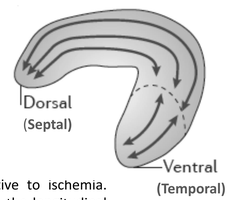
ARE THERE DIFFERENCES IN RESPONSE TO ISCHEMIC INJURY ACROSS THE LONGITUDINAL AXIS OF THE HIPPOCAMPUS?

Sheleza R. Ahad, BSc and John G. Mielke, PhD
School of Public Health and Health Systems, University of Waterloo, Waterloo, ON, Canada N2L 3G1

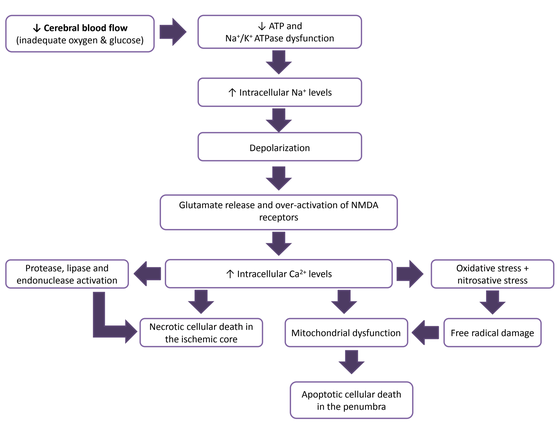
Email: srahad@uwaterloo.ca

INTRODUCTION

- Ischemic strokes occur when a vessel supplying blood to the brain becomes blocked and interferes with the delivery of oxygen and nutrients. Despite being the leading cause of disability and the third leading cause of death in Canada, the ability to treat stroke-related brain injury is limited primarily due to incomplete understanding of the underlying mechanisms.
- The risk of stroke doubles every ten years after the age of 55; although the incidence of stroke is 33% greater in men, during advanced age (>85 years old) the risk of stroke is higher in women (Scott et al., 2012; Heart and Stroke Foundation, 2015).
- The hippocampus, a structure involved in learning and memory, is highly sensitive to ischemia. Experimental literature suggests differences in response to brain injury exist across the longitudinal axis of the hippocampus *(see right)*; however differences in susceptibility to stroke-related damage between the two poles of the axis (septal and temporal) are incompletely understood (Rami et al., 1997).
- As the Canadian population ages, the need for better stroke treatment will only become greater. Our research aims to investigate fundamental factors that may explain the brain's response to injury, in order to assist in the development of improved pharmacotherapies.



PATHOPHYSIOLOGY OF STROKE



OBJECTIVES AND RATIONALE

OBJECTIVES

- To apply a model of stroke (oxygen-glucose deprivation; OGD) to hippocampal slices prepared from male and female rats and to quantify damage using a measure of mitochondrial function (TTC metabolism) and cell membrane integrity (LDH efflux).
- To assess differences in response to OGD-related damage between the septal and temporal poles of the hippocampus.

RATIONALE

- OGD is a well established model of brain injury that mimics the reduction of oxygen and nutrients to the brain seen with ischemia, and permits the assessment of underlying cell death mechanisms.
- The hippocampus is highly sensitive to stroke-related damage, and evidence suggests that differences in response to injury exist across the longitudinal axis.
- Response to ischemic injury between the septal and temporal poles has not been thoroughly investigated.
- Research that contributes to the development of improved stroke treatment is necessary given the aging profile of the Canadian population.

MATERIALS AND METHODS

Acute Hippocampal Slice Preparation

- Male and female rats will be anaesthetized with CO_2 and decapitated. Brains will be rapidly removed (~60 s) and placed in cooled (<4°C) artificial cerebrospinal fluid (ACSF).
- A McIlwain tissue chopper will be used to cut 350 µm thick slices, which will then be placed within separate compartments of an interface incubation chamber (2-4 slices per platform). ACSF will be continuously gassed with carbogen and the incubation chamber will be kept at 35.0 ± 0.5°C *(see figure 1)*.

Oxygen-Glucose Deprivation

- Oxygen-glucose deprivation (OGD) will be applied by transferring platforms containing slices to a separate incubation chamber filled with ACSF (kept at 35.0 ± 0.5°C) in which sucrose has been substituted for glucose and then saturated with 95% N_2/5% CO_2.

Lactate Dehydrogenase Release Assay

- LDH release will be measured using an LDH cytotoxicity kit provided by *Cayman Chemical.*
- Following a 30 minute incubation at 37°C the overall conversion of lactic acid to a highly-coloured formazan product will be measured at 490 nm using spectrophotometry.

2,3,5-Triphenyltetrazolium Chloride (TTC) Metabolism Assay

- Following recovery, slices will be transferred to glass vials containing a 2% (w/v) TTC solution in ACSF aerated vigorously for 10-15 minute with anoxic gas, and incubated for 1 h at 35 ± 0.5°C.
- Slices will be washed with carbogenated ACSF before being placed in extraction buffer (DMSO and 100% ethanol prepared 1:1) overnight at room temperature in the dark.
- Extracted formazan will be measured via spectrophotometry at 485 nm.

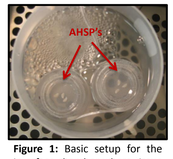
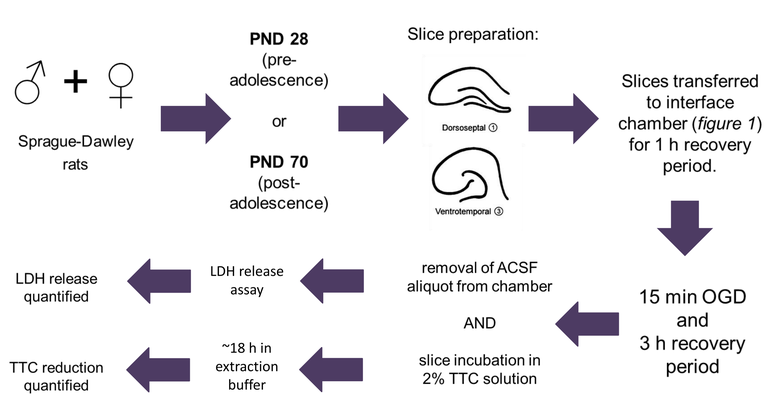
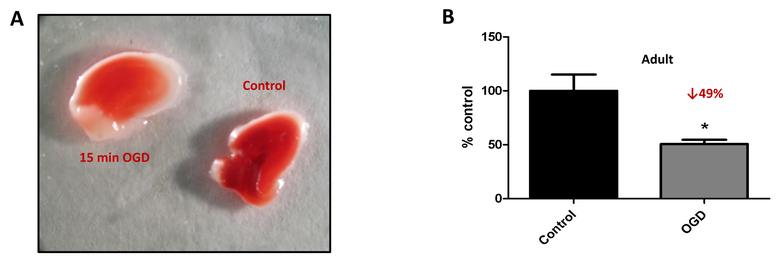


Figure 1: Basic setup for the interface chamber where tissue slices will be maintained. The chamber is filled with ACSF saturated with carbogen and held at 35°C.

EXPERIMENTAL DESIGN



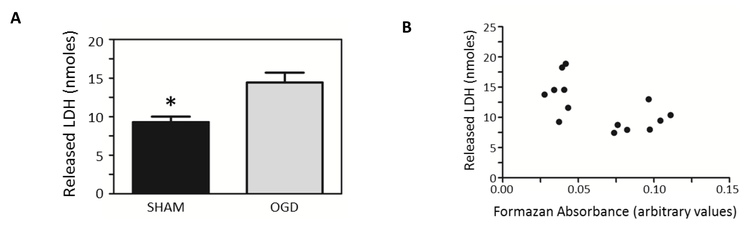
TTC METABOLISM IS AN ACCURATE, HIGH-THROUGHPUT MEASURE OF SLICE VIABILITY



(A) Level of formazan production and corresponding slice viability (colour change) in both control slices (right) and slices subject to 15 min OGD (left) after 1 hour submerged in TTC.

(B) Relative levels of extracted formazan from control and OGD slices harvested from adult animals; data were expressed as percent of control values, and are presented as mean ± SEM. *p < 0.05 compared to control using the Student's t-test.

TTC METABOLISM AND LDH EFFLUX ARE NEGATIVELY CORRELATED AFTER OXYGEN-GLUCOSE DEPRIVATION



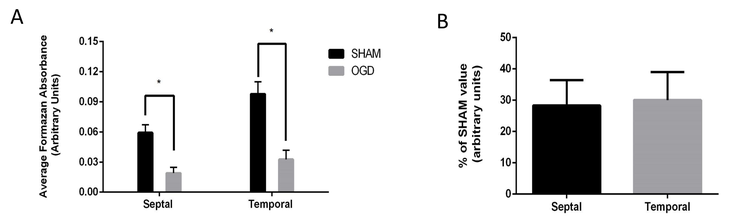
(A) A moderate length of OGD (15 min) leads to a significant increase in LDH efflux (released LDH, nmoles: SHAM, 9.29 ± 0.73; OGD, 14.4 ± 1.29; n = 3 slices from each of N = 7 animals; *p = 0.0023, one-tailed, unpaired Student's t test). Each bar represents the mean ± SEM.

(B) Formazan absorbance and LDH efflux were assessed (n = 3, N = 7), and a significant, negative relationship was found between the two measures of slice viability (Pearson r = -0.5906; 95% CI = -0.8537 to -0.08720; p = 0.026).

OGD = oxygen-glucose deprivation; LDH = lactate dehydrogenase; SHAM = non-OGD treatment.

PRELIMINARY RESULTS

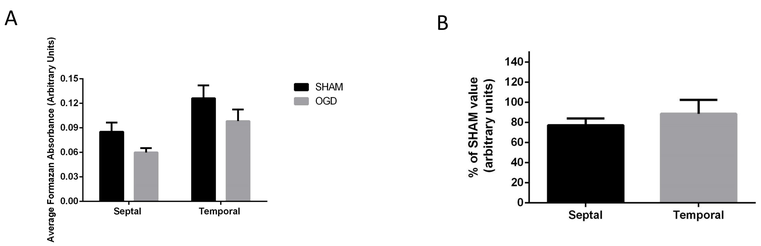
15 Minute OGD Challenge Does Not Cause Changes in TTC Metabolism Across the Longitudinal Axis



(A) 15 minute OGD challenge significantly reduces the level of TTC metabolism in both septal (0.019 ± 0.006, n = 2 slices, taken from N =12 animals) and temporal (0.033 ± 0.009, n = 2, N = 12) slices, relative to SHAM slices (septal: 0.059 ±0.008, n = 2, N = 12; temporal: 0.098 ± 0.012, n = 2, N = 12). Level of significance: p < 0.05 using Student's t-test.

(B) The percent of TTC metabolism in septal (28% ± 8%, n = 2, N = 11) and temporal (30% ± 9%, n = 2, N = 11) slices following 15 minutes of OGD, relative to SHAM slices, were not significantly different from one another (p = 0.850).

10 Minute OGD Challenge Does Not Cause Changes in TTC Metabolism Across the Longitudinal Axis



(A) 10 minute OGD challenge does not significantly reduce the level of TTC metabolism in either septal (0.060 ± 0.005 n = 2 slices, taken from N =13 animals), or temporal (0.098 ± 0.014, n = 2, N = 13) slices, relative to SHAM slices (septal: 0.085 ± 0.011, n = 2, N = 13; temporal: 0.126 ± 0.016, n = 2, N = 13). Level of significance: p < 0.05 using Student's t-test.

(B) The percent of TTC metabolism in septal (77% ± 7%, n = 2, N = 13) and temporal (89% ± 14%, n = 2, N = 13) slices following 10 minutes of OGD, relative to SHAM slices, were not significantly different from one another (p = 0.472). Level of significance: p < 0.05 using t-test analysis.

WATERLOO | APPLIED HEALTH SCIENCES

NSERC CRSNG