Impact of SNCA A53T and GBA Gene Mutations on Neurite Outgrowth and Response to Parkinson's Disease Stressors in IPSC-Derived Neurons

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Background

Specific mutations in the SNCA gene, which encode α-synuclein, increase the risk of familial Parkinson's disease (PD). Additionally, GBA gene mutations, which affect glucocerebrosidase enzyme activity, are associated with sporadic and familial forms of PD, impacting lysosomal function and causing neurodegeneration. This study characterizes iPSC-derived glutamatergic neurons with SNCA A53T mutations and various GBA gene defects, comparing them to wild type (WT) control neurons. The focus is on assessing differences in neurite outgrowth and responses to PD-inducing agents.

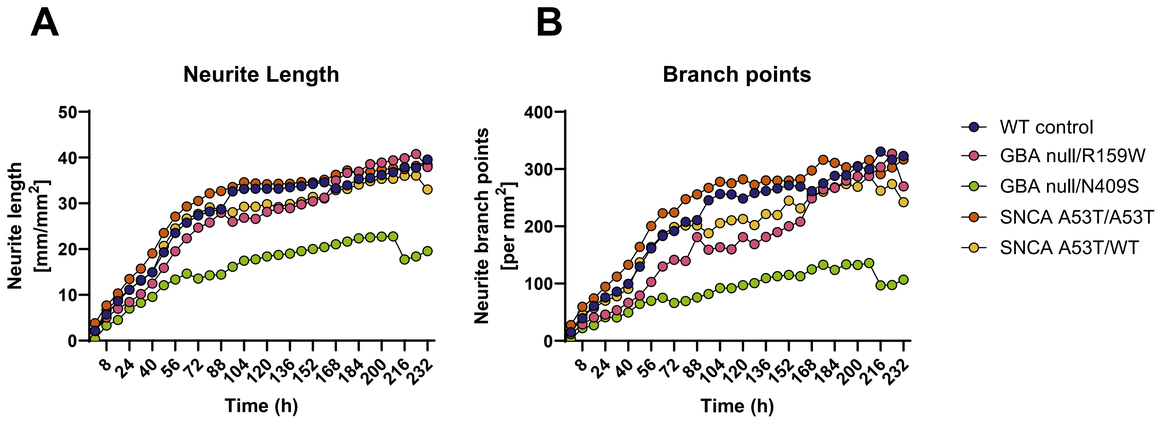
Materials and Methods

iPSC-derived glutamatergic neurons with SNCA A53T mutations (A53T/A53T and A53T/WT) and GBA gene defects (null/null, null/N409S, and null/R159W), along with WT neurons, were purchased from bit.bio Ltd., thawed and cultured following the manufacturers protocol. Neurite outgrowth was monitored over time using imaging and morphometric analysis. On day 10 of *in vitro* culture, neurons were exposed to the PD-inducing agents 1-methyl-4-phenylpyridinium (MPP+) or rotenone. Mitochondrial activity was assessed over time using the TMRM assay, and viability was measured by MTT assay. GCase activity was also measured.

Results

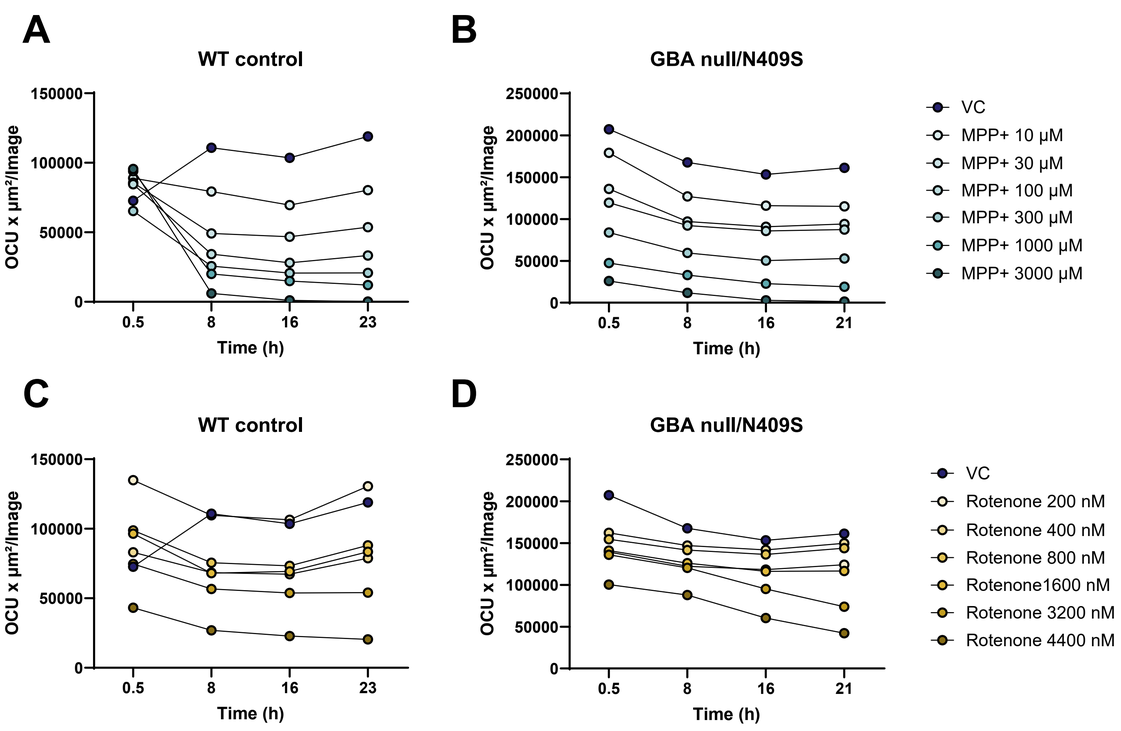
Neurite outgrowth analysis from DIV0 to DIV10 revealed that the GBA null/N409S glutamatergic cell line exhibited shorter neurite length and fewer branch points compared to WT neurons (Fig.1). Assessment of mitochondrial activity revealed a dose-dependent effect of both PD-inducing lesion agents, rotenone and MPP⁺ (Fig.2). Among them, MPP⁺ induced the most consistent effects (Fig.2B). The cell viability assay showed that the GBA null/N409S glutamatergic cell line is significantly affected by rotenone and although with MPP+ a decrease in cell viability was observed, significance was not reached (Fig.3). The GCase activity assay confirmed that iPSC-derived glutamatergic neurons with GBA gene defects, null/null, null/N409S, and null/R159W, showed significantly reduced GCase activity compared to WT neurons (Fig.4).

Neurite Outgrowth



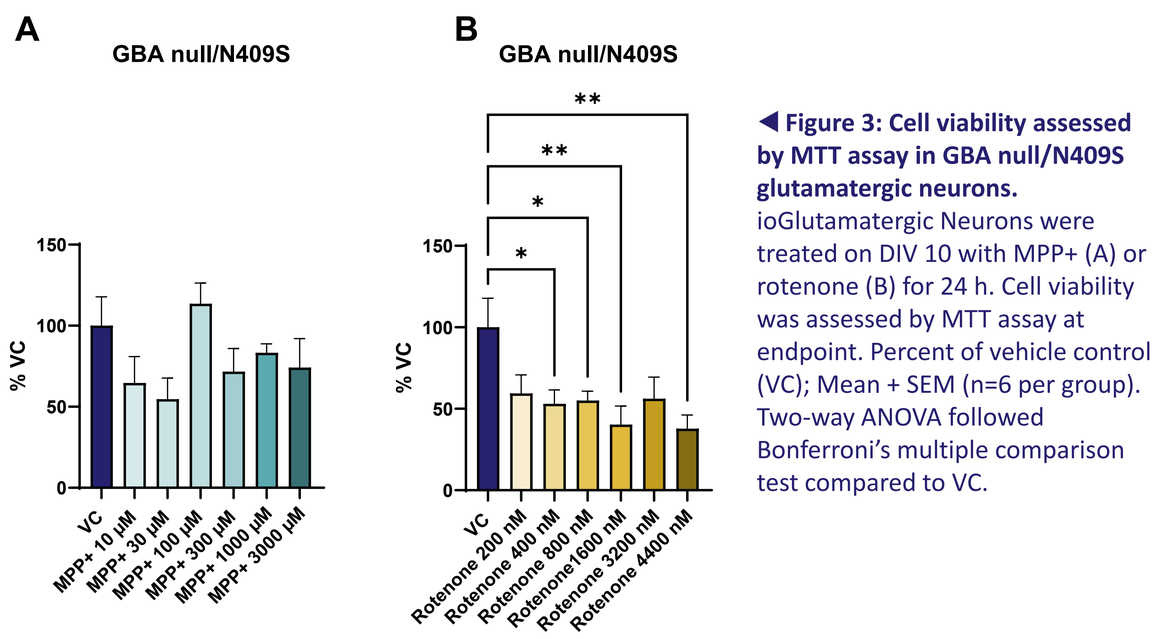
▲ **Figure 1. Neurite outgrowth assessed over time in ioGlutamatergic Neurons.** Neurite length (A) and branch points (B) were assessed over time (from DIV 0 to DIV10) by IncuCyte® Live-Cell analysis system in different types of ioGlutamatergic Neurons. Mean (n=6 per group). Two-way ANOVA followed by Dunnet's multiple comparison test versus wild type (WT).

Mitochondrial Activity



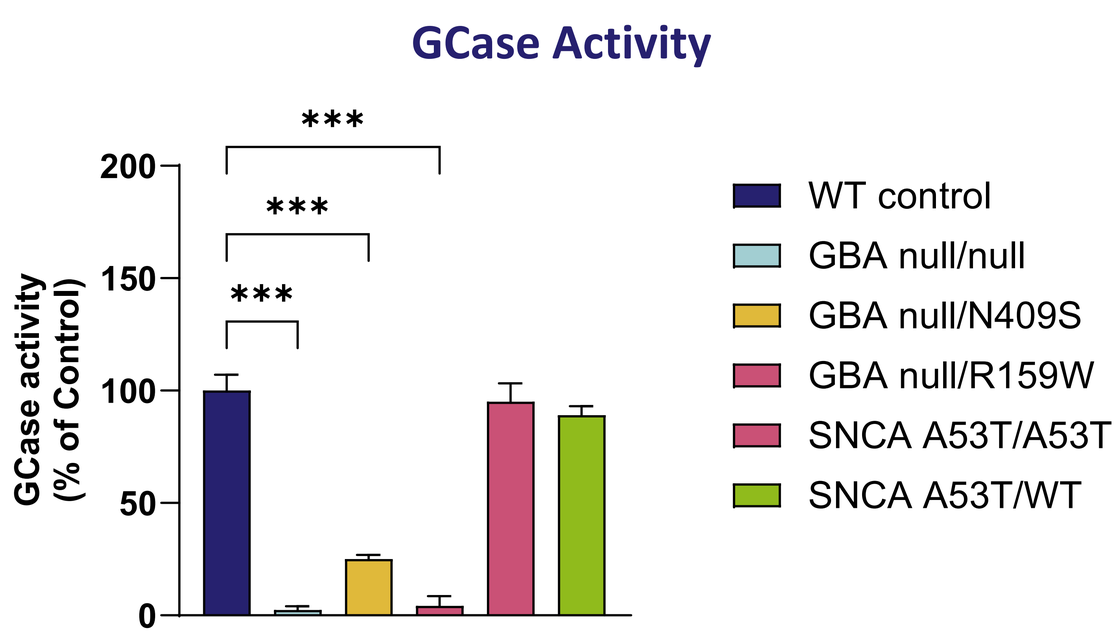
▲ **Figure 2: Mitochondrial activity assessed over time by TMRM assay in Incucyte® Live-Cell analysis system in isogenic control and GBA null/N409S cell lines.** ioGlutamatergic Neurons were treated on DIV 10 with MPP+ or rotenone at 6 different concentrations for 24 h. Total intensity (OCU X µm²/image) as mean (n=6 per group). Two-way ANOVA followed Bonferroni's Multiple comparison test compared to vehicle control (VC). OCU: Total orange object integrated intensity.

Cell Viability



◀ **Figure 3: Cell viability assessed by MTT assay in GBA null/N409S glutamatergic neurons.** ioGlutamatergic Neurons were treated on DIV 10 with MPP+ (A) or rotenone (B) for 24 h. Cell viability was assessed by MTT assay at endpoint. Percent of vehicle control (VC); Mean + SEM (n=6 per group). Two-way ANOVA followed Bonferroni's multiple comparison test compared to VC.

GCase Activity



▲ **Figure 4: GCase activity ioGlutamatergic Neurons.** GCase activity was measured on DIV9 on different types of ioGlutamatergic Neurons (25,000 cells/well) with SNCA A53T mutations: SNCA A53T/A53T and SNCA A53T/WT as well as GBA gene defects (GBA null/null, GBA null/R159W and GBA null/N409S), wild type control neurons were also included in the experiment. One-way ANOVA followed by Dunnett's multiple comparison test versus wild type (WT). ***p<0.001.

Conclusion

iPSC-derived glutamatergic neurons with PD-associated mutations are key tools for understanding the disease and developing new therapeutic strategies. Our preliminary findings suggest that iPSC-derived glutamatergic neurons with GBA defects potentially exhibit distinct characteristics compared to wild type controls with the GBA null/N409S mutation showing the highest vulnerability. In case of mitochondrial activity-related phenotypes, longer timeframe may be required for full manifestation of the pathology. Further research is needed to fully characterize these cell models. However, these results support the models' validity and suitability for evaluating developmental compounds and investigating PD mechanisms.

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