

# Suven Microdialysis Services

***In-Vivo* Brain Microdialysis Studies in Rodents**

**Measuring the Neurotransmitters in Microdialysis Samples**

**Acetylcholine; Histamine and Metabolite; GABA and Glutamate**

**Monoamines (NE, DA, 5HT & Metabolites)**

**Neurotransmitters/ Unbound Drug Concentrations**

**In Microdialysate Samples**

**Mechanism of Action and PK/PD Studies**

**CSF Pharmacokinetics and Neurodegenerative models**

---

**Suven Life Sciences Ltd**

**Serene Chambers, Road-5, Avenue-7, Banjara Hills, Hyderabad – 500 034, India**

**Phone: 91-40-23556039, Fax: 91-40-23541152**

**E-Mails: [nvsrk@suven.com](mailto:nvsrk@suven.com); [knvishu@suven.com](mailto:knvishu@suven.com)**

## **Suven *In-Vivo* Brain Microdialysis**

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

### **List / Type of Studies being offered**

Microdialysis team has expertise in conducting brain microdialysis studies to measure neuronal changes in freely moving animals. Using brain microdialysis, we investigate the highly focused neurotransmitters (and/or test compound) in most of the brain areas of freely moving animal. The neurotransmitters and test compounds are quantified by sensitive analytical techniques.

#### **Infrastructure**

- Four Microdialysis Workstations
- Eight Animals undergo Experimental in Parallel
- Automated Sample Collection under Refrigeration over 24 hours
- Well trained six brain microdialysis study scientists
- 2-3 Weeks Turnover Time for a Typical Study

#### **Species Available**

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

#### **Neurotransmitters:**

- Acetylcholine
- Glutamate
- Histamine
- Serotonin and Metabolite
- Dopamine and Metabolite
- Gamma-amino butyric acid (GABA)

#### **Study Designs**

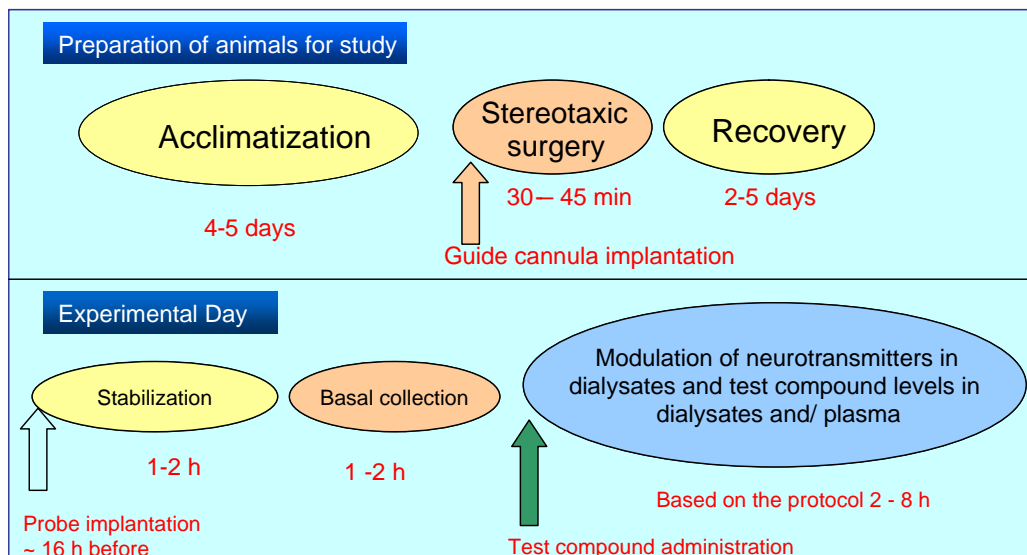
- Parallel Treatment Groups

- Cross-over Treatment Design
- Mechanism of Action Studies using various Antagonists/Blockers.
- Pharmacokinetic, Pharmacodynamic (PK/PD) Studies *in vivo*, by simultaneous monitoring of neurotransmitters and drug concentrations.

### Routes of administration

- Systemic (*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:



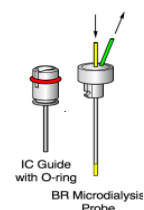
Flow rate : 1.0 – 2.0  $\mu\text{L}/\text{min}$

Sample collection intervals:

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

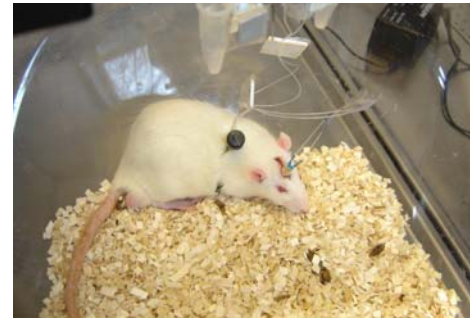
Microdialysis Guide cannulae and probes:

- CMA/11 or CMA/12 (PAES) – 1, 2, 3 and 4 mm
- BASi (CA) – 2 and 4 mm



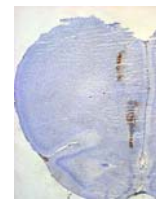
#### Fractions collectors:

- Samples are collected at 4 °C using CMA/170 refrigerated fraction collectors. (up to 24 h post treatment also possible)

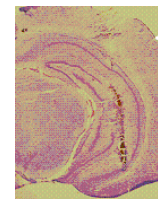


#### Histological Probe placement verification:

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.



(a)



(b)

### Overview of Analytical Methods:

#### ▪ Microdialysis samples:

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

#### ▪ CSF samples:

- Monoamines (5-HT & 5-HIAA) : HPLC + ECD (BASi or ESA)
- Histamine and alpha methyl histamine : HPLC + Fluorescence

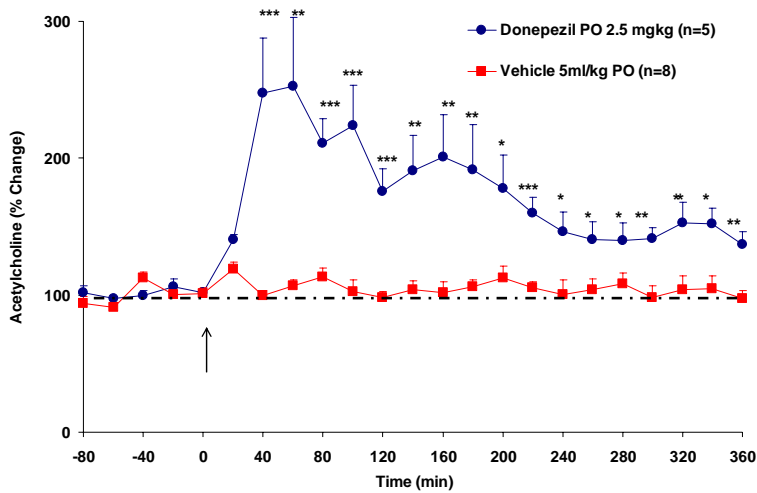
#### ▪ Plasma and Brain tissue samples:

- Monoamines (DA, DOPA, 5-HT & 5-HIAA) : HPLC + ECD (BASi or ESA)
- Test compound : LC-MS/MS (API-4000 Q-Trap)

## Summary of Validation Experiments

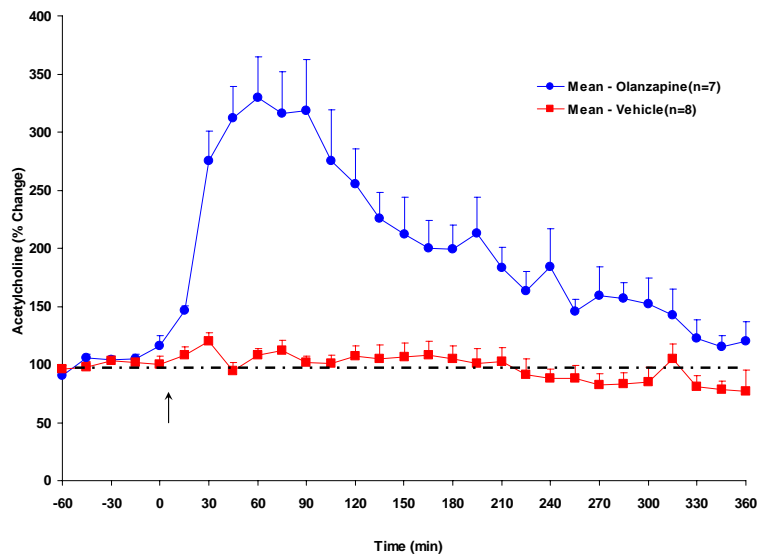
### Acetylcholine

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



*Perfusion fluid: aCSF*  
*Flow rate: 1.5  $\mu$ L/min*  
*Sampling duration: 20 min*

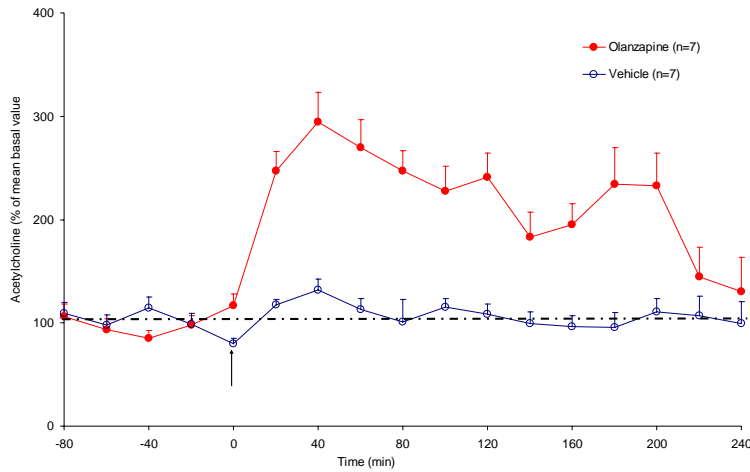
#### ii. Modulation of acetylcholine in ventral hippocampus by olanzapine in male Wistar rats.



*Perfusion fluid: aCSF*  
*containing 0.1  $\mu$ mol*  
*neostigmine*  
*Flow rate: 2.4  $\mu$ L/min*  
*Sampling duration: 15 min*

### iii. Modulation of acetylcholine by olanzapine in prefrontal cortex of Dunkin-Hartley guinea pigs.

pigs.



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

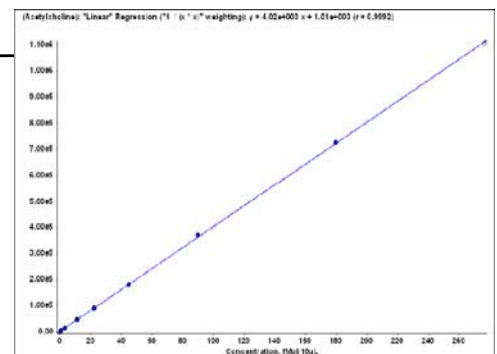
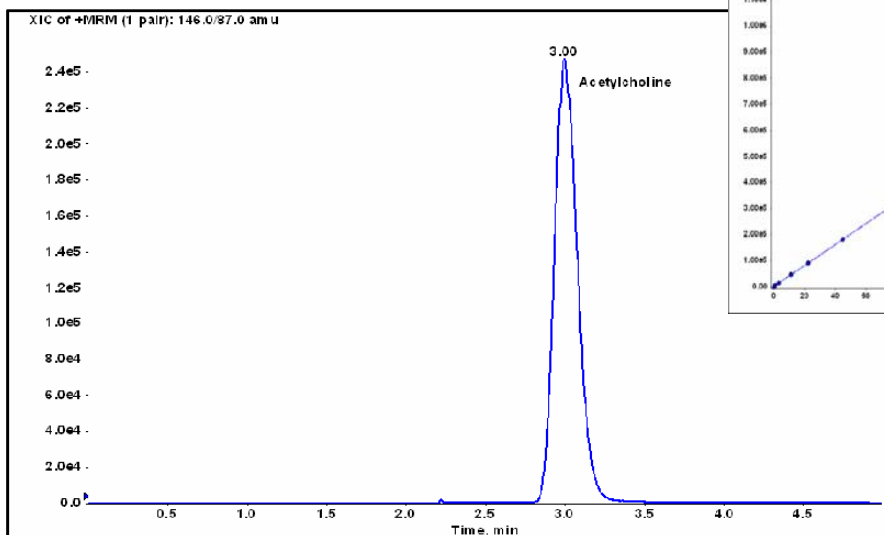
### Quantification of Acetylcholine

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

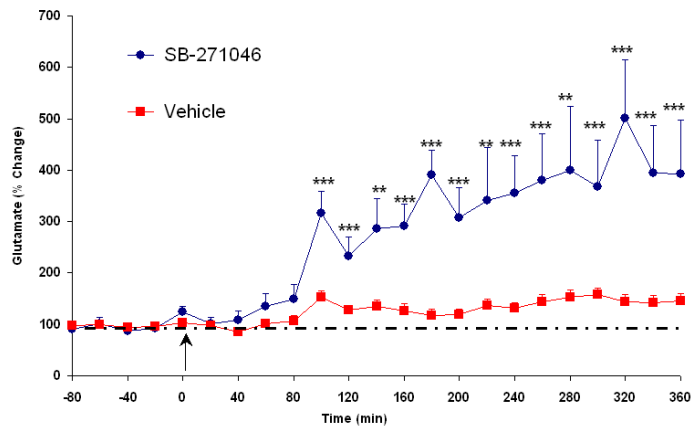
### Calibration curve

### Typical chromatogram of acetylcholine



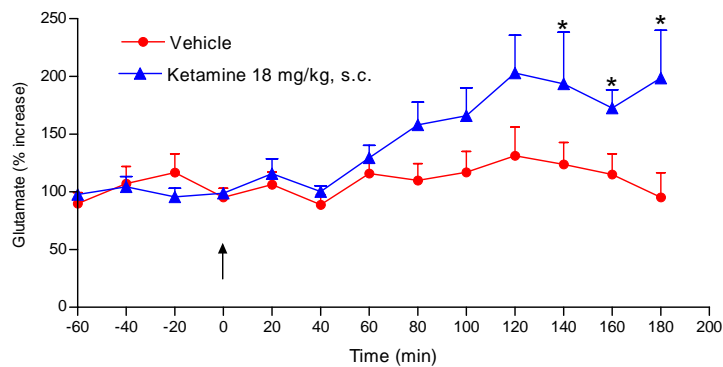
## Glutamate

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



**Perfusion fluid: aCSF**  
**Flow rate: 1.25  $\mu$ L/min**  
**Sampling duration: 15 min**

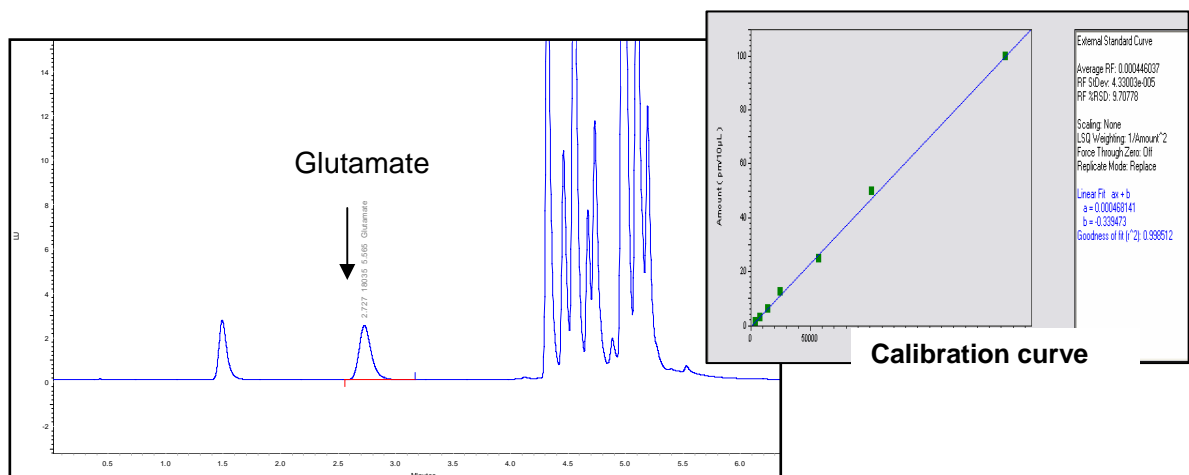
### ii. Ketamine: modulation of glutamate in prefrontal cortex of male Sprague-Dawley rats.



**Perfusion fluid: aCSF**  
**Flow rate: 3.0  $\mu$ L/min**  
**Sampling duration: 20 min**

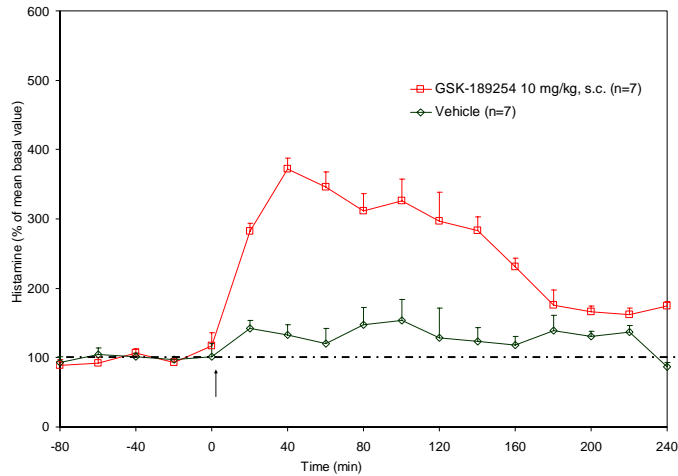
## Quantification

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



## Histamine

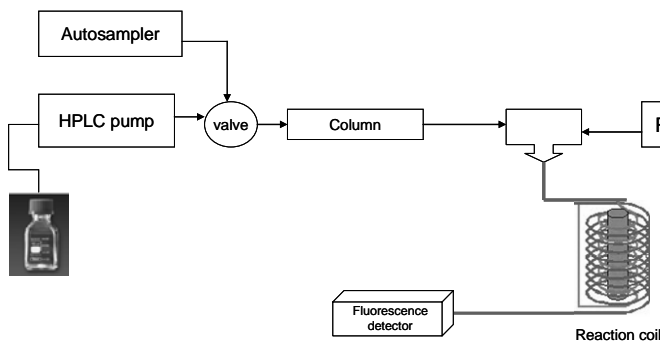
### i. Modulation of histamine by GSK-189254 in prefrontal cortex of male Sprague-Dawley rats.



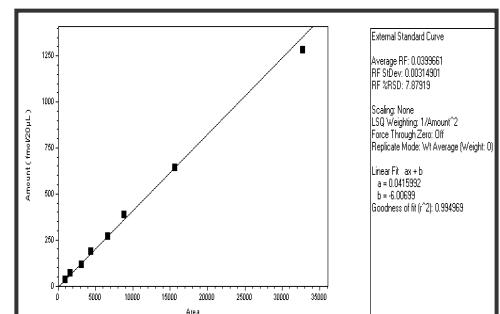
**Perfusion fluid: aCSF**  
**Flow rate: 1.5  $\mu$ L/min**  
**Sampling duration: 20 min**

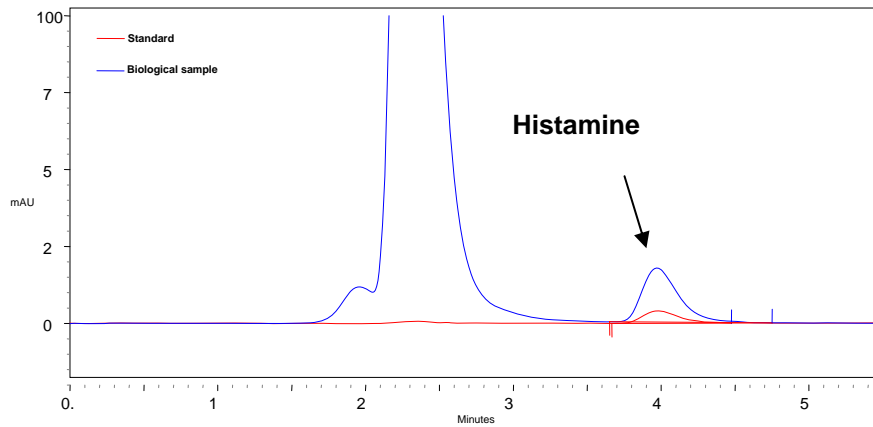
### Quantification

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.



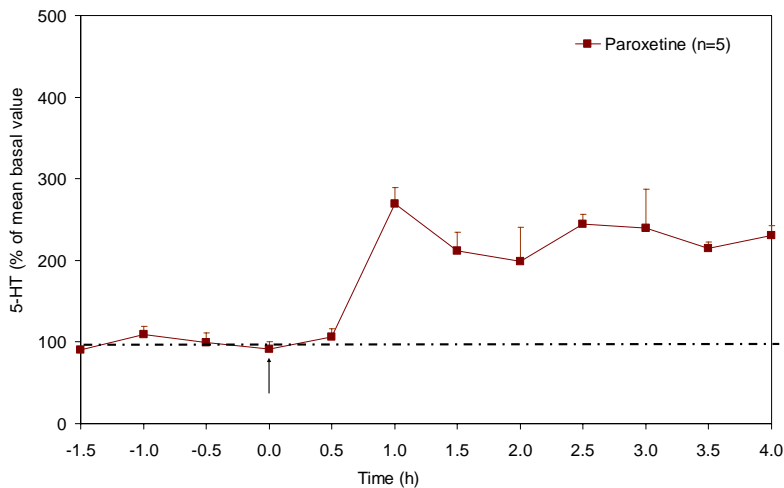
### Calibration curve





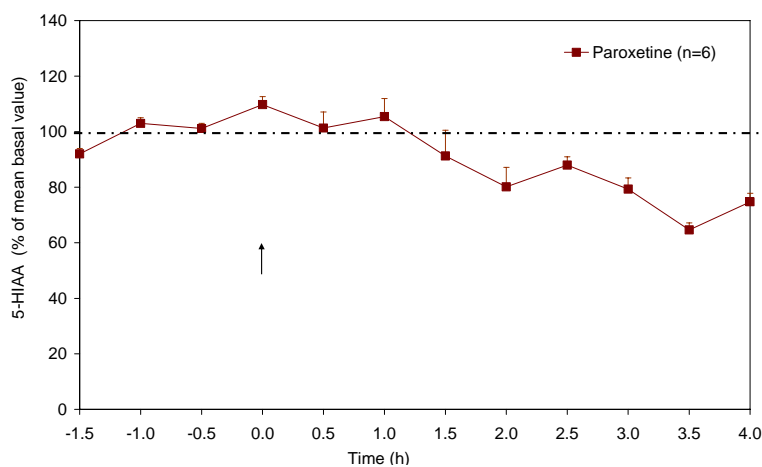
## Serotonin (5-HT) and 5-Hydroxy indole acetic acid (5-HIAA)

### i. Modulation of 5-HT by paroxetine in prefrontal cortex of male Sprague-Dawley rats.



*Perfusion fluid: aCSF*  
*Flow rate: 1.0  $\mu$ L/min*  
*Sampling duration: 30 min*

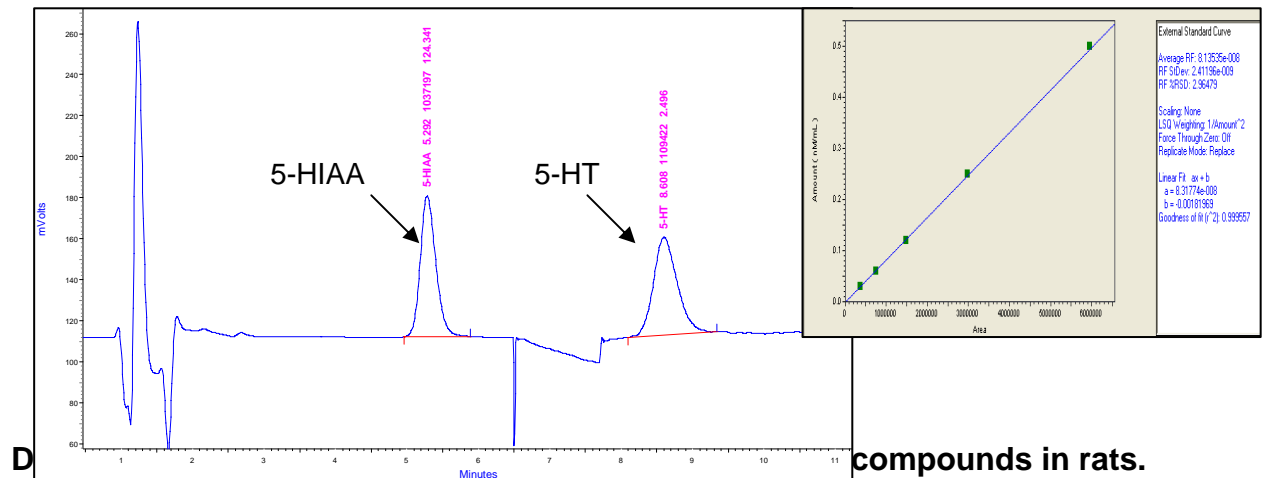
### ii. Modulation of 5-HIAA by paroxetine in prefrontal cortex of male Sprague-Dawley rats.



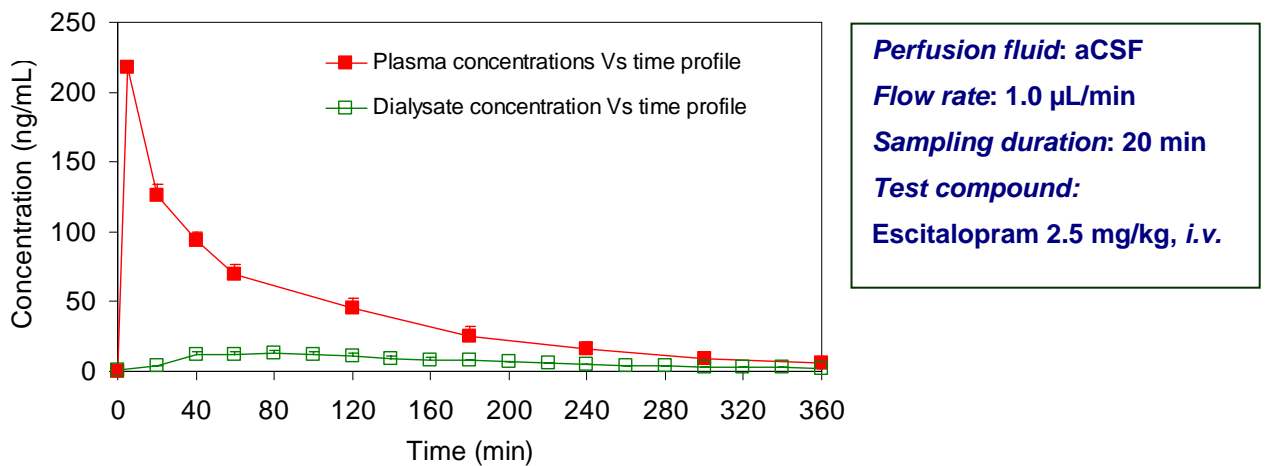
*Perfusion fluid: aCSF*  
*Flow rate: 1.0  $\mu$ L/min*  
*Sampling duration: 30 min*

## Quantification

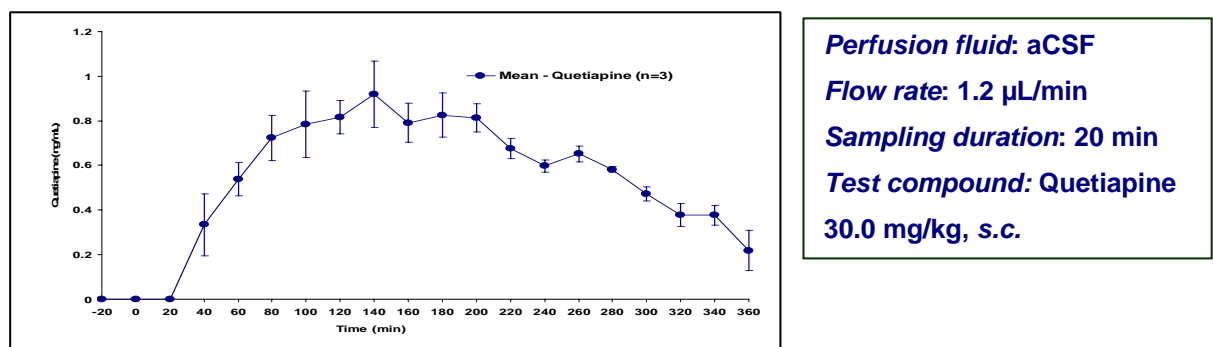
Concentrations of 5-HT and 5-HIAA in dialysates are determined by electrochemical detection.



### i. Unbound concentration of escitalopram in prefrontal cortex

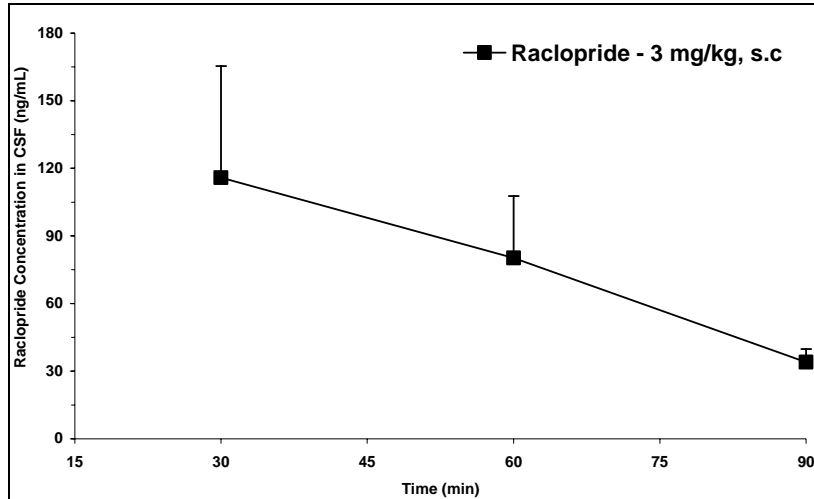


### ii. Unbound brain concentration of quetiapine in striatum



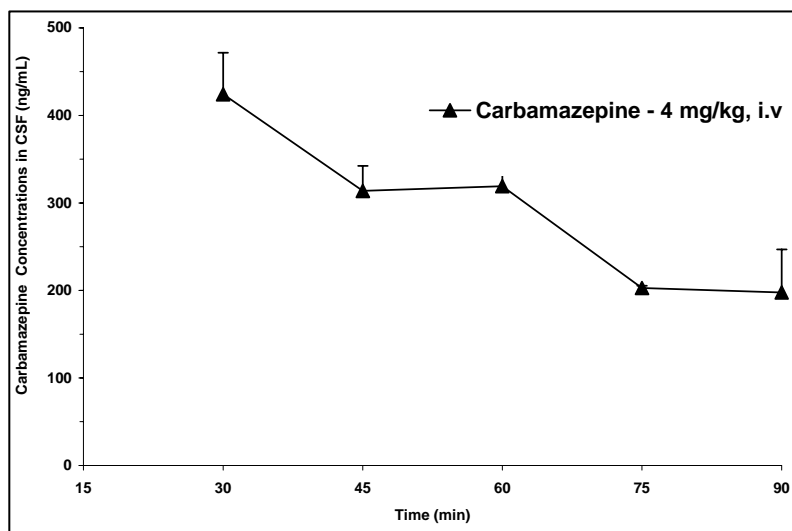
## CSF and Brain Tissue Pharmacokinetic.

### i. CSF concentrations of Raclopride



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

### ii. CSF concentrations of Carbamazepine



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