NOD-LIKE RECEPTOR PROTEIN 3 (NLRP3)

NLRP3 has arisen as a target of interest for Parkinson's disease (PD) given increased levels seen in patient brain and preclinical studies showing inhibition of NLRP3 decreases pathology and motor deficits in preclinical models of PD. A number of models have been used in preclinical studies of NLRP3 in PD. Below you will find a summary of those that are commonly used. Please note, this list is not comprehensive.

SUB-ACUTE MPTP MODEL

- **Description**: The most commonly used model for studying NLRP3 elevation in PD pathology is the sub-acute MPTP model which involves injection of 30mg/kg MPTP for 5 days in mice. This model results in nigrostriatal degeneration, including degeneration of striatal terminals and loss of dopamine neurons in the substantia nigra. Motor deficits are commonly observed as a result of this degeneration, although some groups report absence of motor phenotypes as well. The model also presents in increased astrocytic and microglial reactivity in the striatum and substantia nigra. NLRP3, cleaved Casp1, IL-1β, GSDMD, and ASC are elevated in the substantia nigra and striatum. This model also has reports of increased total aSyn, total tau, phospho-aSyn, and phospho-tau in the substantia nigra and hippocampus.
- **Recommended Use**: In this model, MPTP is converted to MPP+ by astrocytes which is internalized into dopamine neurons causing mitochondrial dysfunction. Given the number of reports of increased NLRP3 in this model across research groups, this is the most commonly used and consistent model for studying NLRP3 in PD. Given MPTP's mechanism of action, this model is good for testing NLRP3-related interventions but is not recommended for studies probing the biology of NLRP3 in the context of idiopathic PD.
- **Helpful Resources**:
  - CRO Recommendations for the MPTP Model - Atuka, Charles River Labs, Psychogenics
  - Sample MPTP-NLRP3 Publications - Han et al (2019) *Autophagy*. Lee et al (2019) *Cell Diff & Death*. Rui et al (2020) *Int J Neuropsychopharmacol*. Que et al (2021) *Frontiers in Immunology*.

LPS MODEL

- **Description**: The administration of lipopolysaccharide (LPS) has long been a method to induce inflammation. In studies of NLRP3, LPS has been delivered through intraperitoneal injection, intranasal injection, and intranigral injection to trigger nigrostriatal degeneration and NLRP3 activation in mice and rats. Regardless of route of administration, LPS induces loss of nigral dopamine neurons, increase microglial activation in the substantia nigra, and increase levels of NLRP3, cleaved Casp1, IL-1β, and GSDMD in the substantia nigra. LPS models also commonly report motor deficits resulting from the nigrostriatal degeneration and the presence of aSyn pathology.
- **Recommended Use**: The LPS model is a "sledgehammer" model whereby huge inflammatory changes/cascades are initiated through LPS administration. While it is a good model for studying extreme inflammation, it should not be the only model selected for studying NLRP3 in PD due to the lack of disease relevance.
- **Helpful Resources**:
  - Recommended Reading
    - Review on LPS models – Deng et al (2020) *Brain, Behavior, and Immunity*.
    - Sample NLRP3-LPS publications – Zhao et al (2020) *J Neuroinflam* (mouse), Sun et al (2020) *J Functional Foods* (mouse), Han et al (2019) *Autophagy* (mouse), Mao et al (2017) *Neurochem Res* (rat)

ICON KEY										
Protein Expression Level			**Protein/Gene Species**		**Mutation**	**Pathology**				
Endogenous Expression	Over-expression	Knockout	Human	Rodent	Mutant	Nigrostriatal Degeneration	α-Synuclein Pathology	Inflammation	Motor Impairments	Cognitive Impairments

* = Robustness of NLRP3 activation in this model is not well understood.

ASYN PFF MOUSE MODEL*

- **Description:** This model uses intrastriatal injection of recombinant aSyn amyloid that are 50nm or smaller (known as preformed fibrils or PFFs) into the mouse. Injection of these PFFs results in templating of the endogenous synuclein to induce pathological modifications. Two groups have reported NLRP3 activation in this model, with increased NLRP3, cleaved Casp1, IL-1β, and ASC in the nigrostriatal system. Synuclein pathology is present in this model, as is nigrostriatal degeneration at late timepoints (6 months post-injection). Motor dysfunction has been reported but is generally not a reliable readout in this model due to relatively minimal loss of nigral dopamine neurons.
- **Recommended Use:** This model should be used for studies investigating the role of aSyn in NLRP3 activation or could be used in studies on NLRP3 inhibitors for PD. While two independent groups have reported NLRP3 activation in this model, the robustness of this effect is still in question. Importantly, the aSyn PFF protein is derived from bacterial expression systems and therefore requires endotoxin removal to ensure the effects are not due to this contaminant. In addition, aSyn monomer should be used as the appropriate control rather than PBS.
- **Helpful Resources:**
  - Commercial aSyn PFF sources – MJFF aSyn PFFs (sold as monomer) or StressMarq aSyn PFFs.
  - CRO Recommendations – Atuka, Psychogenics
  - Sample PFF-NLRP3 publications – Gordon (2018) *Sci Transl Med*. Samidurai (2021) *Front Aging Neurosci*

"M83" HUMAN A53T ASYN MOUSE*

- **Description:** This transgenic mouse model overexpresses human A53T mutant aSyn under the prion promotor. In homozygous mice, robust aSyn pathology and motor dysfunction (paralysis) are observed, but the nigrostriatal system generally remains intact and synuclein pathology is not robust in dopaminergic neurons of the substantia nigra. Ren et al (2022) reports increased astrogliosis, microglial activation, and increased NLRP3 in the hippocampus along with cognitive deficits in 4 month old mice. Han et al (2019) reports increased NLRP3 and cleaved Casp-1 in the midbrain, along with nigral neuron loss in 7 month old mice.
- **Recommended Use:** This model should be used for studies investigating the role of aSyn in NLRP3 activation, especially in the context of widespread synuclein pathology in the CNS or peripheral tissues. While two independent groups have reported NLRP3 activation in this model, the robustness of this effect is still in question.
- **Helpful Resources:**
  - Model information at JPND
  - Commercial Availability – this line is available at JAX (#004479)

6-OHDA MODEL*

- **Description:** Injection of 6-OHDA into the striatum results in loss of nigral dopaminergic neurons, motor deficits, and inflammation. This model is most often performed in rats as the effects are robust and reproducible, but some have used 6-OHDA in mice. Two studies in mice and one in rat have reported elevated NLRP3, ASC, Casp-1, and IL-1β in the striatum following intrastriatal injection of 6-OHDA.
- **Recommended Use:** 6-OHDA is a potent inhibitor of mitochondrial respiration complexes I and IV. Given this, the model may be good for testing NLRP3-related interventions but is not recommended for understanding NLRP3 in idiopathic PD. While a few labs have reported NLRP3 activation, the robustness of this effect is still in question.
- **Helpful Resources:**
  - CRO Recommendations for the 6-OHDA Model - Atuka, Charles River Labs, Psychogenics
  - Sample 6-OHDA-NLRP3 Publications – Gordon et al (2018) *Sci Transl Med* (mouse), Han et al (2020) *Brain & Behavior* (mouse), Mao et al (2017) *Neurochem Res* (rat).

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