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Alterations in hippocampal excitability, synaptic transmission and synaptic plasticity in a neurodevelopmental model of schizophrenia

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ABSTRACT

The risk of developing schizophrenia has been linked to perturbations in embryonic development, but the physiological alterations that result from such insults are incompletely understood. Here, we have investigated aspects of hippocampal physiology in a proposed neurodevelopmental model of schizophrenia, induced during gestation in rats by injection of the antimitotic agent methylazoxymethanol acetate (MAM) at embryonic day 17 (MAM_{E17}). We observed a reduction in synaptic innervation and synaptic transmission in the dorsal hippocampus of MAM_{E17} treated rats, accompanied by a pronounced increase in CA1 pyramidal neuron excitability. Pharmacological investigations suggested that a deficit in GABAergic inhibition could account for the increase in excitability; furthermore, some aspects of the hyper-excitability could be normalised by the $GABA_A$ receptor ($GABA_AR$) potentiator diazepam. Despite these alterations, two major forms of synaptic plasticity, long-term potentiation (LTP) and long-term depression (LTD) could be readily induced. In contrast, there was a substantial deficit in the reversal of LTP, depotentiation. These findings suggest that delivering neurodevelopmental insults at E17 may offer insights into some of the physiological alterations that underlie behavioural and cognitive symptoms observed in schizophrenia.

This article is part of a Special Issue entitled 'Schizophrenia'.

1. Introduction

The aetiology of schizophrenia is only partially understood, but it is widely assumed to be a developmental disease that becomes evident in adulthood, with both genetic and environmental risk factors playing a role during embryogenesis (Jaaro-Peled et al., 2009; Lewis and Levitt, 2002). In particular, schizophrenia may be caused by subtle alterations in neurodevelopment that result in erroneous neuronal connectivity (Stephan et al., 2009). This may then contribute to the positive, negative and cognitive symptoms that are central to the overall behavioural problems and functionality of patients (Elvevag and Goldberg, 2000; Thaker and Carpenter, 2001). Neurodevelopmental disruption induced in rats by MAM_{E17} treatment results in behavioural and histopathological changes consistent with those observed in schizophrenia (Lodge and Grace, 2009). These changes include alterations in a range of behaviours such as sensorimotor gating, hyper-locomotion, social interactions and reversal learning (Featherstone et al., 2007; Flagstad et al., 2004; Moore et al., 2006; Penschuck et al., 2006) which are accompanied by reductions in hippocampal and prefrontal cortex size (Flagstad et al., 2004; Moore et al., 2006), and reductions in neuronal oscillatory activity (Lodge et al., 2009; Penschuck et al., 2006).

Here, we have investigated neuronal excitability, synaptic transmission, and synaptic plasticity in MAM_{E17} rats, focusing on the CA1 sub-region of the hippocampus. Our aim was to evaluate the pathophysiological deficits that may underlie the observed behavioural alterations in this proposed model of schizophrenia. We found a schizophrenia-like pattern of pathophysiological abnormalities including hippocampal shrinkage and pyramidal neuronal hyper-excitability. We found that this hyper-excitability occludes that induced by exogenous application of the $GABA_AR$

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antagonist picrotoxin, and that some aspects of the MAM_{E17} induced hyper-excitability can be normalised by the $GABA_AR$ potentiator diazepam. Surprisingly, given these pronounced differences in excitability and synaptic properties, no abnormalities were found in LTP or LTD. However, we did identify a specific deficit in depotentiation, the reversal of LTP. This is the first description of impaired depotentiation in a neurodevelopmental model of schizophrenia and we speculate that this type of alteration in synaptic plasticity may contribute to the symptoms observed in schizophrenic patients.

2. Materials and methods

2.1. Model preparation

All procedures were conducted in full compliance with the UK Home Office Guidance (UK animals Scientific Procedures Act, 1986) and the ethical policies of Eli Lilly and Co. Ltd. Pregnant Sprague Dawley dams (Charles River, Margate, UK) were injected intraperitoneally with MAM (26 mg/kg) or vehicle (0.9% saline) on day E17 of pregnancy, where E0 is taken as the day the mating pair were put together and E1 is taken as the day the plug was found. Male pups were weaned and re-housed in a temperature-controlled room (21 ± 1 °C) on a 12:12 h light:dark cycle. Food and water were available *ad libitum*. Nineteen cohorts of MAM_{E17}/saline rats were used in this study, each the result of 3 MAM_{E17} treated and 3 saline treated dams.

2.2. Immunohistochemistry

Three month old, MAM_{E17} or sham animals were transcardially perfused with PBS followed by 4% paraformaldehyde, the brains were removed and post-fixed in buffered formalin. For measurement of hippocampal area, brains were freeze-sectioned into 30 μm thick coronal slices and every 5th free-floating section was Nissl-stained (NSALabs®, Knoxville, TN, USA). For neuronal quantification by NeuN staining brains were embedded in paraffin and 6 μm thick coronal sections were cut on a microtome. Sections were sampled from between Bregma −2.20 and Bregma −6.60 for immunohistochemistry, such that the entire dorsal or ventral hippocampus was covered. A total of 797 ± 59 and 766 ± 16 (mean ± SEM) sections were obtained per sham and MAM_{E17} animal respectively. After antigen retrieval in 100 °C citrate buffer, the endogenous peroxidase activity was quenched in 0.3% hydrogen peroxide solution for 30 min. Unspecific binding was blocked by incubating the slides for 1 h at room temperature in solution containing bovine serum albumin and normal horse serum before incubating overnight at 4 °C with the anti-NeuN primary antibody (dilution 1:10,000, Millipore, Billerica, MA, USA). Slices were then rinsed, and incubated successively in a secondary antibody solution and an avidin-biotin-horseradish peroxidase complex (VECTASTAIN Elite ABC Kit Mouse IgG, Vector Labs, Burlingame, CA, USA). The immunostain was revealed using 3, 3′-diaminobenzidine as chromogen (DAB peroxidase substrate kit, Vector Labs, Burlingame, CA, USA). The slices were then dehydrated in ethanol/xylene and cover slipped using xylene-based Shandon Consul-Mount (Thermo Fisher Scientific, Waltham, MA, USA).

Immune-stained slices were scanned with Aperio ScanScope®XT and the digital images were stored in a virtual space provided by Spectrum v1.2.2.2317. Slices were evaluated using the Aperio Scanscope viewer and then representative slices were carefully selected that were between bregma −3.24 mm and −3.36 mm (corresponding to the dorsal hippocampus), between −3.83 mm and −4.08 mm (corresponding to the lateral ventricles) and between −5.16 mm and −5.40 mm (corresponding to the ventral hippocampus). At these stereotaxic levels the outline of the hippocampus and lateral ventricles were clear and easy to delineate due to the level of staining compared to surrounding areas. We then traced round these structures using the Aperio Scanscope image analysis software and this automatically calculated the area of the dorsal and ventral hippocampus, and the lateral ventricles. NeuN-positive cell number was determined using ImageScope software v1.2.2.2319 (Aperio Technologies, Inc., Vista, CA, USA). An automated algorithm allowed us to quantify the total number of NeuN positive cells within several delineated regions throughout the dorsal and ventral hippocampus and this was then converted to density by dividing the cell number by the sample volume.

2.3. Hippocampal slice preparation and extracellular electrophysiology

Transverse hippocampal slices (400 μm) were prepared from the offspring of MAM_{E17} treated dams when they reached 3–4 months of age. Animals were anaesthetized (isofluorane) and sacrificed by cervical dislocation. Brains were then removed and placed in ice cold artificial cerebrospinal fluid (aCSF) aerated with 95% O_2 and 5% CO_2. The aCSF contained (in mM): NaCl, 124; KCl, 3; $NaHCO_3$, 26; NaH_2PO_4, 1.25; $MgSO_4$, 10; glucose, 10; $CaCl_2$, 1. Hippocampi were removed from the brain and

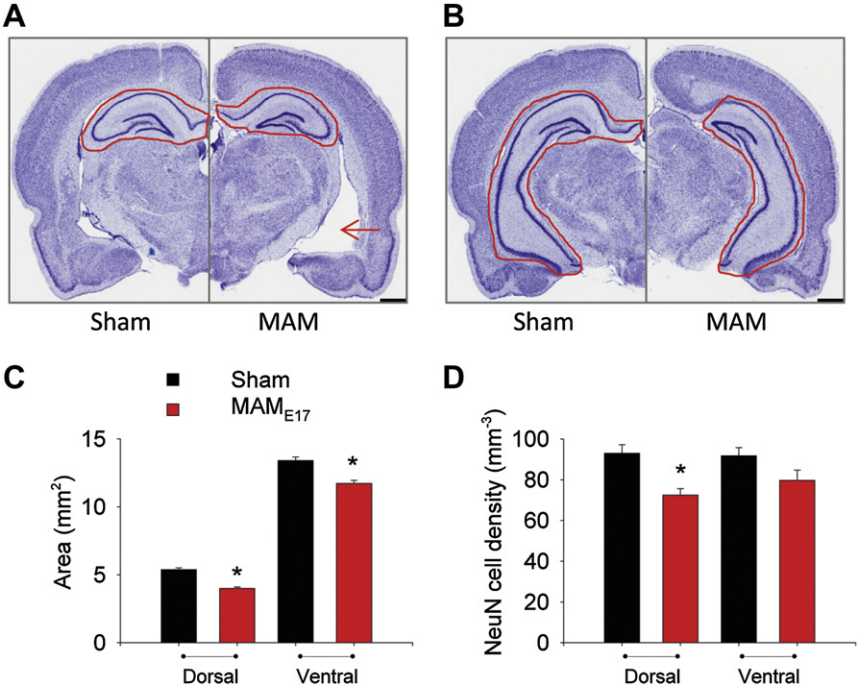

Fig. 1. Pathological changes in the dorsal and ventral hippocampus of sham and MAM_{E17} treated rats. (A) Representative images of coronal sections −2.92 mm from Bregma, stained with cresyl violet (Nissl) stain from sham and MAM_{E17} treated rats. The dorsal hippocampus is outlined in red. Calibration scale is 1 mm. There is a clear reduction in the hippocampal area and an increase in the size of the lateral ventricles (red arrow). (B) Representative images of coronal sections −3.73 mm from Bregma, stained with Nissl stain from sham and MAM_{E17} treated rats. The ventral hippocampus is outlined in red. Calibration scale is 1 mm. (C) Quantification of brain structure area in sham ($n = 8$) and MAM_{E17} ($n = 8$) rats shows that the areas of the dorsal and ventral hippocampus are significantly reduced. (D) Quantification of NeuN staining from sham ($n = 6$) and MAM_{E17} ($n = 6$) rats. The number of NeuN positive neurons was significantly reduced in the dorsal hippocampus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)