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Maternal immune activation alters glutamic acid decarboxylase-67 expression in the brains of adult rat offspring

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Abstract

Activation of the maternal innate immune system, termed “maternal immune activation” (MIA), represents a common environmental risk factor for schizophrenia. Whereas evidence suggests dysregulation of GABA systems may underlie the pathophysiology of schizophrenia, a role for MIA in alteration of GABAergic systems is less clear. Here, pregnant rats received either the viral mimetic polyriboinosinic-polyribocytidilic acid or vehicle injection on gestational day 14. Glutamic acid decarboxylase-67 (GAD_{67}) mRNA expression was examined in male offspring at postnatal day (P)14, P30 and P60. At P60, GAD_{67} mRNA was elevated in hippocampus and

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Contributors

K.B. Seroogy and N.R. Richtand designed the study and methods and supervised the research. S.N. Cassella, A.M. Hemmerle, K.H. Lundgren, T.L. Kyser, R. Ahlbrand and S.L. Bronson carried out the experiments. S.N. Cassella and A.M. Hemmerle analyzed data, conducted the statistical analyses and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest in relation to this study.

thalamus and decreased in prefrontal cortex of MIA offspring. MIA-induced alterations in GAD expression could contribute to the pathophysiology of schizophrenia.

Keywords

Schizophrenia; GAD; GABA; Poly I:C; mRNA; Inflammation; MIA

1. Introduction

Schizophrenia is a neurodevelopmental disorder affecting 1% of the population (Knapp et al., 2004). The etiology of schizophrenia requires a combination of genetic and environmental factors acting in concert. Epidemiological evidence demonstrates that maternal bacterial and viral infection during pregnancy is associated with increased risk of schizophrenia (Mednick et al., 1988; Brown, 2006; Clarke et al., 2009).

In animal models, prenatal exposure to maternal immune activation (MIA) is used to recapitulate this effect (Shi et al., 2003). One such model utilizes the synthetic double-stranded RNA polyriboinosinic-polyribocytidilic acid (Poly I:C). Injection of Poly I:C to pregnant dams activates the maternal innate immune response, stimulating pro-inflammatory cytokine systems (Fortier et al., 2004; Smith et al., 2007). This produces cellular, neurochemical, and behavioral alterations in the offspring attributable to the MIA rather than the virus itself (Zuckerman et al., 2003; Shi et al., 2005; Richtand et al., 2012; Missault et al., 2014; Vorhees et al., 2015) and of relevance to schizophrenia (Meyer et al., 2005, 2009; Brown and Derkits, 2010).

MIA offspring exhibit alterations in several neurotransmitter systems of relevance to schizophrenia including dopamine, glutamate, and γ-aminobutyric acid (GABA) systems (Samuelsson et al., 2006; Lanté et al., 2007; Meyer et al., 2008, 2009; Ibi et al., 2009; Bitanihirwe et al., 2010; Escobar et al., 2011; Roenker et al., 2011; Richetto et al., 2014). As the chief inhibitory neurotransmitter in the central nervous system, GABA is found in numerous locations including neocortical regions, the hippocampus and the thalamus. Imbalance between excitatory glutamate and inhibitory GABA function has been implicated in the pathophysiology of schizophrenia (Roberts, 1972; Huguenard and Prince, 1992; Hashimoto et al., 2003; Lewis et al., 2005; Woo et al., 2008; Chiang et al., 2012; Nakazawa et al., 2012). GABAergic systems are also critical in proper brain development and, therefore, a point of convergence and target in the study of schizophrenia (Wassef et al., 2003; Cellot and Cherubini, 2013; Schmidt and Mirnics, 2015). Modulation of GABA transmission in the brain is often monitored via glutamate decarboxylase isoform 67 kDa (GAD_{67}), the rate-limiting enzyme in GABA synthesis. Here, we examined the consequences of MIA via Poly I:C exposure on GAD_{67} mRNA expression at multiple postnatal time points of the developing rat brain.

2. Methods

2.1 Poly I:C treatment

Female Sprague Dawley rats from Harlan Laboratories (Indianapolis, IN), aged 3–5 months, and males produced within the animal facility were paired for breeding. Animals were housed under standard conditions with access to food and water *ad libitum*. The Poly I:C treatment protocol was performed as previously described (Bronson et al., 2011; Hemmerle et al., 2015). Briefly, on gestational day 14 pregnant dams were injected with Poly I:C (8 mg/kg i.p.; Sigma, St. Louis, MO) dissolved in saline or with saline vehicle (1 ml/kg). On postnatal day (P) 14, P30 and P60 male offspring were sacrificed and their brains were subsequently processed for in situ hybridization. All experimental procedures were approved by the Institutional Animal Care and Use Committee.

2.2 In situ hybridization

Fresh-frozen brains (n=6/condition) were serially sectioned (at 10-μm thickness) throughout the forebrain using a cryostat, thaw-mounted onto Superfrost plus microslides (VWR, Batavia, IL), and stored at −20°C until hybridization. Semi-adjacent sections were processed for the *in situ* hybridization localization of GAD_{67} mRNA using a ^{35}S-labeled cDNA probe, as previously described (Seroogy and Herman, 1997; Hemmerle et al., 2012, 2015; Makinson et al., 2015). Briefly, slides were pretreated, dehydrated and delipidated prior to hybridization. The hybridization probe was prepared from a linearized cDNA plasmid using T3 RNA polymerase and labeled with ^{35}S-UTP (PerkinElmer, Boston, MA). The GAD_{67} plasmid (a generous gift from Dr. James Herman, University of Cincinnati) was contained in a Bluescript SK vector that consisted of 3086 bases (GenBank Gene ID: 24379). Sections were hybridized overnight, washed, treated with RNase, rinsed, air-dried, and exposed to BioMax MR film (Kodak, Rochester, NY) for 7 days. The films were developed with Kodak GPX developer and fixer.

2.3 Analysis

Analysis of the film autoradiograms was performed by taking densitometry measurements utilizing Scion Image software (NIH) as described previously (Numan et al., 2005; Hemmerle et al., 2012, 2015). At least six sections per area per animal were measured from the following regions: prefrontal cortex (PFC, including prelimbic, infralimbic and anterior cingulate cortex subdivisions), frontal, parietal and piriform cortices, striatum, hippocampus, and thalamus. Brain regions were selected for study based upon their participation in circuitry implicated in schizophrenia abnormalities modeled by MIA (Volk and Lewis, 2013). Boundaries of brain regions analyzed were determined using the Paxinos and Watson rat brain atlas (2007). Background measurements were taken from an unlabeled region of each section and subtracted from each optical density (OD) value to give a corrected OD value. The experimental data are shown as a percentage of the control group. Graph Pad Prism was used to determine group differences via t-test and results were considered significant when $p < 0.05$.

3. Results

3.1 Increased GAD_{67} mRNA expression in adult MIA offspring

Hybridization for GAD_{67} mRNA was increased in multiple regions of adult (P60) MIA compared to control offspring, but not in young or adolescent offspring (P14 and P30, respectively). In the hippocampus, levels of GAD_{67} mRNA were elevated in the granule cell layer of the dentate gyrus (DG) ($t(4) = 3.169$, $p < 0.05$) and in region CA2 of the pyramidal cell layer ($t(4) = 5.546$, $p < 0.01$) (Figs. 1A–B, 2). A trend towards an increase in expression was detected in hippocampal region CA3 ($t(4) = 2.606$, $p < 0.06$). In the thalamic reticular nucleus, GAD_{67} mRNA levels were also upregulated in MIA compared to controls ($t(4) = 0.415$, $p < 0.01$) (Figs. 1C, 2). Increased expression of GAD_{67} was not seen in any other forebrain region evaluated, including region CA1 of the hippocampus.

3.2 Decreased GAD_{67} mRNA expression in adult MIA offspring

Levels of GAD_{67} mRNA were decreased ($t(4) = 3.156$, $p < 0.05$) in the prelimbic region of the PFC in adult MIA offspring (P60), but again not at the earlier P14 and P30 time points (Figs. 1D, 2). Additionally, the infralimbic region at P60 exhibited a strong trend towards decreased expression ($p = 0.0504$). In hippocampal region CA2, there was a significant decrease in hybridization for GAD_{67} mRNA at the P30 time point ($t(4) = 3.228$, $p < 0.05$) in MIA offspring. Analysis of other forebrain regions revealed no significant alterations in mRNA expression in the anterior cingulate cortex, in the other cortical regions, or in the striatum.

4. Discussion

The present findings demonstrate differential, long-term alterations in GAD gene expression in multiple forebrain regions in MIA offspring, extending previous studies describing effects of maternal immune activation upon GABAergic systems (see Samuelsson et al., 2006; Meyer et al., 2008, 2009; Oskvig et al., 2012; Richetto et al., 2013, 2014; Tang et al., 2013; Volk and Lewis, 2013). The changes in GAD mRNA levels were not unidirectional; multiple forebrain regions displayed either decreases or increases in expression. Moreover, MIA offspring did not exhibit alterations in GAD_{67} expression until the later developmental time points, corresponding to the emergence of relevant behavioral alterations in the MIA model (Shi et al., 2003; Patterson, 2009). Though GABA levels were not directly measured, the current results may have important implications, as schizophrenia is a developmental disorder with initial overt symptom manifestation in late adolescence or early adulthood.

In studies of schizophrenia patients, changes in the GABAergic system are consistently found in the prefrontal cortex and hippocampus. Hippocampal abnormalities of the GABA system, such as reductions in parvalbumin-positive interneurons and increased GABA receptor expression, are observed in schizophrenia post-mortem tissue (Benes et al., 1998; Heckers and Konradi, 2010; Coyle et al., 2012). An imbalance between excitatory glutamatergic and inhibitory GABAergic neurotransmission is proposed to underlie hippocampal dysfunction in schizophrenia (Heckers and Konradi, 2010).

Similarly, in MIA models, GABAergic alterations have been consistently observed. These observations are perhaps not surprising given that cortical GABAergic neurons are born at the same time points the developing brains are exposed to MIA (Jakovcevski et al., 2011; Volk and Lewis, 2013). Studies have observed reduced GABAergic content in the hippocampus and PFC (Bitanihirwe et al., 2010), decreased GAD_{67} in the dorsal hippocampus and PFC (Dickerson et al., 2014; Richetto et al., 2014), reduction of parvalbumin-positive neurons in the PFC, hippocampus and entorhinal cortex (Meyer et al., 2008; Wischhof et al., 2015), and alterations in $GABA_A$ receptor subunits in select regions of the limbic system (Nyffeler et al., 2006; Samuelsson et al., 2006; Richetto et al., 2014, 2015). However, these GABAergic alternations are not universal in MIA models, emphasizing how variations in design paradigm can affect outcome (Winter et al., 2009; Jing et al., 2013). The data in these current experiments provide further evidence of the vulnerability of the GABAergic system to developmental disturbances.

Our findings of decreased levels of GAD_{67} mRNA in the prelimbic cortex of the offspring at P60 are in agreement with previous studies of the PFC in the MIA mouse model (Richetto et al., 2013, 2014; Labouesse et al., 2015). However, here we add regional specificity by localizing the decrease to the prelimbic subdivision of the PFC, as opposed to other PFC subregions. Inhibitory GABA signaling in the prelimbic cortex is believed to play a role in many cognitive functions known to be impaired in neuropsychiatric disorders, including the fear response and emotionality, acute stress response, aversive learning and working memory (Daviss and Lewis, 1995; Joshi et al., 2012; McKlveen et al., 2013; Piantoadosi and Floresco, 2014). Of note, our findings are also similar to observations in schizophrenia post-mortem studies in the PFC (Guidotti et al., 2000; Hashimoto et al., 2003; Lewis et al., 2005; Straub et al., 2007; Thompson Ray et al., 2011; Kimoto et al., 2014).

Consonant with previous findings suggesting decreases or no change in GAD_{67} mRNA expression in brains of schizophrenia patients and also in relevant animal models (Heckers et al., 2002; Thompson Ray et al., 2011), we found a significant reduction in expression of GAD_{67} mRNA at P30 in hippocampal region CA2, as well as no alterations of GAD_{67} levels in any other hippocampal (or extra-hippocampal) region at the earliest two time points (see Fig. 1). A reduction in GAD_{67} hippocampal mRNA expression was also observed in region CA2/3 of schizophrenia patients using laser-capture microdissection techniques (Benes et al., 2007). Other studies have found that two environmental insults, not simply one, were required to decrease the number of parvalbumin-positive GABA neurons in animals (Giovanoli et al., 2014). However, at the adult P60 time point and in contrast to the schizophrenia findings listed above, we observed increased GAD_{67} mRNA in the hippocampal formation, specifically in the DG and region CA2. The physiological implications for these increases are unknown. Other studies have found that GABAergic neuronal circuitry is vulnerable during adolescence, with effects persisting into adulthood (Guo et al., 2013).

Interestingly, region CA2 exhibited a transient decrease in GAD expression at the adolescent time point (P30), but a robust increase at the later adult time point (P60). It is possible the increase we observed in GAD_{67} mRNA is a compensatory response to the loss of inhibitory interneurons as mentioned above. Recent studies suggest that the CA2 region plays a role in

social memory (Hitti and Siegelbaum, 2014). Specifically with respect to schizophrenia, imaging studies observed reduced non-pyramidal neurons, as well as decreased parvalbumin neuronal density in the CA2 region of schizophrenic patients (Benes et al., 1998; Knable et al., 2004). The GABA inhibitory neurons in the CA2 region and the hippocampus in general regulate the glutamatergic output of pyramidal neurons (Benes and Berretta, 2001) indicating that GAD_{67} expression changes could affect hippocampal excitatory output. It is also possible that the developmental alteration in hippocampal GAD_{67} expression observed in our study reflects a relative imbalance of hippocampal excitatory/inhibitory function. Previous studies have consistently identified altered indices of hippocampal function in the MIA model (Lanté et al., 2007; Lowe et al., 2008; Bitanihirwe et al., 2010; Oh-Nishi et al., 2010; Escobar et al., 2011; Ducharme et al., 2012; Dickerson and Bilkey, 2013; Patrich et al., 2016a,b).

Expression of GAD_{67} mRNA was also increased at P60 in the thalamic reticular nucleus, a GABAergic region implicated in schizophrenia and a major contributor to sensory gating, attentional processing and other reciprocal interactions between the thalamus and cortex (Ferrarelli and Tonoi, 2011; Pratt and Morris, 2015). Damage to the thalamic reticular nucleus results in atrophy of neurons in the PFC, hippocampus and nucleus accumbens and reduces exploratory behavior (Torres-García et al., 2012), suggesting a role for dysfunction of the thalamic reticular nucleus in alterations observed in the PFC of schizophrenia patients (Pratt and Morris, 2015).

The cellular mechanisms behind these differential long-term alterations in GAD expression in MIA offspring remain to be determined, though they could be related, for example, to neuroinflammatory-induced transcription factor modification, or changes in actual cell number. Determination of GAD_{67} protein levels in the present model is also necessary to further elucidate how MIA affects GABAergic gene regulation. Future investigations will explore whether the observed differential regulation of GAD/GABA homeostasis in MIA offspring has a role in the behavioral effects observed in MIA models, including fear conditioning and emotionality, as well as in attention and memory deficits (Meyer et al., 2008; Kranjac et al., 2012; Richtand et al., 2012; Vorhees et al., 2015).

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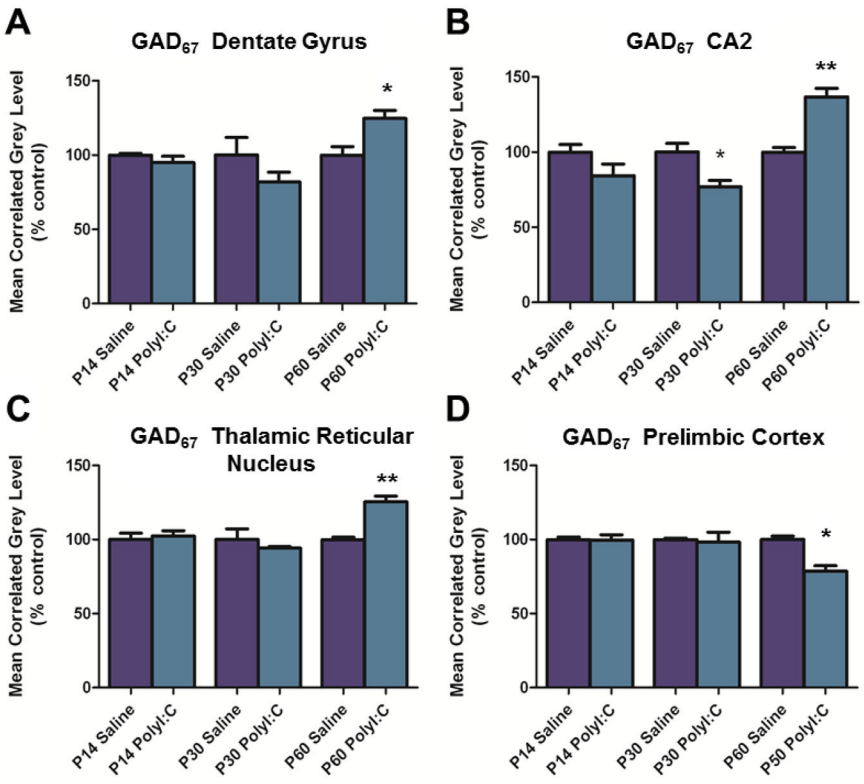

Figure 1.

A. Quantification of GAD_{67} mRNA hybridization signal demonstrates increased levels in the dentate gyrus granule cell layer of Poly I:C (maternal immune activation) animals at P60. **B.** Quantification of GAD_{67} mRNA labeling in the CA2 region of the hippocampus revealed decreased expression at P30 and, in contrast, increased expression at P60 in Poly I:C offspring. **C.** Measurement of GAD_{67} mRNA expression in the thalamic reticular nucleus revealed an increase in hybridization signal at P60. **D.** Decreased cRNA-labeling for GAD_{67} mRNA was found in the prelimbic region of the medial prefrontal cortex of Poly I:C-treated animals at P60. Data are expressed as mean ± SEM. *$p < 0.05$, ** $p < 0.01$ compared to respective control values.

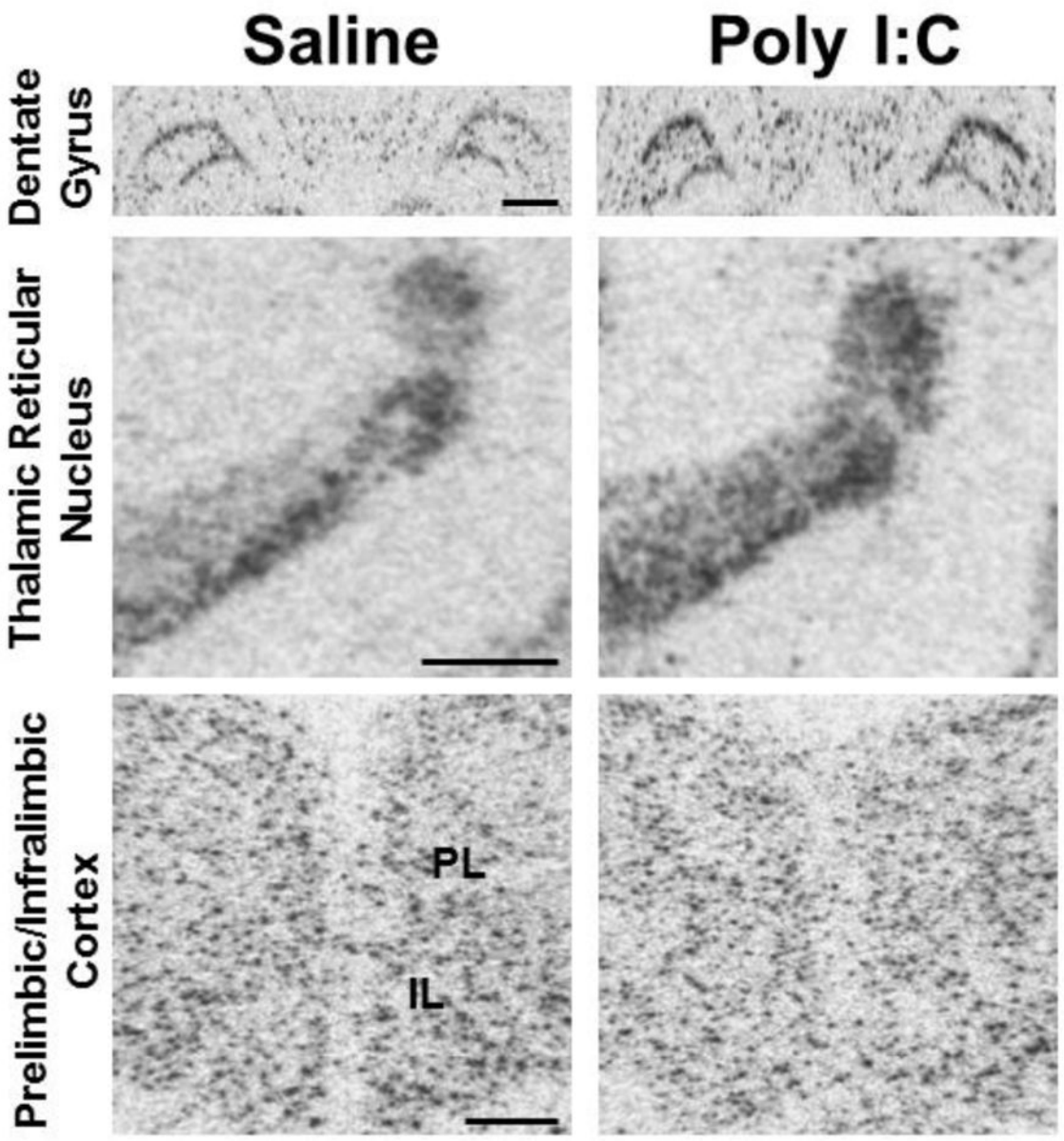

Figure 2.

Representative autoradiograms of GAD_{67} mRNA labeling for several forebrain regions in saline (control) versus Poly I:C (maternal immune activation) conditions at P60. Top row: cRNA-labeling of GAD_{67} mRNA is increased in the dentate gyrus granule cell layer in Poly I:C animals compared to saline controls. Middle row: Increased hybridization signal for GAD67 mRNA is observed in the thalamic reticular nucleus under Poly I:C conditions. Bottom row: Decreased levels of GAD_{67} mRNA expression are present in the prelimbic (PL) region of the medial prefrontal cortex in Poly I:C animals. Note that levels in the infralimbic (IL) cortex also appear decreased, but this did not reach significance when quantified. Scale bar in top row and middle row = 1000 μm; scale bar in bottom row = 500 μm.