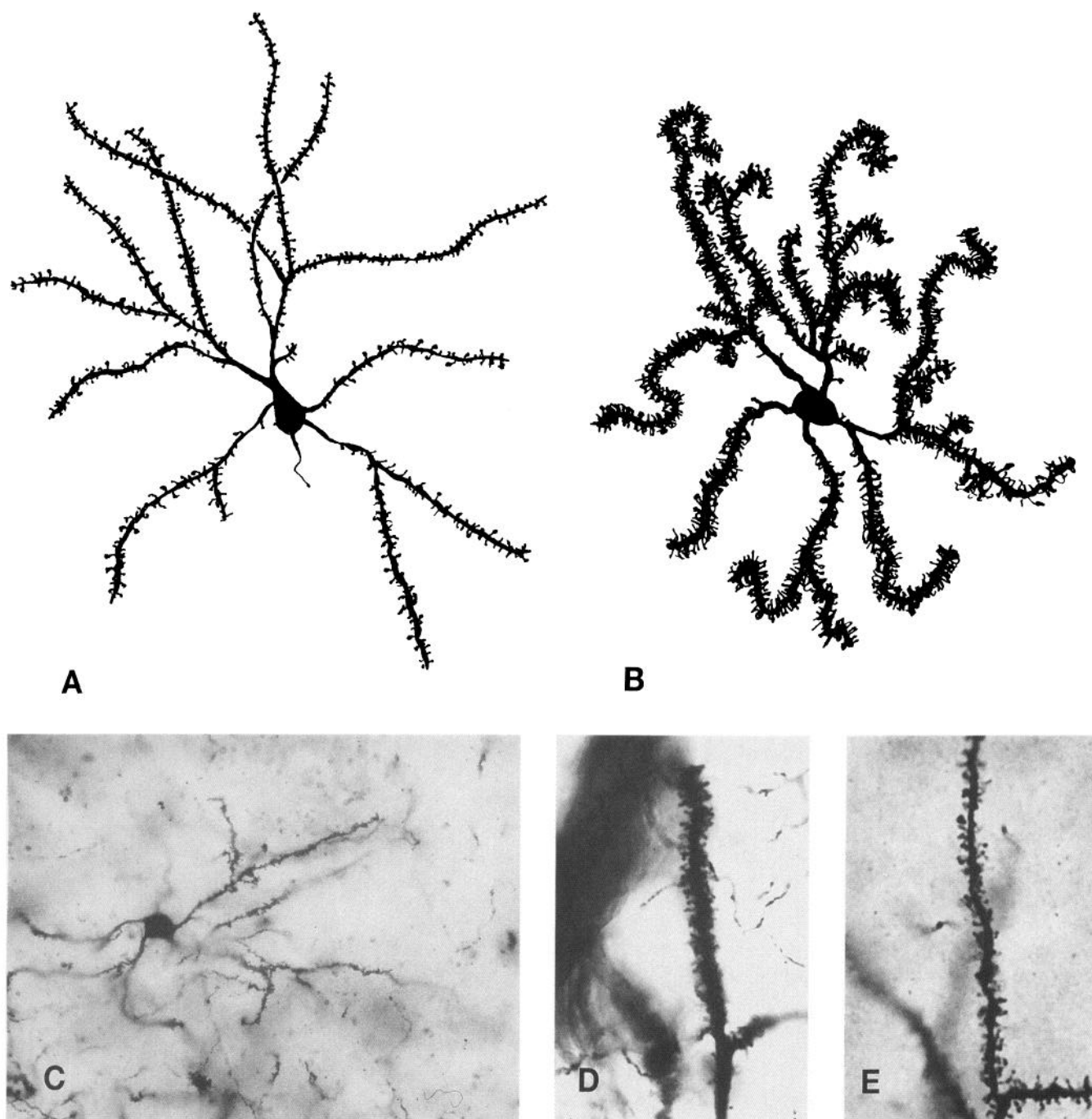
** $p < 0.001$.

Figure 10. Camera lucida drawings of spiny striatal neuron from a chronic 3-NP–treated animal in *B* and normal control in *A*. Morphologic alterations of spiny striatal neurons in the 3-NP–treated animals consist of recurving dendritic segments (*A*), short-segment branching (*C*), and marked increases in spine density with abnormal dendritic growth (*D*) as compared to the normal (*E*).

in spiny striatal neurons in HD were present within the caudate putamen of the chronic 3-NP–treated animals. These alterations were characterized by changes in the dendritic branching pattern and recurving of dendrites (Fig. 10*A,B*). As compared to the saline-treated animals there was short-segment branching along dendrites, with increased dendritic spine density and thickness of dendrites (Fig. 10*C–E*). Immunocytochemistry for NCAM, a marker for neurite outgrowth, was increased in and around the lesion site. It was found in both neurons and immunoreactive puncta within the neuropil (Fig. 11).

Discussion

In the present study we examined whether either intrastriatal or systemic administration of the known mitochondrial toxin 3-NP could induce excitotoxic neuronal death in rats. 3-NP is a plant- and fungal-derived neurotoxin that is an irreversible inhibitor of succinate dehydrogenase (Alston et al., 1977; Coles et al., 1979). Ingestion of 3-NP has been linked to toxicity in both livestock and in human (Ludolph et al., 1991). Chronic poisoning in livestock leads to labored respiration, generalized
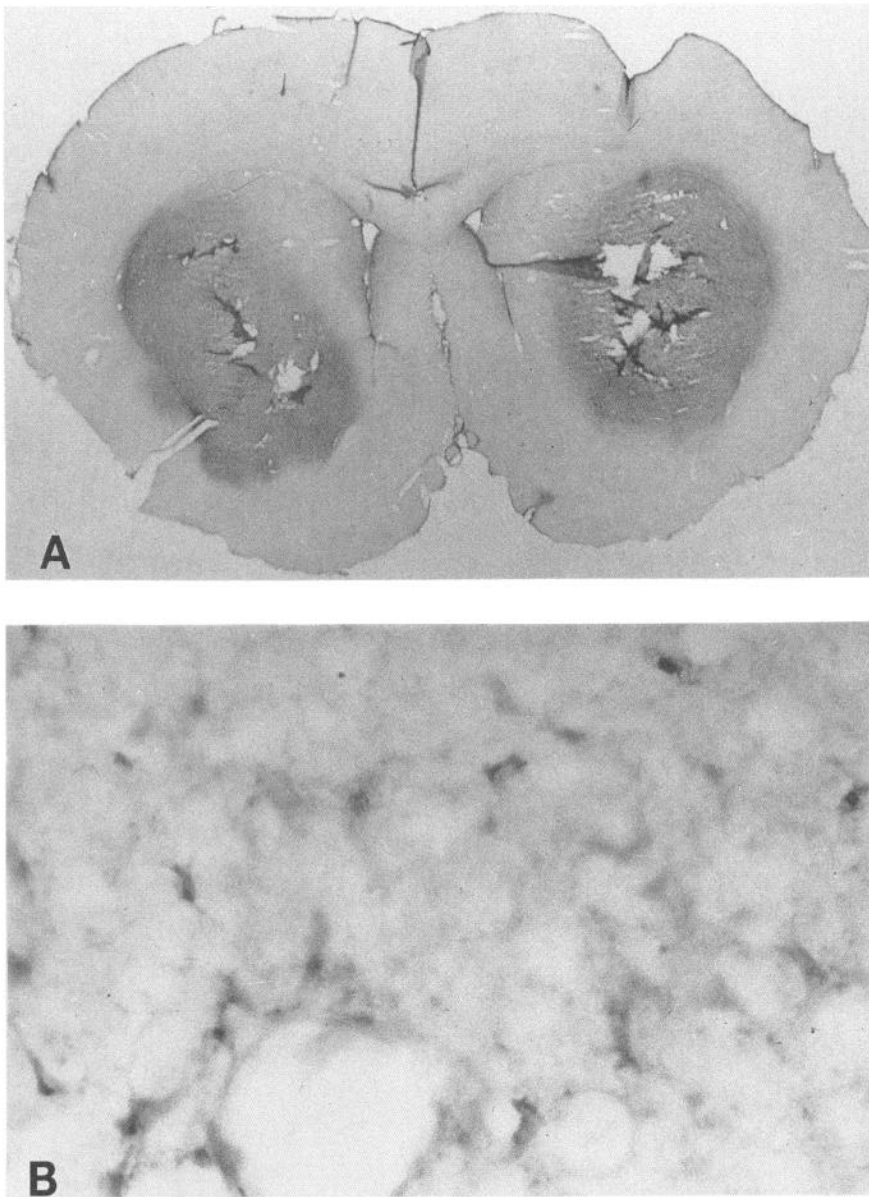
Figure 11. Immunocytochemical staining for NCAM in chronic 3-NP–treated rat. On gross inspection (*A*), intense immunoreactivity is observed throughout the caudate putamen and is present in both neurons and immunoreactive puncta within the neuropil (*B*).

weakness, abnormal gait, and hind limb paralysis. Exposure in human has occurred in China where children have ingested sugar cane contaminated with the fungus *Arthrinium* (Ludolph et al., 1991). The clinical picture consists of an acute encephalopathy followed by appearance of a delayed onset dystonia 11–60 d after ingestion. The patients experience torsion spasms, torticollis, facial grimacing, and jerk-like movements. CT scans show bilateral hypodensities in the putamen and to a lesser extent the globus pallidus. It is of interest that a similar distribution of lesions has been seen in human following cyanide overdoses (Uitti et al., 1985; Carella et al., 1988; Rosenberg et al., 1989).

We characterized both the neurochemical features and the neuronal specificity of striatal lesions induced with 3-NP. Intrastriatal injections of 3-NP resulted in dose-dependent significant reductions in markers for both striatal intrinsic neurons such as GABA, SPLI, SLI, and NPYLI, as well as a reduction in markers for striatal afferents such as dopamine and its me-

tabolites. The effect on dopamine is consistent with those we have found after intrastriatal injections of aminooxyacetic acid, manganese, and 1-methyl-4-phenylpyridinium (Beal et al., 1991; Storey et al., 1992; Brouillet et al., 1993), which also impair mitochondrial energy metabolism. In contrast, systemic administration of 3-NP spared dopamine concentrations and resulted in increased concentrations of DOPAC and HVA, consistent with increased dopamine turnover. Subacute systemic administration of 3-NP resulted in a pattern of neuronal selectivity similar to that seen following intrastriatal injection, with decreases in GABA, substance P, somatostatin, and neuropeptide Y. These lesions showed a marked age dependence, with 4-month-old animals being much more vulnerable than 1-month-old animals. This is consistent with observations we have made studying the age dependence of intrastriatal injections of 3-NP (Brouillet et al., 1993).

Following local intrastriatal injection, both the freeze-clamp technique and death by focused microwave irradiation showed

significant reductions in ATP and increases in lactate. These observations were also confirmed in real time using water-suppressed proton chemical shift magnetic resonance imaging. At 3 hr following 3-NP injections localized accumulations of lactate were seen in the striatum. Similarly, following systemic administration of 3-NP for 3 d localized accumulations of lactate were found in the striata, but not in other brain regions. The increases in lactate preceded the observation of lesions on T_2-weighted images. Subsequent imaging of the lesions using T_2-weighted magnetic resonance imaging showed focal increases in signal in the striatum, similar to observations made in human following a cyanide overdose (Rosenberg et al., 1989).

Histologic examination of the lesions showed dose-dependent striatal lesions characterized by neuronal loss and extensive gliosis. A similar pattern of neuronal loss was seen after either intrastriatal or subacute systemic administration of 3-NP. There was a comparable loss of spiny neurons stained with CaBP and medium-sized aspiny neurons containing somatostatin and NADPH-diaphorase. There was some sparing of large neurons within the lesioned area. The lesions following systemic administration of 3-NP preferentially affected the striatum. With the smaller lesions there was no gliosis or neuronal loss observable in other brain regions, although with very large lesions neuronal loss and gliosis was observed in the hippocampus and cerebral cortex.

The neurochemical and histologic features of the intrastriatal and subacute systemic 3-NP lesions were similar to those we found with striatal lesions produced by non-NMDA agonists such as kainate and AMPA (Beal et al., 1989). There was a comparable loss of markers for both spiny neurons (GABA, SPLI, CaBP) and medium-sized aspiny neurons (SLI, NPYLI, somatostatin, NADPH-diaphorase). Prior decortication, which removes the corticostriatal glutamatergic input, attenuated the lesions, consistent with reports that prior decortication blocks kainic acid striatal lesions (McGeer et al., 1978; Biziere and Coyle, 1979). The effects of decortication on dopamine depletion following local 3-NP injection were only partial, consistent with findings following intrastriatal injection of either aminooxyacetic acid or 1-methyl-4-phenylpyridinium (Storey et al., 1992; M. F. Beal, unpublished observations). Striatal lesions produced by local intrastriatal injection of 3-NP were not blocked by MK-801, using a paradigm that is effective in blocking lesions produced by 1-methyl-4-phenylpyridinium (Storey et al., 1992), consistent with activation of non-NMDA receptors. These results are consistent with a recent report showing that although MK-801 conferred some protection against 3-NP neurotoxicity in cortical explants, more robust protection was obtained by a combination of MK-801 and the non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (Ludolph et al., 1992).

The present studies are consistent with earlier observations of Gould and Gustine (1982). They found that systemic administration of 3-NP to mice resulted in swollen mitochondria and a reduction of brain mitochondrial succinate dehydrogenase activity to 20% of control values. Electron microscopy showed marked swelling of neuronal processes and cell bodies, similar to observations made following excitotoxic lesions. Hamilton and Gould (1987a,b) hypothesized that 3-NP produced lesions by inducing histotoxic hypoxia, since in their studies systemically administered 3-NP induced increased arterial blood pressure and arterial blood oxygen as compared with controls. In contrast, prior reports of cyanide intoxication had considered it necessary to induce systemic hypotension to produce brain lesions (Brierley et al., 1976, 1977). Our results, showing that intrastriatal injection of 3-NP produces lesions, suggest that histotoxic hypoxia in the absence of systemic hypotension is sufficient to produce nerve cell death.

We observed similar behavioral observations to those reported by Hamilton and Gould (1987a). Following subacute intraperitoneal systemic administration of 3-NP, rats showed transient lethargy after the second or third dose. They then developed mild incoordination and stiffness of their hind limbs. At the most advanced stage the hind limbs were rigidly extended and rats were recumbent on their sides or stomach. With time, animals that survived showed variable recovery and some rats were able to resume walking, yet remained wobbly. Since lesions were not observed in either the spinal cord or other brain regions, it appears that the motor symptoms represent a striatal-induced dystonia.

The possibility that impairment of energy metabolism might result in secondary excitotoxic lesions was first proposed by Henneberry and associates (Novelli et al., 1988). They hypothesized that a depletion of energy stores may lead to relief of the voltage-dependent Mg^{2+} block of the NMDA receptor, leading to persistent receptor activation. Zeevalk and Nicklas (1990) found that partial inhibition of energy metabolism with either iodoacetate or cyanide alone resulted in NMDA receptor–dependent neuronal lesions, which were not accompanied by increases in extracellular glutamate. With increasing intensity or duration of metabolic stress there was activation of non-NMDA receptors as well, as indicated by the observation that the addition of the non-NMDA antagonist CNQX in combination with MK-801 produced a greater protection than when either antagonist was given alone (Zeevalk and Nicklas, 1991). Furthermore, they showed that graded titration of membrane potential with potassium or relief of the Mg^{2+} block of the NMDA receptor mimicked the effects of metabolic inhibition (Zeevalk and Nicklas, 1992).

We believe that our results with 3-NP in vivo are consistent with these results. Microdialysis studies showed that a single dose of 3-NP does not result in increased glutamate concentrations for at least 5 hr after systemic administration, a time point (4 hr) at which we found a twofold increase in striatal lactate concentrations. These results are therefore consistent with a postsynaptic mechanism by which energy depletion results in neurons becoming vulnerable to endogenous concentrations of glutamate. Previous work showed that 3-NP has no direct depolarizing effects in hippocampal slices (Riepe et al., 1992). Consistent with our observations, Ludolph et al. (1992) found that 3-NP produces energy depletion and neuronal degeneration in cortical explants that was attenuated by pretreatment with glutamate antagonists.

Both intrastriatal and subacute administration of 3-NP are likely to produce profound energy deficits within the cell, since 3-NP irreversibly halts both the Krebs cycle and complex II activity. We reasoned, however, that a chronic low-grade administration of 3-NP might produce a less severe metabolic compromise resulting in partial neuronal depolarization and selective activation of NMDA receptors. We therefore administered 3-NP at a dose of 12 mg/kg for 1 month by Alzet pump. Animals that received 3-NP using this paradigm showed variable susceptibility, with only half the animals surviving for the entire month. In these animals there were selective striatal lesions characterized by neuronal loss and astroglial proliferation,

Table 2. Comparison of Huntington's disease and 3-Nitropropionic acid striatal lesions

Huntington's disease	3-Nitropropionic acid
Young adult onset	Vulnerability of young adult animals
Striatal vulnerability	Striatal vulnerability
Movement disorder chorea	Movement disorder dystonia
Sparing of striatal afferents	Sparing of striatal afferents
Loss of spiny projection neurons	Loss of spiny projection neurons
Spiny neuron dendritic abnormalities	Spiny neuron dendritic abnormalities
Sparing of NADPH-diaphorase neurons	Sparing of NADPH-diaphorase neurons

with no evidence of pathology elsewhere in the brain. In two of the animals the lesions were not apparent on Nissl stains, but neuronal depletion was confirmed with quantitative cell counts. The lesions showed selective sparing of both NADPH-diaphorase and large neurons (presumed cholinergic) as compared with Nissl- and CaBP-stained neurons. This is a pattern we previously found is associated with NMDA excitotoxic lesions (Beal et al., 1986a, 1989). In addition, we found that this paradigm produces proliferative dendritic changes in striatal spiny neurons that closely resemble those seen in HD (Ferrante et al., 1991; Brouillet et al., 1993). Although some increases in spiny dendrites occur with normal aging (Rafols et al., 1989), they are not as marked as those observed in the present study. In the present study we also show that these lesions result in the induction of other growth-related markers such as NCAM. Both intrastriatal and systemic administration of 3-NP appear to cause striatal lesions by a secondary excitotoxic mechanism due to compromised energy production. In the case of intrastriatal or subacute administration there is severe tissue ischemia with glutamate release and activation of non-NMDA receptors, whereas mild metabolic compromise by chronic administration leads to selective NMDA receptor activation by postsynaptic mechanisms.

The present results have implications for the pathogenesis of striatal degeneration associated with both known mitochondrial defects and neurodegenerative diseases. Symmetric basal ganglia lesions are found in patients with defects in succinate oxidation, in Leigh's disease, and in some families with Leber's optic atrophy (Martin et al., 1988; Fujii et al., 1990; Bruyn et al., 1991; Larsson et al., 1991). Chronic systemic administration of 3-NP also produces lesions that share many of the characteristic histologic and neurochemical features of HD as summarized in Table 2. We have recently obtained evidence of impaired oxidative metabolism in HD using proton chemical shift magnetic resonance imaging (Koroshetz et al., 1992). Chronic 3-NP lesions appear to be an improved model of HD pathology and may prove useful in evaluating potential therapies for HD.

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