available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Persistent Borna Disease Virus infection changes expression and function of astroglial gap junctions *in vivo* and *in vitro*

*Christiane Köster-Patzlaff, Seyed Mehdi Hosseini, Bernhard Reuss**

Center for Anatomy, Neuroanatomy, University of Göttingen, Kreuzbergring 36, 37075 Göttingen, Germany

ARTICLE INFO

Article history:
Accepted 24 September 2007
Available online 3 October 2007

Keywords:
Astrocytes
Gap junctions
Viral infections
Dentate gyrus
Borna Disease Virus

ABSTRACT

Neonatal Borna Disease Virus (BDV) infection of the Lewis rat brain leads to dentate gyrus (DG) degeneration, underlying mechanisms are not fully understood. Since astroglial gap junction (GJ) coupling is known to influence neurodegenerative processes, the question arose whether persistent BDV infection influences astroglial connexins (Cx) Cx43 and Cx30 in the hippocampal formation (HiF) of Lewis rats. RT-PCR and Western blot analysis of forebrain (FB) samples revealed a virus dependent reduction of both Cx types 8 but not 4 weeks post infection (p.i.). Immunohistochemistry revealed an increase of Cx43 in the DG and a decrease in the CA3 region 4 and 8 weeks p.i. Cx30, which was detectable only 8 weeks p.i., revealed a BDV dependent increase in DG and CA3 regions. BDV dependent astrogliosis as revealed by immunodetection of glial fibrillary acidic protein (GFAP) correlated not with astroglial connexin expression. With regard to functional coupling as revealed by scrape loading, BDV infection resulted in increased spreading of the GJ permeant dye Lucifer yellow in primary hippocampal astroglial cultures, and in increased expression of Cx43 and Cx30 as revealed by immunocytochemistry. In conclusion, persistent BDV infection of the Lewis rat brain leads to changes in astroglial Cx expression both *in vivo* and *in vitro* and of functional coupling *in vitro*. Distribution and time course of these changes suggest them to be a direct result of neurodegeneration in the DG and an indirect effect of neuronal deafferentiation in the CA3 region.

1. Introduction

Borna Disease Virus (BDV) is a non-segmented, negative-stranded, non-lytic RNA virus which is found to infect occasionally the brains of adult sheep and horses (for a review see Boucher et al., 1999). BDV can also cause persistent infections of the human brain (Miranda et al., 2006; Matsunaga et al., 2005; Terayama et al., 2003), which are thought to be able to cause neuropsychiatric diseases such as schizophrenia, bipolar disorder and autism (for a review see Hornig et al., 2003). However, the role of BDV infections for human pathology is still under debate (for a review see Lieb and Staeheli, 2001). A widely used model system for BDV persistence is neonatal infection of the Lewis rat brain, which leads to characteristic structural and behavioral deficits like degeneration of DG granule cells, as well as altered learning and playing behavior (Rubin et al., 1999; Pletnikov et al., 2001, 2002; Lancaster et al., 2007).

Besides the direct neuronal effects of persistent BDV infection of the rat brain, also astrocytes seem to be affected, since the astroglial markers vimentin and GFAP are increased (Carbone

* *Corresponding author*. Fax: +49 551 39 91208.
E-mail address: breuss@gwdg.de (B. Reuss).

Abbreviations: BDV, Borna Disease Virus; BDV-p40, BDV nucleoprotein 40; CA, cornu ammonis; CdP, caudatoputamen; Cx, connexin; DG, dentate gyrus; FB, forebrain; GFAP, glial fibrillary acidic protein; GJ, gap junction; GL, granular layer; GFAP, glial fibrillar acidic protein; HI, hilus; HiF, hippocampal formation; Hyt, hypothalamus; ML, molecular layer; p.i., post infection; PL, pyramidal layer; Tha, thalamus

0006-8993/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2007.09.062

et al., 1989, 1991). Astrocytes are important regulators of CNS homeostatic parameters, like extracellular pH, K^+ concentration and neurotransmitter levels (Anderson and Swanson, 2000; Ransom and Orkand, 1996; Ransom, 2000). They are also involved in the propagation of Ca^{++} waves over long distances (Scemes et al., 1998; Schipke et al., 2002). In the hippocampus many synapses are associated with astrocytic processes which are thought to modulate synaptic transmission (Ventura and Harris, 1999).

Astrocytes are extensively coupled by GJ, and therefore, most of the features mentioned above are affected by changes in astroglial GJ coupling (Charles et al., 1992; Farahani et al., 2005; Froes and de Carvalho, 1998; Naus and Bani-Yaghoub, 1998; Naus et al., 1999; Rozental et al., 2000; Spray et al., 1999; Stout et al., 2002; Ye et al., 2003). GJ are assemblies of protein transmembrane channels, mediating the direct cytoplasmic contact between adjacent cells. By this, GJs integrate astrocytes into a functional syncytium within which transmitters, metabolites, waste products and ions are exchanged (Froes and de Carvalho, 1998; Spray et al., 1999). Each GJ channel is formed by two hemichannels (connexons) provided by adjacent cells, with each connexon being composed of six protein subunits called connexins. The most prominent astroglial connexin types are Cx43 and Cx30 (Altevogt and Paul, 2004; Dermietzel et al., 2000; Nagy et al., 2001, 2003).

Although astrocytes are known to be major sites for BDV replication (Carbone et al., 1989, 1991) effects of BDV infections on astroglial GJ coupling have not yet been investigated. We therefore analyzed expression of astroglial GJ connexins Cx43 and Cx30 in the rat FB and hippocampus during BDV dependent degeneration of DG granule cells by Western blot, RT-PCR and immunohistochemistry. In addition, we investigated the effects of BDV infections on functional coupling in primary hippocampal astroglial cultures, by the scrape loading technique.

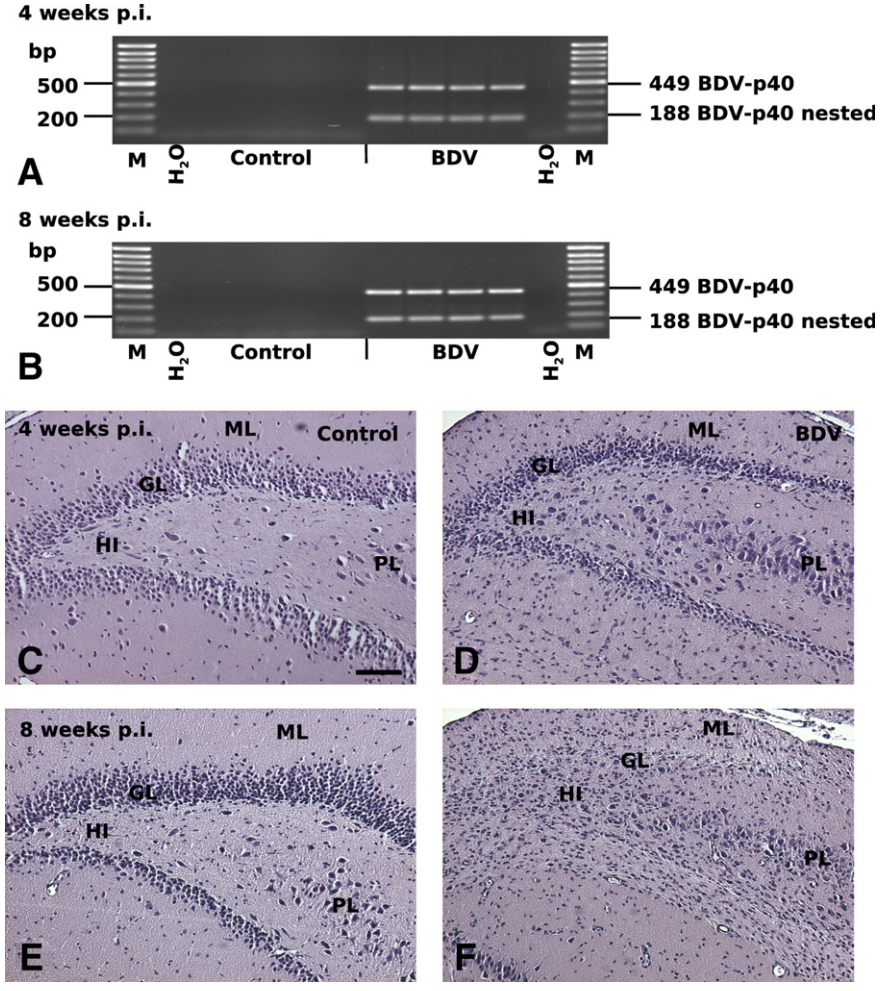

Fig. 1 – Detection of BDV infections by standard and nested RT-PCR. (A) As demonstrated on this fluorescence image of an ethidium bromide stained agarose gel 4 weeks p.i. BDV-p40 specific RNA bands can be detected by standard and nested RT-PCR of RNA samples from brains of rats neonatally inoculated with a BDV containing brain homogenate, whereas samples from control treated animals were negative. (B) Also 8 weeks p.i., only BDV infected samples show BDV-p40 specific PCR bands. (C–F) Morphological changes in the hippocampus of control treated (C, E) and BDV infected (D, F) Lewis rats 4 (C, D) and 8 (E, F) weeks p.i., demonstrating a nearly complete BDV dependent degeneration of DG granule cells 8 weeks p.i. Scale bar = 100 μm; bp = base pairs; GL = granular layer; HI = hilus; kDa = kilodalton; M = 100 bp marker; ML = molecular layer; p.i. = post infection; PL = pyramidal layer.