ing CNS tissue would alter phenotypically to become cerebral-like or impermeable. I also found (*1*) that the observed permeability to protein in CNS grafts is *variable*, even when the identical experiments are performed. This finding suggests that the neovascularization process may not be a predictable one.

In fact we indicated that leaky vessels in the choroid may contribute to leakiness in an adjacent graft and stated this in our paper (*1*, page 773, paragraph 7, lines 1–12; see also *3*, *5*). Our autoradiographic studies (*5*) show that vessels can arise directly from the choroid plexus or area postrema to enter and anastomose with the nascent graft vessels. Since these normally leaky vessels do not change phenotypically, large protein exudations occur which subsequently diffuse through the graft neuropil. I believe he describes an identical physiological situation in his third-ventricle grafts (*2*) by stopping protein movement by 1 minute.

Broadwell's term "intimate contact" between graft and choroid or median eminence may not be anatomically correct. The permeable vessels of these tissues are covered by specialized epithelial cells tethered by tight junctions—the basis of the blood-cerebrospinal fluid (CSF) barrier. Assuming that there was no direct mechanical trauma (which does not occur in fourth-ventricle grafts), it is an intriguing question as to how the leaky vessels emerged from between their united cellular covering to enter the graft. Angiogenic factors emanating from the graft tissue might induce endothelial cell movement. An additional important event consequent to the vascular invasion is the potential alteration of the blood-CSF barrier (*3*). With regard to dural vessels, the issue is clearly more complicated since the dura and arachnoid are compromised. Although the arachnoid is a tightly bound layer, perhaps the dural vessels grow into the graft tissue before the tight junctions can reform (if, in fact, they do).

Contrary to what Broadwell suggests, the correct key control experiment to determine the physiological state of the vasculature and to *exclude* the possibility of altered vessels caused by exogenous protein infusion is to stain immunocytochemically for endogenous rat serum protein in the grafts, as was done in my study. The distribution of serum albumin in the graft, a well-known neuropathological test for the potential presence of vasogenic edema, mimicks that of exogenous protein in the grafts (*1*, see also *6*).

With respect to Broadwell's comment on the mechanism of tracer leakage, I suggested, but did not prove, along with several other possibilities for protein leakage, such as ischemia or immunological or astrocytic reactions, that there could be some increased endothelial transport. I believe we agree that, if some endothelial transport of protein occurs, it probably represents only a minor contribution to the observed permeability, although species differences (rat and mouse) could be involved.

With regard to the highlighting of the blood vessels, I refer the reader to the original and elegant work of Rennels *et al.* (*7*) showing outlining after *ventricular* but not vascular horseradish peroxidase (HRP) administration. In rats bearing permeable autonomic grafts, the brain vessels are lightly highlighted after short HRP circulation and much more heavily so at longer times (*8*). Because Rennels *et al.* used ventricular injection to show outlining identical to that reported to occur by Broadwell after vascular injection, it might seem logical to conclude that the precipitate of the tetramethylbenzidine method results from delivery of the material by both vascular and ventricular routes, particularly since HRP adjacent to permeable areas such as median eminence can enter the CSF.

From an experimental perspective, if CNS grafts will be used clinically, the placement is of importance in determining whether a compromised BBB will be present. My findings indicate the *possibility* of BBB permeability—a high probability in ventricular grafts and a considerably lower and variable one in parenchymal grafts. On this point, Broadwell and I are in some agreement since, in his note 7, he states there exist occasional extravasations in parenchymal grafts.

A BBB dysfunction should not necessarily be viewed in a detrimental context, but as an experimental condition in which the neural grafting paradigm can be further explored. This is suggested since it is not known directly if lack of a BBB plays a role in cerebral dysfunction (*9*). One could deliver exogenous compounds that do not cross the BBB directly to specific grafts (*10*) for a variety of biochemical or metabolic reasons. In addition, from a neuropathological perspective grafting induces extensive neovascularization, which does not occur in normal mature brain (but does, for instance, in brain tumor formation). The study of angiogenesis and permeability could yield significant data concerning mechanisms of graft function and might also be useful for the study of neurological disorders where the BBB is compromised. Finally, ventricular and parenchymal grafts (which differ in permeability) or in their neuroactive mechanisms may also differ in effectiveness in treatment of disorders.

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Model of Huntington's Disease

In her article "Animals yield clues to Huntington's disease" (Research News, 11 Dec. 1987, p. 1510), Jean L. Marx reiterates the interpretation and subsequent dismissal by M. F. Beal *et al.* of our recent data presented in *Nature* (*1*). We wish to reaffirm our belief [and that of others (*2*)] that quinolinic acid demonstrates a spectrum of neurotoxicity similar to that of other excitotoxins, there being no preferential sparing of neurons containing somatostatin, neuropeptide Y, or NADPH (nicotinamide adenine dinucleotide phosphate, reduced)-diaphorase. In their original paper, Beal *et al.* (*3*) reported a study of the effects of intrastriatal injection of quinolinic acid, kainic acid, and *N*-methyl-D-aspartic acid on a variety of histochemical and neurochemical parameters. Their principal conclusion was that quinolinic acid uniquely among the excitotoxins spares somatostatin-containing neurons in a "transition zone peripheral to the injection site." GABAergic [as measured by a 50% decrease in GABA (γ-aminobutyric acid) levels (*4*)] and cholinergic neurons (as measured by a 51% decrease in the number of acetylcholinesterase-positive neurons) were similarly affected. The extent of sparing of the cells containing somatostatin was such that homogenates of whole striatum showed a 70% reduction in GABA, a 75% reduction in substance P, but no significant

decrease in somatostatin, neuropeptide Y, or NADPH-diaphorase (*3*). These observations do not appear to be consistent with a sparing of peptidergic neurons within a peripheral transition zone. In recent studies (*1*, *2*, *5*) it has been shown that low doses of excitotoxins will selectively spare striatal cholinergic neurons. Indeed, in striata showing a 26% reduction in cholinergic neurons there is an accompanying 82% reduction in NADPH-diaphorase–containing cells (*5*) [see also Boegman *et al.* (*2*), where a 32% decrease in acetylcholinesterase-containing cells was accompanied by a 61% decrease in cells containing neuropeptide Y].

Thus, while Beal *et al.* reported a relative sparing of neurons containing somatostatin, a finding which has not been substantiated by subsequent studies, they did not detect the sparing of cholinergic neurons, which seem to be a prominent feature of excitotoxin-induced degeneration of the striatum.

We therefore wish to caution against either adopting the animal lesioned with quinolinic acid as an appropriate model of Huntington's disease or, perhaps more important, the use of this model to predict a possible strategy for preventing the disease; despite suggestions to the contrary, major differences between these two examples of striatal nerve cell degeneration appear to remain.

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Response: Davies and Roberts disagree with our observations on effects of quinolinic acid lesions in striatum. Their objections are inconsistent with their own reported data on several points. Moreover, they base their criticism on morphologic studies and have not reported confirmatory biochemical data. They contend first that we did not detect a sparing of cholinergic neurons after the striatal excitotoxin lesions were made, which they now seem to have found. This is at variance with their initial report (*1*), in which they stated, "The intrastriatal injection of 120 nmol of quinolinic acid completely eliminated both AChE- and diaphorase-containing perikarya throughout the . . . lesion area as seen with nissl staining." They also presented a graph in figure 2 and stated "There was no significant difference between the cell losses for both cell types at any position within the striatum." It is therefore surprising to learn that they now report sparing of cholinergic neurons in the context of challenging our results.

Indeed we have already presented data showing a preservation of acetylcholinesterase neurons at the lesion core and have confirmed this with choline acetyltransferase staining (*2*, *3*). At the lesion core these neurons are preserved out of proportion to NADPH (nicotinamide adenine dinucleotide phosphate, reduced)-diaphorase neurons. This relative sparing occurs with excitotoxins acting at all three subtypes of glutamate receptors (*3*). This provides a further parallel with Huntington's disease, in which we have also demonstrated preserved cholinergic neurons (*4*).

We do not agree with Davies and Roberts that "quinolinic acid demonstrates a spectrum of neurotoxicity similar to that of other excitotoxins, there being no preferential sparing of neurons containing somatostatin, neuropeptide Y, or NADPH . . . diaphorase." We have reexamined this question with both biochemical measures and novel histochemical procedures (*3*). We have performed full dose-response curves using quinolinic acid, *N*-methyl-D, L-asparate, L-homocysteic acid, kainic acid, quisqualic acid, and AMPA (D,L-α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid). At doses that resulted in equivalent 50% reductions in GABA (γ-aminobutryic acid), only NMDA (*N*-methyl-D-aspartic acid) agonists (quinolinic acid, *N*-methyl-D, L-aspartate, L-homocysteate) showed sparing of somatostatin–neuropeptide Y concentrations, while kainic acid, quisqualic acid, and AMPA resulted in significant depletions of both somatostatin and neuropeptide Y. At high doses, NMDA agonists caused significant depletions of somatostatin and neuropeptide Y, which indicates a relative but not absolute sparing of these neurons. Although we initially did not find sparing with NMDA, only a single high dose was examined. We have recently developed techniques for double staining for enkephalin and NADPH-diaphorase. Using this technique we have counted directly the number (ratio) of NADPH-diaphorase neurons to enkephalin neurons in a single section at the transition zone (the border between the lesion core and normal striatum) where we consistently find selective neuronal sparing. We have demonstrated that there is relative sparing of NADPH-diaphorase neurons with several NMDA agonists, whereas preferential loss of this cell class is seen with agonists acting at the kainate and quisqualate receptors (*3*).

We have recently extended our studies to 1-year quinolinic acid lesions (*5*). These experiments provide even more convincing evidence of sparing of somatostatin–neuropeptide Y neurons. One year after the acute lesions were made, the striatum had shrunk to about 50% of its normal volume. At that time the number of NADPH-diaphorase neurons on the lesion side had increased more than twofold per unit area in a region in which there was a 64% depletion of enkephalin neurons. Neurochemical measurements confirm this finding, showing significant increases in both somatostatin and neuropeptide Y concentrations.

Our studies therefore demonstrate (i) a selective sparing of cholinergic neurons with excitotoxins acting at all three subtypes of glutamate receptors and (ii) a relative but not absolute sparing of NADPH-diaphorase neurons, with excitotoxins acting at the NMDA receptor subtype but not with those acting at quisqualate or kainate receptors. Our results have been confirmed by Choi and his colleagues in both cortical and striatal cell cultures (*6*).

We maintain that Davies and Roberts have only provided histochemical studies of quinolinic acid lesions at the lesion core. We believe our detailed studies comparing the effects of a variety of excitotoxins with the use of both neurochemical and histologic parameters provide compelling data which show that quinolinic acid and other NMDA agonists result in a pattern of neuronal sparing that closely mimics that of Huntington's disease. We believe this model may prove useful in evaluating potential treatments of Huntington's disease (*7*).

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