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Endocannabinoid dysregulation in cognitive and stress-related brain regions in the *Nrg1* mouse model of schizophrenia

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ABSTRACT

The endocannabinoid system is dysregulated in schizophrenia. Mice with heterozygous deletion of neuregulin 1 (*Nrg1* HET mice) provide a well-characterised animal model of schizophrenia, and display enhanced sensitivity to stress and cannabinoids during adolescence. However, no study has yet determined whether these mice have altered brain endocannabinoid concentrations. *Nrg1* application to hippocampal slices decreased 2-arachidonoylglycerol (2-AG) signalling and disrupted long-term depression, a form of synaptic plasticity critical to spatial learning. Therefore we specifically aimed to examine whether *Nrg1* HET mice exhibit increased 2-AG concentrations and disruption of spatial learning. As chronic stress influences brain endocannabinoids, we also sought to examine whether *Nrg1* deficiency moderates adolescent stress-induced alterations in brain endocannabinoids. Adolescent *Nrg1* HET and wild-type (WT) mice were submitted to chronic restraint stress and brain endocannabinoid concentrations were analysed. A separate cohort of WT and *Nrg1* HET mice was also assessed for spatial learning performance in the Morris Water Maze. Partial genetic deletion of *Nrg1* increased anandamide concentrations in the amygdala and decreased 2-AG concentrations in the hypothalamus. Further, *Nrg1* HET mice exhibited increased 2-AG concentrations in the hippocampus and impaired spatial learning performance. Chronic adolescent stress increased anandamide concentrations in the amygdala, however, *Nrg1* disruption did not influence this stress-induced change. These results demonstrate for the first time in vivo interplay between *Nrg1* and endocannabinoids in the brain. Our results demonstrate that aberrant *Nrg1* and endocannabinoid signalling may cooperate in the hippocampus to impair cognition, and that *Nrg1* deficiency alters endocannabinoid signalling in brain stress circuitry.

1. Introduction

The endocannabinoid system is dysregulated in schizophrenia. Cannabinoid CB1 receptor expression is reduced in schizophrenia brain, and cerebrospinal fluid and blood concentrations of the endogenous cannabinoid, anandamide, are elevated in schizophrenia patients (De Marchi et al. 2003; Giuffrida et al. 2004; Leweke et al. 1999; Newell et al. 2006; Ranganathan et al. 2015; Zuardi et al. 2011). In addition, schizophrenia brain has distorted concentrations of the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG), as well as other related lipids such as palmitoylethanolamide (PEA) and dihomo-γ-linolenoylethanolamine (LEA) (Muguruza et al. 2013). Interestingly, the elevated 2-arachidonoylglycerol (2-AG) concentrations in the hippocampus of schizophrenia brain was normalised by antipsychotic drug treatment. Thus the endocannabinoid system may then provide a novel therapeutic target for antipsychotic drugs. Indeed cannabidiol reduced schizophrenia symptoms in patients that was associated with elevated brain anandamide concentrations (Leweke et al. 2012).

Animal models are needed to advance our understanding of the mechanisms responsible for endocannabinoid system dysregulation in schizophrenia. Such models will provide a platform to explore the potential antipsychotic efficacy of novel drugs that modulate endocannabinoid signalling. Neuregulin 1 is a neurotrophic factor implicated in the pathophysiology of schizophrenia (Mei and Nave 2014) and mice with heterozygous deletion of the neuregulin 1 gene (*Nrg1* HET mice) display face, predictive, and construct validity in modelling the disorder (Arnold et al. 2012; Karl 2013; Karl and Arnold 2013). Nrg1 HET mice display classic schizophrenia-relevant phenotypes such as sensorimotor gating deficits and locomotor hyperactivity that is reversed by clozapine administration (Boucher et al. 2007a; Chohan et al. 2014a; Long et al. 2010; Stefansson et al. 2002). Moreover, they exhibit stereotypic behaviours including repetitive teeth chattering which may have relevance to orofacial dyskinesia observed in schizophrenia patients (Tomiyama et al. 2009). The mice also display increased sensitivity to schizophrenia-relevant environmental factors such as stress and cannabinoid exposure (Boucher et al. 2007a;

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Boucher et al. 2007b; Boucher et al. 2011; Chesworth et al. 2012b; Chohan et al. 2014a; Desbonnet et al. 2012; Long et al. 2013; Spencer et al. 2013).

While we have shown that *Nrg1* HET mice have reduced CB1 receptor expression in the brain (Long et al. 2013), no study has yet examined brain endocannabinoid concentrations in these mice. There is evidence that Nrg1 regulates function of the endocannabinoid system in vitro, as exogenous Nrg1 application to hippocampal brain slices decreased long-term depression (LTD) that was explained by decreased retrograde 2-AG signalling (Du et al. 2013). This finding implies that *Nrg1* might disrupt memory function, particularly spatial memory, which critically depends upon hippocampal LTD (Han et al. 2015; Xu et al. 1997). We therefore aim to examine whether *Nrg1* HET mice exhibit distorted brain endocannabinoid concentrations which would confirm in vivo the interplay between *Nrg1*-ErbB and endocannabinoid signalling systems. We specifically hypothesise that partial genetic deletion of *Nrg1* will increase 2-AG levels in hippocampus. Moreover, that *Nrg1* HET mice will display a functional spatial learning impairment in the Morris Water Maze, as this task is dependent on normal hippocampal function and endocannabinoid signalling (Dong et al. 2013; Ge et al. 2010; Vorhees and Williams 2006).

Nrg1 HET mice display altered sensitivity to the effects of adolescent stress on behaviour and neurobiology (Chohan et al. 2014a; Chohan et al. 2014b; Desbonnet et al. 2012). Moreover, there is emerging evidence of interplay between *Nrg1* and stress systems in the brain. *Nrg1* hypomorphic rats exhibit changes in glucocorticoid receptor expression in the hypothalamus and hippocampus (Taylor et al. 2011), and stress exposure affected *NRG1*-ErbB pathway components in the prefrontal cortex and hippocampus (Brydges et al. 2014; Dang et al. 2016). Stress exposure also alters brain concentrations of endocannabinoids such as 2-AG and anandamide in the amygdala, prefrontal cortices and hippocampus (Hill and McEwen 2010; Hill et al. 2004; Hillard 2014; McLaughlin et al. 2014; Patel and Hillard 2008; Patel et al. 2009; Patel et al. 2005; Rademacher et al. 2008; Sumislawski et al. 2011). Chronic restraint stress in adult mice resulted in reduced anandamide concentrations in the amygdala (Hill et al. 2013). However, no studies have assessed the effects of this widely used restraint stress paradigm in adolescent animals. Given the well established role of endocannabinoids in stress regulatory systems, additional aims of the present study were to discern whether partial genetic deletion of *Nrg1* affects endocannabinoid concentrations in stress-related brain regions and whether these signalling molecules play a role in the interaction between *Nrg1* deficiency and adolescent stress.

2. Materials and methods

2.1. Mice

Nrg1 HET mice and wild-type (WT) were used as previously described (Boucher et al. 2011; Chohan et al. 2014a) (58 mice in total). A heterozygous line is used as homozygous deletion of *Nrg1* in mice is embryonically lethal as Nrg1 is critical to cardiac development (Stefansson et al. 2002). Mice were housed on a reverse light/dark cycle in cages of up to 6 mice per cage. The mice had free access to food and water and environmental enrichment (Boucher et al. 2011; Chohan et al. 2014a). All research and animal care procedures were approved by the University of Sydney's Animal Ethics Committee and were in agreement with the Australian Code of Practice for the Care and use of Animals for Scientific Purposes.

2.2. Chronic adolescent restraint stress paradigm

Adolescent male mice were subjected to restraint within a clear plastic tube as previously described (Chohan et al. 2014a). Restraint stress sessions started in adolescence on post-natal day (PND) 35, and were 6 h in duration and occurred daily for 21 days (PND 57) (Bloss et al. 2010; Chohan et al. 2014a; Hill et al. 2013). At PND 58 the mice were sacrificed via cervical dislocation, the brain extracted and then dissected into hypothalamus, hippocampus, amygdala, and prefrontal cortex. Tissues were immediately snap frozen in liquid nitrogen before being stored in a −80 freezer.

2.3. Endocannabinoid analysis

Homogenised brain tissue underwent solid phase extraction to isolate the lipids, anandamide, 2-AG, PEA, oleoylethanolamide (OEA), and LEA. A Shimadzu 8030 triple quadrupole mass spectrometer was used to ionize the sample using positive electrospray ionization (ESI) through a multiple reaction monitoring (MRM) method. For more detailed methods see Stuart et al. (2013).

2.4. Morris Water Maze experiment

WT and *Nrg1* HET mice of both genders were used in these experiments (PND 180). A 120 cm diameter pool was filled with water containing a nontoxic acrylic white paint to obscure the platform location. The platform was placed slightly submerged in the middle of one quadrant. The protocol was the same as that used by Boucher et al. (2009), although an additional reversal phase was tested. The protocol ran over 12 days starting with a cue phase consisted of the first 2 trials, where the platform was marked with a visible cue. The acquisition phase commenced on day 1 and was repeated daily through to day 7. The acquisition phase assessed performance in finding the hidden platform in the target quadrant. In the probe phase on day 8, the platform was removed and the mice swam freely for 60 s. In reversal 1, conducted on days 9 to 10, the platform was moved to the opposite quadrant and in reversal 2 on day 12, the platform was moved back to the target quadrant. Mice swimming behaviour was recorded using ceiling mounted cameras directly over the pool and quantified with video tracking software (Motion Mensura, NSW, Australia).

2.5. Statistical analysis

Statistical analyses were performed using SPSS (IBM) or Staview (SAS Institute Inc) software. All lipid concentration data were analysed using a 2 factor ANOVA with genotype and stress condition as factors. All Morris Water Maze data was initially analysed with a 2 factor repeated measures ANOVA with gender and genotype as factors. Should no gender differences be observed, the male and female data will be consolidated and a 1 factor repeated measures ANOVA performed with genotype as the sole between subjects factor. Subsequent two-tailed t-tests will be performed on individual days. The results of all analyses were deemed significant at $P < 0.05$.

3. Results

3.1. Adolescent restraint stress increased concentrations of anandamide in the amygdala that were not influenced by partial genetic deletion of Nrg1

Chronic restraint stress in adolescent mice increased anandamide concentrations in the amygdala (2-way ANOVA main effect of stress, $F_{1,27} = 7.25$, $P < 0.05$, Fig. 1B). However, it did not influence anandamide in the other brain regions examined (Fig. 1A, C and D). Nor did stress affect concentrations of 2-AG or other lipid mediators, namely OEA, LEA and PEA in any brain region (Fig. 2, Table 1). *Nrg1* genotype did not influence stress-induced changes in endocannabinoid mediators, as 2-way ANOVA did not show any significant genotype by stress interactions for anandamide, 2-AG, OEA, LEA and PEA in any brain region examined (Fig. 1, Fig. 2, and Table 1).