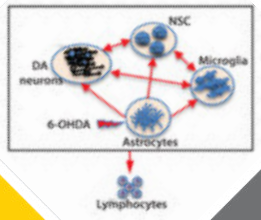
Brain Diseases

PARKINSON'S DISEASE



Schematic Representation of Cell-Cell Interaction Complexity Recreated In Vitro

Background

The precise cause of Parkinson's Disease (PD) is unknown, but there is a consensus that an inflammatory event is involved in the initiation of neurodegeneration, and that chronic neuroinflammation is a sustaining and exacerbating reason for the loss of the dopaminergic neurons.

Recent findings have revealed that the functional interaction between astrocytes, microglia and neurons govern both the sequence of inflammatory events (i.e. cascades of inflammatory mediators) and the pathological outcome (damage or absence of damage) to neurons.

Among the proinflammatory molecules, cytokines play a central role in the self-propagation of neuroinflammation in PD.

In spite of the evidence indicating that inflammation might influence the pathogenesis of PD, there is considerable debate concerning which molecules are synthesized and released, how astrocytes and microglia interact reciprocally and with neuronal cells within the neurovascular unit, and how the kinetic responses and the precise connectivity of the inflammatory cascades are regulated.

Pathology Model

In order to evaluate the CLIENT's compound modulatory activity of the neuroinflammatory events leading to dopaminergic neuron degeneration, physiologically relevant cell cultures of dopaminergic (DA) neurons will be either directly intoxicated with 6-OHDA or exposed to 6-OHDA primed glial medium. Both the direct effects on dopaminergic neurons as well as the microglial-mediated effects will be taken into consideration.

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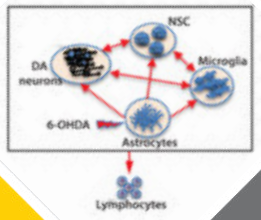

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Readouts

After reoxygenation the following parameters will be evaluated:

Step 1 - Direct Neuronal Damage

4. Morphological Characterization
    - Qualitative evaluation of neuronal cytoskeletal disruption
    - Quantitative evaluation of dendritic branching
    - Quantitative evaluation of neurite elongation modulation
5. Biochemical Characterization
    - Quantitative evaluation of neuronal cell death (i.e. PI/DAPI/Calcein AM)
    - Quantitative evaluation of caspase activation (i.e. 3 or 8 or 9)
    - Quantitative evaluation of DNA degradation (i.e. TUNEL staining)
6. Analysis of Oxidative Stress
    - Quantitative evaluation of total ROS production
    - Quantitative evaluation of NO production

Step 2 - Glial Pro-Inflammatory Phenotype

1. Biochemical characterization
    - Quantitative evaluation of metabolic activity
    - Inflammatory cytokine production: (i.e. IL1 beta, TNFalpha, IL6)
    - Total ROS production
    - Quantification of NO production

2. Functional characterization
    - Quantitative evaluation of phagocytic potential
    - Quantitative evaluation of membrane permeability
    - Quantitative evaluation of microvesicle shedding