An Investigation of Hypofrontality in an Animal Model of Schizophrenia Using Real-Time Microelectrochemical Sensors for Glucose, Oxygen, and Nitric Oxide

Niall J. Finnerty,[†] Fiachra B. Bolger,[†] Erik Pålsson,[‡] and John P. Lowry*,[†]

[†]Department of Chemistry, National University of Ireland Maynooth, Co. Kildare, Ireland

[‡]Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at University of Gothenburg, PO Box 431, 405 30 Gothenburg, Sweden

ABSTRACT: Glucose, O_2, and nitric oxide (NO) were monitored in real time in the prefrontal cortex of freely moving animals using microelectrochemical sensors following phencyclidine (PCP) administration. Injection of saline controls produced a decrease in glucose and increases in both O_2 and NO. These changes were short-lived and typical of injection stress, lasting ca. 30 s for glucose and between 2 and 6 min for O_2 and NO, respectively. Subchronic PCP (10 mg/kg) resulted in increased motor activity and increases in all three analytes lasting several hours: O_2 and glucose were uncoupled with O_2 increasing rapidly following injection reaching a maximum of 70% (ca. 62 μM) after ca. 15 min and then slowly returning to baseline over a period of ca. 3 h. The time course of changes in glucose and NO were similar; both signals increased gradually over the first hour post injection reaching maxima of 55% (ca. 982 μM) and 8% (ca. 31 nM), respectively, and remaining elevated to within 1 h of returning to baseline levels (after ca. 5 and 7 h, respectively). While supporting increased utilization of glucose and O_2 and suggesting overcompensating supply mechanisms, this neurochemical data indicates a hyperfrontal effect following acute PCP administration which is potentially mediated by NO. It also confirms that long-term in vivo electrochemical sensors and data offer a real-time biochemical perspective of the underlying mechanisms.

KEYWORDS: Hypofrontality, brain, electrochemical sensors, real-time monitoring, metabolism, schizophrenia
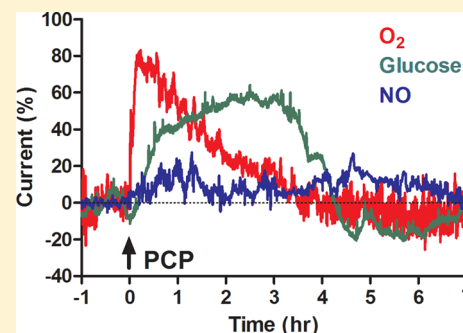
Functional imaging studies in schizophrenic patients have suggested reduced frontal lobe activity, that is, reduced glucose utilization and decreased regional cerebral blood flow (rCBF),[1−4] especially during cognitive tasks involving the prefrontal cortex such as working memory or verbal fluency.[5,6] This hypofrontality was first report by Ingvar and Franzén in 1974[1] and has been highly controversial since then with conflicting findings being reported in the literature right up to the present day.[5−7] While neuroimaging measures of metabolism and rCBF traditionally reflect the average activity across an anatomically defined "region of interest", or more recently identification of clusters of significant activation using voxel-by-voxel comparisons across the entire brain,[4] they do not provide direct in situ neurochemical information on the potential molecular underpinnings of hypofrontality. In addition, the technique, while ideally suitable to cognitive testing in humans, is not amenable to such testing in rodents due to the need to anaesthetize the experimental subjects.

The aim of the current study, therefore, was to investigate whether these specific issues could be addressed using long-term in vivo electrochemistry. LIVE involves the detection of substances in brain extracellular fluid (ECF) using electrochemistry with implanted amperometric sensors. By implanting a microelectrode (sensor) in a specific brain region, applying a suitable potential profile and recording the resulting Faradaic current, changes in the concentration of a variety of ECF species can be monitored. This allows investigations of the functions of specific chemicals in neuronal signaling, drug actions, and well-defined behaviors. It offers excellent spatial (e.g., 10−200 μm) and temporal (millisecond) resolutions, and a major advantage of long-term stability (continuous monitoring in vivo over several weeks).[8−10]

We have previously reported the in vitro[11−17] and in vivo[13,15,17−19] characterization of several sensors (e.g., glucose, O_2 and nitric oxide (NO)) developed to study brain energy metabolism. Operational characteristics such as sensitivity, selectivity, response time, limit of detection, etc., were characterized in detail for each device and their target substrate. The sensors were then subsequently used to study brain function in different application (e.g., pharmacological and behavioral) studies.[20−24] We have also shown that the measured tissue O_2 signal can serve both as an index of changes in blood flow,[25] and of the magnitude of the blood oxygenation level dependent (BOLD) fMRI response, thus providing a reliable awake animal surrogate of human fMRI experimentation, and an effective translational tool which can

better enable the comparison of preclinical and clinical research.[26] In the present study we have explored the effects of hypofrontality induced using phencyclidine (PCP), a noncompetitive inhibitor of the NMDA receptor, on extracellular glucose, O_2 and NO monitored in real-time in freely moving animals using our previously developed LIVE sensors.

■ RESULTS AND DISCUSSION

LIVE Neurochemical Monitoring. LIVE sensors enable continuous real-time monitoring of a variety of neurochemicals in freely moving behaving animals where both gross and fine details can be recorded. For example, typical prolonged recordings of tissue O_2 during day and night are shown in Figure 1. Observed changes can be rapid, occurring over
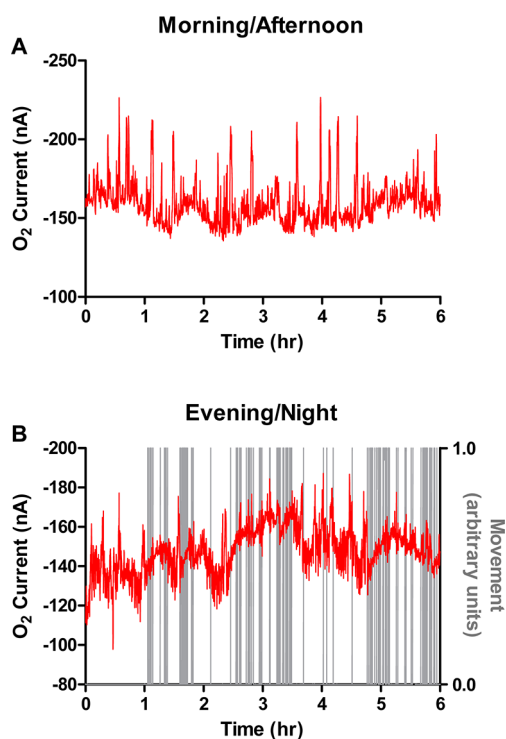
Figure 1. Typical examples of continuous real-time in vivo recordings of brain tissue O_2 during six-hour periods covering morning/afternoon (A, 10 am to 4 pm) and evening/night (B, 6 pm to 12 am, gray lines represent simultaneously monitored motor activity).
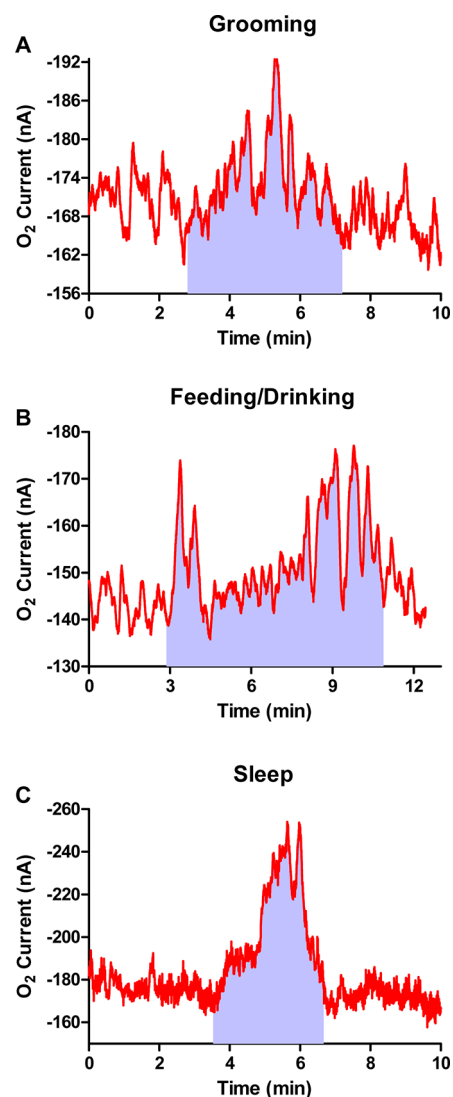

Figure 2. Typical examples of changes (shaded areas) in baseline in vivo brain tissue O_2 signals associated with naturally occurring grooming (A), feeding/drinking (B), and sleep (C). All data recorded during morning/afternoon periods (10 am to 4 pm).

periods ranging from seconds to minutes, or more prolonged, lasting one or more hours. The former tends to be associated with physiological phenomena such as grooming, feeding, and even sleep (see Figure 2), while the latter occurs mainly with periods of intense activity (see Figure 1B). For O_2, both are reflective of the fact that the measured real-time $[O_2]$ is the dynamic balance between supply and utilization. Such changes in signal (nA) can be converted to units of pressure (mmHg, often used to represent pO_2,[15,27] using post in vivo calibration data and literature reported concentration and pressure data associated with the air-saturated $[O_2]$ at 37 °C:[28] the maximum increases of 22 nA (grooming, Figure 2A), 26 nA (feeding/drinking, Figure 2B) and 76 nA (sleep, Figure 2C) correspond to ca. 25 μM O_2, 29 μM O_2 and 84 μM O_2, or 18, 24, and 63 mmHg, respectively.

The recent finding that brain tissue O_2 monitored using a microelectrochemical sensor in behaving animals can act as a surrogate for BOLD fMRI responses in humans[26,29,30] opens the possibility of studying, in animal models, controversial hypotheses or paradigms where data has primarily come from clinical studies. Hypofrontality is one such example. Ingvar and Franzén reported rCBF measurements in their original paper and found a hypofrontal rCBF distribution pattern at rest in chronic deteriorated schizophrenics.[1] Since then, while resting hypofrontality has been replicated in a number of studies,[31−33] there have also been many conflicting reports.[34,35] This may in part be explained by the potential for substantial variance in the resting state from individual to individual because of the heterogeneity of patient samples.[36,37] Also, some reports suggest that the ability of schizophrenic patients to recruit the prefrontal cortex decreases when they were stimulated with a frontal cognitive challenge.[36,38] However, like resting hypofrontality, this task-related or activation hypofrontality has not been consistently replicated.[39] While the debate has primarily concentrated on issues such as lack of standard resting

374.7 nA × min (PCP, $n = 15$); NO -0.045 ± 0.008 nA x min (saline, $n = 5$), 23.4 ± 1.4 nA × min (PCP, $n = 5$). These results clearly show that LIVE sensors can provide continuous real-time metabolic data in an animal model of psychosis. Not only that, they also enable simultaneous recording of multiple data sets facilitating a direct comparison and investigation of their inter-relationship.

The observed increase in O_2 suggests an initial rapid increase in rCBF,[40] and given that the changes were coincident with increased locomotor activity it is likely to be due to increased delivery to cope with the increased energy demand. The slower increase in glucose is also more likely to reflect increased supply rather than a decrease in metabolic activity, and hence glucose utilization as observed in resting state hypofrontality. Since the O_2 signal represents the balance between supply from the vasculature and utilization, the subsequent declining signal suggests a decrease in supply rather than increased utilization, given the continuous locomotor hyperactivity and the fact that the glucose signal remains elevated during this declining period. The uncoupling and the difference in the time scales for the changes in glucose and O_2 is something we have observed previously with anesthetics[40] and during mild neuronal activation induced by a 5 min period of stimulation (tail pinch).[22] While some of the supplied glucose may be coming from the increased blood flow it is likely that there is a significant contribution from another source or intermediate compartment. Evidence suggests that this is likely to involve astrocytes, which now appear to play a central role in the delivery of metabolic substrates.[44,45]

One of the current theories of hypofrontality suggests that a hyper-glutamatergic state of the PFC corresponds to hypo-activation which is caused by a disruption of glutamatergic signaling of the NMDA receptor complex.[5] This acute NMDA receptor antagonist model is in apparent contradiction to the profound increases in cortical excitatory activity observed following NMDA receptor blockade, that is, hyperfrontality, or increases in glucose utilization and blood flow in the PFC.[46−49] However, recent animal studies using techniques directly analogous to those used in patients have demonstrated that repeated or chronic treatment with PCP evokes neuroadaptive processes more relevant to schizophrenia than acute treatment and that these processes manifest as hypofrontality.[50] The glucose and O_2 data presented herein support a hyperfronal effect following subchronic treatment. The NO data suggests a potential role for this gaseous second messenger of the NMDA receptor in mediating this process. Indeed, it has previously been implicated in the mechanism of PCP psychosis[23,51,52] as well as cognitive dysfunction.[53,54] It has also recently been reported that the hyperactive state of glutamatergic neurons in the PFC may be mediated through inhibition of GABAergic interneurons by NO.[5] One must also remember that NO interacts with the dopaminergic as well as the serotonergic systems, thereby linking glutamatergic to monoaminergic signaling. In fact disruption in the latter suggests that hypofrontality is a delayed consequence of PCP administration; repeated PCP exposure has been reported to induce a selective reduction in basal and stress-evoked dopamine utilization in the PFC, and rats previously subchronically treated with PCP were found to be impaired in a spatial working memory task.[55] Interestingly, these dopaminergic and cognitive deficits were observed following withdrawal from PCP, and as such were reported to be due to drug-induced neurochemical changes rather than direct drug effects.

CONCLUSIONS

This study presents real-time neurochemical measurements of glucose, O_2, and NO recorded using microelectrochemical sensors in an animal model of schizophrenia known to induce hypofrontaility. Injection of saline produced small short-lived changes in all three analytes while PCP resulted in locomotor hyperactivity and increased signals lasting several hours. The time course of changes in glucose and NO were similar; both signals increased gradually over the first hour post injection and remained elevated to within an hour of returning to baseline levels (after ca. 5 and 7 h, respectively). Oxygen on the other hand increased rapidly following injection reaching a maximum after ca. 15 min and then slowly returning to baseline over a period of ca. 3 h. While the presented results provide direct in situ real-time neurochemical and behavioral information on the effects of PCP administration validating the application of LIVE sensors in such studies, they do not support a hypofrontal effect for subchronic doses and reinforce the notion of disturbed prefrontal NO neurotransmission playing a role in schizophrenia. Future work will investigate whether a metabolic hypofunction exits under chronic conditions and if post chronic PCP administration is a better model for schizophrenia, as has been suggested for dopamine.

METHODS

Chemicals and Solutions. Saline solutions (0.9%) were prepared by dissolving 0.9 g of NaCl in 100 mL of doubly distilled water. Phencyclidine hydrochloride (PCP, Sigma Chemicals, St. Louis, MO) was dissolved in saline and administered via systemic (intraperitoneal, glucose and O_2; subcutaneous, NO) injection. A dose of 10 mg/kg in a volume of 2 mL was used based on previously published studies.[43,55] All standard chemicals (NaCl, NaH_2PO_4, and NaOH) were used as received and purchased from Sigma-Aldrich Ireland Ltd. (Dublin).

Sensor Preparation. Sensors for O_2 (carbon paste disk electrodes; 8T Teflon-coated Ag wire, 200-μm bare diameter, 270-μm coated diameter, Advent Research Materials, Suffolk, U.K.), glucose (5T Teflon-coated Pt disk electrodes modified with poly(o-phenylenedi-amine) and glucose oxidase; 125 μm bare diameter, 160 μm coated diameter, Advent Research Materials), and NO (5T Teflon-coated Pt disk electrodes modified with Nafion) were prepared following previously reported protocols.[15,18,19] In vitro calibrations were performed over physiologically relevant concentrations (glucose, 0−10 mM; O_2, 0−240 μM; NO, 0−1 μM) in a standard three-electrode glass electrochemical cell containing 15 mL of phosphate buffer saline (PBS) solution, pH 7.4 (0.15 M NaCl, 0.04 M NaH_2PO_4, and 0.04 M NaOH). A saturated calomel electrode (SCE) was used as the reference electrode, and a Pt wire served as the auxiliary electrode.

Instrumentation and Software. Constant potential amperom-etry (CPA; −650 mV (O_2), +700 mV (glucose), and +900 mV (NO)) was performed in all electrochemical experiments using a low-noise custom designed potentiostat (Biostat IV, ACM Instruments, Cumbria, U.K.). Data acquisition was performed with a notebook PC, a PowerLab interface system (ADInstruments Ltd., Oxford, U.K.), and LabChart for Windows software (ADInstruments Ltd.).

All data are presented as mean ± standard error (SEM). Data is reported as baselines, maximum/peak responses (currents) and durations (time). To facilitate ease of comparison area under curve (AUC) analysis was performed to quantify the observed changes in the sensor signals. For PCP the AUC was calculated by integrating the current for each analyte over the period during which it deviated from the baseline. For saline the AUC was calculated for a fixed period of 10 min. For presentation (in Figures 3 and 4), data was normalized to the respective baseline levels for each sensor. All analysis was performed using Microsoft Excel 2007 and the commercial packages Prism (version 5.01) and InStat (GraphPad Software Inc., La Jolla, CA). The statistical significance of differences observed was calculated using Student's t tests (two-tailed paired or unpaired observations where

828

dx.doi.org/10.1021/cn4000567 | ACS Chem. Neurosci. 2013, 4, 825−831

appropriate). Values of $P < 0.05$ were considered to indicate statistical significance.

Surgical Procedures. Male Wistar rats weighing 200−300 g were anesthetized with the volatile anesthetic isoflurane (4% in air for induction, 1.5−3.0% for maintenance; IsoFlo, Abbott, U.K.) using a Univentor 400 Anaesthesia Unit (AgnTho's AB, Sweden). Once surgical anesthesia was established, animals were placed in a stereotaxic frame and the sensors implanted following a previously described procedure.[22] Coordinates for the prefrontal cortex with the skull leveled between bregma and lambda were as follows: A/P + 3.2, M/L ± 0.8 from bregma, and D/V −4.2 from dura. Sensors were implanted bilaterally in each animal in either glucose/O_2 or NO/NO combinations. A reference electrode (8T Ag wire, 200 μm bare diameter; Advent Research Materials) was placed in the cortex and an auxiliary electrode (8T Ag wire) attached to one of the support screws (see below). The reference potential provided by the Ag wire in brain tissue is very similar to that of the SCE. The electrodes were fixed to the skull with dental screws and dental acrylate (Associated Dental Products Ltd., Swindon, U.K.). Postoperative analgesia was provided in the form of a single injection (0.1 mg/kg, s.c.) of Vetergesic (buprenorphine hydrochloride, Reckitt and Colman Pharmaceuticals, Hull, U.K.) given immediately following the surgery. Animals were allowed to recuperate for 24 h after surgery and were assessed for good health according to published guidelines[56] immediately after recovery from anesthesia and at the beginning of each day. This work was carried out under license in accordance with the European Communities Regulations 2002 (Irish Statutory Instrument 566/2002, Amendment of Cruelty to Animals Act 1876).

Experimental Conditions in Vivo. Rats were housed in large plastic bowls, in a windowless room under a 12 h light, 12 h dark cycle, lights coming on at 8 a.m., with free access to water. Food was available ad libitum. All experiments were carried out with the animal in its home bowl. Implanted sensors were connected to the potentiostat after the 24 h recuperation period, through a six-pin Teflon socket (MS363, Plastics One, Roanoke, VA), and a flexible screened six core cable (363−363 6TCM, Plastics One) which was mounted through a swivel (SL6C, Plastics One) above the rat's head. This arrangement allowed free movement of the animal which remained continuously connected to the instrumentation. After application of the appropriate applied potential each animal was given a further 24 h before experiments were begun in order to ensure that the background currents for the electrodes were completely stabilized. A low-pass digital filter (50 Hz cutoff) was used to eliminate mains AC noise and all data was recorded at 4 Hz.

Animal motor activity (movement) was recorded using a passive infrared (PIR) detector (Gardscan QX PIR, Gardiner Technology, Queensway, Rochdale, OL11 1TQ, UK) positioned directly over the home bowl and connected to a dedicated channel in the data acquisition system (PowerLab/LabChart). Movement originating in the field of view of the sensor activates the "alarm" function of the detector thereby sending a count (voltage trigger) to the PowerLab which appears as a 0/1 (off/on) spike in the LabChart trace. This facilitates the direct correlation of movement data with the in vivo data from the implanted sensors.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: John.Lowry@nuim.ie.

Notes
The authors declare no competing financial interest.

■ REFERENCES

(1) Ingvar, D. H., and Franzén, G. (1974) Abnormalities of cerebral blood flow distribution in patients with chronic schizophrenia. *Acta Psychiatr. Scand. 50*, 425−462.

(2) Buchsbaum, M. S., Nuechterlein, K. H., Haier, R. J., Wu, J., Sicotte, N., Hazlett, E., Asarnow, R., Potkin, S., and Guich, S. (1990) Glucose metabolic rate in normals and schizophrenics during the continuous performance test assessed by positron emission tomography. *Br. J. Psychiatry 156*, 216−227.

(3) Bullmore, E., Brammer, M., Williams, S. C., Curtis, V., McGuire, P., Morris, R., Murray, R., and Sharma, T. (1999) Functional MR imaging of confounded hypofrontality. *Hum. Brain Mapping 8*, 86−91.

(4) Hill, K., Mann, L., Laws, K. R., Stephenson, C. M. E., Nimmo-Smith, I., and McKenna, P. J. (2004) Hypofrontality in schizophrenia: a meta-analysis of functional imaging studies. *Acta Psychiatr. Scand. 110*, 1−14.

(5) Reif, A., Schecklmann, M., Eirich, E., Jacob, C. P., Jarczok, T. A., Kittel-Schneider, S., Lesch, K.-P., Fallgatter, A. J., and Ehlis, A.-C. (2011) A functional promoter polymorphism of neuronal nitric oxide synthase moderates prefrontal functioning in schizophrenia. *Int. J. Neuropsychopharmacol. 14*, 887−897.

(6) Walter, H., Wunderlich, A. P., Blankenhorn, M., Schäfer, S., Tomczak, R., Spitzer, M., and Grön, G. (2003) No hypofrontality, but absence of prefrontal lateralization comparing verbal and spatial working memory in schizophrenia. *Schizophr. Res. 61*, 175−184.

(7) Gur, R. C., and Gur, R. E. (1995) Hypofrontality in schizophrenia: RIP. *Lancet 345*, 1383−1384.

(8) Stamford, J. A., and Justice, J. B. (1996) Probing brain chemistry. *Anal. Chem. 68*, A359−A363.

(9) O'Neill, R. D., Lowry, J. P., and Mas, M. (1998) Monitoring brain chemistry in vivo: voltammetric techniques, sensors and behavioral applications. *Crit. Rev. Neurobiol. 12*, 69−127.

(10) Lowry, J. P. and O'Neill, R. D. (2005) Neuroanalytical chemistry *in vivo* using biosensors. In *Encyclopedia of Sensors* (Grimes, C. A. and Dickey, E. C., Eds.), pp 501−524, American Scientific Publishers, Stevenson Ranch, CA.

(11) Lowry, J. P., and O'Neill, R. D. (1993) Partial characterization *in vitro* of glucose oxidase-modified poly(phenylenediamine)-coated electrodes for neurochemical analysis *in vivo*. *Electroanalysis 6*, 369−379.

(12) Lowry, J. P., McAteer, K., El Attash, S. S., and O'Neill, R. D. (1994) Efficient glucose detection in anaerobic solutions using an enzyme-modified electrode designed to detect H_2O_2: Implications for biomedical applications. *J. Chem. Soc., Chem. Commun. 21*, 2483−2484.

(13) Lowry, J. P., McAteer, K., El Attash, S. S., Duff, A., and O'Neill, R. D. (1994) Characterization of glucose oxidase-modified poly-(phenylenediamine)-coated electrodes *in vitro* and *in vivo*: Homogeneous interference by ascorbic acid in hydrogen peroxide detection. *Anal. Chem. 66*, 1754−1761.

(14) Lowry, J. P., Boutelle, M. G., O'Neill, R. D., and Fillenz, M. (1996) Characterisation of carbon paste electrodes *in vitro* for simultaneous amperometric measurement of changes in oxygen and ascorbic acid concentrations *in vivo*. *Analyst 121*, 761−766.

(15) Bolger, F. B., McHugh, S. B., Bennett, R., Li, J., Ishiwari, K., Francois, J., Conway, M. W., Gilmour, G., Bannerman, D. M., Fillenz, M., Tricklebank, M., and Lowry, J. P. (2011) Characterisation of carbon paste electrodes for real-time amperometric monitoring of brain tissue oxygen. *J. Neurosci. Methods 195*, 135−142.

(16) Brown, F. O., and Lowry, J. P. (2003) Microelectrochemical Sensors for In Vivo Brain Analysis: An Investigation of Procedures for Modifying Pt Electrodes Using Nafion. *Analyst 128*, 700−705.

(17) Brown, F. O., Finnerty, N. J., and Lowry, J. P. (2009) Nitric oxide monitoring in brain extracellular fluid: Characterisation of Nafion-modified Pt electrodes in vitro and in vivo. *Analyst 134*, 2012−2020.

(18) Lowry, J. P., Miele, M., O'Neill, R. D., Boutelle, M. G., and Fillenz, M. (1998) An amperometric glucose-oxidase/poly(o-phenylenediamine) biosensor for monitoring brain extracellular glucose: In vivo characterization in the striatum of freely-moving rats. J. Neurosci. Methods 79, 65−74.

(19) Finnerty, N. J., O'Riordan, S. L., Brown, F. O., Serra, P. A., O'Neill, R. D., and Lowry, J. P. (2011) In vivo characterisation of a Nafion-modified Pt electrode for real-time nitric oxide monitoring in brain extracellular fluid. Anal. Methods 4, 550−557.

(20) Lowry, J. P., O'Neill, R. D., Boutelle, M. G., and Fillenz, M. (1998) Continuous monitoring of extracellular glucose concentrations in the striatum of freely moving rats with an implanted glucose biosensor. J. Neurochem. 70, 391−396.

(21) Fillenz, M., and Lowry, J. P. (1998) Studies of the source of glucose in the extracellular compartment of the rat brain. Dev. Neurosci. (Basel, Switz.) 20, 365−368.

(22) Lowry, J. P., and Fillenz, M. (1997) Evidence for uncoupling of oxygen and glucose utilisation during neuronal activation in rat striatum. J. Physiol. (London, U.K.) 498, 497−501.

(23) Pålsson, E., Finnerty, N., Fejgin, K., Klamer, D., Wass, C., Svensson, L., and Lowry, J. (2009) Increased Cortical Nitric Oxide Release After Phencyclidine Administration. Synapse 63, 1083−1088.

(24) McHugh, S. B., Fillenz, M., Lowry, J. P., Rawlins, N. P., and Bannerman, D. M. (2010) Brain tissue oxygen amperometry in behaving rats demonstrates functional dissociation of dorsal and ventral hippocampus during spatial processing and anxiety. Eur. J. Neurosci. 33, 322−37.

(25) Lowry, J. P., Boutelle, M. G., and Fillenz, M. (1997) Measurement of brain tissue oxygen at a carbon paste electrode can serve as an index of increases in regional cerebral blood flow. J. Neurosci. Methods 71, 177−182.

(26) Lowry, J. P., Griffin, K., McHugh, S. B., Lowe, A. S., Tricklebank, M., and Sibson, N. R. (2010) Real-time electrochemical monitoring of brain tissue oxygen: a surrogate for functional magnetic resonance imaging in rodents. NeuroImage 52, 549−55.

(27) Piilgaard, H., and Lauritzen, M. (2009) Persistent increase in oxygen consumption and impaired neurovascular coupling after spreading depression in rat neocortex. J. Cereb. Blood Flow Metab. 29, 1517−27.

(28) Forstner, H. and Gnaiger, E. (2010) Calculation of equilibrium oxygen concentration. In Polarographic oxygen sensors. Aquatic and physiological applications (Gnaiger, E. and Forstner, H., Eds.), pp 321−336, Springer-Verlag, Berlin, Heidelberg.

(29) Francois, J., Conway, M. W., Lowry, J. P., Tricklebank, M., and Gilmour, G. (2012) Changes in reward-related signals in the rat nucleus accumbens measured by in vivo oxygen amperometry are consistent with fMRI BOLD responses in man. NeuroImage 60, 2169−2181.

(30) McHugh, S. B., Marques-Smith, A., Li, J., Rawlins, J. N., Conway, M., Gilmour, G., Tricklebank, M., and Bannerman, D. M. (2013) Hemodynamic responses in amygdala and hippocampus distinguish between aversive and neutral cues during Pavlovian fear conditioning in behaving rats. Eur. J. Neurosci. 37, 498−507.

(31) Farkas, T., Wolf, A. P., Jaeger, J., Brodie, J. D., Christman, D. R., Fowler, J. S., MacGregor, R. R., deLeon, M. J., deFina, P., Goldman, A., Yonekura, Y., Brill, A. B., Schwartz, M., Logan, J., and Cancro, R. (1984) Regional brain glucose metabolism in chronic schizophrenia. A positron emission transaxial tomographic study. Arch. Gen. Psychiatry 41, 293−300.

(32) Wolkin, A., Jaeger, J., Brodie, J. D., Wolf, A. P., Fowler, J., Rotrosen, J., Gomez-Mont, F., and Cancro, R. (1985) Persistence of cerebral metabolic abnormalities in chronic schizophrenia as determined by positron emission tomography. Am. J. Psychiatry 142, 564−571.

(33) Buchsbaum, M. S. (1990) The frontal lobes, basal ganglia, and temporal lobes as sites for schizophrenia. Schizophr. Bull. 16, 379−389.

(34) Gur, R. E., Skolnick, B. E., Gur, R. C., Caroff, S., Rieger, W., Obrist, W. D., Younkin, D., and Reivich, M. (1983) Brain function in psychiatric disorders. I. Regional cerebral blood flow in medicated schizophrenics. Arch. Gen. Psychiatry 40, 1250−1254.

(35) Ebmeier, K. P., Lawrie, S. M., Blackwood, D. H. R., Johnstone, E. C., and Goodwin, G. M. (1995) Hypofrontality revisited: a high resolution single photon emission computed tomography study in schizophrenia. J. Neurosurg. Psychiatry 58, 452−456.

(36) Andreasen, N. C., Rezai, K., Alliger, R., Swayze, V. W., 2nd, Flaum, M., Kirchner, P., Cohen, G., and O'Leary, D. S. (1992) Hypofrontality in neuroleptic-naive patients and in patients with chronic schizophrenia. Arch. Gen. Psychiatry 49, 943−958.

(37) Hoshi, Y., Shinba, T., Sato, C., and Doi, N. (2006) Resting hypofrontality in schizophrenia: A study using near-infrared time-resolved spectroscopy. Schizophr. Res. 84, 411−420.

(38) Steinberg, J. L., Devous, M. D., Sr., and Paulman, R. G. (1996) Wisconsin card sorting activated regional cerebral blood flow in first break and chronic schizophrenic patients and normal controls. Schizophr. Res. 19, 177−187.

(39) Callicott, J. H., Bertolino, A., Mattay, V. S., Langheim, F. J. P., Duyn, J., Coppola, R., Goldberg, T. E., and Weinberger, D. R. (2000) Physiological dysfunction of the dorsolateral prefrontal cortex in schizophrenia revisited. Cereb. Cortex 10, 1078−1092.

(40) Lowry, J. P., and Fillenz, M. (2001) Real-Time Monitoring of Brain Energy Metabolism In Vivo using Microelectrochemical Sensors: The Effects of Anesthesia. Bioelectrochemistry 54, 39−47.

(41) Vahabzadeh, A., and Fillenz, M. (1994) Comparison of stress-induced changes in noradrenergic and serotonergic neurons in the rat hippocampus using microdialysis. Eur. J. Neurosci. 6, 1205−1212.

(42) Freed, W. J., Bing, L. A., and Wyatt, R. J. (1984) Effects of neuroleptics on phencyclidine (PCP)-induced locomotor stimulation in mice. Neuropharmacology 23, 175−81.

(43) Bristow, L. J., Hutson, P. H., Thorn, L., and Tricklebank, M. D. (1993) The glycine/NMDA receptor antagonist, R-(+)-HA-966, blocks activation of the mesolimbic dopaminergic system induced by phencyclidine and dizocilpine (MK-801) in rodents. Br. J. Pharmacol. 108, 1156−1163.

(44) Magistretti, P. J., and Pellerin, L. (1996) Cellular bases of brain energy metabolism and their relevance to functional brain imaging: evidence for a prominent role of astrocytes. Cereb. Cortex 6, 50−61.

(45) Fillenz, M., Lowry, J. P., Boutelle, M. G., and Fray, A. E. (1999) The role of astrocytes and noradrenaline in neuronal glucose metabolism. Acta Physiol. Scand. 167, 275−284.

(46) Weissman, A. D., Dam, M., and London, E. D. (1987) Alterations in local cerebral glucose utilization induced by phencyclidine. Brain Res. 435, 29−40.

(47) Gao, X. M., Shirakawa, O., Du, F., and Tamminga, C. A. (1993) Delayed regional metabolic actions of phencyclidine. Eur. J. Pharmacol. 241, 7−15.

(48) Duncan, G. E., Leipzig, J. N., Mailman, R. B., and Lieberman, J. A. (1998) Differential effects of clozapine and haloperidol on ketamineinduced brain metabolic activation. Brain Res. 812, 65−75.

(49) Homayoun, H., and Moghaddam, B. (2007) NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurones and pyramidal neurones. J. Neurosci. 27, 11496−11500.

(50) Pratt, J. A., Winchester, C., Egerton, A., Cochran, S. M., and Morris, B. J. (2008) Modelling prefrontal cortex deficits in schizophrenia: implications for treatment. Br. J. Pharmacol. 153, S465−S470.

(51) Fejgin, K., Pålsson, E., Wass, C., Svensson, L., and Klamer, D. (2008) Nitric oxide signaling in the medial prefrontal cortex is involved in the biochemical and behavioral effects of phencyclidine. Neuropsychopharmacology 33, 1874−1883.

(52) Fejgin, K., Pålsson, E., Wass, C., Finnerty, N., Lowry, J. P., and Klamer, D. (2009) Prefrontal GABA$_B$ receptor activation attenuates phencyclidine-induced impairments of prepulse inhibition: involvement of nitric oxide. Neuropsychopharmacology 34, 1673−1684.

(53) Wultsch, T., Chourbaji, S., Fritzen, S., Kittelt, S., Grünblatt, E., Gutknecht, L., Chizat, F., Golfier, G., Schmitt, A., Gass, P., Lesch, K. P., and Reif, A. (2007) Behavioural and expressional phenotyping of nitric

oxide synthase-I knockdown animals. *J. Neural Transm., Suppl. 72*, 69–85.

(54) Harooni, H. E., Naghdi, N., Sepehri, H., and Rohani, A. H. (2009) The role of hippocampal nitric oxide (NO) on learning and immediate, short- and long-term memory retrieval in inhibitory avoidance task in male adult rat. *Behav. Brain Res. 201*, 166–172.

(55) Jentsch, J. D., Tran, A., Le, D., Youngren, K. D., and Roth, R. H. (1997) Subchronic phencyclidine administration reduces mesoprefrontal dopamine utilization and impairs prefrontal cortical-dependent cognition in the rat. *Neuropsychopharmacology 17*, 92–99.

(56) Morton, D. B., and Griffiths, P. H. M. (1985) Guidelines on the recognition of pain and discomfort in experimental animals and an hypothesis for assessment. *Vet Rec. 116*, 431–6.