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# Simultaneous recording of hippocampal oxygen and glucose in real time using constant potential amperometry in the freely-moving rat

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## HIGHLIGHTS

- We show that average concentrations of hippocampal oxygen and glucose are 100.26 ± 5.76 μM and 0.60 ± 0.06 mM respectively.
- We show that there are uncoupled changes in oxygen and glucose during neuronal activation.
- Anaesthesia and carbonic anhydrase inhibition both significantly increase hippocampal oxygen.
- Anaesthesia, dimethyl sulfoxide administration and carbonic anhydrase inhibition significantly increase hippocampal glucose.
- We show that changes in hippocampal metabolism can be detected in real time using constant potential amperometry.

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## ABSTRACT

Amperometric sensors for oxygen and glucose allow for real time recording from the brain in freely-moving animals. These sensors have been used to detect activity- and drug-induced changes in metabolism in a number of brain regions but little attention has been given over to the hippocampus despite its importance in cognition and disease. Sensors for oxygen and glucose were co-implanted into the hippocampus and allowed to record for several days. Baseline recordings show that basal concentrations of hippocampal oxygen and glucose are 100.26 ± 5.76 μM and 0.60 ± 0.06 mM respectively. Furthermore, stress-induced changes in neural activity have been shown to significantly alter concentrations of both analytes in the hippocampus. Administration of $O_2$ gas to the animals' snouts results in significant increases in hippocampal oxygen and glucose and administration of $N_2$ gas results in a significant decrease in hippocampal oxygen. Chloral hydrate-induced anaesthesia causes a significant increase in hippocampal oxygen whereas treatment with the carbonic anhydrase inhibitor acetazolamide significantly increases hippocampal oxygen and glucose. These findings provide real time electrochemical data for the hippocampus which has been previously impossible with traditional methods such as microdialysis or *ex vivo* analysis. As such, these sensors provide a window into hippocampal function which can be used in conjunction with behavioural and pharmacological interventions to further elucidate the functions and mechanisms of action of the hippocampus in normal and disease states.

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## 1. Introduction

Using implantable sensors allows for stable, long-term recording of a number of common analytes in the extracellular fluid (ECF) of the brain including oxygen (Lowry et al., 1996, 1997; Lowry and Fillenz, 2001; Bolger and Lowry, 2005), glucose (Hu and Wilson, 1997; Fillenz and Lowry, 1998a; Lowry et al., 1998a,b,c; Lowry and Fillenz, 2001; Dixon et al., 2002), nitric oxide (Brown et al., 2009) and glutamate (Kulagina et al., 1999; McMahon et al., 2006a, 2006b, 2007; Qin et al., 2008; Tian et al., 2009). Unlike other methods such as microdialysis, electrochemical sensors are small, can record at a sub-second temporal resolution and cause less disruption in surrounding tissue compared to microdialysis guide cannulae and probes (Bungay et al., 2003; Borland et al., 2005). While limited in terms of what analytes can be measured at any one time, the high specificity and temporal resolution of sensors make them ideal for measuring the relationships between neurochemistry, metabolism and neural activity (Lowry and O'Neill, 2006).

Tissue levels of oxygen in the brain have been traditionally measured indirectly by jugular bulb oximetry (Andrews et al., 1991; Feldman and Robertson, 1997; De Deyne et al., 1998) or by using functional magnetic resonance imaging (fMRI) to monitor the blood-oxygen-level dependent (BOLD) signal (Ogawa et al., 1990; Menon et al., 1992; Glover, 2011). Oxygen can also be measured more directly by voltammetry using Clark-type electrodes

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implanted into the brain tissue (Clark et al., 1958; Krolicki and Leniger-Follert, 1980; Doppenberg et al., 1998; Gupta et al., 1999). Classical Clark electrodes are restrictive due to their large size; they have poor spatial resolution for measuring specific brain regions and are too bulky to be used reliably in freely-moving animals. More recently, these restrictions have been overcome by the development of sensors utilising constant potential amperometry (CPA) that can measure tissue oxygen levels from specific brain regions (Bolger et al., 2011a). Carbon paste electrodes (CPEs) have been shown to detect oxygen with high sensitivity and selectivity at a sub-second temporal resolution (Bolger et al., 2011b). Changes in tissue oxygen levels measured by CPEs are related to changes in regional cerebral blood flow (Lowry et al., 1997) and these changes are directly comparable to the BOLD signal from fMRI studies (Lowry et al., 2010; Francois et al., 2012). As such, tissue oxygen levels measured using CPEs can be used to assess neural activity from localised regions of the brain in freely-moving animals (Li et al., 2011; Russell et al., 2012).

Similarly, measuring tissue levels of glucose in the brain has traditionally relied on a number of indirect and direct techniques. While non-invasive measurements of brain glucose using MRI technology is possible (Choi et al., 2001), its use is not as widespread as the use of the BOLD signal in fMRI. Direct sampling of cerebral spinal fluid using microdialysis allows for local ECF levels of glucose to be determined (Sandberg et al., 1986) and for activity-related changes in glucose concentrations to be assessed (Fellows et al., 1992; McNay et al., 2000, 2001; De Bundel et al., 2009). Microdialysis is limited by its poor temporal resolution with most collection methods limited to the range of minutes, though higher temporal resolution is possible with recent advances in dialysate collection and analysis (Morales-Villagrán et al., 2008; 2012). However, the real time data acquisition made possible by glucose biosensors allow for the kind of analysis that is not currently possible using microdialysis (Lowry et al., 1998a,b,c). A number of different glucose biosensors have been developed and have been shown to be able to rapidly detect changes in cerebral glucose (Boutelle et al., 1986; Shram et al., 1997). Measuring glucose in real time with CPA can be achieved using platinum/poly(*o*-phenylenediamine)/glucose oxidase (Pt/PPD/GOx) biosensors (Lowry et al., 1994, 1998b; Dixon et al., 2002).

Sensors have been previously used to show the varied metabolic characteristics of different parts of the brain including the striatum (Lowry et al., 1998b; Bazzu et al., 2009; Brown et al., 2009; Calia et al., 2009), nucleus accumbens (Finnerty et al., 2012; Francois et al., 2012), motor cortex (Lowry et al., 2010), prefrontal cortex (Finnerty et al., 2012) and whisker barrel cortex (Li et al., 2011). However, there has been less attention has been afforded to the hippocampus (Freund et al., 1989; Hu and Wilson, 1997) despite its pivotal roles in declarative memory (Scoville and Milner, 1957; Cohen et al., 1999; Eacott and Easton, 2011), spatial navigation (O'Keefe and Nadel, 1978; Morris et al., 1982; D'Hooge and De Deyn, 2001) and its links to various neurodegenerative/psychiatric disorders (Heckers and Konradi, 2010; Marlatt and Lucassen, 2010; Bast, 2011; Dhikav and Anand, 2011; Bonilha et al., 2012). Therefore, there is a need for sensor data recorded in real-time to supplement the large amount of electrophysiological data (Bliss and Lomo, 1973; Martin et al., 2000; Morris et al., 2003; Colgin and Moser, 2010), microdialysis data (McNay et al., 2001; Gold, 2003; De Bundel et al., 2009; López-Pérez et al., 2012) and molecular data from tissue samples (Gooney et al., 2002; Minichiello, 2009; Barry and Commins, 2011) already published from work in the hippocampus. Some behavioural work with hippocampal sensors has been performed; it has been recently shown that oxygen sensors can be used to differentiate between the activity of the dorsal and ventral regions of the hippocampus in a spatial memory task (McHugh et al., 2011). Yet, there has been little work done using sensors to measure basal levels of oxygen and glucose in the hippocampus or to determine how basic behavioural and pharmacological interventions can alter levels of these particular analytes.

Hence, in this paper, we describe a number of experiments where tissue levels of hippocampal oxygen and glucose are simultaneously measured in the rat using CPEs and Pt/PPD/GOx sensors respectively. Alterations in hippocampal oxygen and glucose levels are detected during behavioural-induced changes in neural activity using the tail pinch and restraint stress paradigms and during periods of mild hyperoxia and hypoxia. Levels of hippocampal oxygen and glucose are also monitored following treatment with saline, the anaesthetic chloral hydrate, the commonly-used vehicle dimethyl sulfoxide and the carbonic anhydrase inhibitor acetazolamide (Diamox®).

## 2. Materials and methods

### 2.1. Subjects

Male Sprague Dawley rats (250–300 g; Charles River Laboratories International, Inc.; U.K.) were housed in a temperature-controlled facility with a 12 h light/dark cycle (lights on at 07:00) with access *ad libitum* to food and water. All procedures were performed under license in accordance with the European Communities Regulations 2002 (Irish Statutory Instrument 566/2002).

### 2.2. Data acquisition and statistical analysis

All electrochemical experiments were performed using a low noise potentiostat (Biostat IV, ACM Instruments, Cumbria, U.K.). Data acquisition was performed using a PowerLab® interface system (ADInstruments Ltd., Oxford, U.K.) and a Logiq laptop or Mac. The software packages used were LabChart for Windows and Mac (Version 6) and EChem for Windows Version 1.5.2 (ADInstruments Ltd., Oxford, U.K.).

All data was preliminarily processed in Microsoft® Excel® for Mac 2011 (Version 14.2.2) before being exported to GraphPad Prism® 5 for Mac OS X (Version 5.0a) for statistical analysis and plotting of graphs. Data was either normalised to baseline levels for ease of comparison or area under curve (AUC) analysis was performed to quantify any observed changes in the sensor signals for statistical analysis. For multiple comparisons, repeated-measures and mixed-factorial analysis of variance tests (ANOVAs) with Bonferroni *post hoc* analysis were used as appropriate. Paired *t*-tests were also when comparing results from two different time points. $p < 0.05$ was considered to be significant and all data is presented as the mean ± standard error of the mean (SEM).

### 2.3. Working electrode preparation and surgery

Carbon paste (O'Neill et al., 1982) was prepared by thoroughly mixing 0.71 g of graphite powder (1–2 μm, Aldrich) with 250 μl of silicone oil (high temperature, Aldrich). CPEs were made from Teflon®-coated silver wire (8 T, 200 μm bare diameter, 256 μm coated diameter; Advent Research Materials, Suffolk, U.K.) as reported previously (Lowry et al., 1997). Pt/PPD/GOx sensors were made by immobilising GOx (from *Aspergillus niger*; EC 1.1.3.4, type VII-S; Sigma) in a poly(*o*-phenylenediamine) (PPD) film by potentiometric electropolymerisation of the monomer *o*-phenylenediamine (Sigma; 300 mmol/l; Geise et al., 1991) on the bare disc end of a freshly cut Teflon®-coated platinum wire (5 T, 125 μm bare diameter, 175 μm coated diameter; Advent Research Materials, Suffolk, U.K.). A deoxygenated solution of

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