the interval (Fig. 1). Data from one (previously unpublished) family of southern Bavarian origin showed positive lod scores with a maximum multipoint score of 1.5 (family K, Fig. 1). This lod score is close to the theoretical maximum in this relatively small family.

In six families (FR-041, FR-722, FR-727, FR-755, UK-A, and UK-B), only the two polymorphic markers most closely linked to PD1 (D4S1647 and D4S2380) have been analysed. Obligate recombinations (no allele shared by all affecteds) were observed in five of these families either for each of the markers individually (three families), or for the haplotype of both markers (two families), again strongly arguing against linkage with the PD1 locus. In one family (FR-041), a positive pairwise lod score was obtained for D4S2380 (0.29 at Theta = 0). Positive lod scores in families K and FR-041 may reflect true linkage, but they may also be a result of random fluctuations, because the relatively small size of these families precludes definite proof of linkage.

We conclude that mutations at the PD1 locus are probably a rare cause of autosomal-dominant parkinsonism. The role of the PD-1 gene in sporadic PD is still to be determined. T. Gasser, Department of Neurology, Klinikum Großhadern, 81377 Munich, Germany; B. Müller-Myhsok, Bernhard-Nocht-Institute for Tropical Medicine, Hamburg 20359, Germany; Z. K. Wszolek, Section of Neurology, University of Nebraska, Omaha, NE 68198–2045, USA; A. Dürr, INSERM U289, Paris 75013, France; J. R. Vaughan, Institute of Neurology, The National Hospital Queen Square, London WC1N 3BG, UK; V. Bonifati and G. Meco, Dipartimento di Scienze Neurologiche, Università “La Sapienza,” Rome 00168, Italy; B. Berezina, Department of Neurology, Klinikum Großhadern; R. Oehlmann, Bernhard-Nocht-Institute for Tropical Medicine; Y. Agid and A. Briese, INSERM U289; N. Wood, Institute of Neurology, The National Hospital Queen Square, and the European Consortium on Genetic Susceptibility in Parkinson’s Disease (GSPD) (10).

**Experiments in a Parkinson’s Rat Model**

Derek L. Choi-Lundberg et al. present evidence (1) that a replication-defective adeno-viral (Ad) vector that encodes human glial cell line–derived neurotrophic factor (GDNF) protects dopaminergic neurons in substantia nigra (SN) in rats from progressive degeneration induced by the neurotoxin 6-hydroxydopamine (6-OHDA) that has been injected into the striatum. These results are important because of possible applications of Ad vector–mediated GDNF gene therapy in patients with Parkinson’s disease. The experimental design used by Choi-Lundberg et al. however, raises some concerns.

Choi-Lundberg et al. (1) injected 6-OHDA into the striatum of rats 7 days after labeling SN neurons with the retrograde fluorescent tracer fluororogol (FG). Thus, the neurotoxin acted mainly on SN neurons that were loaded with FG. Because of neuronal death and membrane disruption, the fluorescent tracer diffused in the extracellular space, from where it might have been incorporated by other cells. That such an uptake of tracer really occurred in the experiment by Choi-Lundberg et al. is demonstrated by figure 2, C through G, in their report, showing that microglia and other non-neuronal cells in the SN have been labeled with FG. Similar to non-neuronal cells, SN neurons that survived the neurotoxin might have incorporated the tracer through their cell membranes (2).

To conclude, the finding (1) of a reduced loss of FG-labeled neurons in the SN of GDNF-treated rats does not necessarily imply a neuroprotective action of GDNF. A control in which the injection of FG is made after the complete or nearly complete degeneration of the SN neurons would seem to be necessary to definitely support the conclusions made by Choi-Lundberg et al. 

**REFERENCES**

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**TECHNICAL COMMENTS**

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**REFERENCES AND NOTES**

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**Experiments in a Parkinson’s Rat Model**

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**REFERENCES**


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Response: In our study (1), we injected FG into the striatum in order to retrogradely label a subpopulation of dopaminergic (DA) neurons in the SN. We injected 6-OHDA into the same site 7 days later, so that the FG-labeled DA neurons would be those most susceptible to the lesion. This research design allowed us to identify DA neurons that project specifically to this site without relying on their phenotype. Relevant to this rationale are studies showing that DA neurons, as identified solely by tyrosine hydroxylase (TH), “disappear” when lesions are induced with 6-OHDA and then “reappear” after injection of GDNF protein (2). In our study, the reduction of FG* neurons in the SN in control groups after treatment with 6-OHDA confirmed that these cells died and did not merely lose phenotypic marker expression. Microglia and other small cells were labeled with FG, which suggests either that these were cells that had phagocytosed degenerating FG* neuronal debris or were shrunken, degenerating DA neurons. Rats treated with an adenoviral vector (Ad) that encodes GDNF had significantly more large FG* neurons remaining in the SN 42 days after injection of 6-OHDA than were found in control rats—an average of 79% (Ad GDNF) instead of 31% (controls) [see figure 3 in (1)]. If neurons had degenerated with subsequent uptake by other DA neurons, then the FG labeling would have been in more dorsal neurons, with some FG* neurons remaining in the ventral portion of the SN (some FG* neurons survived in the ventral SN in control groups [figure 2D in (1)]. However, this distribution was not observed, which suggests that FG* neurons did not degenerate in rats treated with Ad GDNF. Another observation refuting this possibility is that almost no small FG* cells were present in sections of the SN in Ad GDNF–treated rats: If FG* DA neurons had degenerated, some microglia would likely have phagocytosed FG* DA neuronal debris.

Pallini et al. also suggest that injection of FG, after the degeneration of SN neurons had occurred, would demonstrate the protective effect of Ad GDNF. However, we did not observe any obvious differences among the various treatment groups in the size of the 6-OHDA lesion in the striatum, as indicated by the density of TH fiber staining (1). This suggests that Ad GDNF delivered near the SN did not protect DA nerve terminals from striatal 6-OHDA, as was also reported after injection of GDNF protein near the SN (3). Injection of FG after 6-OHDA would likely lead to little retrograde transport to the SN as DA nerve terminals would have been destroyed. If the striatal lesion volume had been reduced by GDNF treatment, this could have been caused by protection of nerve terminals or induction of sprouting into the denervated area. These possibilities might be distinguished by labeling with FG before the lesion, with subsequent injection of another tracer after the lesion. Consequently, if FG had been injected at the end of the experiment as suggested by Pallini et al., this would have labeled only those DA neurons whose fibers had sprouted or remained in the lesion site, defeating the original purpose of the labeling.

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REFERENCES

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Experiments in a Parkinson's Rat Model
Roberto Pallini, Alessandro Consales, Liverana Lauretti and Eduardo Fernandez

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