Suven Microdialysis Services

*In Vivo* Brain Microdialysis Studies in Rodents for Monitoring Changes in Neurotransmitters
Acetylcholine
Histamine and Metabolite
GABA and Glutamate
Monoamines (NE, DA, 5HT & Metabolites)

Simultaneous Monitoring of Neurotransmitters and Unbound Test Compound Concentrations

*In Vivo* Spinal Microdialysis in Rodents for Monitoring Changes in Inflammatory Biomarkers and Neurotransmitters Responsible for Pain

Mechanism of Action and PK/PD Studies
CSF Pharmacokinetics
Neurodegenerative models
Suven Life Sciences Limited

Suven Life Sciences Limited is a Bio-pharmaceutical Company in existence since 1989 based at Hyderabad, India. Suven Drug Discovery & Development Support Services (DDDSS) is providing research services to global Pharma and Biotech companies.

**Suven In-Vivo Microdialysis**

**List / Type of Studies being offered**

Microdialysis team has expertise in conducting brain and spinal microdialysis studies to measure neuronal changes in freely moving animals.

Using brain microdialysis, we evaluate the modulation of neurotransmitters (and/or test compound) in most of the brain areas of freely moving animals.

Spinal microdialysis evaluates modulation of inflammatory biomarkers and neurotransmitters in spinal regions.

Neurotransmitters and test compounds are quantified by specific and sensitive analytical techniques.

**Infrastructure**

- Six Microdialysis Workstations
- Twelve Animals Can Undergo Dialysis Simultaneously
- Automated and Programmed Sample Collection under Refrigeration Over 24 hours
- Well Trained and Team of Scientists
- 2-3 Weeks Turnover Time for a Typical Study

**Preclinical Species**

- Rat (Wistar/Sprague Dawley)
- Guinea pig (Dunkin Hartley)
- Mouse

**Neurotransmitters**

- Acetylcholine
- Glutamate
- Gamma-amino butyric acid (GABA)
- Histamine
- Dopamine, Norepinephrine and Serotonin
- Monoamine Metabolites
Study Designs

- Parallel Treatment Groups
- Cross-over Treatment Design
- Mechanism of Action Studies using various Antagonists/Blockers
- PharmacoKinetic, PharmacoDynamic (PK/PD) Studies \textit{in vivo}, by simultaneous monitoring of neurotransmitters and drug concentrations

Routes of administration

- Systemic (\textit{p.o., i.p., s.c., i.v.} – bolus and infusion)
- Prolonged infusion using osmotic infusion pumps
- Local application (retrodialysis)
- Intracerebroventricular (ICV) injection

Typical experimental protocol

<table>
<thead>
<tr>
<th>Preparation of animals for study</th>
<th>Acclimatization 4-5 days</th>
<th>Stereotaxic surgery 0.5-0.75 h</th>
<th>Recovery 2-5 days</th>
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<tbody>
<tr>
<td>Stereotaxic surgery</td>
<td>Guide cannula implantation</td>
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<table>
<thead>
<tr>
<th>Experimental Day</th>
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<tr>
<td>Stabilization 1-2 h</td>
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<tr>
<td>Basal collection 1-2 h</td>
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<tr>
<td>Modulation of neurotransmitters in dialysates and test compound levels in dialysates and plasma</td>
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<td>Probe implantation - 16 h before</td>
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<td>Test compound administration</td>
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<tr>
<td>Based on the protocol</td>
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Flow rate: 1.0 – 2.0 \(\mu\text{L/min}\)

Sample collection intervals:

- Microdialysates: \(15 - 30 \text{ min.}\)
- Blood sampling (through jugular vein) from same animal: \(6 – 8 \text{ points during absorption, distribution and elimination phase (or) same animal will be used after wash out period for exposure in plasma, brain and CSF (based on neurotransmitter profile)}\)
Fractions collectors:

- Samples are collected at 4 °C using programmed, refrigerated fraction collectors (up to 24 h post treatment).

Histological Probe placement verification:

At the end of the experiment, probe placement is verified in each brain. Representative probe placement tracks in mPFC (a) and ventral hippocampus (b) of rat respectively.

Overview of Analytical Methods:

- Microdialysis samples:
  - Acetylcholine: LC-MS/MS (API-4000)
  - Amino acids: Glutamate and GABA – HPLC + Fluorescence
  - Histamine: HPLC + Fluorescence
  - Monoamines: LC-MS/MS (API-4000 Q-Trap)
  - Monoamine metabolites: HPLC-ECD (BASi Epsilon)
  - Test compounds: LC-MS/MS (API-4000 Q-Trap)
  - Prostaglandin E2: ELISA

- CSF samples:
  - Monoamines (5-HT & 5-HIAA): HPLC + ECD (BASi or ESA)
  - Histamine and alpha methyl histamine: HPLC + Fluorescence
  - Prostaglandin E2: ELISA
  - Interleukin-1β: ELISA
Plasma and Brain tissue samples:
- Monoamines: HPLC + ECD (BASi or ESA)
- Test compound: LC-MS/MS (API-4000 Q-Trap)

Summary of Validation Experiments

**Acetylcholine**

i. Modulation of acetylcholine in ventral hippocampus by **donepezil** in male Wistar rats

![Graph showing modulation of acetylcholine in ventral hippocampus by donepezil](image)

- Perfusion fluid: aCSF
- **Flow rate**: 1.5 µL/min
- **Sampling duration**: 20 min

ii. Modulation of acetylcholine in ventral hippocampus by **Olanzapine** in male Wistar rats

![Graph showing modulation of acetylcholine in ventral hippocampus by olanzapine](image)

- Perfusion fluid: aCSF containing 0.1 µmol neostigmine
- **Flow rate**: 2.4 µL/min
- **Sampling duration**: 15 min
iii. Modulation of acetylcholine by Olanzapine in prefrontal cortex of Dunkin-Hartley guinea pigs

Quantification of Acetylcholine

Acetylcholine concentrations in microdialysate samples are quantified by using HPLC coupled with tandem mass spectrometry (LC-MS/MS) without any sample pretreatment.

- Quantitation range: 0.05-103.50 nM

Typical chromatogram of acetylcholine

**Perfusion fluid:** aCSF containing 0.1 µmol neostigmine
**Flow rate:** 1.5 µL/min
**Sampling duration:** 20 min
Glutamate

i. SB-271046: modulation of glutamate in frontal cortex of male Sprague-Dawley rats

![Graph showing glutamate changes over time for SB-271046 and Vehicle.

Perfusion fluid: aCSF  
Flow rate: 1.25 µL/min  
Sampling duration: 15 min]

ii. Ketamine: modulation of glutamate in prefrontal cortex of male Sprague-Dawley rats

![Graph showing glutamate changes over time for Vehicle and Ketamine 18 mg/kg, s.c.

Perfusion fluid: aCSF  
Flow rate: 3.0 µL/min  
Sampling duration: 20 min]

Quantification

Concentrations of glutamate in dialysates are determined by pre-column derivatization using HPLC-fluorescence method.

![Calibration curve for glutamate analysis.]

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Histamine

Modulation of histamine by **GSK-189254** in prefrontal cortex of male Sprague-Dawley rats

![Graph showing histamine levels](image)

**Perfusion fluid:** aCSF  
**Flow rate:** 1.5 µL/min  
**Sampling duration:** 20 min

Quantification

Concentrations of histamine in microdialysates are analyzed by HPLC and fluorometric detection after post-column derivatization with O-phthalaldehyde (OPA) reagent, which is delivered by a secondary flow system.

![HPLC and fluorometric detection diagram](image)
Dopamine, Norepinephrine and Serotonin

i. Modulation of monoamines by venlafaxine in prefrontal cortex of male Sprague-Dawley rats

Perfusion fluid: aCSF
Flow rate: 1.0 µL/min
Sampling duration: 30 min

ii. Modulation of monoamines by atomoxetine in prefrontal cortex of male Wistar rats
Quantification

The catecholamine neurotransmitters dopamine, norepinephrine and serotonin were subjected for derivatization with dansyl chloride and following precursor-product ion pairs were monitored with m/z 853.1–170.1, m/z 869.2–170.1 and m/z 643.3-170.1 for the dansylated dopamine, norepinephrine and serotonin respectively. The analytes were quantified using triple quadrupole tandem mass spectrometer in positive ionisation mode using atmospheric pressure ionization source (Ref: Nirogi et al. 2013 Journal of Chromatography B. 913-914, p. 41-47). The test samples were quantified against a calibration curve prepared for each of the neurotransmitter using artificial cerebrospinal fluid in the calibration range of

- Dopamine 0.066-14.835 nmol
- Norepinephrine 0.066-14.728 nmol
- Serotonin 0.066-14.689 nmol
Determination of unbound brain concentrations of test compounds in rats.

i. Unbound concentration of escitalopram in prefrontal cortex

![Graph showing plasma and dialysate concentrations of escitalopram over time.]

**Perfusion fluid:** aCSF  
**Flow rate:** 1.0 µL/min  
**Sampling duration:** 20 min  
**Test compound:** Escitalopram 2.5 mg/kg, i.v.

ii. Unbound brain concentration of quetiapine in striatum

![Graph showing plasma and dialysate concentrations of quetiapine over time.]

**Perfusion fluid:** aCSF  
**Flow rate:** 1.2 µL/min  
**Sampling duration:** 20 min  
**Test compound:** Quetiapine 30.0 mg/kg, s.c.
CSF and Brain Tissue Pharmacokinetic

i. CSF concentrations of Raclopride

![Graph showing the concentration of Raclopride in CSF over time.]

- **Raclopride - 3 mg/kg, s.c**

**Group size:** n=5/ time point
**Test compound:** Raclopride 5.0 mg/kg, i.p.

ii. CSF concentrations of Carbamazepine

![Graph showing the concentration of Carbamazepine in CSF over time.]

- **Carbamazepine - 4 mg/kg, i.v**

**Group size:** n=5/ time point
**Test compound:** Carbamazepine 4.0 mg/kg, i.v.