Brain Research 1650 (2016) 125–133

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres

Research report

# Acute axonal damage in three different murine models of multiple sclerosis: A comparative approach

Katharina Marie Höflich [a], Cordian Beyer [a], Tim Clarner [a], Christoph Schmitz [b], Stella Nyamoya [a,b], Markus Kipp [b], Tanja Hochstrasser [b,*]

[a] Institute of Neuroanatomy, Faculty of Medicine, RWTH Aachen University, 52074 Aachen, Germany
[b] Department of Neuroanatomy, Ludwig-Maximilians-University of Munich, 80336 Munich, Germany

ARTICLE INFO

*Article history:*
Received 6 June 2016
Received in revised form
30 August 2016
Accepted 31 August 2016
Available online 1 September 2016

*Keywords:*
Axonal damage
APP
Experimental autoimmune encephalomyelitis
Cuprizone
Lysolecithin

ABSTRACT

Axonal damage has been identified as a significant contributor to permanent clinical disability in multiple sclerosis. In the context of demyelinating disorders, this destructive event can be the result of inflammation, demyelination and/or the activation of innate defense cells such as microglia or monocytes. The relative contribution of each of these variables to acute axonal injury is, however, unknown.

In the present study, we compared the extent of acute axonal damage in three different murine demyelination models using anti-amyloid precursor protein (APP) immunohistochemistry. T cell dependent ($MOG_{35-55}$-induced experimental autoimmune encephalomyelitis (EAE)) as well as T cell independent demyelination models (cuprizone- and lysolecithin-induced demyelination) were used.

$APP^+$ spheroids were present in all three experimental demyelination models. The number of $APP^+$ spheroids was highest within LPC-induced lesions. Equal amounts were found in the spinal cord of $MOG_{35-55}$-EAE animals and the corpus callosum of cuprizone-intoxicated animals. Moreover, we detected increased immunoreactivity of the pre-synaptic protein vesicular glutamate transporter 1 (VGluT1) in demyelinated foci. VGluT1-staining revealed long stretched, ovoid-like axonal structures which co-localized with APP.

In summary, we showed that acute axonal damage is evident under various experimental demyelination paradigms. Furthermore, disturbed axonal transport mechanisms, which are responsible for intra-axonal APP accumulation, do not only disturb APP, but also the transport of other synaptic proteins. These results indicate that, despite differences in their characteristics, all three models may serve as valid and suitable systems for investigating responsible mechanisms of axonal damage and potential protective strategies.



## 1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). At the histopathological level, lesions are characterized by demyelination, inflammation (i.e. peripheral immune cell recruitment), gliosis and axonal loss. Axonal pathology has already been described by Charcot in 1868 (Charcot, 1868). Currently, it is strongly believed that accumulating axonal damage and loss critically contribute to permanent neurological deficits in MS patients (Kornek et al., 2000; Trapp et al., 1999). Axonal damage can be visualized in fixed tissues by immunohistochemical staining against amyloid precursor protein (APP) (Bitsch et al., 2000; Kuhlmann et al., 2002; Pfeifenbring et al., 2015; Schirmer et al., 2013). APP is a membrane-spanning glycoprotein which is transported from neuron cell bodies to axon terminals by fast anterograde axonal transport. In healthy axons, APP is below the immunohistochemical detection limit. Under pathological conditions, e.g. cytoskeletal disruption, anterograde axonal transport is disturbed and APP accumulates as ovoid spheroids (Kuhlmann et al., 2002).

In MS patients, axonal damage occurs early during lesion formation (Bitsch et al., 2000; Khademi et al., 2013). Interestingly, acute axonal damage, as defined by the accumulation of APP, was found to occur not only in active demyelinating but also in remyelinating and inactive demyelinated MS lesions with a large inter-patient variability (Bitsch et al., 2000). From a pathophysiological point of view, infiltrating T cells and monocytes as well as activated microglia cells might play a role during the development and progression of axonal injury in MS. Acute axonal damage has

* Corresponding author.
*E-mail address:* tanja.hochstrasser@med.uni-muenchen.de (T. Hochstrasser).

http://dx.doi.org/10.1016/j.brainres.2016.08.048
0006-8993/

also been shown in MS animal models, such as experimental autoimmune encephalomyelitis (EAE) and toxin models (Gilgun-Sherki et al., 2003; Herrero-Herranz et al., 2008; Irvine and Blakemore, 2006; 2008; Kornek et al., 2000; Lindner et al., 2009; Nikic et al., 2011; Woodruff and Franklin, 1999).

EAE models the autoimmune aspects of MS with T cell infiltration into the CNS, recruitment of monocytes, macrophages and neutrophils and subsequently destruction of myelin sheaths, oligodendrocytes and axons. Comparable to what can be observed in histopathological sections in MS lesions (Kornek et al., 2000; Kuhlmann et al., 2002), $MOG_{35-55}$-induced EAE in C57BL/6 mice leads to early axonal loss in white matter and gray matter lesions (Gilgun-Sherki et al., 2003; Herrero-Herranz et al., 2008). In contrast to EAE, pathology driven by the copper-chelator cuprizone is T cell independent (Kipp et al., 2009). In the cuprizone model, demyelinating lesions are characterized by severe oligodendrocyte loss and demyelination with concomitant activation of microglia and astrocytes, while lacking the characteristic T cell infiltration, and hence the autoimmune component of the disease. Also in this model, axonal damage occurs in demyelinated foci (Goldberg et al., 2015; Slowik et al., 2015). A third method to induce demyelination is the focal application of lysolecithin, also called lysophosphatidyl choline (LPC). It is believed that focal administration by stereotactic surgery induces demyelination by disrupting myelin membranes. Macrophage infiltration and microglia activation with minimal T cell involvement has been described in this model as a consequence of experimental demyelination (Imai et al., 2008; Waxman et al., 1979). Although this model is frequently considered to be axonal sparing (Wessig et al., 2007; Woodruff and Franklin, 1999), axonal loss was shown distal to the site of injection in primate centrum semiovale, as well as in rabbit corpus callosum (Dousset et al., 1995; Waxman et al., 1979), but, to the best of our knowledge, was not systematically examined in mice.

Reports about mechanisms leading to axonal injury under demyelinating conditions are manifold and include an attack of encephalitogenic T cells (Shriver and Dittel, 2006), as well as microglia-mediated (Mahad et al., 2015), monocyte-mediated (Bitsch et al., 2000; Hendriks et al., 2005) or astrocyte-mediated (Correale and Farez, 2015) mechanisms. Furthermore, focal energy failure due to myelin membrane destruction broadens the list of plausible factors leading to acute axonal injury in the brains and spinal cords of MS patients (Haider, 2015). Most importantly, it has been shown that acute axonal injury is a reversible event (Nikic et al., 2011) and, therefore, may be subject to therapeutic intervention.

Due to the diverse effects causing axonal damage in demyelinating disorders and the different animal models available to study this important aspect of MS pathology, we aimed in this study to conduct a side-by-side comparison of the extent and distribution of acute axonal damage in three different demyelination animal models using anti-APP immunohistochemistry.

## 2. Results

### 2.1. Demyelination as a hallmark of MS animal models

To verify demyelination in all three animal models (i.e. $MOG_{35-55}$-EAE, global cuprizone-induced and focal LPC-induced demyelination), we performed histochemical staining for myelin using Luxol fast blue (LFB)/Periodic Acid Schiff (PAS).

In spinal cords of $MOG_{35-55}$-EAE animals, lesions were randomly distributed throughout the entire spinal cord. On the histopathological level, such lesions were hypercellular, and LFB staining intensity was focally reduced (Fig. 1A). Lesions were found in all three funiculi and at all three levels included in the study. Of note, lesions did not appear to be focused around parenchymal vessels, but rather originated from subpial areas and extended into the deeper white matter parts of the spinal cord (Alvord et al., 1985). As reported previously, demyelinating foci were virtually absent in the forebrain of $MOG_{35-55}$-induced EAE mice (data not shown).

In the cuprizone model, demyelination was observed in various brain regions including the hippocampus, striatum and corpus callosum. Additionally, anti-PLP immunohistochemical stains revealed demyelination of grey matter structures such as the cortex or striatum matrix region (data not shown). As demonstrated in Fig. 1B, the midline of the corpus callosum was severely affected, whereas the underlying dorsal fornix appeared to be somewhat less sensitive to the cuprizone intoxication.

Analysis of brains which were focally injected with LPC revealed a nearly complete demyelination in the corpus callosum next to the injection site (Fig. 1C). Notably, demyelination was not restricted to the injection side, appearing to spread along the callosal fiber pathway to the contralateral side. Furthermore,

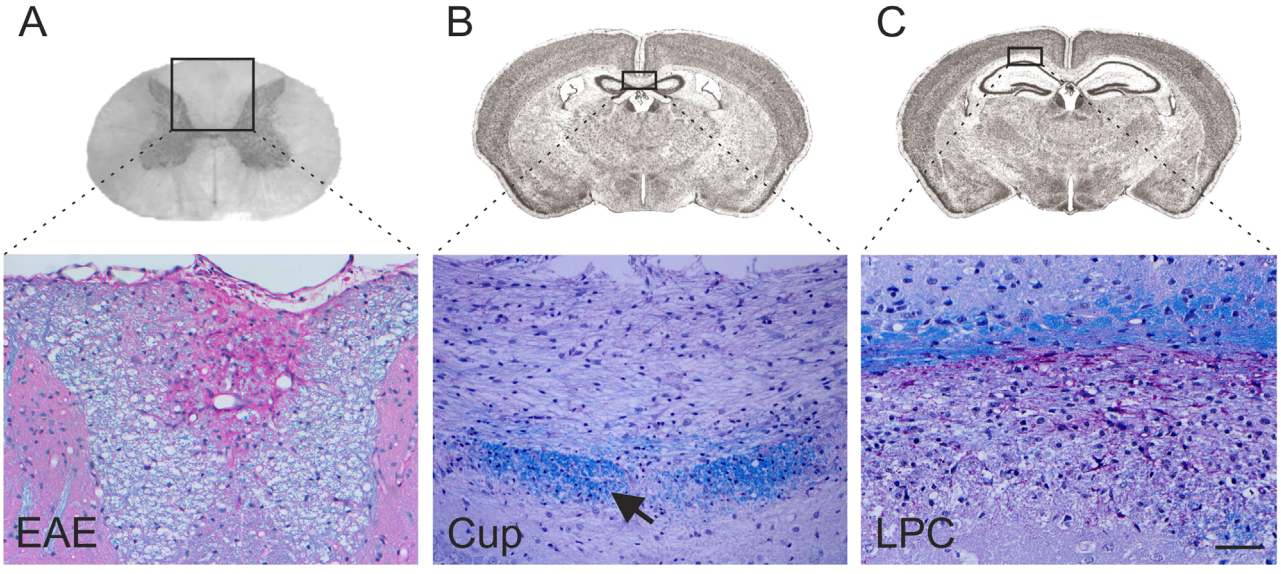


**Fig. 1.** Representative LFB/PAS staining of the spinal cord of $MOG_{35-55}$-EAE mice (A), of the medial corpus callosum (CC) of cuprizone-fed mice (B) and of the lateral CC of LPC mice (C). The arrow indicates the dorsal fornix. (Scale bar = 50 μm).