Standard Operating Procedure
For

Positron Emission Tomography (PET) monitoring of stem cell therapy.

PURPOSE

The effects of modeled diseases and therapies can be monitored as a function of time in in vivo using in noninvasive measures of the brain activity using micro-PET

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

Assessment: The animals will be monitored repetitively by micro-PET, and behavioral at different time after 4 and 8 weeks of diseases induction and therapeutic intervention to monitor development brain degenerative and restorative changes.

3.0 PROCEDURE

3.1 Micro-PET (only on C57Bl/6J mice): this procedure will be performed by **Dr. Robert Miletich** (Dept. of Nuclear Medicine, member of SCEF) and by trained LAF personnel. Mouse under isofluorane gas inhalation anesthesia will be positioned in the micro PET camera. Anesthesia is administered for analgesia, anxiolysis and chemical restraint. Anesthesia induction is in a separate plastic cage. After induction, mice are quickly moved to the scan table, their bodies properly positioned and their snouts placed inside a plastic Isoflurane gas delivery system, with their snout placed through a plastic membrane which imparts no damaging force on the head soft tissues. Body heat is maintained by a heated water pad. Anesthesia is titrated by level of arousal and regularity and ease of respiration. Body temperature, respiration and level of arousal are constantly monitored. The scan time is only one hour. After scanning, anesthesia is immediately removed and water p.o. is available ad libitum. Also, SC fluids (1-2 ml) be administered following the MicroPET procedure for better recovery.
PET scanning will be done with the cerebral blood flow tracer, [^{15}O]-water (H2O), and the dopamine (DA) D2/D3 receptor ligand, [^{11}C]raclopride (RAC). H2O will provide a regional measure of overall regional cerebral function or physiology. RAC will assay changes of receptor function and in particular DA receptor density[76]. Since D2 receptors reside on intrinsic striatal neurons, including medium spiny interneurons, the decreased density of D2 receptors is a direct marker for cellular viability. Altered regional function and decreased D2 receptor density have been well-characterized in Huntington's disease[77-80] and in animal models of the disease[81-83]. In addition to measure density of dopamine terminals the dopamine transporter ligand 11C-Methylphenidate at a radioactivity dose of 0.5-1 mCi will

be injected into penile venous cistern. At the same time PET data acquisition will be started. The data will be acquired in the list mode for 45 min. It will be ensured that the ligand is injected at high specific activity (>250 mCi/micromol) to reduce the volume of injected fluid and also to make sure that only a very small amount (Tracer dose) of methylphenidate is injected. The injection volume will not exceed 1% of the body weight.

These in vivo changes have even been proposed as diagnostic markers[80]. By measuring the basal physiologic processes of cerebral blood flow with [^{15}O]-H_2O PET (H_2O), we will simultaneously derive measures of regional function and of cellular viability. Regional blood flow reflects in major part the amounts of synaptic activity present, as well as the densities of regional cell populations[84]. Sequential, near simultaneous, acquisition of regional blood flow and D2/3 receptor densities will help disentangle function from cell viability. D2/3 receptor determinations also provides specificity to the viability question, as the main cellular elements which express D2 receptors in the striatum are neurons. In the current project we will be measuring both regional blood flow with H_2O-PET and regional RAC-PET to establish the effects of our interventions on both basal physiologic processes and neuronal cellular viability.

PET by its nature is a relatively non-invasive technique which can effectively probe just about any physiologic or chemical process in the body with the appropriate positron-emitting molecular probe. MicroPET, or the ability to image small animals, including mouse, rat and rabbit, has extended this opportunity of using PET to small animal models of human disease (for example, Fig. 9). This not only allows a detailed examination of the relation between molecular biology assays and more basal measures of physiology, as are currently acquired in the clinical PET, but will facilitate transitional research in applying small animal model discoveries to develop improved diagnostic and therapeutic clinical interventions, including gene therapy. This current project is just such transitional research. By using microPET, we are providing a proof in principle of PET use in humans, if the current interventions and monitoring methods prove efficacious. This project will effectively use this technology to follow cellular and tissue restoration.

After the scan, data will be preprocessed and images will be reconstructed using filtered back projection and analyzed using various software systems including Amide, Matlab and SPM. The analysis will provide qualitative and quantitative estimates of the ligand binding. Since methylphenidate is a specific ligand for dopamine transporter, its signal will indicate the density of dopamine terminals in the mouse brain. After PET session animal will be transferred to an adjacent Radioactivity Recovery Room, placed in a recovery cage for at least 10 half-lives (for C-11, that is 200 minutes), but usually up to 24 hours. This is sufficient for complete decay of the radioactive material. Subsequently the mice will be returned to their housing room. This procedure will be repeated three times for an individual mouse, 3-5 weeks apart.