# 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

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## 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**

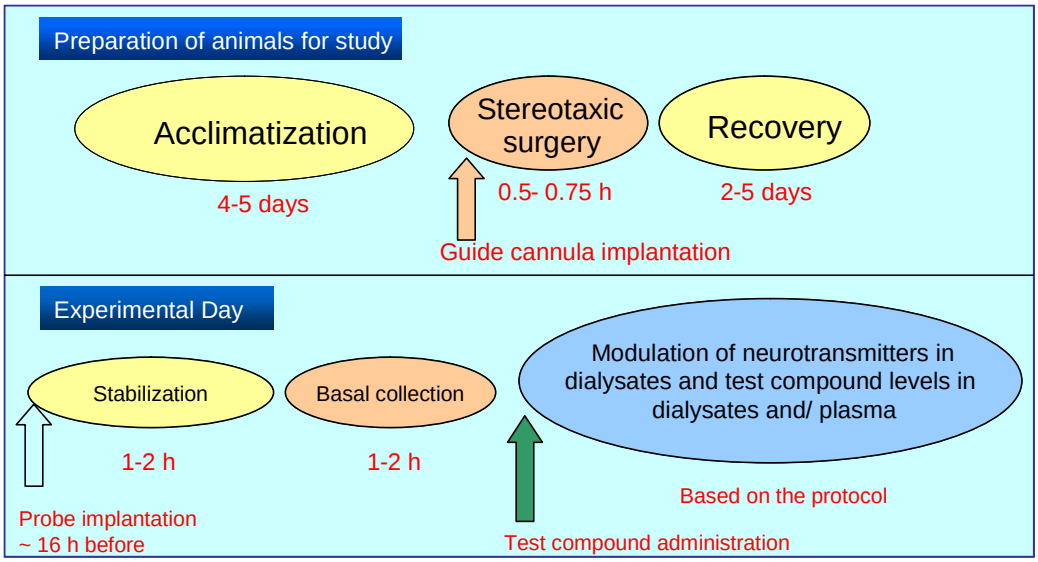
## Study Designs

- **Parallel Treatment Groups**
- **Cross-over Treatment Design**
- **Mechanism of Action Studies using various Antagonists/Blockers**
- **PharmacoKinetic, PharmacoDynamic (PK/PD) Studies *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 µL/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 wash out period for exposure in plasma, brain and CSF (based on neurotransmitter profile)**

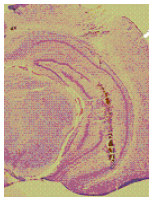
**Fractions collectors:**

- **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.**

**(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 : 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**

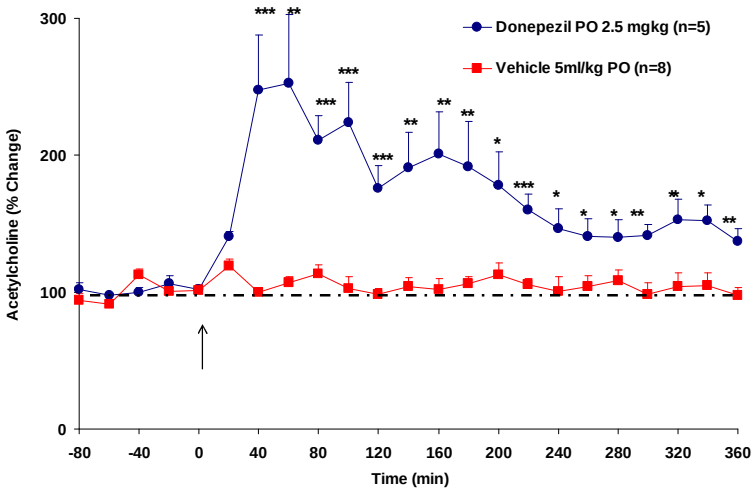
- **CSF samples:**
  - **Monoamines (5-HT & 5-HIAA) : HPLC + ECD (BASi or ESA)**
  - **Histamine and alpha methyl histamine : HPLC + Fluorescence**
  - **Prostaglandin E2 : ELISA**
  - **Interleukin-1β : ELISA**

- **Plasma and Brain tissue samples:**
  - **Monoamines : HPLC + ECD (BASi or ESA)**
  - **Test compound : LC-MS/MS (API-4000 Q-Trap)**
  -

# Summary of Validation Experiments

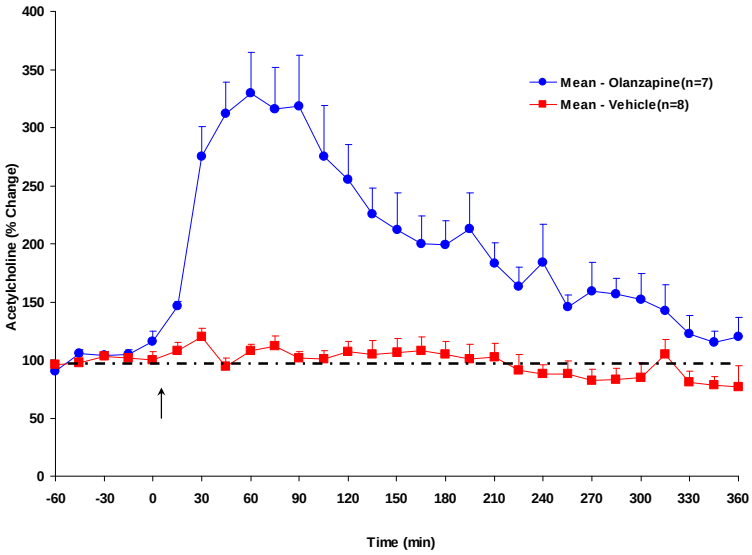
## Acetylcholine

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




***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**




***Perfusion fluid*: aCSF containing 0.1 µmol neostigmine**
***Flow rate*: 2.4 µL/min**
***Sampling duration*: 15 min**

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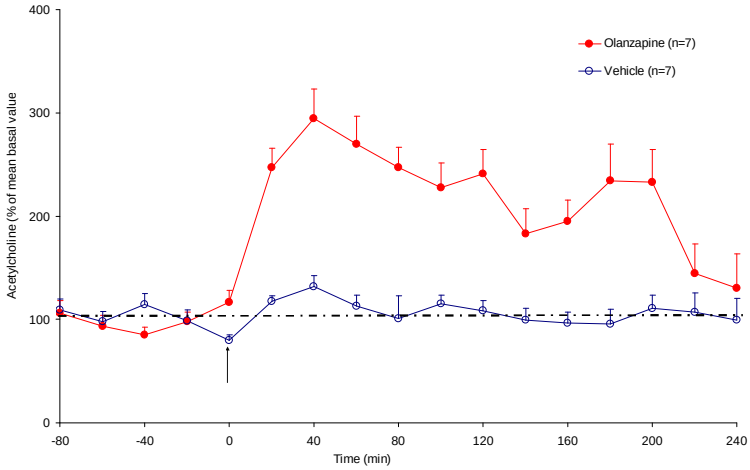
**iii. Modulation of acetylcholine by Olanzapine in prefrontal cortex of Dunkin-Hartley guinea pigs**



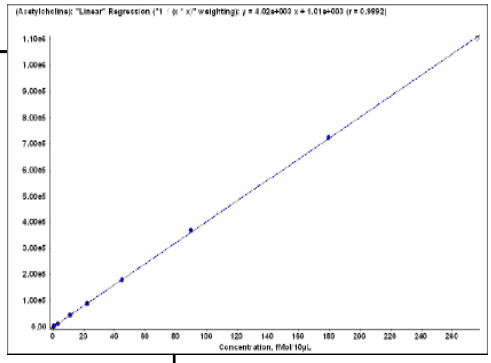

***Perfusion fluid*: aCSF containing 0.1 µmol neostigmine**
***Flow rate*: 1.5 µL/min**
***Sampling duration*: 20 min**

## Quantification of Acetylcholine

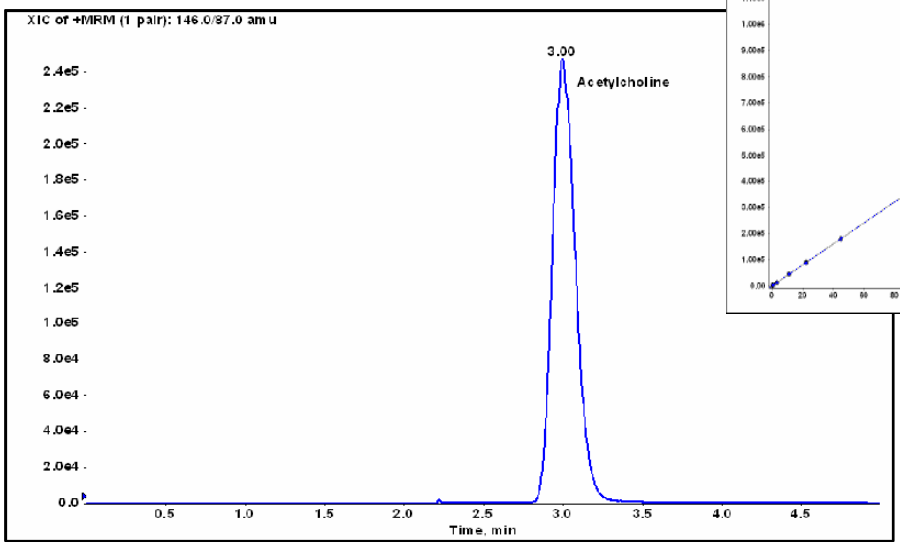
Acetylcholine concentrations in microdialysate samples are quantified by using HPLC coupled with tandem mass spectrometry (LC-MS/MS) without any sample pretreatment.

- Quantitation range: 0.05-103.50 nM

**Calibration curve**



**Typical chromatogram of acetylcholine**

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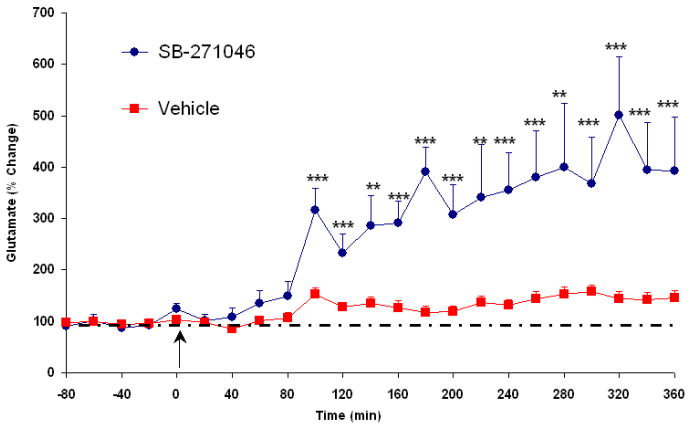
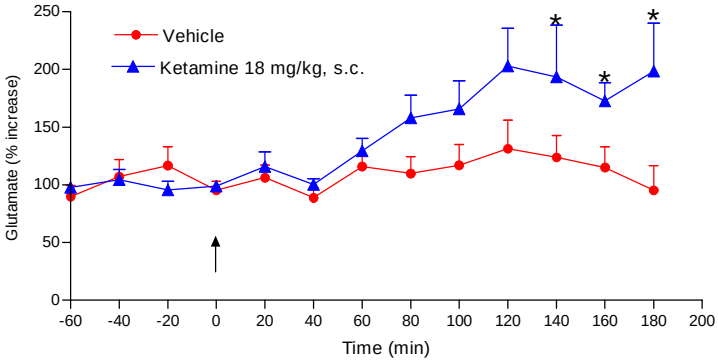
## Glutamate

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




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

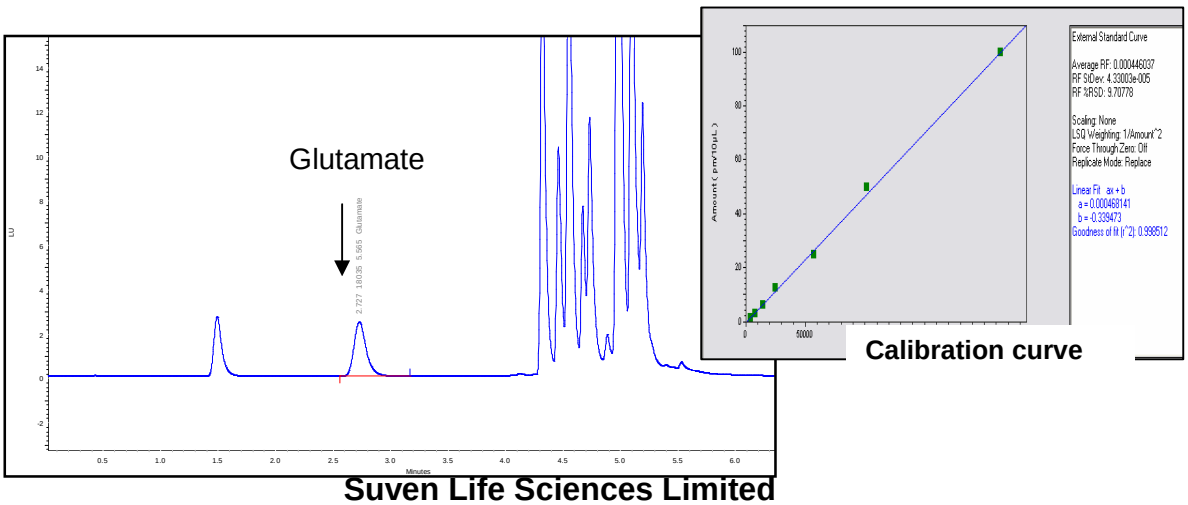
**ii. Ketamine: modulation of glutamate in prefrontal cortex of male Sprague-Dawley rats**




***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.

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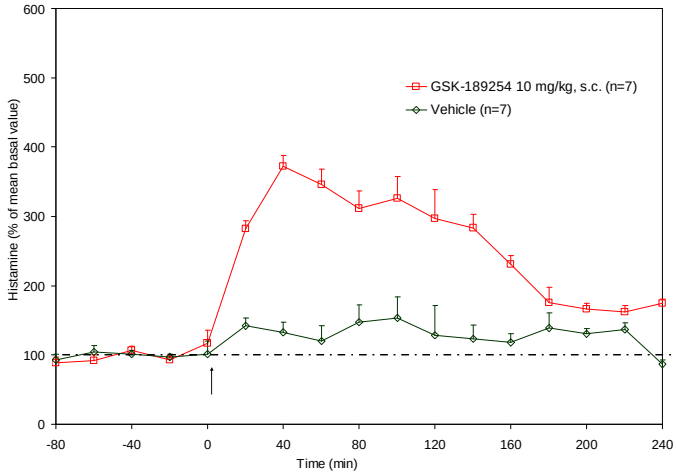
# Histamine

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



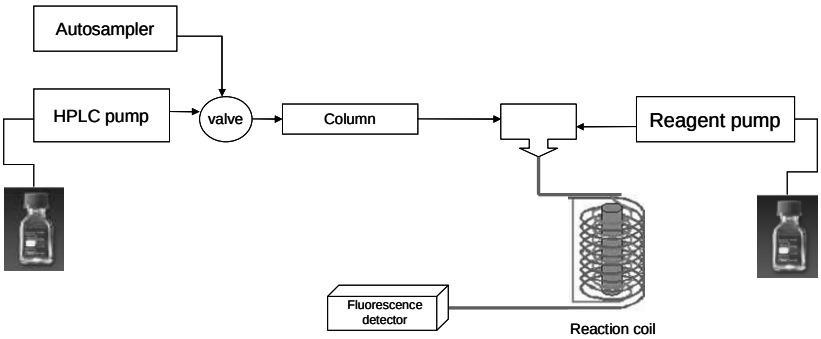

***Perfusion fluid*: aCSF**

***Flow rate*: 1.5 µL/min**

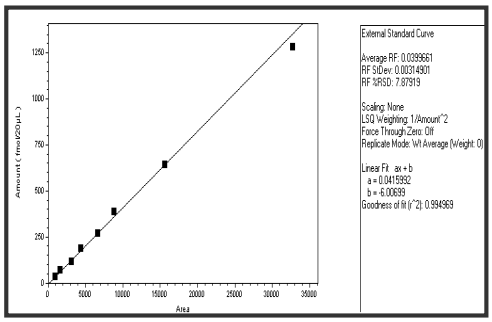
***Sampling duration*: 20 min**

## Quantification

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




**Calibration curve**



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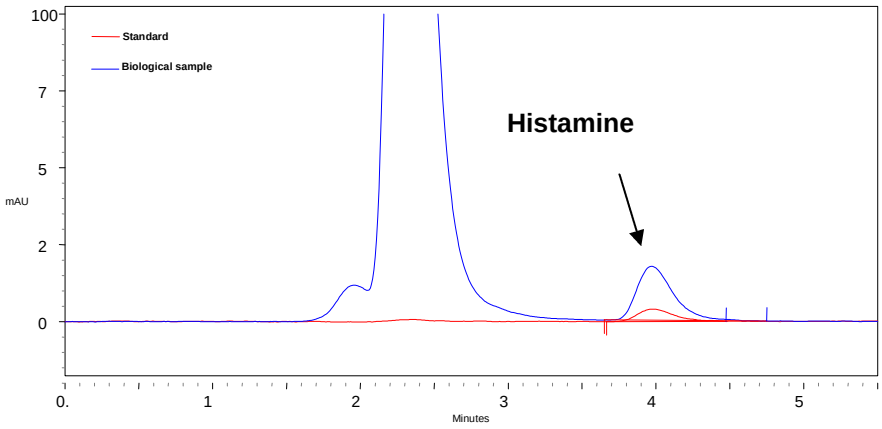


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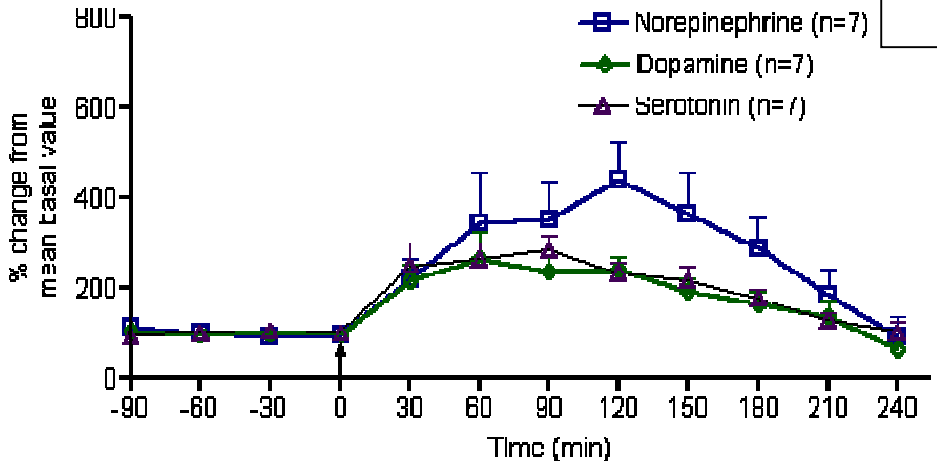
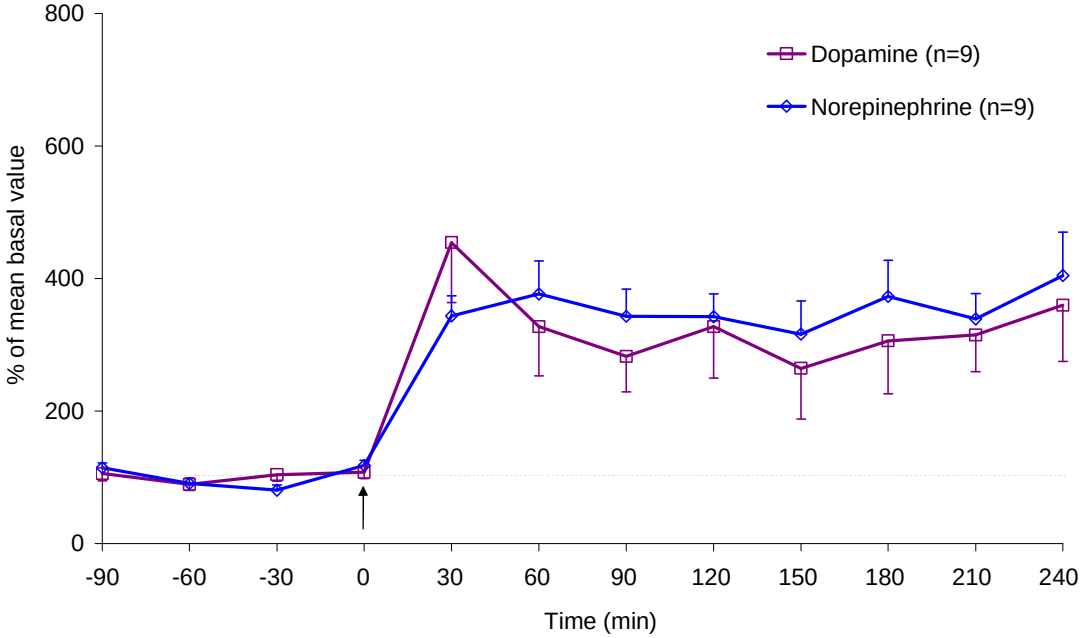
## Dopamine, Norepinephrine and Serotonin

**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**

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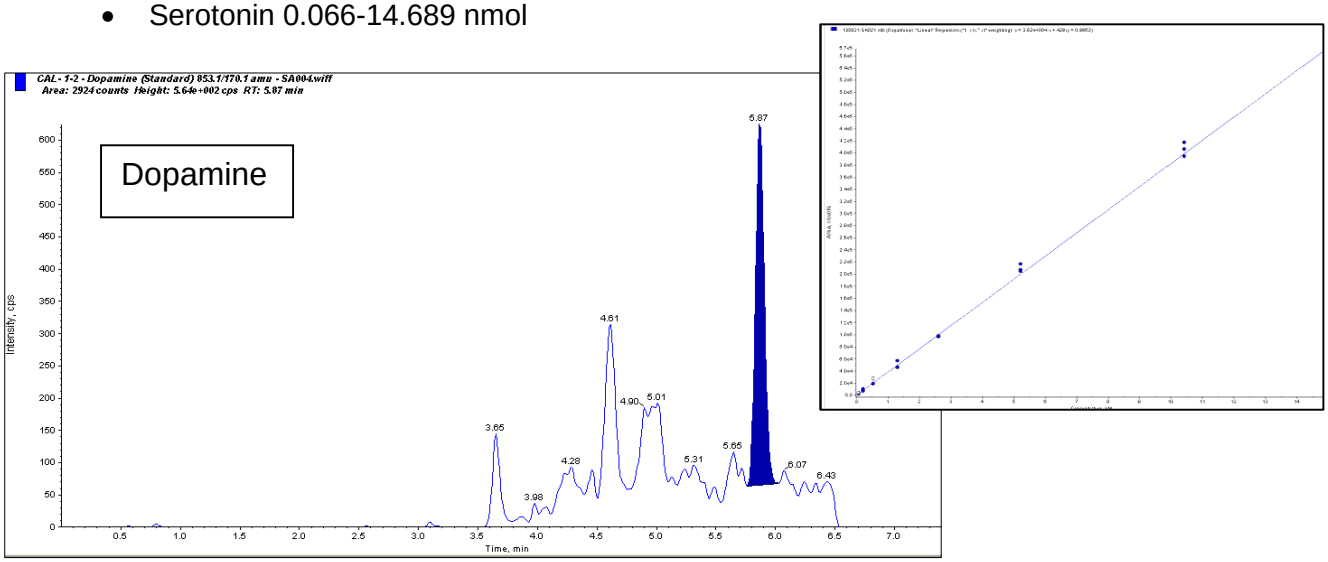
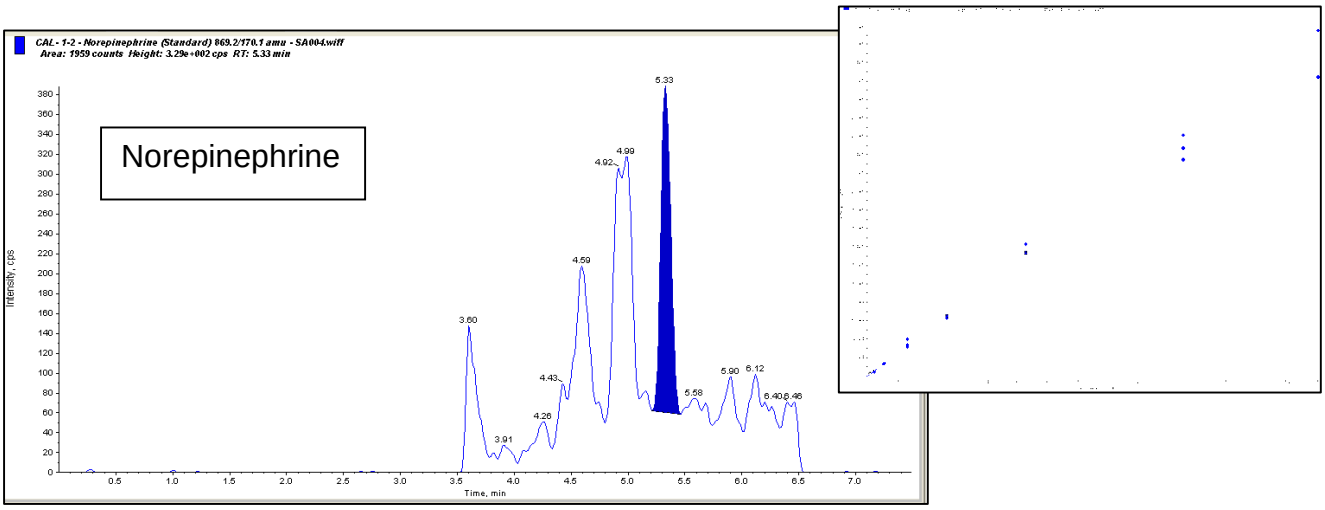
## Quantification

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

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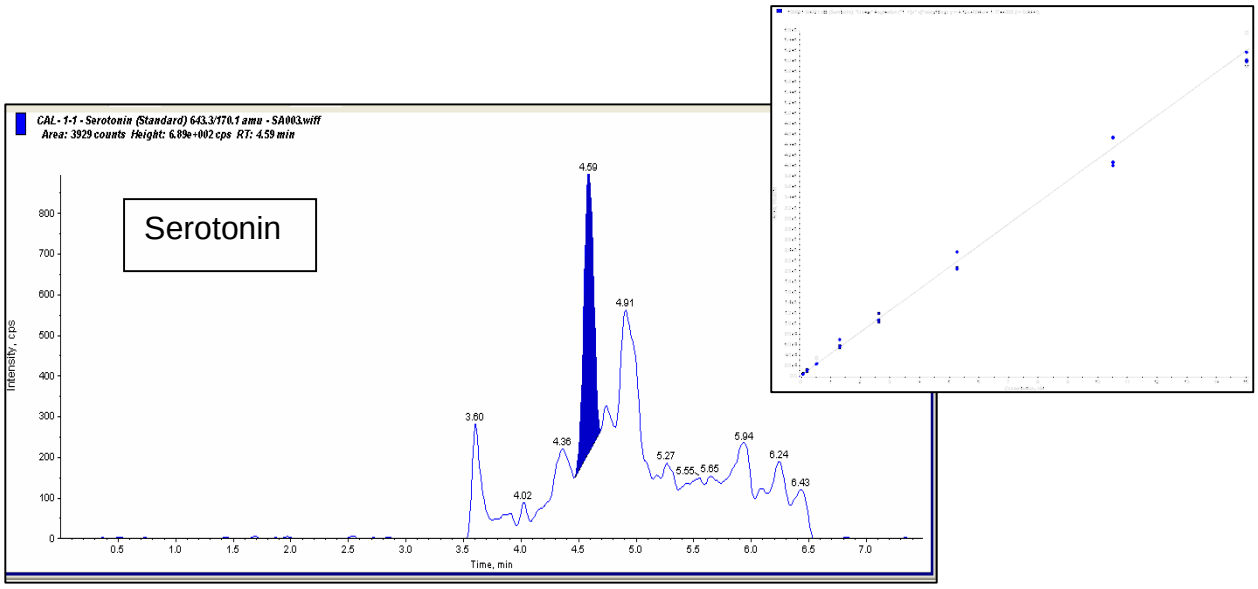


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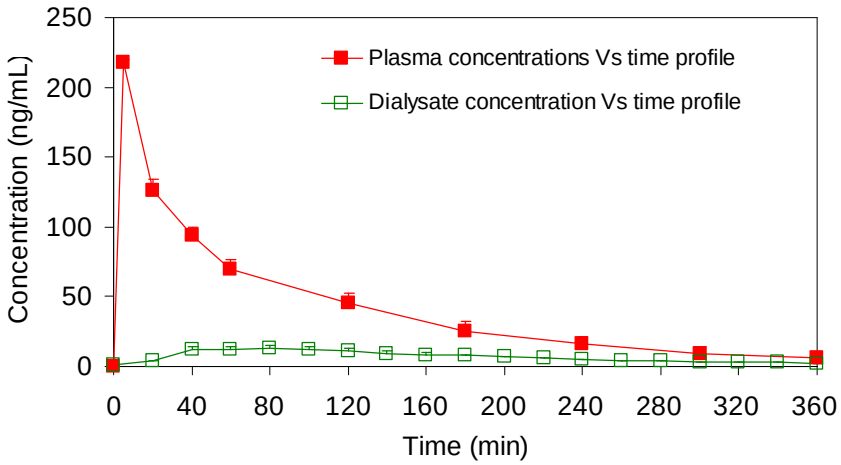
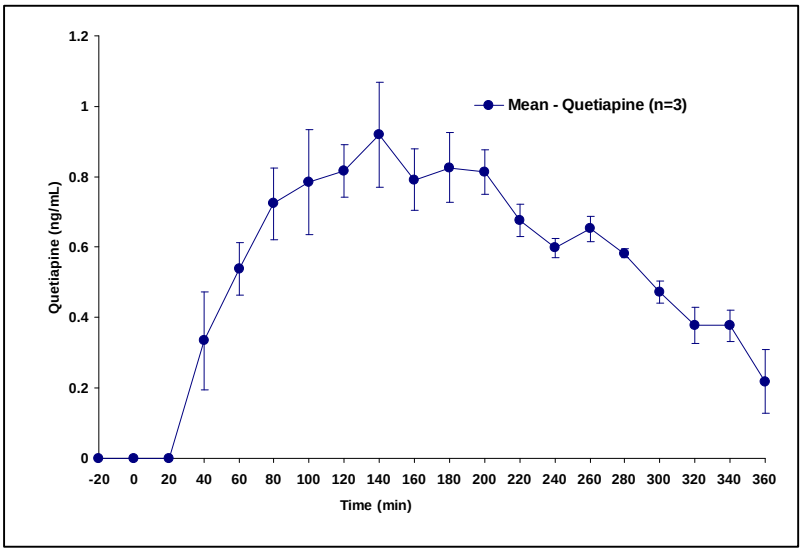
**Determination of unbound brain concentrations of test compounds in rats.**

## i. Unbound concentration of escitalopram in prefrontal cortex




***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




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

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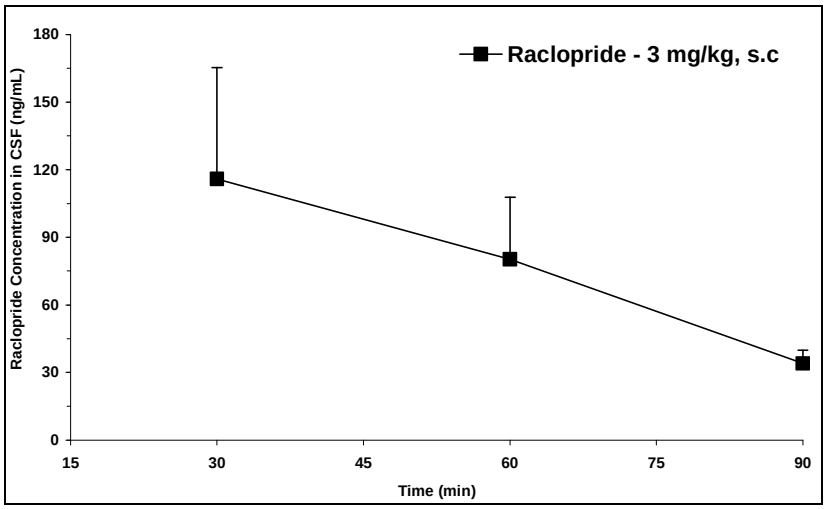
## CSF and Brain Tissue Pharmacokinetic

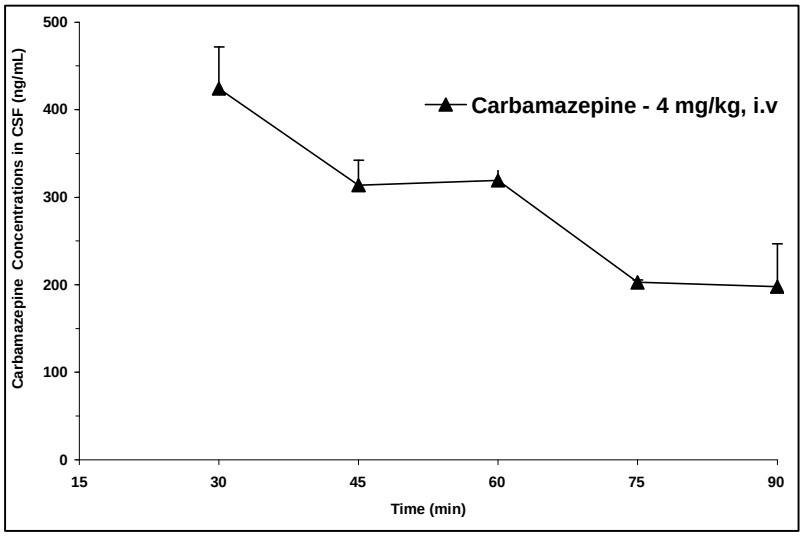
### i. CSF concentrations of Raclopride




***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:*** **Carabamazepine 4.0 mg/kg, *i.v.***

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