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Commentary

Bad News for Neuroprotective Therapies in PD?

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While the etiopathogenesis of Parkinson's disease (PD) continues to fuel intense debate [1], there is little doubt that the motor features of PD are caused by the degeneration of nigrostriatal dopamine (DA) neurons [2]. Clinical symptoms have been estimated to appear when at least 50% nigral DA neurons and 70% putaminal DA tissue contents are lost [3, 4]. Dopaminergic imaging suggests that at least 30% DA storage capacity (^{18}F-dopa PET) and 50% of putamen DA transporters (DAT SPECT) have been lost by the onset of contralateral limb bradykinesia and rigidity [5–7]. Parkinsonian motor features are highly responsive to DA replacement therapy, which provides a stable symptomatic improvement for approximately 4–7 years. After this "honeymoon" period, the patients' motor response to levodopa starts to fluctuate, and other complications, such as involuntary movements (dyskinesias), start to appear (reviewed in [8, 9]. The clinical history outlined above has assumed a continuing, gradual degeneration of nigrostriatal DA projections causing a progressive loss of DA terminals and DA storage capacity in the striatum (reviewed in [10]. The steady progression of this process is, however, challenged by a pathological investigation by Kordower and coworkers, published in the August issue of Brain [11].

In this study, putaminal and nigral sections from 28 subjects affected by idiopathic PD and 9 age-matched controls were immunohistochemically stained for the dopaminergic markers tyrosine hydroxylase (TH) and the dopamine transporter (DAT). The PD cases ascertained had survived for 1–27 years after clinical diagnosis, so the authors were able to cross-sectionally estimate TH and DAT loss in the putamen along with the loss of TH-positive and melanised cells in the substantia nigra at different stages of the disease. At 1 year, the dorsal putamen had lost 50% of the dopaminergic markers, while these were preserved in head of caudate and ventral striatum, a pattern in keeping with the results of previous investigations on end-stage cases [12]. However, a novel finding was the observation that loss of DAT and TH in the dorsal putamen appeared to have become complete by 4-5 years from clinical diagnosis. Under high power microscopy, intact DA fibers were still seen in the dorsal striatum in PD cases with clinical disease duration of 3 years or less. By 4 years, however, only a few TH- or DAT-positive fibers could be detected in the dorsal putamen. Morphologically, they had thick and swollen varicosities, suggesting ongoing degeneration. The comprehensive loss of DA markers in the dorsal putamen by 4-5 years was observed in two independent cohorts of PD patients, an elderly cohort from Arizona and a younger Australian cohort. Whether this severe reduction of DA markers represented fibre loss or phenotypic downregulation could not be discriminated.

In the substantia nigra pars compacta (SNpc), the reduction in TH-immunoreactive neurons was marked (50–90%), but highly variable even at the earliest time points examined. Unexpectedly, several of the short duration subjects showed a loss of TH-positive neurons

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comparable to that seen in the 20 years post-diagnosis group. Thus, also in the SNpc, the loss of dopaminergic markers seemed to occur very rapidly after the diagnosis. At all time points, however, the SNpc contained more melanized cells than TH-positive cells. Since the melanin pigment only forms in catecholaminergic neurons, these data suggest that the loss of TH and DAT reflects, in part, phenotypic downregulation of these markers along with the neurodegenerative process - the former could be potentially reversible.

The main conclusion of Kordower and colleagues is that there are negligible fibers expressing TH and DAT in the dorsal putamen after 4-5 years of clinical disease, suggesting "complete degeneration of dopamine terminals and a time point at which no benefit could be expected, even from a potentially effective (neuroprotective) agent". According to the authors, these findings indicate that there may be significant challenges for neuroprotective therapies directed at patients with a disease duration longer than 4 years. Such a conclusion could dismay the laboratories and pharmaceutical companies currently engaged in developing neurorestorative or neuroprotective treatments for PD.

Given this, it is important to consider the findings in the light of other observations available. Molecular imaging studies using ^{18}F-dopa positron emission tomography (PET) and DAT imaging have shown that significant DOPA decarboxylase and DAT activities still persist in the PD putamen for five years after diagnosis [13–16]. This suggests that dopaminergic function is still present in terminals and potentially available to rescue. It is also important to consider Kordower's conclusion in the light of both PET and pathological findings from PD patients who received putaminal GDNF infusions. In all cases receiving putamen infusions of the active agent, ^{18}F-dopa uptake was locally increased by around 25% suggesting that dopaminergic terminal function could in part be restored [17]. When the GDNF infusions were withdrawn for safety concerns, the increased putamen ^{18}F-dopa uptake persisted for up to 3 years [18].

In the one pathological study available [19], researchers performed TH immunohistochemistry on striatal sections from a PD patient enrolled in an open label GDNF infusion trial. The patient had received unilateral GDNF infusions into his right putamen for 43 months starting at 5 years after his PD diagnosis. The infusion had resulted in improved motor scores off-medication on the contralateral side of the body, whereas worsening had occurred on the ipsilateral side. Post-mortem immunohistochemical analyses showed very clear TH staining in the right putamen, much above the levels found in the untreated left putamen. This data suggests that, in face of an advanced disease process, GDNF might have induced dopaminergic fiber sprouting, as it has been well documented to do both in rodent and in non-human primate models of PD [20–22]. An alternative explanation is that GDNF can induce expression of dopaminergic markers and DA synthesis in cells other than the nigrostriatal neurons. Although yet unexplored, this possibility would be consistent with the presence of highly plastic neural precursors and perivascular cells, capable of responding to neurotrophins, also in the adult human brain [23–25]. In either case, trophic factor therapy may be able to restore some degree of dopaminergic function in a severely denervated striatum.

Kordower and colleagues suggest that future trials of putative neuroprotective and restorative agents should be directed at very early or even prodromal PD cases. If such agents were non-toxic and efficacious this would indeed be a very rational strategy. Unfortunately, to date, all candidates tested in randomised controlled double blind trials have failed to meet their primary endpoints [26]. At present, it is therefore difficult to justify exposure of PD patients to agents with potential untoward effects during the first years of their disease, when symptoms can be well controlled with dopaminergic medications. For this reason, neuroprotection trials are likely to continue targeting patients with established disease, where the risk- benefit analysis is more favourable.

On the other hand, the realization that a substantial amount of healthy-looking dopaminergic fibers is still present at the time of PD diagnosis should call for an early introduction of life style measures that are likely to increase the endogenous production of neurotrophic factors and so boost compensatory mechanisms in monoaminergic pathways. These include specific regimens of physical exercise and enriched environment (reviewed in [27]), interventions that are probably both safe and pleasant.

On a scientific level, the study offers one additional very important implication. Based on the time-course analysis of dopaminergic markers, Kordower and colleagues suggest that the clinical deterioration occurring from four years into PD reflects either a loss of compensatory mechanisms or degeneration of non-dopaminergic neurons. The latter possibility is worth a lot of attention, not least because it challenges the way in which neuroprotective and neurorestorative treatments for PD are currently assessed in animal models. If not the nigrostriatal neuron, then what cell types must be protected in order to halt a further

clinical progression of moderate-advanced PD? This question can only be answered by a new wave of studies establishing first a correlation, and then a causal relationship, between symptom development and pathophysiological events in specific cells and circuits within the parkinsonian brain.

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