EFFECTS OF DISEASE-RELEVANT ALPHA-SYNUCLEIN- AND LRRK2 - MUTANTS ON NEURITAL DYNAMICS IN PRIMARY MIDBRAIN DOPAMINERGIC NEURONS

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One of the main hallmarks of Parkinson's disease (PD) is the progressive loss of dopaminergic neurons in the substantia nigra pars compacta. Clinical symptoms, however, do not appear until a large fraction of nigral neurons is degenerated. In normal ageing, the number of dopaminergic neurons also decreases over time, which however does not result in clinical symptoms. This is suggestive of compensatory mechanisms in normal ageing, which may be dysfunctional in PD. Axonal regeneration and sprouting may be part of such compensatory mechanisms. Since alpha-synuclein and LRRK2 represent the two most commonly PD-associated proteins, we set out to study their role in neurital dynamics, such as growth, regeneration and vesicular transport.

We generated primary rat embryonic midbrain cultures (E14) containing ~5-10% dopaminergic neurons. Cultures were then transfected using the nucleofection technique with plasmids expressing either human alpha-synuclein (WT, A30P, A53T), LRRK2 (WT, KD, G2019S, R1441C, R1441C-KD) or EGFP (control). Cotransfection with an EGFP-expressing plasmid allowed the identification of transfected neurons. Neurite morphology was evaluated after immunocytochemical staining and vesicular transport was monitored via life-imaging of synaptophysin in vitro.

Here, we present data on neurite outgrowth, branching behaviour, regeneration, growth cone morphology and vesicular transport in dopaminergic and non-dopaminergic neurons. Our data demonstrate differential effects of alpha-synuclein, LRRK2 and their disease-relevant mutants on neurital dynamics, suggesting new roles of these proteins in the pathogenesis of Parkinson's disease.