

PARKINSON'S DISEASE MODELS

There are three current techniques for producing in vivo models of Parkinson's disease: unilateral lesioning with 6-hydroxydopamine (rats), systemic injection of 1-methyl 4-phenyltetrahydropyridine (MPTP)(mice and monkeys), and systemic injection of rotenone (rats).

6-OHDA RAT MODEL

This is the traditional model for testing Parkinson's therapies, especially those intended to increase dopamine levels in the striatum. The principal advantage of this model is that it is very sensitive to dopamine agonists. The toxin 6-hydroxydopamine (6-OHDA) is injected on one side of the rat, while the opposite side serves as an intra-animal control. This injection produces DA neuron loss on the injected side while sparing the contralateral DA neurons.

Neurochemical Assay: *Tyrosine Hydroxylase (TH) Immunocytochemistry (Quantitative Morphology)*

A standard conjugated secondary antibody method is used to mark TH (tyrosine hydroxylase) immunoreactive neurons in the substantia nigra pars compacta (A9). These neurons are counted, on both right and left sides, using standard stereological techniques. Labeled cells are considered neurons if they possess at least two but no more than six processes. Counts of neurons expressing detectable levels of TH activity are a measure of functionality of substantia nigra DA neurons that survive the lesion.

Neuroprotection Assay

DA neurons in the substantia nigra are labeled with a retrograde tracer (Fluoro-Gold), which is injected into the medial forebrain bundle prior to lesioning. This long-lasting tracer remains in neurons that are protected from lesion-induced degeneration by a test compound. These neurons are then counted using stereological counting techniques.

Behavior testing

The standard behavioral test in this model has been amphetamine induced rotation (to the side contralateral to the lesion). The degree to which this

rotational side bias is reduced by a test compound has been considered a measure of the compound's restoration of dopamine function. However it is now widely thought that this behavior is a poor reflection of the Parkinson's condition in humans, and in the rat the extent of turning reflects the animal's compensatory ability to use its intact paws more than anything else. Therefore it is becoming more common to include at least three other behavioral measures: a reaching task (an assay of fine motor function that is only affected by test compounds that either are neuroprotective, or induce sprouting); a forelimb asymmetry test (also known as the cylinder test, in which the animal balances itself while rearing in a confined environment, a behavior that is especially sensitive to dopamine agonists); and tactile placing.

MPTP MOUSE MODEL

The MPTP mouse model of Parkinson's disease is thought to mimic more closely the behavioral pathology of Parkinson's disease, compared to the 6-OHDA rat model, and is currently the model of choice. Mice exhibit Parkinson's-like symptoms following systemic injection of the pyridine toxin MPTP, which produces a loss of striatal dopamine (DA) nerve terminal markers and, at higher doses, death of DA neurons in the substantia nigra. Since the process of terminal loss and degeneration takes 6-9 days following MPTP injection, drugs can be administered both before the MPTP is given (to assay neuroprotection), and/or while the toxin is active in the first week post-injection (to assay preservation of dopamine function).

(The neurochemical and neuroprotection assays are the same as for the 6-OHDA model.)

Behavior Testing

In mice, MPTP injections produce a temporary increase in hyperactivity, a long-lasting increase in wall-supported rearing in the open field enclosure (relative to "free" rearing in the center), a lasting increase in foot slippage (foot faults in a grid-walking test,) and a lasting impairment in treadmill activity (using several measures of gait). The hyperactivity returns to baseline after 2 weeks but the other three measures of impaired balance remain abnormal indefinitely. Improvements in the latter measures following drug treatment correlate with increased dopamine neuron presence (increased numbers of TH-positive cells) in the substantia nigra and increased dopamine activity in the striatum.

MPTP PRIMATE MODEL

Similar to the MPTP mouse model, this model is most often used as a final study of a test compound's efficacy before testing in humans. The non-human primate species used are either cynomolgus, rhesus, or African green monkeys. Following systemic MPTP administration, the animal's motor and cognitive functions are impaired, as in humans. In particular the animals display muscle rigidity, akinesia, and tremors, along with impairment on a common memory task, delayed match to sample (DMTS).

Neurochemical assay: *Tyrosine Hydroxylase (TH) Immunohistochemistry (Quantitative Morphology) and Dopamine turnover*

Following behavioral testing the animals are sacrificed and their brains sectioned and processed for TH immunohistochemistry through the substantia nigra, as in the comparable mouse model. Counts of TH positive cells in treated and control animals are a measure of the test compound's ability to preserve dopamine function in nigra neurons. In addition dopamine turnover is measured directly in the striatum, using HPLC.

Behavior Testing

A parkinsonism scale has been developed and validated to track the various motor deficits that result from the MPTP lesion, as well as the dyskinesias that result as a side effect of common dopamine-enhancing therapeutics, e.g. L-DOPA.

THE ROTENONE MODEL

The most recent of the Parkinson's models uses systemic injection of rotenone, a member of a class of compounds found in pesticides, and in natural products, that disrupts mitochondrial activity and results in pathology that is strikingly similar to that seen in Parkinson's disease (e.g., accumulation and aggregation of α -synuclein and ubiquitin, progressive oxidative damage, and caspase-dependent death)

Behavior Testing

Only recently have reliable behavior tests become available with this model. This reliability comes from using two distinct protocols, each with different optimal doses of the toxin.

In the first protocol, a lower dose is used that minimizes the loss of animals before the 3-week endpoint of a typical study, yet still produces a decrease in TH in the striatum. The extent of reduced TH activity is variable across rats and there is no correlation between the extent of TH loss and the behavioral deficits.

Thus, this model is appropriate for testing compounds that reduce toxicity and the resultant behavioral deficits, but their effect on dopamine activity is secondary

In the second protocol a higher dose of rotenone is used that produces pronounced neuropathology. In this case the endpoint measure is acute “toxic” behavior that requires perfusion of the animal. These animals have a reliable loss of TH in the striatum and loss of DA neurons in the nigra, both of which can be measured. A neuroprotective compound tested in this version of the model would decrease the number of rats displaying “toxic” behavior at a set post-lesion time point, or prolong the average time before “toxic” behavior. A positive control in this protocol is Coenzyme Q, which is neuroprotective.

IN VITRO MODELS

Dopaminergic neuron cultures are treated with rotenone or MPP+ for 24h, or with Epoxyomicin for 48h.

Rotenone

A botanical compound obtained from the roots of Derris sp., rotenone is an active ingredient in many pesticides. Rotenone blocks complex I of the mitochondrial respiratory chain and thereby induces nigrostriatal degeneration in rodents, thus reproducing the hallmark pathology of Parkinson’s disease.

MPP+

The active metabolite of MPTP, MPP+ is a piperidine derivative which induces a Parkinsonian syndrome in mice and primates. MPP+ selectively enters dopamine neurons via the dopamine transporter and also blocks complex I of the mitochondrial respiratory chain.

Epoxyomicin

Epoxyomicin, a natural product isolated from Actinomyces sp., is a cell-permeable, potent, selective and irreversible proteasome inhibitor. Impairment of the ubiquitin-proteasome system replicates features of Parkinson’s disease.

Neurochemical assay

In all these cultures dopaminergic neurons are visualized by confocal microscopy using a mouse monoclonal antibody anti-Tyrosine Hydroxylase (TH). The number of surviving dopaminergic neurons is determined by counting the number of TH positive neurons in four fields covering the entire surface of the culture spot.



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