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Full-length Article

Alteration of transcriptional networks in the entorhinal cortex after maternal immune activation and adolescent cannabinoid exposure

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ABSTRACT

Maternal immune activation (MIA) and adolescent cannabinoid exposure (ACE) have both been identified as major environmental risk factors for schizophrenia. We examined the effects of these two risk factors alone, and in combination, on gene expression during late adolescence. Pregnant rats were exposed to the viral infection mimic polyriboinosinic-polyribocytidylic acid (poly I:C) on gestational day (GD) 15. Adolescent offspring received daily injections of the cannabinoid HU210 for 14 days starting on postnatal day (PND) 35. Gene expression was examined in the left entorhinal cortex (EC) using mRNA microarrays. We found prenatal treatment with poly I:C alone, or HU210 alone, produced relatively minor changes in gene expression. However, following combined treatments, offspring displayed significant changes in transcription. This dramatic and persistent alteration of transcriptional networks enriched with genes involved in neurotransmission, cellular signalling and schizophrenia, was associated with a corresponding perturbation in the expression of small non-coding microRNA (miRNA). These results suggest that a combination of environmental exposures during development leads to significant genomic remodeling that disrupts maturation of the EC and its associated circuitry with important implications as the potential antecedents of memory and learning deficits in schizophrenia and other neuropsychiatric disorders.

1. Introduction

Schizophrenia is a chronic, severe and debilitating brain disorder that has significant genetic risk associated with its etiology. However, environmental challenges, especially during critical developmental windows, are also significant contributors in the emergence of the disorder. Perhaps the best understood of these environmental stressors is prenatal infection, which confers significant risk in the subsequent development of schizophrenia (Mednick et al., 1994; Brown and Derkits, 2010). These findings also align well with a host of pre-clinical animal studies demonstrating MIA induces long-lasting disruptions in gene expression in the developing brain (Fatemi et al., 2011; Smith et al., 2007). Importantly, these disturbances may underlie aberrant neurodevelopment leading to the emergence of behavioural abnormalities (Meyer et al., 2008; Piontkewitz et al., 2011; Zuckerman et al., 2003; Zuckerman and Weiner, 2005) associated with schizophrenia.

While several brain regions display disturbed function in schizophrenia, the EC may be particularly vulnerable to developmental inflammation. Fetal exposure to increased levels of the maternal cytokine IL-8 was associated with significant decreases in left EC volumes in subjects with schizophrenia (Ellman et al., 2010) and pyknotic-like neuronal profiles accompanied by schizophrenia-associated behavioural abnormalities were observed in the EC of adult rat offspring following MIA (Zuckerman et al., 2003; Zuckerman and Weiner, 2005). The EC is a relevant node in the network mediating learning and memory, therefore malformation in this region could play a crucial role in the development of neuropsychiatric disorders (Eichenbaum et al., 2007; Fransén, 2005; Witter et al., 2000). The involvement of EC pathology is supported in both postmortem brain (Schultz et al., 2010; Jakob and Beckmann, 1986) and neuroimaging data (Schultz et al., 2010; Joyal et al., 2002; Baiano et al., 2008; Prasad et al., 2004) and in animal models with disrupted EC function (Meyer et al., 2009; Goto et al., 2002; Schmadel et al., 2004; Ji and Maren, 2008; Wilson et al., 2013). Collectively these data suggest that developmental disturbance of the EC may play a role in functional deficits that manifest as perceptual, cognitive and behavioural disturbances observed in the disorder.

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While early life infection and inflammation are a significant risk factor for schizophrenia (Brown and Derkits, 2010; Ellman et al., 2010; Brown, 2012), there are many other cofactors that enhance susceptibility. These cofactors amplify or synergize with the primary pathological insult to exacerbate the neurodevelopmental consequences (Bayer et al., 1999; Maynard et al., 2001). Human neurodevelopment is an extended process continuing well into adolescence and early adulthood (Catts et al., 2013). During adolescence and puberty the nervous system is highly plastic, undergoing radical functional changes associated with widespread synaptic remodeling and pruning and altered neuronal signalling. It is also during this period that major neuropsychiatric disorders, including schizophrenia, may become evident. In many individuals this coincides with increased risk-taking behaviors including experimentation with psychotropic drugs, in particular cannabis, that may present a window of amplified susceptibility for the onset of select neuropsychiatric disorders (Spear, 2000; Viveros et al., 2012). Prenatal infection and early adolescent cannabis use confer a two-to-five fold increase in the relative risk of developing schizophrenia in adulthood (Brown and Derkits, 2010; Henquet et al., 2005; Moore et al., 2007; Brown, 2011). It is therefore highly probable that an interaction of the two could amplify changes in the developing brain leading to neuropathology. How infection and cannabis alter brain structure and function during development is not known but may involve epigenetic remodeling. To investigate this mechanism we examined changes in gene expression in the EC of late adolescent rats in response to MIA alone, and in combination with, ACE. We hypothesised that MIA will alter gene expression in the EC, and that this will be exacerbated by ACE. This was supported by our observations, with changes in gene expression occurring predominantly in the combined treatment group with possible downstream effects on neuronal differentiation and development, as well as neurotransmitter systems and synaptic function. We also show that a significant proportion of this change in expression relates to changes in the post-transcriptional regulatory matrix established by small non-coding miRNA.

2. Materials and methods

2.1. Animals

Pregnant Wistar rats were sourced from the Animal Resource Centre, Perth, Australia. The animals were kept at a constant temperature of 22 ± 2 °C on a 12–12 h light–dark cycle with lights on at 09.00am and were handled during the seven days preceding poly I:C treatment. All handling of animals and procedures were approved by the Animal Care and Ethics Committee at the Australian Nuclear Science and Technology Organisation.

2.1.1. Prenatal poly I:C treatment

On GD 15, pregnant rats were placed in a restraint device and received either a single intravenous injection of 4 mg/kg poly I:C (Sigma, Australia) dissolved in phosphate buffered saline (PBS) (n = 4) or an equivalent volume of PBS (n = 4). Injections were administered at a volume of 1 ml/kg. To ensure MIA occurred, blood samples were collected at two hours post-injection for analysis of plasma cytokine levels. Poly I:C at this dose provoked a systemic immune response in the pregnant dams, significantly increasing levels of TNF-α, IL-6 and corticosterone (Dalton et al., 2012). Pups were weaned on PND 21 and littermates left together until PND 27 to prevent heat loss when the mother is removed (Krinke, 2000). On PND 27, pups were sexed and separated into cages containing 3–4 animals of the same gender but different litter to avoid litter effects. Pups from poly I:C and vehicle treated dams were housed separately. In view of the evidence of gender effects on behavioural and functional phenotypes induced by prenatal poly I:C (Piontkewitz et al., 2012), only male rats were used in the present study.

2.1.2. Adolescent HU210 treatment of offspring

On PND 27, male offspring were divided into the following four treatment groups: (1) vehicle only: prenatal PBS with adolescent vehicle exposure (Veh-Veh), (2) adolescent HU210 only: prenatal PBS with adolescent HU210 (Veh-HU210), (3) poly I:C only: prenatal poly I:C with adolescent vehicle (Poly I:C-Veh), (4) "two hit" group: prenatal poly I:C with adolescent HU210 (Poly I:C-HU210). The synthetic cannabinoid, HU210 (Sapphire Laboratories, Australia) was dissolved in a vehicle solution of Tween 80: dimethyl sulfoxide:saline (1:1:98). On PND 35 rats in the adolescent HU210 groups (Veh-HU210 and Poly I:C-HU210) received daily intraperitoneal injections of 100 μg/kg HU210 for 14 days. Rats in the adolescent vehicle groups (Veh-Veh and Poly I:C-Veh) received vehicle solution for the treatment period. Injections were administered at a volume of 1 ml/kg (Dalton et al., 2012; Dalton and Zavitsanou, 2010; Ottani and Giuliani, 2001; Rodriguez de Fonseca et al., 1996). Animals were euthanized on PND 55 (n = 5 per treatment group), brains were removed, frozen in liquid nitrogen and stored at −80 °C. In rats, PND 55 corresponds to the period of human late adolescence (Spear, 2000) and in this animal model has been shown to be consistent with the appearance of structural alterations in the brain (Piontkewitz et al., 2012).

2.2. Neuropathology and imaging

The entorhinal cortex pathology in each treatment group was examined in coronal section postmortem using the 5HT1AR-binding radio ligand [^{3}H]8-OH-DPAT (Dalton et al., 2012). Coronal brain sections (16 μm) were cut with a cryostat and thaw mounted onto microscope slides. Sections were preincubated for 15 min at room temperature in a buffer containing 50 mM Tris HCl (pH 7.4), 120 mM NaCl and 4 mM $CaCl_2$. Sections were then incubated for 60 min at room temperature in the same buffer with the addition of 2 nM [^{3}H]8-OH-DPAT (specific activity 170.2 Ci/mmol, Perkin Elmer, USA). Nonspecific binding was determined by incubating adjacent sections in 2 nM [^{3}H]8-OH-DPAT in the presence of 10 μM MM 77 dihydrochloride. After this incubation, sections were washed twice for 10 min each in ice-cold buffer, followed by one dip in ice-cold distilled water and then dried. Sections were opposed to Kodak Biomax MR films, together with autoradiographic standards ([^{3}H] microscales from Amersham), in X-ray film cassettes. Films were exposed for 49 days and were then developed and fixed using Kodak GBX developer and fixer (Dalton et al., 2012). Custom Matlab software was used to define region of interest (Fig. 1A) from which six sections within this region were cropped (Fig. 1B) then used for further analysis (regions 1–6) for each of the four groups. Autoradiographic images were then analysed using binary image thresholding (Jones et al., 2015; Johnson and Walker, 2015) to calculate pixel intensity in the selected region and quantify group differences in the radioligand labeled sections. Prior to commencing the analysis, the thresholding signal range (the upper and lower bounds at which a thresholding assessment could be sensibly made) was defined on a predetermined set of control images. The mid point of the range was then selected as the subsequent level at which groups would be compared (Johnson and Walker, 2015). All cropped images were then passed into Metamorph Imaging System Software (Version 7.5; Molecular Devices Analytical Technologies) (James et al., 2014; Tynan et al., 2010) to determine the number of pixels that occurred at each of the total 256 pixel intensities. This data was then converted into a cumulative threshold percentage score that involved determining the amount of total material

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