Standard Operating Procedure
For

in vivo microdialysis

1.0 PURPOSE

The ability to measure extracellular basal levels of neurotransmitters in the brain of awake animals allows for the determination of effects of different systemic challenges (pharmacological or physiological) to the CNS. For example, one can directly measure how the animal's midbrain dopamine projections respond to dopamine-releasing drugs like d-amphetamine or natural stimuli like food. As well, precise introduction of drugs through the microdialysis probe allows for refined work on site specificity in a compound s mechanism of action. This technique has excellent anatomical and chemical resolution but only modest time resolution as microdialysis samples are usually processed every 20-30 minutes to ensure detectable neurotransmitter levels. Complementary ex vivo tools (i.e., slice and cell culture electrophysiology) can assist with monitoring real-time neurotransmission.

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

This Procedure is designed to show you how to implant guide cannulae targeting specific sites in the mouse brain, how to insert and implant a microdialysis probe and how to use high performance liquid chromatography coupled with electrochemical detection

3.0 PROCEDURE

3.1 Stereotactic Implantation of Cannulae

The microdialysis procedure will be conducted once, for a period up to 18 hr. Approximately 10 hr before sample collection begins; mice will be moved to a microdialysis area within the LAF facilities where, a microdialysis probe will be inserted through the indwelling guide cannula and will extend 2 mm beyond the tip of the cannula within the target brain region (i.e. striatum).

3.2 Microdialysis of Artificial Cerebrospinal Fluids

The microdialysis probe is essentially a porous tube ('u'-shaped) through which artificial cerebral spinal fluid (aCSF) is perfused. As it passes through the microdialysis probe the aCSF 'picks-up' analytes from the extracellular space in the brain. It does this through the process of diffusion. To push the aCSF through the probe and to collect it after dialysis, two pieces of small diameter Teflon tubing are attached to the

microdialysis probe – an inflow and an outflow tube. The inflow tubing is attached to a syringe pump that pushes the aCSF through the probe at rates less than 2.5µl/min. The outflow tubing ends in a microcentrifuge tube for recapture of the now dialyzed aCSF. The inflow and outflow tubing is protected attaching it to 12" long lightweight metal wire. The metal wire is attached to the tether screw at one end and a liquid swivel at the other end. The swivel is hung on an arm in the center of the top of the cage (~13" above the top of the cage). The wire/swivel/arm assembly allows the mouse free movement around their cage without getting entangled in the probe tubing and without undue pull on the cannula stage. A 8-10 hr period of habituation and equilibration is given before any samples are collected. Samples are collected of 6-8 hrs. In total, the mouse is in the microdialysis chamber, tethered, so that they can move freely around the chamber without damaging the inflow and outflow tubing, for 14-18 hrs. All materials used for microdialysis including the mouse tether system are standards in the field and used in many laboratories. The mice will have a free access to food and water.

Created by Ewa Stachowiak, 15 December 2011