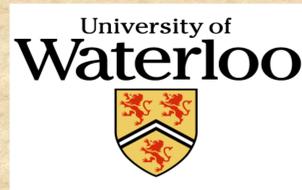
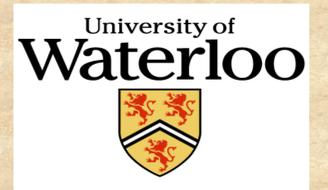


# Solid Phase Microextraction: The Potential for In vivo Monitoring of Endogenous Small Polar molecules in Biological Systems



Erasmus Cudjoe, Clemant Hamani, Ehsanul Hoque, Ines dé Lannoy, Victor Saldivia, Huadong Sun and Janusz Pawliszyn



## Abstract

An advantage of SPME is ability to monitor in real-time biochemical changes in a given biological system (in vivo/in vitro). This potential can be explored to capture global biochemical events of small molecules in cells, tissues, or biological fluids to understand biochemical pathways. This study demonstrates the potential of SPME to simultaneously monitor various neurotransmitters (GABA, Glutamic acid, Dopamine and Serotonin) in freely moving rats subjected deep-brain-stimulation. The technique was validated with independent brain pharmacokinetic studies of carbamazepine using microdialysis. In addition, SPME was also applied to metabolomics studies of human clinical samples. Principal component analysis showed changes in biochemical pathways induced by treatment and individual differences in response to therapy.

## Introduction

Neurotransmitters, small endogenous polar molecules form the core of communication between nerve cells. The transmission of neurocompounds is associated with processes which involves the release of multiple compounds into the ECS. Therefore, the ECS contains vast chemical information and monitoring of its chemical composition *in vivo* could provide very critical information about brain function and neurological disorders. However, monitoring neurocompounds *in vivo* using existing methods continues to pose analytical challenge which includes method selectivity and robustness, interference from endogenous matrix complexities to mention a few. Detection methods like laser induced fluorescence (LIF) and electrochemical use fluorescent and electroactive properties respectively which limits the technique to only specific neurocompounds. Microdialysis (MD) a well-established method for sampling of neurotransmitters is characterized by significant matrix effect which often requires further steps for sample cleanup to ensure reliability. In addition, sample handling in MD is very difficult due to the very small sample volume generated. Solid phase microextraction (SPME) an emerging sample preparation method for *in vivo* analysis of biological system has applied successfully for various applications including clinical, pharmacokinetics, etc. Most of these studies focused on drug bioanalysis and till date SPME has not been used for monitoring endogenous neurochemicals in brain tissue analysis. For the first time, we demonstrate the ability to couple new biocompatible SPME probes to LCMS/MS to monitor changes in neurotransmitters in freely moving rats. The method was validated by simultaneously sampling both hemispheres with MD and SPME. The technique was successfully applied to study effect of deep brain stimulation on neurotransmitters.

## Experimental Procedure



Figure 1 Simultaneous *in vivo* sampling from left and right brain hemispheres of a freely moving rat using microdialysis and SPME probes

## Summary of sampling procedure for neurotransmitters

	Microdialysis	Solid Phase Microextraction
Sampling time	30 min interval	
Brain region sampled	Left hemisphere - striatum	Right hemisphere -striatum
Basal sampling time	Sampling was done every 30 min for 2 hr to obtain basal concentrations	
Sampling after i.v. dose of fluoxetine	Monitored changes in neurotransmitters for 3 hr at 30 min intervals	
Sample analysis	Analyzed with HPLC coupled to ECD system	Analyzed with HPLC-MS/MS system
Compounds monitored	Serotonin (5-HT) and dopamine (DA)	5-HT, DA, glutamic acid and GABA

## Application: Monitoring effect of deep brain stimulation on neurotransmitters

Deep brain stimulation is known therapeutic treatment for various types of neurological disorders such as Parkinson's and Alzheimer's diseases. Despite its success the actual mechanism of the therapeutic effect still remains unknown. Neurotransmitters have been suspected to be involved but still further studies are required to determine the type(s) involved. The new biocompatible SPME probes have shown the potential to monitor changes in multiple neurotransmitters simultaneously in rat *in vivo* brain tissue sampling. Here we apply the method to monitor both amine and amino acid transmitters in the pre-frontal cortex of the rat during deep brain stimulation.

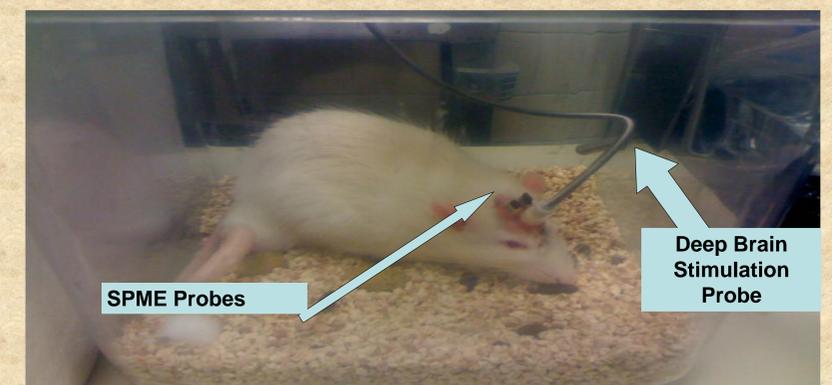
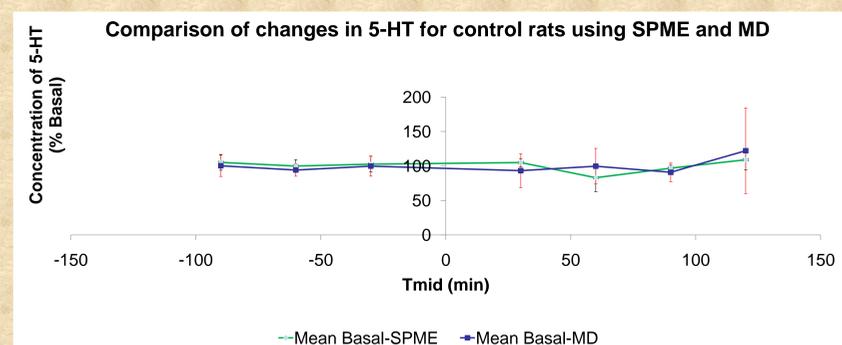
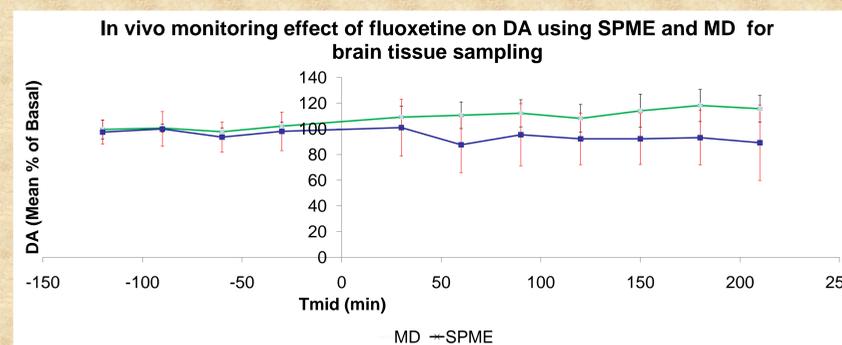
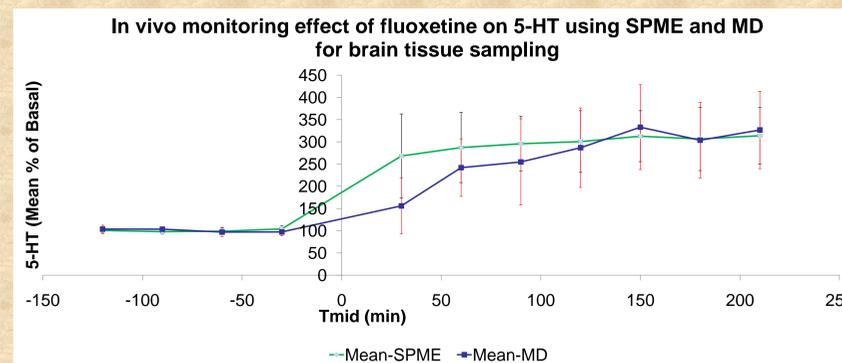
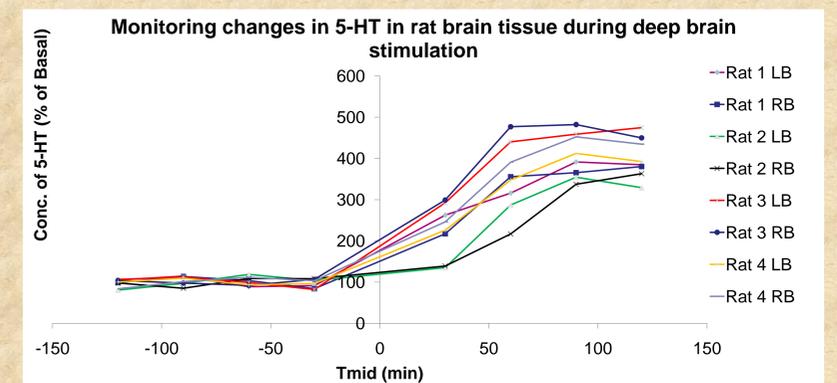


Figure 2 Simultaneous *in vivo* sampling of left and right brain hemispheres of a freely moving rat using SPME probes only

## Results: Comparison of SPME and MD



## Results: Changes in 5-HT levels during DBS



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