available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

A comparison of behavioural and histological outcomes of periventricular injection of ibotenic acid in neonatal rats at postnatal days 5 and 7

*Aiqing Chen, Nicola Dimambro, Gavin J. Clowry**

Institute of Neuroscience and School of Clinical Medical Sciences (Child Health), Newcastle University, Newcastle upon Tyne, UK

ARTICLE INFO

Article history:
Accepted 23 January 2008
Available online 6 February 2008

Keywords:
Cerebral palsy
Corticospinal
Excitotoxicity
Immunohistochemistry
Periventricular leukomalacia
Reaching test

ABSTRACT

Periventricular white matter injury (PVWMI) in premature babies is a major cause of cerebral palsy. Excitotoxic ibotenic acid (IBA) causes PVWMI-like lesions when injected into the white matter of neonatal rodents, however, whether it causes sensorimotor behavioural deficits that could also model cerebral palsy has not been tested. We compared IBA injection at postnatal day 7 (P7) when rodent development is equivalent to the stage of human corticospinal maturation vulnerable to PVWMI and P5 when rodent oligodendrocyte precursor cells are more vulnerable to excitotoxicity. IBA or saline were injected bilaterally into white matter between the external angle of the lateral ventricle and the forelimb sensorimotor cortex. By P14, IBA injection at P5 caused localised hypomyelination and cyst formation in this region, although cortical grey matter remained intact. Treatment at P7 produced less hypomyelination, but more widespread loss of neurofilament immunoreactivity. From P28 onwards, corticospinal function was assessed by testing reaching and retrieval of food rewards. All rats improved with age, but there was a consistent and significant difference between IBA treated rats (P5 and P7) and controls. Histological examination following testing revealed no difference in forebrain cross-sectional area but that the lateral ventricles were significantly larger in IBA treated animals than controls, especially at P7. P5 treatment caused a significantly reduced density of anti-myelin immunoreactivity in the corpus callosum compared to the anterior commissure that was not consistently seen following P7 treatment. We conclude that IBA induced lesions provide a satisfactory model of PVWMI, particularly when made at P5.

1. Introduction

Periventricular white mater injury (PVWMI) is a demyelinating and sometimes cystic lesion of the subcortical white matter that largely leaves the cortical grey matter intact (Marin-Padilla, 1997) although neuroimaging studies in premature infants show some reduction of cerebral cortical gray matter volume (Inder et al., 2003). PVWMI is the most important cause of cerebral palsy in prematurity and its incidence, along with the severity of cerebral palsy, have actually increased over time as medical advances have lead to a greater survival rate for premature infants (Volpe, 1995, 1998). Its etiology is multifactorial, involving both prenatal and perinatal factors that may include genetic causes, ischaemic–reperfusion failure, growth factor deficiency, and infection/inflammation ante- or postnatally (Nelson and Ellenberg, 1986; Murphy et al., 1995; Volpe, 1995).

* *Corresponding author*. Sir James Spence Institute for Child Health, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP, UK. Fax: +44 191 202 3022.
E-mail address: g.j.clowry@ncl.ac.uk (G.J. Clowry).

0006-8993/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2008.01.066

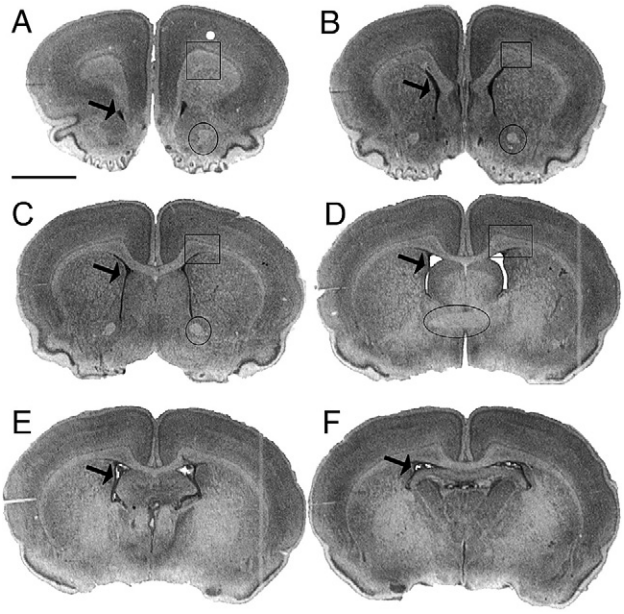

Fig. 1 – Representative, Nissl stained sections from the rat forebrain showing at which levels measurements were made of myelin density and ventricle size. The region of corpus callosum sampled is outlined with a rectangle and anterior commissure with an oval in A–D. Sections A–F were used for measurement of total cross-sectional area, and areas of lateral ventricles (arrows). Scale bar=3 mm.

Age dependent regional susceptibility is a major characteristic of PVWMI. The highest susceptibility in the human brain is between 24 and 32 weeks gestation, a stage of vascular development that leaves the periventricular regions at risk of hypoperfusion and hypoxia (McQuillen and Ferriero, 2004). Coincidently, at this stage of development the subcortical white matter is populated predominantly by premyelinating oligodendrocytes (Kinney and Back, 1998; Back et al., 2001), including both oligodendrocyte precursor cells and immature oligodendrocytes. Both *in vitro* and *in vivo* studies have shown that pre-oligodendrocytes are more vulnerable than mature oligodendrocytes to a variety of hypoxic/ischaemic injury-related insults including glutamate receptor-mediated excitotoxicity (Follett et al., 2000; Itoh et al., 2002; Rosenberg et al., 2003). However the time period of vulnerability to PVWMI also coincides with the timing of corticospinal (Eyre et al., 2000) and thalamocortical and cortico–cortical synaptogenesis (Kostovic and Judas, 2006). Secondary damage to axon tracts, and to subplate neurons which are transiently present in the developing human during this time (Kostovic and Rakic, 1990; Samuelsen et al., 2003) is therefore likely. Subplate neurons play an essential role in the development of connections between thalamus and cortex and of connections within the cortex (Kanold et al., 2003).

Based on the various risk factors, different types of animal models have been developed in different species, including models of hypoperfusion and models using infectious agents, bacterial products, or excitotoxic insults (reviewed by Hagberg et al., 2002; see also McQuillen and Ferriero, 2004; Fan et al., 2005). A successful animal model for testing experimental therapies

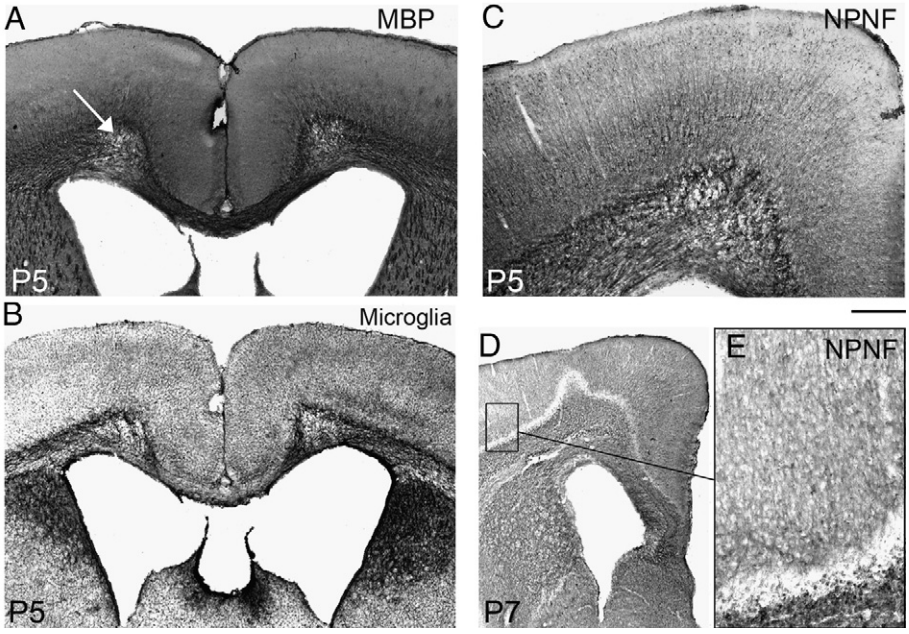

Fig. 2 – On postnatal day 14 (P14), after IBA injection at P5 there is focal loss of myelin basic protein immunoreactivity (MBP) accompanied by the formation of small cysts (arrow) in the corpus callosum near to the injection site below the forelimb sensorimotor cortex (A). The effects upon microglia, visualised by B4-isolectin binding (B) are more widespread with increased density of microglia visible in the white matter and around the walls of the ventricles. Non-phosphorylated neurofilaments (NPNF), a marker for large cortical neurons and their axons at this stage of development, shows a focal depletion of axon staining (arrow) following injection at P5 but with layer V neurons still present (C). Injection at P7 (D) in this case caused a more widespread loss of neurons and axons in both the cortex (E) with a neurofilament free boundary separating cortex from underlying white matter. Scale bar=1000 μm in A, B, D, 600 μm in C and 150 μm in E.