#572 GLIAL-NEURONAL INTERACTION PROMPTED BY RECOMBINANT FIBRILLAR PROTEIN CONSTRUCTS OF ALPHA-SYNUCLEIN – AN IN VITRO CHARACTERISATION

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Aims: Recombinant fibrillar protein constructs of alpha-synuclein have recently been developed in efforts to establish new cell and animal model systems in which representative synucleinopathy-related pathology is generated more rapidly than current models. In the present investigation, we explore in primary cultures the glial-neuronal interactions triggered by the administration of a variety of monomeric, oligomeric, and fibrillar alpha-synuclein preparations provided by StressMarq Biosciences Inc. Primary rat cortical neuronal, mixed glial, enriched microglial, and astroglial cultures are treated for 14 days with monomer-seeded$^{SPR-321}$ preformed fibrils (PFFs); type 1$^{SPR-322}$, type 2$^{SPR-317}$, or type 3$^{SPR-448}$ PFFs; A53T mutant alpha-synuclein PFFs$^{SPR-326}$; soluble filaments$^{SPR-450}$; or dopamine-stabilised oligomers$^{SPR-466}$. Glial conditioned media (CM) is collected, filtered, and used to treat neurons for 48 hours. Delivery of the alpha-synuclein gene using an adeno-associated viral (AAV) vector is an effective means to drive protein expression within neurons over time. Neurons are treated with the AAV5 serotype encoding human wildtype alpha-synuclein (AAV5-CBA-alpha-synuclein$^{1x10^{13}\mu g/ml}$). Following transfection of neurons, microglia are treated with CM of alpha-synuclein-expressing neurons. Immunocytochemistry, qPCR, and multiplex infrared immunoassays are performed to determine cell morphology, activation state, viability, and inflammatory profile. These preparations have shown to generate representative synucleinopathy-related pathology in vitro, informing the study of glial-neuronal interactions associated with alpha-synuclein aggregation. Alpha-synuclein fibrils are shown to "seed" their own monomers into more complex structures during aggregation. As such, we propose the in vitro cellular investigation of fibrillar alpha-synuclein protein constructs to be the optimal approach for exploring in a time frame of days what occurs over years in humans.

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