Behavioral Effects and Gene Delivery in a Rat Model of Parkinson's Disease

A report by M. J. During *et al.* (*1*) raises the possibility of developing a therapeutic gene-delivery system for the L-dopa synthesizing enzyme, tyrosine hydroxylase (TH). However, caution about the interpretations of functional effects in this report is warranted.

During *et al.* used apomorphine-induced body-turning (in circles) in rats with partially denervated striatum, lesioned by 6-hydroxydopamine as a criterion for dopaminergic action on dopamine-supersensitive striatal neurons (*2*). They reasoned that down-regulation of dopamine receptors, resulting from dopamine release generated by the presence of a delivered gene for TH, could be indirectly measured by the reduction in a rat's turning behavior. However, striatal injections of the gene-delivery herpes amplicon vector systems can kill or seriously impair neurons in the striatum (*3*). We have used the same viral stocks, titers, and volumes for injections into the striatum of amplicon virus preparations (pHSVlac) as those used by During *et al.* Our systematic studies of neuronal death resulting from pHSV reveal an average volume of striatal necrosis of 2.2 ± 0.4 mm^3 after amplicon injections containing 25% of the number of particles injected by During *et al.* (*3*). Further studies have revealed that infusions of titers and volumes used by During *et al.* seriously damage or lesion approximately 50% of the neostriatum 1 to 2 weeks after viral infusions. These lesions are sufficient by themselves to reduce turning behavior, as rats with damaged striatal neurons cannot respond adequately to dopamine agonist stimulation (*2*). Consequently, it may not be valid to interpret a reduction in turning behavior as merely a loss of a supersensitivity to dopamine-like drugs in the striatum if any damage or dysfunction of striatal neurons is present.

This critique is further strengthened by the small number of TH expressing neurons (5 to 10) found in animals that showed reduced rotational behavior in the report (*1*). It is unlikely that this number of TH-positive neurons would be sufficient to cause a behavioral reduction by itself (*4*). The impression that TH expression may be extremely limited is not refuted in the report by the polymerase chain reaction (PCR) analysis at 16 months of TH-DNA, as this is not a quantitative technique and could be the result of a minute amount of PCR amplified nonexpressing TH-DNA. The reverse transcriptase–PCR data presented for TH-mRNA expression lack data points beyond 1 month (*1*), and thus does not confirm gene expression of TH at later time points. During *et al.* themselves state that the vector systems down-regulate gene expression over time, and we have also observed rapid in vivo down-regulation of identical vector systems (pHSVlac) to those used by During *et al.* after striatal in vivo injections. As behavioral recovery is constant in their experiments while gene delivery gradually decreases, it is reasonable to assume that other factors (such as neuronal impairment of striatal function) are partially or totally responsible for the behavioral effects observed.

In addition, there have been systematic observations of neuropathological consequences and wild-type pHSV reversion and gene delivery of the vector system used (*3*, *5*). Our studies demonstrate disseminated pHSVlac gene delivery in some animals, with a frequency predicted from the frequency of wild-type virus present in these vector preparations (10^{-5}). This means that, in the experiment reported by During *et al.*, each animal received one wild-type virus (on average) along with a pHVS modified gene contained within virus and helper virus. In our hands, this yields gene delivery that goes across *several* synapses, and while the viruses may still be defective and ultimately go into latency, gene delivery can occur in distant sites such as the contralateral striatum (*3*). During *et al.* likewise detected pHSVth DNA in the contralateral striatum in some animals at 3 months. The most plausible explanation for this is gene delivery (aided by a wild-type virus) to distant sites, as there does not exist a monosynaptic anatomical pathway from the contralateral striatum to either cortex or striatum of the injected side from which retrograde transport of pHSV particles could occur. While slow disseminated gene delivery is potentially damaging, it could also cause a relatively high amount of gene delivery (*3*) and a strong dopamine signal during microdialysis (before viruses go into latency). All of these concerns become compounded by the limited number of experimental animals used by During *et al.* for full comparison or correlations of physiological expression of gene delivery (dopamine-microdialysis) with neuropathological, histological, and behavioral data.

Experiments in other laboratories with reagents similar to those used by During *et al.*, but with different behavioral tests (less prone to nonspecific changes), will determine the validity of the interpretation given by During *et al.* that brain function has been altered by specific actions resulting from the gene delivered.

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Response: We agree with Isacson that significant injury to striatal neurons can result in partial behavioral recovery in the 6-OHDA rat model of Parkinson's disease and that local inflammation due to infiltration of immune cells, release of cytokines, or other inflammatory events can also influence rotational behavior. Although HSV-1 vectors cause cell damage as documented in cell culture (*1*) and in vivo, both the amount of neurotoxicity and the amount of vector spread that we observed are consistent with published results from other investigators and with our own studies in other brain areas, and are less than that observed by Pakzaban *et al.* (*2*) who used a single virus stock. Several types of data, obtained with independent assays, are not consistent with Isacson's interpretation that cell damage caused by the vector system led to the behavioral recovery. Our results suggest that expression of recombinant TH directed the behavioral recovery.

The group of rats that received pHSV-lac, which expresses β-galactosidase, did not show behavioral recovery (*3*). This result was not discussed in Isacson's comment. If the pHSVlac virus caused an amount of cell damage similar to that observed with the single virus stock used by Pakzaban *et al.* (*2*), then it is likely that this experimental group would have displayed at least some behavioral recovery.

The pHSVth group showed biochemical recovery with increased striatal L-DOPA biosynthesis and dopamine (*3*) (consistent with recombinant TH expression, rather than striatal cell death) resulting in behavioral recovery.

In the pHSVth group, most of the cells that contained TH immunoreactivity were located in the striatum, few were found in the projection areas (pallidum and medial agranular cortex). Also, several rats that received pHSVlac were analyzed for expres-

sion of β-galactosidase; X-gal positive cells were found in the injected striatum, but not spread throughout brain (3). Thus, this pattern of expression, observed with several virus stocks, is consistent with limited spread of the virus through retrograde transport rather than the transynaptic spread to multiple brain areas, as seen in only some rats when only a single virus stock was used (2).

The persistence of pHSVth DNA in specific brain areas was detected by PCR, a sensitive assay. Seven out of eight rats had pHSVth DNA in their injected striatum; only two had it in the contralateral striatum, and, in these, only in some of the sections that were analyzed (3). As we discussed in our report, this limited spread of pHSVth DNA could be a result of a projection from the contralateral cortex to the injection site or of a virus rising up the needle track to infect projections from the contralateral striatum. This pattern of persistence of vector DNA is consistent with the pattern of expression of recombinant gene products (TH and β-galactosidase) and with limited spread of the virus through retrograde transport.

We reported a zone of necrosis around the injection site (3) significantly smaller than that reported by Pahzaban *et al.* for the single virus stock (2).

If ongoing virus reactivation resulting in continuous virus spread was occurring, as suggested by Isacson, then HSV-1 particles should be present throughout the 1-year experimental period. In contrast, with the use of both the pHSVth and pHSVlac viruses, HSV-1 particle immunoreactivity was detected at 4 days after gene transfer, but not at longer times (6 to 12 months) after gene transfer (3). Also, ongoing virus reactivation, which Isacson suggests might direct sufficient TH expression to cause biochemical recovery 4 to 6 months after gene transfer, usually results in HSV-1 encephalitis, which would be expected to kill the rats. In contrast, a limited number of rat deaths (<10%) occurred within 2 weeks after gene transfer, and all the remaining rats, in both the pHSVth and pHSVlac groups, survived until deliberately killed (3).

After injection of defective HSV-1 vectors into the hippocampus, Ho *et al.* (4) reported localized recombinant gene expression and limited cell damage, consistent with our results (3).

After injection of an unpurifed preparation of defective HSV-1 vectors packaged with a temperature-sensitive mutant into the hippocampus (5), Pfaff reported an immune response, but limited virus spread (only to mammillary body), consistent with retrograde transport and inconsistent with the transsynaptic spread to multiple brain areas found in only some rats exposed to only a single virus stock (2). Several investigators working with recombinant (whole virus genome) HSV-1 vectors have also reported limited cell damage (6) and limited spread of the virus consistent with retrograde transport. These recombinant HSV-1 vectors contain a different structure, so the results may not be directly applicable to our system. In ongoing work that has used multiple virus stocks, injecting HSV-1 vectors has resulted in only limited cell damage with limited spread of recombinant gene expression (7).

Isacson also questions the capability of the few TH immunoreactive cells we observed to direct behavioral recovery (8). However, in addition to behavioral recovery, we also measured biochemical restoration (3). While the number of cells expressing recombinant TH was relatively low (5 to 10 to several hundred), significant increases in both TH activity and dopamine concentrations were measured, as assayed by in vivo microdialysis, and the pHSVlac group did not show such increases. Striatal TH activity and dopamine concentrations are likely to be capable of directing behavioral recovery in the amounts detected. Results with transplanted dopamine producing cells (8) show a wide range in the number of cells which are sufficient to direct behavioral recovery: for example, Freed obtained behavioral recovery after transplantation of dissociated fetal substantia nigra cells, even though many of these grafts contained fewer than 100 TH-positive cells and several grafts contained five or fewer TH-positive cells (9). There are several critical differences between direct gene transfer into striatal cells as compared with cell transplantation, and these differences may result in more efficient behavioral recovery from direct gene transfer. The extent of diffusion of dopamine from its source is critically important as it is clear that dopamine-mediated functional effects extend well beyond the graft (10). Graft cells are localized, and diffusion may be retarded by extracellular matrix secreted from the graft; in contrast, direct gene transfer delivers the TH gene to relatively widely dispersed cells, and no additional extracellular matrix is produced. Also, graft cells contain dopamine transporters that can result in reuptake of dopamine (11), thereby diminishing the diffusion of dopamine. In contrast, direct gene transfer into nondopaminergic striatal cells should not add any dopamine transporters to the striatum, so diffusion of dopamine may be unimpeded by local dopamine transporters.

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