



Standard Operating Procedure for *Stereotactic injection of 6-Hydroxy dopamine (6-OHDA)* *Acute, unilateral model of Parkinson's Disease*

Our core offers two animal Models of Parkinson's Disease: (1) acute unilateral PD produced by 6-OHDA induced lesion of brain DA neurons and (2) a progressive model of PD induced by chronic treatment with pesticide (Paraquat) and fungicide (Maneb).

1.0 PURPOSE

Parkinson's disease (PD) is a well-characterized disorder with several animal models. These animal models provide an outstanding opportunity test the ability of novel therapies to treat, delay, or cure the disease. The purpose of this procedure is to generate disease model which will be used in stem cell therapy. We will acutely lesion dopamine neurons on one side of the brain to produce quantifiable neuroanatomical changes, behavioral deficits and neurotransmission deficits (measured by PET or microdialysis). Subsequently, the efficacies of different stem cell therapies aiming at preventing or reversing this unilateral Parkinson-like syndrome will be tested

The basic design of all the experiments is:

1. Induce damage and assess deficits (behavioral, neurochemical, neuroanatomical)
2. Administer therapy (drug, stem cells, or gene therapy)
3. Assess recovery of function (behavioral, neurochemical, neuroanatomical)

Each subject will receive ONE form of damage and ONE treatment. Subjects therefore must undergo separate procedures for inducing damage, treatment, and assessing the recovery of function.

2.0 SCOPE (Should include which Cores this SOP applies to)

This procedure applies to all personnel who will use the 6OHDA model of PD in mice on the within the Stem Cells Engraftment and *in vivo* Analysis Core.

3.0 PROCEDURE

3.1 Introduction of Pathology

Standard stereotaxic surgery will be performed and mice will be kept in stereotaxic frame under anesthesia (a mixture of oxygen and Isoflurane). The scalp is scrubbed with soap, then alcohol then betadine). The head is placed in a stereotaxic apparatus for rodents and held in place by ear bars and a nose cone. A midline incision is made on the top of the skull and the skin retracted. Marcaine (0.25%) is applied topically to the fascia. The underlying fascia is then pushed away and a hole is drilled through the skull and the skull and dura overlaying the striatum on one side of the brain. A small bur hole is drilled above the injection site using sterile bit. A 30 gauge stainless steel infusion needle connected to a Hamilton syringe is inserted into the brain by lowering the stereotactic arm to precisely lower the needle into place. The unilateral injection of 6OHDA into Medial Forebrain Bundle (pathway from Dopamine neurons which connects substantia nigra with striatum) will be stereotactically placed using micromanipulator screw at 1 ul/min. Stereotactic injection of 6OHDA (0.5-.7 ug) in 0.5 – 2.0 ul of 0.2% ascorbic acid is performed using micromanipulator screw at 1 ul/min. The wound is closed using standard sutures for the skin and the Marcaine applied to the wound site (see 16F1). This surgery will take less than 20 min. During all stereotaxic surgeries performed in

our lab, as well in this proposed study, we continuously monitor mouse's breathing and reflexes, therefore, we do not cover animal body with a drape. After recovery from anesthesia mice are moved to home cages.

3.2 Evaluation

1. behavioral assessment of motor functions (see SOPs for behavioral tests)
2. In vivo brain Microdialysis – measurement of Dopamine release (see SOP for Microdialysis)
3. Immunocytochemistry – stereology assessment of neuronal loss (see SOP for stereology)

Damage: Mice with unilateral lesion of dopamine neurons by stereotaxic injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle will be tested for behavioral as well as for the physiological and neuroanatomical abnormalities.

Assessment (Damage and Recovery of Function): Animals will be monitored repetitively by micro-PET (SOP – PET) and behavioral testing (SOP behavioral tests) before and after the lesion (at approximately days 7, 14 and 30 post-surgery) to monitor development of deficits, in particular the point at which these deficits stabilize (typically 14 days in 6-OHDA model).

Treatment: This repetitive behavioral and PET monitoring will reduce number of mice needed for these studies. The lesioned and sham-lesioned mice can be subdivided into cohorts which will be subjected to different therapeutic treatments (listed below), behavioral tests, PET imaging and to the end point analyses.

Endpoint: At the end of experiment the lesioned mice and their sham-lesioned control can be subjected to ONE of the following:

- (a) measure DA tissue content (HPLC) and dopamine transporter (DAT) levels
- (b) perfusion so the brain structures can be analyzed using stereological methods (Dopamine neurons, grafted and endogenous drug- or gene therapy-activated brain stem cells).

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The broader goals of the experiments using these models are:

1. Test the therapeutic potential of endogenous and grafted stem cells, and gene-therapy on neurodegenerative diseases
2. Determine the mechanism by which successful therapies ameliorate the symptoms of neurodegenerative diseases

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The basic design of all the experiments is:

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Each subject will receive ONE form of damage and ONE treatment. Subjects therefore must undergo separate procedures for inducing damage, treatment, and assessing the recovery of function.

The 6-OHDA model:

The typical experimental design can be summarized as:

(Surgery 1) (Surgery 2)
Damage -----> Assess Damage -----> Treatment -----> Assess Recovery of Function -----> Endpoint

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- ©microdialysis to evaluate *in vivo* DA release,

All mice will be subjected to stereotaxic 6-OHDA lesion (first surgery). In addition mice used in gene therapy or cell transplantation will be subjected to a second stereotaxic surgery (TOTAL 2 SURGICAL PROCEDURES).

1. 6-hydroxydopamine unilateral lesion of dopamine neurons. The purpose of this procedure is to generate disease model which will be used in stem cell therapy. We will acutely lesion dopamine neurons on one side of the brain to produce quantifiable neuroanatomical changes, behavioral deficits and neurotransmission deficits (measured by PET or microdialysis). Subsequently, the efficacies of different stem cell therapies aiming at preventing or reversing this unilateral Parkinson-like syndrome will be tested.
2. Standard stereotaxic surgery will be performed and mice will be kept in stereotaxic frame under anesthesia (a mixture of oxygen and Isoflurane). The scalp is scrubbed with soap, then alcohol then betadine). The head is placed in a stereotaxic apparatus for rodents and held in place by ear bars and a nose cone. A midline incision is made on the top of the skull and the skin retracted. Marcaine (0.25%) is applied topically to the fascia. The underlying fascia is then pushed away and a hole is drilled through the skull and the skull and dura overlaying the striatum on one side of the brain. A small bur hole is drilled above the injection site using sterile bit. A 30 gauge stainless steel infusion needle connected to a Hamilton syringe is inserted into the brain by lowering the stereotaxic arm to precisely lower the needle into place. The unilateral injection of 6OHDA into

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- **Mice evaluation –**
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