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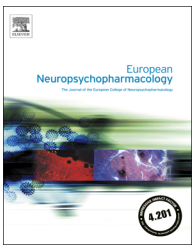

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Longitudinal in vivo maturational changes of metabolites in the prefrontal cortex of rats exposed to polyinosinic-polycytidylic acid in utero

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Abstract
Proton magnetic resonance spectroscopy (^{1}H MRS) studies in schizophrenia patients generally report decreased levels of N-acetyl-aspartate (NAA), glutamate and glutathione, particularly in frontal cortex. However, these data are inconsistent in part due to confounds associated with clinical samples. The lack of validated diagnostic biomarkers also hampers analysis of the neurodevelopmental trajectory of neurochemical abnormalities. Rodent models are powerful tools to address these issues, particularly when combined with ^{1}H MRS (clinically comparable technology). We investigated the trajectory of metabolic changes in the prefrontal cortex during brain maturation from adolescence to adulthood in vivo using ^{1}H MRS in rats exposed prenatally to polyinosinic–polycytidylic acid (POL), a rodent model of maternal immune activation (MIA), an epidemiological risk factor for several psychiatric disorders with a neurodevelopmental origin. Longitudinal in vivo ^{1}H MRS revealed a significant decrease in PFC levels of GSH and taurine in adult, but not adolescent rats. Significant age × MIA interactions for PFC levels of NAA were also observed. These data replicate some deficits observed in the PFC of patients with schizophrenia. There were no significant changes in the

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levels of glutamate or any other metabolite. These data suggest prenatal exposure to POL leads to subtle metabolic perturbations of the normal maturing PFC, which may be related to subsequent behavioural abnormalities. Further work is however required to examine any potential confound of shipping stress on the presumed imbalances in PFC metabolites in POL-exposed offspring. Testing the interactions between MIA with stress or genetic risk variants will also be an important advance.

1. Introduction

Proton magnetic resonance spectroscopy (^{1}H MRS), a non-invasive technique to examine the neurochemistry of the living brain has provided in vivo evidence for neurochemical brain abnormalities in patients with schizophrenia (SCZ) (Brugger et al., 2011). The majority of these studies have focused on the frontal lobe and recent meta-analyses suggest SCZ is associated with both psychotic state and age-dependent decreases (Brugger et al., 2011) in N-acetyl aspartate (NAA), a putative marker of neuronal integrity (Brugger et al., 2011), and the excitatory neurotransmitter glutamate (Marsman et al., 2013). Deficits in the brain anti-oxidant glutathione (GSH) are also reported in the frontal cortex of chronically ill SCZ patients, which is absent in earlier stages of the illness (Do et al., 2000; Monin et al., 2014). However, ^{1}H MRS studies in SCZ patients have also consistently produced inconsistent findings (Uhl et al., 2011). This reflects confounds associated with clinical samples including illness stage at time of scan, symptom severity, and potential effects of antipsychotic medication. In addition, the absence of validated diagnostic biomarkers for disease risk (Jung et al., 2012) limits investigation of the maturational trajectory of abnormalities in brain metabolites in clinical samples, particularly during dynamic periods of brain development from adolescence to adulthood.

Rodent models cannot recapitulate the full phenotypic spectrum of psychiatric disorders, but are powerful tools to dissect mechanisms underlying risk factors for psychiatric illness. Behavioural phenotyping in such models often only reveals subtle deficits that may not be reproducible, nor translate effectively to humans (Nestler and Hyman, 2010). Neuroanatomical, or neurochemical phenotypes, on the other hand, as defined by high-resolution small animal MR imaging, are quite robust and at least technically, directly translational to the clinic (Ellegood et al., 2013; Piontkewitz et al., 2012). In this context, epidemiological evidence consistently supports a link between maternal immune activation (MIA) and an increased risk for the development of multiple psychiatric disorders in the offspring (Atladottir et al., 2012; Atladottir et al., 2009; Brown and Derkits, 2010; Canetta et al., 2014). This can be modelled in rodents providing a powerful translational model system (Meyer, 2014) that does not rely on any presumption of the neural substrates involved in a specific psychiatric disorder (Meyer, 2014). This offers an unbiased way to identify pathological processes underlying the changes in neurodevelopmental trajectories and behavioural function following prenatal immune challenge (Meyer, 2014). Indeed, several studies have consistently shown that the viral mimetic, polyinosinic–polycytidylic acid (POL) administered in middle/late gestation to pregnant rodent dams mimics a broad spectrum of cognitive, behavioural, neuroanatomical, neurotransmitter and pharmacological abnormalities in the offspring relevant to multiple psychiatric disorders (Bitanihirwe et al., 2010; Meyer et al., 2006a, 2008a, 2008b; Winter et al., 2009; Wolff and Bilkey, 2010; Zuckerman et al., 2003).

Whilst there is evidence for the maturational trajectory of neuroanatomical changes following gestational POL exposure (Piontkewitz et al., 2011) no studies have non-invasively examined brain metabolites. We therefore utilised longitudinal in vivo ^{1}H MRS to track maturational changes in the prefrontal cortex (PFC) of male rats exposed to POL in utero, as compared to saline-exposed controls from late adolescence (post natal day 50) into adult life (postnatal day 100 and 180) (Mengler et al., 2014). We chose the PFC since this has been the focus of several clinical ^{1}H MRS studies in SCZ, autism spectrum disorders (ASD) and Bipolar disorder (BD) and abnormalities in this region are thought to be central to the pathology of these disorders (Antonova et al., 2004; van Haren et al., 2008). Based on available clinical evidence, we predicted that prenatal POL treatment would lead to specific alterations in the trajectory of NAA, glutamate and GSH during brain maturation.

2. Experimental procedures

2.1. Animals

Animals were treated in accordance with the guidelines approved by the Home Office Animals (Scientific procedures) Act, UK, 1986 and European Union Directive 2010/63/EU. All animal experiments were given ethical approval by the ethical committee of King's College London (United Kingdom). Eleven male and eleven female Sprague-Dawley rats (Charles River Laboratories, UK, 3 months of age) were used for timed mated breeding. Dams were housed individually under standard laboratory conditions in a temperature – (22±2 °C) and humidity – (55±10%) controlled room on a 12 h light-dark cycle (lights on at 6:00 am) with standard food and water available ad libitum.

2.2. Maternal immune activation (MIA)

Time-mated breeding and MIA procedures were performed at Charles River Laboratories UK in their dedicated animal facility. MIA was induced in pregnant rats in mid/late pregnancy (GD15) by intravenous (i.v.) injection into the tail vein of 4 mg/kg polyinosinic-polycytidylic acid potassium salt (POL; n=8; P9582 Sigma-Aldrich, UK). Control dams received an injection of 0.9% saline (SAL; n=3). Prospective epidemiological studies have highlighted that infections in early gestation (i.e., in the first trimester of human pregnancy) are associated with the highest risk for schizophrenia and related disorders in the offspring (Meyer et al., 2007). Modelling this epidemiological