

# Monitoring Molecules in Neuroscience



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release *in vivo*. Moreover, dopamine release was decreased in HDtg rats compared to WT control rats, and the HDtg rats showed a distinctly different behavioral response to amphetamine compared to WT rats. These results suggest that impairments in dopamine release may contribute to the altered behavioral response to dopamine uptake inhibition. Nevertheless, additional studies are required to confirm both of these conclusions.

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## Endogenous efflux of D-ser, L-glu, and adenosine in response to modeled ischemia using microperfusion sampling of single acute hippocampal slices

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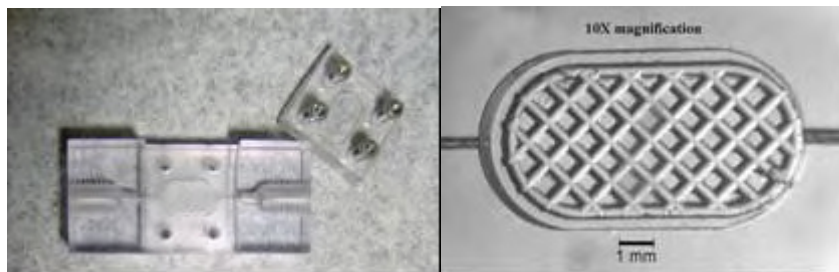
### Introduction

Acute hippocampal slices are commonly used to investigate various physiological functions and disorders in the CNS and are generally believed to closely represent *in vivo* biology. Here we report on a microperfusion chamber developed in-house for sampling endogenous transmitters from single (1-3 mg) acute hippocampal slices of rat without the need for sample cleanup via microdialysis. The utility of this offline collection and quantification approach is demonstrated in acute hippocampus of Sprague Dawley rat by investigating endogenous efflux of L-glutamate (L-glu), D-serine (D-ser), and adenosine (Ado) in response to oxygen glucose deprivation (OGD), an *in vitro* model of ischemic stroke.

### Methods

Microperfusion chambers were constructed in-house from polycarbonate. The design of the chamber is shown in Figure 1 and was a significantly modified version of the retinal chamber originally developed by O'Brien and colleagues [1]. The internal chamber volume is estimated at ~35  $\mu$ L and chambers were constructed to include a built-in microchannel support. A syringe pump connected to a micro switching valve was used to perfuse chamber with artificial cerebral spinal fluid (aCSF) and treatment. Treatment consisted of a 24 min exposure to OGD (glucose free aCSF equilibrated with N<sub>2</sub>).

Male Sprague Dawley rats were used for all experiments in accordance with the Institutional Animal Care and Use Committee at the University of Alaska Fairbanks. Transverse 400  $\mu$ m acute hippocampal slices were obtained at 2°C using a Vibratome and allowed to recover for 1 h at room temperature. Slices were transferred to microchambers and perfused at 35°C with aCSF using a flow rate of 7  $\mu$ L/min. Treatment was administered after basal levels had equilibrated and sampling continued for another 45-50 min after reperfusion. Samples were collected and stored at -80°C until quantification. Reversed polarity chiral capillary electrophoresis was used to quantify amino acids as described previously [2]. Adenosine was quantified by HPLC-UV using the method described by Porkka-Heiskanen and colleagues [3]. Typical requirements for the CE-LIF and HPLC-UV assays were 1  $\mu$ L and 1-5 $\mu$ L sample respectively.



**Figure 1. Microperfusion chamber design.** Left image shows the polycarbonate microperfusion chamber with leak-free chamber lid. The chamber is engineered for single acute hippocampus slices of rats. Right image shows the built-in micro-channel support that the acute slice rests on during micro-perfusion. The sealed chamber volume is approximately 35  $\mu$ L when an average slice is in the chamber.

## Results and Discussion

Significant increases in efflux of D-ser, L-glu, and Ado were observed in response to OGD. Response of L-glu to OGD corresponds well to published *in vivo* ischemia data supporting the notion that 1) acute slices remain sufficiently viable in our microchambers and 2) that our OGD preparation may closely represent ischemic conditions. Adenosine efflux also corresponds well to literature suggesting that the known inhibitory effects of Ado on L-glu neurotransmission during model ischemia can be further investigated pharmacologically using this technique. In addition, rapid dynamics in D-ser efflux during OGD was demonstrated for the first time here and this data suggests that OGD induced efflux can provide additional N-methyl-D-aspartate receptor (NMDAR) coagonist which may potentially exacerbate NMDAR induced neurotoxicity.

## References

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