Near Real-Time Measurement of Glutamate Concentration Changes Using Biosensors in Place of Traditional Methodologies

D. Aillon[1] D. Johnson[1], S. Gabbert[1], E. Naylor[1], and G.S. Wilson[2], D.A. Johnson[1], 1. Pinnacle Technology Inc., Lawrence, KS, USA 66046 2. Dept. of Chemistry, University of Kansas, Lawrence, KS, USA 66045

PINNACLE TECHNOLOGY INC.

Introduction

This study demonstrates the ease with which enzymatic glutamate biosensors and wireless technologies can be employed for the near real-time measurement of glutamate in untethered animals. Razoux et al (2007) used MD techniques to measure changes in extracellular glutamate concentrations in the rat brain stimulated by systemic injection of ketamine (25 mg/kg, ip). In this study, we used biosensors to replicate the MD experiments by Razoux.

Methods

Sensor Construction

Sensors were constructed similarly to Hu et al (1994). Guide cannulas were used to allow for post surgical insertion of the glutamate biosensor.

Surgery

Adult male Sprague Dawley rats used.

Rats were anesthetized with ketamine/xylazine cocktail (70mg/kg, 5mg/kg) and mounted on stereotaxic frame.

During surgery, an intracerebral guide cannula (implantation coordinates: 1.6, 0.8, -4.4) and surrounding enclosure were installed onto the head of the rat, secured by bone screws and dental cement.

Rats were allowed at least five days to recover from surgery.

Sensor Insertion

Following recovery period, rats were restrained, without the use of anesthesia, and the dummy probe inside of the guide cannula was replaced with a glutamate biosensor.

The biosensor was then connected to a wireless potentiostat within the head-mounted enclosure.

Recording of the biosensor output began immediately and the rat was allowed to return to its cage overnight.

Dosing

Approximately 12 to 14 hours after sensor insertion, drug dosing began.

Animals were first dosed with 0.5 mL of saline (IP).

At least one hour after the initial saline dose, animals were given a sub-anesthetic dose of ketamine (25 mg/kg, IP).

Recordings were continued for at least one hour following the ketamine dose, when animals were euthanized and the glutamate biosensor explanted for immediate post-calibration.

Results

Saline dose:

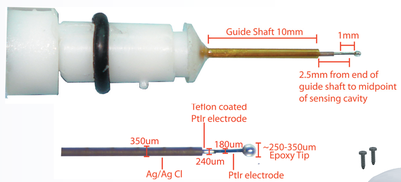
Little change in animal activity.

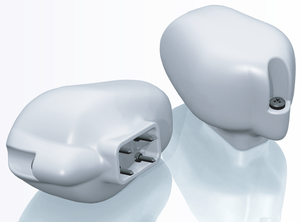
Little change in sensor signal.

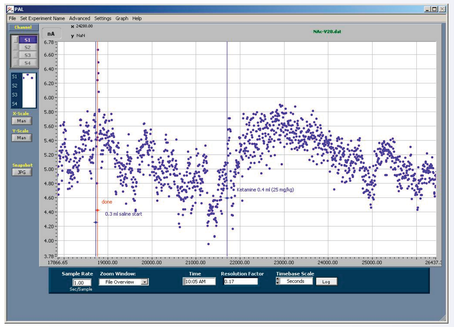
Ketamine dose:

Rat displayed increased locomotor activity lasting approximately one hour.

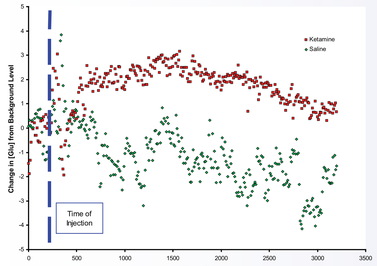
Sensor measured a sustained rise in glutamate levels, reaching approximately 3uM over the course of an hour.



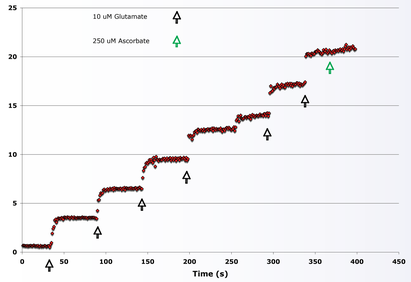




Snapshot of real-time data acquisition software. Data shown includes delivery of saline and ketamine doses (ip).



Glutamate response in the Nucleus Accumbens to an injection of ketamine (25 mg/kg, ip) or saline (ip).



Calibration of the glutamate biosensor immediately following explantation.

Discussion

Biosensors can be used to measure drug induced changes in glutamate levels.

Wireless data transmission frees the animal from the tethered connection.

Less signal noise.

Reduces the impact on the animals behavioral response.

Less stress on the animal.

Real time measurements offer more insight into the second by second changes of neurotransmitters than can be achieved through technologies with slower sample times such as microdialysis.

MD results show a sharp rise in glutamate levels after the injection, which is then sustained for approximately two hours and then falls off abruptly.

The biosensor data show a fast increase in glutamate levels after the ketamine injection which quickly achieves a maximum change (~3 uM) and then begins a gradual return to baseline levels over the course of one hour from the time of injection.

References

1. Razoux, F, Garcia R, Lena I (2007) Ketamine at a Dose that Disrupts Motor Behavior and Latent Inhibition, Enhances Prefrontal Cortex Synaptic Efficacy and Glutamate Release in the Nucleus Accumbens, Neuropsychopharmacology 31: 719-727.

2. Hu Y, Mitchell KM, Fakhrildeen NA, Albahadily FN, Michaelis EK, Wilson GS (1994) Direct measurement of glutamate release in the brain using a dual enzyme-based Velectrochemical sensor Brain Research 659: 117-125.

Pinnacle greatfully acknowledges NIH funding 5R 44NS37608-03