Age-Dependent Differences in Survival of Striatal Somatostatin–NPY–NADPH–Diaphorase-Containing Interneurons versus Striatal Projection Neurons after Intrastriatal Injection of Quinolinic Acid in Rats

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INTRODUCTION

Huntington’s disease (HD) is a hereditary neurodegenerative disorder characterized by choreiform movements, cognitive decline, and personality disturbance (55). The progressive deterioration, particularly in movement control, is associated with progressive loss of striatal neurons. Within the striatum, however, there is a selective loss of neuronal types. HD is characterized by a complete sparing of several types of striatal interneurons and a gradual loss of projection neurons. Among the interneurons preserved are those that contain the neuropeptides somatostatin (SS) and neuropeptide Y (NPY) and the enzyme neuronal nitric oxide synthase (the latter of which can be visualized by NADPH–diaphorase, or NADPHd, enzyme histochemistry, 32, 40, 47, 76, 78, 89) (4, 9, 14, 15, 35) and those containing acetylcholine (17, 36, 52).

The mechanism underlying the selective neurodegeneration in HD is still unknown, but it has been proposed that an N-methyl-D-aspartate (NMDA) receptor-mediated excitotoxic process may be involved (14, 17, 34, 98). Consistent with this hypothesis, some experimental studies in rats have shown that intrastriatal injections of the tryptophan metabolite, quinolinic acid (QA), which is an endogenous agonist of NMDA receptors, produces a pattern of striatal cell loss that mimics the depletion of striatal projection neurons and the relative sparing of striatal interneurons observed in HD (14, 17, 18, 79). In a number of other studies, however, neither an absolute nor a relative sparing of SS/NPY/NADPHd interneurons was observed after intrastriatal injections of QA in rats (22, 23, 30, 39). This discrepancy has left uncertain the claim that NMDA receptor-mediated excitotoxicity is involved in HD with a role of excitotoxicity in HD pathogenesis, and they also have implications for the basis of the more pernicious nature of striatal neuron loss in juvenile onset HD. © 1997 Academic Press
pathogenesis and the claim that intrastratal QA injections produce an animal model of HD.

This discrepancy among different research groups may be related to subtle experimental differences, since the methods used by the opposing research groups differed in several parameters. In an effort to find the basis of the discrepancy, we explored the influence of these technical differences on the relative survival of striatal SS/NPY/NADPHd interneurons relative to striatal projection neurons. The parameters examined were (1) the injection speed (fast vs slow); (2) the concentration of QA injected (50 vs 225 mM) injected; and (3) the age of the rats used. We also used either of two different markers for the SS/NPY/NADPHd neurons to explore the influence of marker used on outcome (SS/NPY immunohistochemistry vs NADPHd histochemical labeling). Our results show that the age of the rats and the speed of the QA injection are key to the relative survival of SS/NPY/NADPHd interneurons versus projection neurons in rats. In particular, we found that injection protocols yielding brief exposure to QA in young rats do indeed produce a pattern of projection neuron versus SS/NPY/NADPHd interneuron loss that resembles that in HD.

MATERIALS AND METHODS

Subjects. Results for 40 male Sprague–Dawley rats (Harlan Bioproducts, Indianapolis, IN) weighing between 150 and 500 g are presented here. Note that additional animals were used but are not presented here because the lesions or histology were not adequate for inclusion in this study. All animals were housed under a normal diurnal light–dark cycle and fed and watered ad libitum. Because their rearing and feeding conditions were normal, their weight was correlated with their age. Half of the rats weighed less than 315 g, and the remaining half weighed over 400 g. Based on normal growth charts for Harlan Sprague-Dawley rats, 315-g rats are typically 76 days old, while 400-g rats are typically greater than 120 days old (12, 57). For this reason, the rats in the <315 g group were considered young rats, while those in the >400 g group were considered mature rats. The mean weight of the young rats was 225 g (equivalent to 55 days old), while the mean weight for the mature rats was 446 g (equivalent to greater than 120 days old) (57).

Experimental design. An injection of 1 µl of QA was made into the right striatum in each rat with either a fast or a slow injection protocol. In the fast injection protocol, the QA injection was made over 1 min, followed by withdrawal of the syringe after a 2-min postinjection waiting period. In the slow injection protocol, the QA injection was made over 10 min, followed by a 5-min waiting period before the syringe was slowly withdrawn. Half of the rats receiving either a fast or slow injection received a low concentration of QA (50 mM), while the other half received a high concentration of QA (225 mM). Half of the members of each of these four groups were young rats and half were mature rats. Thus, eight groups of rats were studied (five rats per group), with each group being distinct in its combination of age, injection speed, and QA concentration. The brains were fixed by transcardial perfusion, removed, and sectioned, and adjacent series were stained for NADPHd, SS/NPY, and cresyl violet. Since over 90% of striatal neurons are projection neurons (68), cresyl violet staining was used as a simple and convenient method for studying projection neuron abundance, as in previous studies by others (17, 18). Immunohistochemistry for SS/NPY was used to detect striatal SS/NPY interneurons, while NADPHd histochemistry was used to label these same neurons for NADPHd. The SS/NPY neurons were simultaneously labeled for both SS and NPY, since these two neuropeptides are cocontained in the vast majority of the neurons in which either is found (40, 76, 82, 88, 91) and because we wished to detect all interneurons of this type. A section from each series at a level just posterior to the injection center was used for analysis of neuronal survival. The level chosen showed approximately 50% survival of cresyl violet-stained neurons (projection neurons) by eyethrough a large area of the striatum. Camera lucida drawings of cresyl violet-stained neurons were made for the lesioned and control sides in this section, and NADPHd and SS/NPY neurons in the adjacent sections were similarly drawn. For each case, the drawn part of the striatum was divided into the same 0.2-mm-wide and 1-mm-high zones for each drawing and the number of neurons in each zone counted on control and lesion side. Zones showing 40–60% cresyl violet neuron survival were used in further data analysis. Details for each of these steps are presented below.

Surgery. The animals were anesthetized with ketamine (0.33 ml/500 g) and xylazine (0.16 ml/500 g) and positioned in stereotaxic apparatus. A 1-µl Hamilton syringe with a stainless needle was used to inject QA into the right striatum at the coordinates: AP: +0.4 mm; ML: −2.5 mm; DV: −4.5 mm, using the bregma-based coordinate system from the stereotaxic atlas of Paxinos and Watson (63). The QA (Sigma Chemical, St. Louis) was dissolved in 0.1 M sodium phosphate buffer (PB) at pH 7.4 and 1 µl of QA was injected manually into the striatum, using either the slow or fast injection protocol noted above. Either a 50 or a 225 mM QA concentration was injected. We have observed that the slow procedure results in little or no efflux of injected QA, while the rapid injection protocol does result in visually evident QA efflux upon syringe withdrawal. Following a 2-week survival period, animals were sacrificed and processed for staining as described below.
Tissue processing. Under deep anesthesia (0.5 ml of 35% chloral hydrate), rats were perfused transcardially through the ascending aorta with 60 ml of 6% dextran in pH 7.4 PB, followed by 200 ml of 4% paraformaldehyde, 0.1 M lysine–0.1 M sodium periodate in 0.1 PB. The brains were removed and postfixed overnight at 4°C and then stored for 24 h in 20% sucrose–10% glycerol solution at 4°C until sectioned. The fixed brains were then frozen with dry ice and sectioned on a sliding microtome in the transverse plane at 40 μm. Each brain was collected as 12 separate series in 0.1 M PB–0.02% sodium azide. Adjacent series of sections for each rat were stained with cresyl violet, stained immunohistochemically for SS/NPY, and stained histochemically for NADPHd, respectively. For cresyl violet staining, sections were mounted onto gelatin-coated slides, dried and defatted in xylene, rehydrated and stained with cresyl violet, and then dehydrated, cleared in xylene, and coverslipped with Permount.

Immunohistochemistry and histochemistry. For SS/NPY immunolabeling, the peroxidase–antiperoxidase (PAP) procedure was used (7, 39). Sections were incubated for 72 h at 4°C in a mixture of anti-SS (INCFSTAR) and anti-NPY (Accurate Chemical Co.) primary antisera, each at a 1:500 dilution using PB–0.02% sodium azide–0.3% Triton X-100 as diluent. Both primary antisera were raised in rabbits, and their specificity has been demonstrated previously (8, 40, 90). After incubation in the primary antisera, sections were washed and incubated in donkey anti-rabbit IgG diluted 1:100 in Triton X-100/sodium azide/PB for 1 h. They were then washed again and incubated in rabbit PAP diluted 1:100 in Triton X-100/sodium azide PB. Incubations in secondary antisera and PAP were carried out at room temperature for 1 h, and all incubations were carried out in microcentrifuge tubes on a rotator. Sections were washed after PAP incubation by three 10-min rinses in distilled water. To visualize the peroxidase, sections were incubated in a solution of 50 mg diaminobenzidine HCl (DAB) in 50 ml of 0.05 M imidazole–0.05 M cacodylate buffer (pH 7.2) for 10 min, followed by incubation in this same solution for an additional 10 min after the addition of 200 μl of 3% hydrogen peroxide. Sections were then washed in distilled water, placed in PB, mounted onto gelatin-coated slides, dried, dehydrated, and coverslipped with Permount. For NADPHd histochemistry, sections were rinsed three times and then incubated in 10 ml of 0.1 M Tris–HCL buffer (pH 7.4) containing 10 mg NADPH (Sigma), 4 mg nitroblue tetrazolium, 12.5 mg monosodium malate, and 0.03 ml of 0.3% Triton at 37°C for 30–60 min (88).

Cell counting procedure. Cresyl violet-labeled sections posterior to the injection site were examined and for each rat a section in which a large portion of the lesioned striatum showed approximately 50% neuronal survival (i.e., the apparent lesion transition zone) was chosen for analysis. The SS/NPY-immunolabeled and the NADPHd-labeled sections adjacent to this chosen cresyl violet-stained section were also analyzed. The stained striatal neurons through the apparent transition zone and the comparable striatal region on the normal side were drawn using a light microscope (Olympus B-II) equipped with a camera lucida, at 125× magnification. Each drawn QA-injected striatum was divided into 0.2-mm-wide and 1-mm-high zones parallel to the ventricular edge of the striatum. For convenience, an acetate overlay with the zones drawn on it was created for each case to delineate the striatal regions to be counted for each zone. In this fashion, the striatum at the level of the drawing was divided into 10–12 zones. The number of stained neurons in each zone was counted on the lesioned and normal sides, and neuronal survival on the lesioned side was expressed as the percentage of neuronal abundance found in the matching zone on the control side. Zones showing 40–60% survival of cresyl violet-stained neurons on the lesioned side were considered to be within the transition zone. For each drawn lesioned striatum, three to five of the 0.2-mm-wide zones fell within the transition zone. Data from these zones were pooled for each rat and used for analysis. The same zones from the SS/NPY and NADPHd sections were also used for analysis.

Data analysis. The mean relative survival of each neuron type was tabulated and graphed. The results were analyzed using a four-way factorial ANOVA with repeated measures followed by a priori planned comparisons to test the relative differences in mean survival of the cresyl violet, SS/NPY, and NADPHd neurons within group and between groups (83). We also evaluated the sizes of the lesions in the various groups by measuring in a cresyl violet-stained section through the center of the lesion for each case the transverse diameter of the lesioned area in which survival was less than 50%. The lesion size data were analyzed using a three-way factorial ANOVA with repeated measures followed by a priori planned comparisons to test the relative differences in mean lesion size between different groups.

RESULTS

Lesion size and qualitative effect of lesions on neuronal survival. The striatal lesions were in either the middle or the lateral part of the rostral striatum. Mean lesion size through the center of the lesion for each group is shown in Table 1. The 225 mM QA injections yielded lesions that were two to three times the diameter of 50 mM QA injections, and the 50 mM QA injections produced lesions of similar size irrespective of animal age or injection speed. The fast high concentration QA injections, however, yielded significantly larger lesions in young than in mature rats (P ≤ .05).
About 50% of the projection neurons survived (i.e., transinterneurons in the area of the lesioned striatum in which NADPHd and SS/NPY interneurons versus projection neurons showed survival across all animals, (P \geq 0.0061), although not on the survival of the SS/NPY interneurons (P \geq 0.1581). Additionally, the survival of SS/NPY neurons was significantly different in the transition zone than that of NADPHd neurons across age and concentration with both slow QA injection (P \leq 0.0276) and fast QA injection (P \leq 0.0438). Similar comparisons revealed that there was no significant effect across animals of QA concentration on SS/NPY neuron survival (P \geq 0.9751), but the survival of NADPHd neurons was significantly better in the transition zone with 225 mM QA than with 50 mM QA (P \leq 0.0059). Because the effects of age and injection speed on the survival of NADPHd and/or SS/NPY neurons appeared more prominent than the effects of QA concentration, the groups were collapsed across QA concentration for further analysis. The data for the resulting four groups are presented in Table 3 and the relative survival of NADPHd neurons and/or SS/NPY neurons in these four groups are analyzed statistically in the next section.

The effects of age and injection speed on interneuron survival—Group by group comparisons. Figure 3 and Table 3 show relative NADPHd and SS/NPY neuron survival in the transition zone following slow QA injection in young and mature rats, collapsing the data across concentration. Although SS/NPY neurons show slightly better survival (69.5% of normal) than cresyl violet neurons (46.7% of normal) in the young rats with slow QA injection, neither NADPHd neurons nor SS/NPY interneurons were significantly different in their survival from the cresyl violet neurons in the young rats with slow QA injection (NADPHd neurons, P \geq 0.6916; SS/NPY neurons, P \geq 0.1805). Similarly, although NADPHd neurons showed slightly poorer survival than cresyl violet neurons in the older rats with slow QA injection (29.7% for NADPHd neurons versus 52.5% for cresyl violet neurons), neither NADPHd nor SS/NPY interneurons were significantly different in their survival from the cresyl violet neurons (NADPHd neurons, P \geq 0.2023; SS/NPY neurons, P \geq 0.9469). There were also no significant differences between the young and old rats with slow QA injection in survival of NADPHd interneurons (P \geq 0.6612) or in the survival of SS/NPY interneurons (P \geq 0.3673). In contrast, Fig. 4 and Table 3 reveal that there were significant effects on interneuron survival relative to cresyl violet neuron survival in the transition zone of both the young and mature rats after fast QA injection. For example, in the young rats with fast QA injection, NADPHd interneuron survival (105.8% of normal) was significantly better than cresyl violet neuron survival (49.1% of normal) (P \leq 0.0021). Although SS/NPY interneuron survival (74.5% of normal) also appeared better

### Table 1

<table>
<thead>
<tr>
<th>Injection (mM)</th>
<th>Young (mm)</th>
<th>Mature (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow injection: 50</td>
<td>1.894 ± 0.158</td>
<td>1.848 ± 0.113</td>
</tr>
<tr>
<td>225</td>
<td>3.184 ± 0.176</td>
<td>3.320 ± 0.334</td>
</tr>
<tr>
<td>Fast injection: 50</td>
<td>1.892 ± 0.114</td>
<td>2.116 ± 0.140</td>
</tr>
<tr>
<td>225</td>
<td>4.226 ± 0.313</td>
<td>3.556 ± 0.395</td>
</tr>
</tbody>
</table>

Note. Table 1 shows the mean diameter of lesion (measuring the zone of greater than 50% neuronal loss) for each of the eight groups of rats that differed systematically in age, QA injection speed, and QA concentration. The mean lesion sizes ± SEM are shown for each group. The results show a major effect of QA concentration on lesion size irrespective of animal age and injection speed. The results also show that with high concentration QA injection there is an effect of injection speed and age on lesion size.

No such effect of age was, however, observed with the slow high concentration QA injections. Additionally, fast high concentration QA injections appeared to yield larger lesions in general than did slow high concentration QA injections, and this effect was significant for the young rats (P \leq .05). Thus, the largest lesions were observed with fast, high concentration QA injection in young rats, while the smallest were observed with slow, low concentration QA injections in mature rats.

Qualitative examination of the three types of labeled sections revealed that there appeared to be a clear influence of animal age on the relative survival of NADPHd and SS/NPY interneurons versus projection neurons in the area of the lesioned striatum in which about 50% of the projection neurons survived (i.e., transition zone). The survival of these interneurons was generally much better in the younger rats than in the older rats, particularly with fast high concentration QA injection in young rats compared to slow low concentration QA injection in mature rats (Figs. 1 and 2).

Quantitative analysis of the effects of QA injection speed, concentration, and animal age—overall comparisons. We quantified the survival of the NADPHd interneurons, SS/NPY interneurons, and cresyl violet neurons (among which projection neurons predominate) in the transition zone for the rats in each of the eight groups. The mean (±SEM) survival for these neuron types for each group are presented in Table 2. Because we studied the transition zone, the tabulated survival for the cresyl violet neurons for all eight groups was about 50%. As can be seen, both types of interneurons generally survived better in the transition zone of young rats than they did in the mature rats. This trend appeared accentuated in the young rats with the fast QA injection irrespective of QA concentration. There was a significant overall effect on neuron survival of age (P = 0.0084), but not of injection speed (P \geq 0.5007) or QA concentration (P \geq 0.1947). There was, however, a significant effect of injection speed on the survival of the NADPHd neurons across all animals (P = 0.0061), although not on the survival of the SS/NPY interneurons (P \geq 0.1581). Additionally, the survival of SS/NPY neurons was significantly different in the transition zone than that of NADPHd neurons across age and concentration with both slow QA injection (P \leq 0.0276) and fast QA injection (P \leq 0.0438). Similar comparisons revealed that there was no significant effect across animals of QA concentration on SS/NPY neuron survival (P \geq 0.9751), but the survival of NADPHd neurons was significantly better in the transition zone with 225 mM QA than with 50 mM QA (P \leq 0.0059). Because the effects of age and injection speed on the survival of NADPHd and/or SS/NPY neurons appeared more prominent than the effects of QA concentration, the groups were collapsed across QA concentration for further analysis. The data for the resulting four groups are presented in Table 3 and the relative survival of NADPHd neurons and/or SS/NPY neurons in these four groups are analyzed statistically in the next section.

The effects of age and injection speed on interneuron survival—Group by group comparisons. Figure 3 and Table 3 show relative NADPHd and SS/NPY neuron survival in the transition zone following slow QA injection in young and mature rats, collapsing the data across concentration. Although SS/NPY neurons show slightly better survival (69.5% of normal) than cresyl violet neurons (46.7% of normal) in the young rats with slow QA injection, neither NADPHd neurons nor SS/NPY interneurons were significantly different in their survival from the cresyl violet neurons in the young rats with slow QA injection (NADPHd neurons, P \geq 0.6916; SS/NPY neurons, P \geq 0.1805). Similarly, although NADPHd neurons showed slightly poorer survival than cresyl violet neurons in the older rats with slow QA injection (29.7% for NADPHd neurons versus 52.5% for cresyl violet neurons), neither NADPHd nor SS/NPY interneurons were significantly different in their survival from the cresyl violet neurons (NADPHd neurons, P \geq 0.2023; SS/NPY neurons, P \geq 0.9469). There were also no significant differences between the young and old rats with slow QA injection in survival of NADPHd interneurons (P \geq 0.6612) or in the survival of SS/NPY interneurons (P \geq 0.3673). In contrast, Fig. 4 and Table 3 reveal that there were significant effects on interneuron survival relative to cresyl violet neuron survival in the transition zone of both the young and mature rats after fast QA injection. For example, in the young rats with fast QA injection, NADPHd interneuron survival (105.8% of normal) was significantly better than cresyl violet neuron survival (49.1% of normal) (P \leq 0.0021). Although SS/NPY interneuron survival (74.5% of normal) also appeared better
than cresyl violet neuron survival in the young rats with fast QA injection, this effect was not statistically significant ($P \geq 0.1549$). For the mature rats, survival of both types of interneurons (33.0% of normal for NADPHd and 13.0% of normal for SS/NPY) was poorer than that for cresyl violet neurons (48.9% of normal), although the differences between interneuron and cresyl violet neuron survival were statistically significant only for SS/NPY neurons (NADPHd, $P \approx 0.3703$; SS/NPY, $P \approx 0.0460$). Comparing between young and old rats with fast QA injection, survival of both the NADPHd interneurons and the SS/NPY interneurons was significantly better for the young than the mature rats (NADPHd, $P \approx 0.0001$; SS/NPY, $P \approx 0.0009$). Thus, both NADPHd and SS/NPY interneurons appeared to survive better than cresyl violet neurons in young rats with fast QA injection and more poorly than cresyl violet neurons in mature rats with fast QA injection.

Comparison of results with fast high concentration QA injection in young rats to results with slow low concentration QA injection in mature rats. Previous studies demonstrating a preferential survival of the NADPHd type of interneuron have used young rats and fast, high concentration QA injections, as in one of our eight original groups. In contrast, studies observing relatively poor survival of this type of interneuron have used slow, low concentration QA injection and/or mature rats, as in another of our original eight groups. For this reason, a comparison of the results for these two groups in the present study is of interest. These results are shown in Table 2 and Fig. 5. In brief, the results of this comparison are consistent with those of the previous studies. Fast, high concentration QA in young rats yields remarkably good survival of NADPHd interneurons (149.5% of normal) compared to cresyl violet neurons (46.9% of normal). This difference is highly
significant ($P \leq 0.0001$). In these same rats, however, SS/NPY neuron survival (53.4% of normal) was very similar to cresyl violet neuron survival. The results for the mature rats with slow, low concentration QA injection were fundamentally the opposite. For example, NADPHd interneuron survival (23.7% of normal) was poorer than cresyl violet neuron survival (52.5%) in mature rats with fast low concentration QA injection and was significantly poorer ($P = 0.0001$) than that for NADPHd interneurons in the young rats with high concentration, fast QA injection. The survival of the SS/NPY interneurons (46.9% of normal) in these mature rats, however, was only slightly less than for the cresyl violet neurons. Survival of the SS/NPY interneurons in these mature rats was not significantly different than for the cresyl violet neurons in these rats ($P = 0.8275$), and it was not different than for the SS/NPY interneurons in the young rats with high concentration, fast QA injection ($P \geq 0.7947$).

**DISCUSSION**

We found that the age of the rat and to a lesser extent the speed with which QA is injected have significant effects on the survival of striatal SS/NPY/NADPHd interneurons compared to striatal projection neurons following intrastriatal QA injection. The NADPHd- and SS/NPY-labeled neurons survived better than did projection neurons in young rats and more poorly in old rats. This trend was greatly accentuated with fast QA injection. Since rapid injections result in QA efflux, we interpret these results to suggest that brief intense QA exposure may produce a different pattern of striatal NADPHd/SS/NPY interneuron versus striatal projection neuron survival than does prolonged QA exposure. Age-related differences may be attributable to declines in projection neuron sensitivity to QA with age. Our findings reconcile the discordant results found by previous authors and suggest that under some circum-

![FIG. 2. Photomicrographs of corresponding striatal fields from the normal side (A, C) and lesion transition zone on the lesioned side (B, D) from a young rat with a fast injection of 225 mM QA, showing NADPH-d-labeled neurons in normal striatum (A) and in the lesion transition zone (B) and SS/NPY-labeled neurons for normal striatum (C) and in the lesion transition zone (D). For the normal side the ventricle is to the right, while for the lesioned side the ventricle is to the left. The NADPHd are no less abundant than normal in the lesion transition zone of this mature rat. Arrows in A–D indicate some of the labeled neurons present in the fields shown. Scale bar for A–D, 200 µm. Abbreviation: CTR, control; Lx, lesion.](image)
The Relative Percent Survival (Compared to Control Side) of Cresyl Violet-Stained Neurons (the Vast Majority of Which Are Projection Neurons) Compared to SS/NPY/NADPHd Interneurons Labeled by NADPHd Histochemistry and SS/NPY Immunohistochemistry in the Lesion Transition Zone for Each of the Eight Groups of Rats That Differed Systematically in Age, QA Injection Speed, and QA Concentration.

### TABLE 2

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Young</th>
<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mM Slow injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nissl</td>
<td>46.2 ± 2.1</td>
<td>52.5 ± 2.2</td>
</tr>
<tr>
<td>NADPH-d</td>
<td>34.3 ± 14.4</td>
<td>23.7 ± 5.6</td>
</tr>
<tr>
<td>SS/NPY</td>
<td>53.2 ± 19.0</td>
<td>46.9 ± 14.2</td>
</tr>
<tr>
<td>50 mM Fast injection</td>
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<tr>
<td>Nissl</td>
<td>51.3 ± 3.3</td>
<td>48.4 ± 2.4</td>
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<tr>
<td>NADPH-d</td>
<td>62.2 ± 15.3</td>
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<tr>
<td>SS/NPY</td>
<td>95.5 ± 25.4</td>
<td>15.3 ± 7.4</td>
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<tr>
<td>225 mM Slow injection</td>
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<tr>
<td>Nissl</td>
<td>47.1 ± 1.7</td>
<td>52.4 ± 3.0</td>
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<tr>
<td>NADPH-d</td>
<td>40.1 ± 14.7</td>
<td>35.6 ± 13.2</td>
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<tr>
<td>SS/NPY</td>
<td>85.9 ± 37.4</td>
<td>60.2 ± 24.6</td>
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<tr>
<td>225 mM Fast injection</td>
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<tr>
<td>Nissl</td>
<td>46.9 ± 2.0</td>
<td>49.4 ± 3.2</td>
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<tr>
<td>NADPH-d</td>
<td>149.5 ± 64.2*</td>
<td>52.8 ± 19.5</td>
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<tr>
<td>SS/NPY</td>
<td>53.4 ± 7.8</td>
<td>10.7 ± 6.9</td>
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Note. The mean percent survival ± SEM for each cell type for each group is shown. Asterisks indicate cell types whose survival is significantly less than that for the cresyl violet neurons within the same group. The results show a major effect of age and a lesser effect of injection speed on relative interneuron survival.

The Relative Percent Survival (Compared to Control Side) of Cresyl Violet-Stained Neurons (the Vast Majority of Which Are Projection Neurons) Compared to SS/NPY/NADPHd Interneurons Labeled by NADPHd Histochemistry and SS/NPY Immunohistochemistry in the Lesion Transition Zone for Each of the Four Groups of Rats That Differed Systematically in Age and QA Injection Speed, Having Collapsed the Data across QA Concentration.

### TABLE 3

<table>
<thead>
<tr>
<th>Cell type</th>
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<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nissl</td>
<td>46.7 ± 1.3</td>
<td>52.5 ± 1.8</td>
</tr>
<tr>
<td>NADPH-d</td>
<td>37.2 ± 9.7</td>
<td>29.7 ± 7.1</td>
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<tr>
<td>SS/NPY</td>
<td>69.5 ± 20.5</td>
<td>53.6 ± 13.6</td>
</tr>
<tr>
<td>Fast injection</td>
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<tr>
<td>Nissl</td>
<td>49.1 ± 2.0</td>
<td>48.9 ± 1.9</td>
</tr>
<tr>
<td>NADPH-d</td>
<td>105.8 ± 34.3*</td>
<td>33.0 ± 11.7</td>
</tr>
<tr>
<td>SS/NPY</td>
<td>74.5 ± 14.3</td>
<td>13.0 ± 4.8*</td>
</tr>
</tbody>
</table>

Note. The mean percent survival ± SEM for each cell type for each of the four resultant groups is shown. Asterisks indicate cell types whose survival is significantly less than that for the cresyl violet neurons within the same group. The results show a major effect of age and a lesser effect of injection speed on relative interneuron survival.

FIG. 3. Histograms showing the relative percent survival (compared to normal striatum) of cresyl violet, NADPHd, and SS/NPY neurons in young and mature rats with slow QA injection, collapsing the data across QA concentration. Although interneuron survival is seemingly slightly poorer in the mature rats than in the young rats, there are no statistically significant differences between the two groups.

FIG. 4. Histograms showing the relative percent survival (compared to normal striatum) of cresyl violet, NADPHd, and SS/NPY neurons in young and mature rats with fast QA injection, collapsing the data across QA concentration. Survival of both interneuron types is significantly poorer in the mature rats than in the young rats. Asterisks indicate survival is significantly different than for the cresyl violet neurons within group.

stances QA injected into rat striatum does reproduce the preferential survival of SS/NPY/NADPHd interneuron over projection neurons observed in HD. Our results are of interest in the light of the possible involvement of excitotoxicity in the neuronal death in striatum in HD. These issues are further discussed below.
Comparison to Results of Previous Similar Studies

SS/NPY/NADPHd interneurons versus projection neurons. In a series of papers, Beal and co-workers reported that striatal SS/NPY/NADPHd interneurons (typically using NADPHd as a marker) are less vulnerable to QA than striatal projection neurons in rats (14, 17, 18). In their initial papers using primarily biochemical methods, these authors claimed that SS/NPY/NADPHd striatal interneurons were, in fact, highly resistant to QA (14, 15).

Following subsequent studies by others that used anatomical methods to reveal extensive loss of striatal SS/NPY/NADPHd interneurons in rats after intrastriatal QA or NMDA injections (22, 23, 29), Beal and co-workers amended their claim and reported that while SS/NPY/NADPHd striatal interneurons are, in fact, vulnerable to QA, they are less vulnerable than striatal projection neurons (17, 18). Beal and co-workers have employed fast injections (completed in 3 min) of a high concentration of QA (120–240 mM) in young animals (1–2 months of age). As found in our study, this paradigm does favor the survival of SS/NPY/NADPHd striatal interneurons over projection neurons. These authors interpreted these results and their finding that striatal damage with QA could be antagonized with an NMDA receptor antagonist (MK-801) to suggest that NMDA receptor selective excitotoxicity might be involved in the pathogenesis of HD (15). Some authors, however, reported that intrastriatal injection of QA does not yield preferential survival of SS/NPY/NADPHd striatal neurons over projection neurons (30, 39). These studies have employed mature rats (39) and/or slow QA injections (30, 39). As also found here, these conditions favor projection neuron over SS/NPY/NADPHd interneuron survival or no major difference in survival. Thus, differences in the age of the rats used and the injection speed seem to be key variables that account for the discrepant prior results.

Effect of sampling method. One prior study has suggested that the methods used for sampling the striatum for neuron type-specific loss after intrastriatal QA injection might account for the reported discrepancies in the literature (74). In particular, they emphasized that the transition zone must be analyzed in order to show preferential survival of striatal SS/NPY/NADPHd interneurons. While there seems little doubt that differences in sampling methods can produce different results, sampling methods do not appear to account for the discrepancies between the results of Beal and co-workers (17, 18) and the results of Davies and Roberts (30) and Figuredo-Cardenas et al. (39). Both of the latter authors evaluated SS/NPY/NADPHd neuron and projection neuron survival in successive steps away from the QA lesion center and found no region of preferential SS/NPY/NADPHd interneuron survival. Sampling method also does not explain the differences in results between the different experimental groups in the present study, since we used the same sampling method for all of our groups. Thus, we believe that the most likely explanation for the discrepant results in previous studies examining the effects of acute intrastriatal QA injection on relative SS/NPY/NADPHd interneuron survival lies in age differences and injection speed differences.

Effect of marker used. The marker used for SS/NPY/NADPHd interneurons might also affect the apparent relative survival of these striatal interneurons compared to projection neurons. We found that across rats in general, as well as for many of our individual groups, there was a dissociation between apparent survival of these neurons using SS/NPY immunolabeling and NADPHd histochemistry. For example, in both young and mature rats, fast high concentration QA yielded much better NADPHd than SS/NPY interneuron survival. Qiu et al. (66) have reported a similar result after injecting 120 nmol of QA in young rat striatum. They also reported that such an injection yielded preferential survival of NADPHd interneurons over striatal projection neurons but not of SS/NPY interneurons (labeled by in situ hybridization histochemistry for prosomatostatin mRNA) over projection neurons. In all of our rat groups with slow injection of QA and in our mature and young rats with fast injection of low concentration QA, SS/NPY interneuron survival tended to be slightly
better than NADPHd interneuron survival. The basis of the variation in apparent SS/NPY/NADPHd neuron survival depending on whether SS/NPY labeling or NADPHd histochemistry is used is uncertain. One possibility stems from the recent observation that SS, NPY, and NADPHd do not co-occur in all striatal neurons in which any one of them is found (40, 76). While all NPY neurons in rats contain SS and NADPHd, 5–10% of SS neurons do not contain NADPHd and 5–10% of NADPHd neurons do not contain SS. Thus, disparities between SS neuron and NADPHd neuron abundance after QA could stem from QA affecting the SS-only population differently than the NADPHd-only population. It is also possible that in some cases QA induces NADPHd expression in striatal neurons not containing NADPHd. Induction by injury of NADPHd expression in neurons normally devoid of it has been reported previously in other systems (75). This might account for the above-normal abundance of NADPHd neurons in some of our rat groups. In any case, these various considerations make it uncertain which labeling approach yields the more accurate estimate of SS/NPY/NADPHd neuronal morbidity after intrastriatal QA injection. Irrespective of this issue, the variation in apparent SS/NPY/NADPHd neuron survival depending on the marker used clearly suggests this as a possible factor in discrepancies among prior studies differing in the use of these markers.

Effect of QA dose. Qin et al. (66) have found that variation in the amount or concentration of QA injected into the striatum can also affect the apparent pattern of survival of SS/NPY/NADPHd interneurons compared to striatal projection neurons. In our present study, however, we did not find a major effect of QA concentration on the survival of SS/NPY/NADPHd interneurons compared to striatal projection neurons in the lesion transition zone, although lesion size was greatly affected by QA concentration.

Effect of anesthetic. In the present study, we used ketamine to anesthetize the rats during the QA injection. Since ketamine is a weak noncompetitive antagonist of NMDA receptors (54), the possibility can be raised that the anesthetic was neuroprotective against QA and affected the pattern of neuron survival. Additionally, the possibility could be raised that differences among prior studies in the anesthetic used might contribute to observed discrepancies in SS/NPY/NADPHd neuron versus projection neuron survival, since some studies used ketamine (39, 72), while others used pentobarbital (17, 18, 30). Nonetheless, it is unlikely that the use of ketamine as an anesthetic was neuroprotective against QA, and the use of ketamine as an anesthetic also does not account for the differences among prior studies in relative SS/NPY/NADPHd neuron survival. Ketamine is only a very weak NMDA antagonist and at the dose used here does not provide evident neuroprotection (54, 56), in contrast to other more potent noncompetitive NMDA antagonists, such as MK-801, that do readily provide neuroprotection against QA (17, 56). Additionally, prior studies using pentobarbital anesthesia also obtained discrepant results on the relative survival of SS/NPY/NADPHd neurons versus projection neurons (17, 18, 30). Finally, we used ketamine as an anesthetic for all groups in the present study and obtained different relative patterns of SS/NPY/NADPHd neuron versus projection neuron survival depending on animal age and QA injection speed, replicating prior published results for each parameter irrespective of the anesthetic used in the prior study.

Chronic QA infusion. Our finding that slow low concentration QA injections yield no preferential survival of SS/NPY/NADPHd interneurons over projection neurons suggests that a similar result might be obtained using chronic intrastriatal infusion of QA. The three studies using this approach have, however, yielded seemingly discordant results. Susel et al. (84) reported loss of striatal NADPHd neurons in the lesion core with chronic infusion of 270 nmol/day in young rats, but did not compare survival of NADPHd neurons to striatal projection neurons. Forloni et al. (42) did make such comparisons and reported no significantly preferential survival of SS/NPY/NADPHd neurons over projection neurons after 1–2 weeks of chronic QA infusion at 96–240 nmol/day in young rats. Both SS immunolabeling and NADPHd histochemistry were used to identify the SS/NPY/NADPHd interneurons. Cholinergic interneurons were, however, spared. In contrast, Bazzett et al. (13) reported that in both young and aged rats (although the results were not shown separately), chronic infusion of QA at a rate of 160 nmol/day yielded preferential sparing of NADPHd neurons in the lesion transition zone. The seemingly discordant results of Forloni et al. and Bazzett et al., however, may be more similar than the authors appear to claim. Bazzett et al. actually find no preferential survival except in the transition zone of subtotal striatal lesions. Forloni et al. actually show that with their smaller lesions (i.e., 96–144 nmol QA/day) the SS/NPY/NADPHd interneuron survival is better than striatal projection neuron survival in the lesion zone showing 30–64% projection neuron survival (i.e., what could be called the transition zone). The effects are small, however, and with the number of animals used are not statistically significant. Nonetheless, these results suggest the conclusion that slow chronic infusion of QA at a concentration that produces subtotal striatal damage does yield some degree of preferential SS/NPY/NADPHd interneuron survival, at least in the young animals used in both studies. Although we found fast infusions to have a more marked tendency in young rats to accen-
tial SS/NPY/NADPHd interneuron survival with slow high concentration QA injection, particularly in young rats using SS/NPY as the marker. Thus, while fast QA injection in young rats greatly accentuates preferential SS/NPY/NADPHd interneuron survival, this tendency may still be slightly evident with slow high concentration QA injection in young rats.

Intrastratial QA injection in monkeys. Ferrante et al. (37) have reported that fast injection of a large amount of QA into striatum in adult monkeys also yields preferential SS/NPY/NADPHd interneuron survival over projection neuron survival. Thus, while intrastratial QA injections must be done in young rats (preferably using fast injection) to yield a survival pattern strongly mimicking HD, fast intrastratal QA injection yields HD-like survival in adult monkeys. In contrast, fast high concentration injection in mature rats in our study yielded relatively poor survival of the SS/NPY/NADPHd interneurons. There are several possible non-mutually exclusive explanations for the apparent discrepancy between adult rats and monkeys. First, mature rat and adult monkey striatal neurons may differ in their sensitivity to QA, with adult monkey striatal neurons being more akin to young rat striatal neurons. Second, the adult monkeys may have not been truly adults. They may have been similar to our young rats in developmental stage (i.e., sexually mature juveniles). Third, technical differences may explain the apparent differences between adult monkeys and mature rats. In particular, in the Ferrante et al. (37) study, each striatum was injected with 10 separate fast injections of 360 nmol of QA. Injection of this much QA into the striatum may have resulted in a large enough residual high concentration of QA in the monkey striatum that it acted as a slow injection. As seen in our own study in rats, slow injection of a large amount of QA does produce hints of preferential SS/NPY/NADPHd interneuron survival in mature rats, as least with SS/NPY as the marker.

Basis of Age and Injection Speed Effects on Survival after Intrastratal QA

The precise basis of QA excitotoxicity in the striatum is uncertain. Although the excitotoxicity initiated by QA can be antagonized by NMDA receptor antagonists (15, 37), it is not certain if QA excitotoxicity at the level of postsynaptic neurons is exclusively mediated by NMDA receptors. For example, several studies indicate that striatal QA excitotoxicity is dependent on the integrity of the cortical input to the striatum (43, 80, 96). This raises the possibility that the excitotoxic action of QA may be indirect and depend on the accumulation of glutamate released by corticostriatal terminals. QA might have this effect by a direct action on corticostriatal terminals that promotes release of glutamate (80) or it might act by impairing the ability of glial cells to take up the extracellular glutamate released by corticostriatal terminals (53, 92). Consistent with the latter possibility, glial cells appear to possess NMDA receptors (1, 46). In either case, QA might therefore act by yielding an excess of extracellular glutamate in the striatum and QA excitotoxicity may be mediated at the postsynaptic neuronal level by NMDA and non-NMDA type glutamate receptors.

The effect of age on vulnerability to QA could be due therefore to age-related changes in either NMDA or non-NMDA-type glutamate receptors, both of which are present on striatal SS/NPY/NADPHd interneurons and projection neurons (28, 85). Additionally, striatal neurons are vulnerable to both NMDA and non-NMDA glutamate receptor-mediated excitotoxicity (27, 38, 44, 50, 59). Both NMDA and non-NMDA receptors have been reported to change in subunit configuration (21, 33, 60, 64, 93, 97, 99), change in conductance properties (45, 87), and decline in abundance (10, 62, 64, 86, 94) from youth to senescence in rodents, cats, and primates in diverse brain regions, including striatum. Consistent with this observation, lesions with a given amount of excitotoxin have been reported to be larger in juveniles than in mature and aged animals (41, 58), as also found here. If age-related changes in glutamate receptors do account for the differences between our young and mature rats in relative survival of SS/NPY/NADPHd interneurons and projection neurons, then there must be a greater age-related decline in glutamate sensitivity among projection neurons than among SS/NPY/NADPHd neurons. Injection speed may affect neuronal survival because fast injection results in transient exposure of neurons to a high concentration of QA, followed by apparent rapid efflux. Previous in vitro studies of QA excitotoxic effects on cortical and striatal neurons have indicated that brief exposures to high QA concentrations cause rapid neuronal death after the cessation of the exposure (44, 48, 49). In contrast, slow injections may yield more prolonged exposure because the QA remains at the injection site (11). Nonetheless, slow infusion yields smaller lesions than rapid QA infusion. This may be the case because of the low toxicity of low concentration QA (48, 49) and because with slow infusion effective QA concentration at the injection site may be reduced by: (1) diffusion from the injection site (11); (2) inactivation by catabolic enzymes (51); and/or (3) neuronal adaptation (95). Brief exposure in young animals may favor projection neuron death because projection neurons may be more sensitive than interneurons in animals of this age. In mature animals, fast injections may no longer yield such preferential death of projection neurons because of the putative age-related loss in glutamate receptors they have experienced. Slow injection may yield no clear evidence of differential vulnerability to QA of projection and SS/NPY/NADPHd neurons because the
prolonged exposures with slow injection are adequate to kill both types of neurons in comparable numbers.

Implications for Mechanism of Cell Death in HD

HD is a disease of typically midlife onset (26, 55) that progresses gradually over a 15- to 20-year period, implicating some slow, chronic degenerative process. A number of studies comparing the types of neurons that die in HD to those that die in the striatum following intrastriatal QA injection in rats and monkeys have shown prominent similarities in the pattern of neuronal types affected. Cholinergic striatal interneurons appear to resist both HD and QA extremely well (30, 31, 39, 52, 72). Our present results and those of previous authors also show that it is possible to model the preferential survival of SS/NPY/NADPHd interneurons over projection neurons observed in HD (4, 16, 35, 52) by using fast high concentration QA injection in young rats (17, 18). As we noted in a prior study (39), slow QA injections in mature rats also mimic the preferential survival of striatoentopeduncular (i.e., striatal neurons projecting to the internal pallidal segment in primates) over other projection neurons that is observed in HD (4, 5, 6, 67, 69, 71, 77). Thus, there is evidence consistent with the idea that excitotoxicity is involved in striatal neuron death in HD. If the pathogenetic process in HD involves excitotoxicity, however, the current results suggest that it is unlikely that adult onset HD involves chronic exposure to low levels of an endogenously produced NMDA receptor excitotoxin. The relatively poor survival of SS/NPY/NADPHd striatal interneurons when exposed to QA in mature animals does not match the high survival of these neurons observed in adult onset HD. Our data also suggest that it is unlikely that age-related increases in excitotoxic vulnerability account for midlife onset in adult onset of HD. Our findings on the effects of age on lesion size and on relative survival of projection neurons versus SS/NPY/NADPHd neurons both suggest that projection neurons appear to become less vulnerable to excitotoxins with age. A similar conclusion was reached previously by Finn et al. (41). Thus, our results suggest that excitotoxic destruction of striatal neurons in HD may not be mediated via chronic exposure to low levels of an excitotoxin such as QA, as some had proposed (73, 96). Direct measurements of QA levels in HD brain also do not unequivocally implicate QA in HD pathogenesis (70, 81). Rather, excitotoxicity may be mediated by defects in energy metabolism engendered by the HD gene defect (2, 19, 61). Destruction of neurons by this indirect excitotoxic mechanism is, in fact, enhanced in aged animals (20, 24, 25).

Finally, our finding that striatal projection neurons appear to be more highly vulnerable to excitotoxic destruction in young rats than mature rats may be relevant to juvenile onset HD. In this form of HD, striatal projection neuron loss proceeds much more rapidly and less differentially than in adult onset HD (4). Interneurons are nonetheless spared in juvenile onset HD (4). Our present results are consistent with the possibility that while a high number of CAG repeats in the HD gene may cause the onset of HD during the juvenile years (3), the more pernicious nature of juvenile onset HD may stem from the greater vulnerability of juvenile striatal neurons to excitotoxic destruction. In light of this notion, it would be interesting to determine if QA injection in young rats leads to more uniform loss of striatal projection neuron types than observed in mature rats (39).

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