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The quality of cortical network function recovery depends on localization and degree of axonal demyelination

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ABSTRACT

Myelin loss is a severe pathological hallmark common to a number of neurodegenerative diseases, including multiple sclerosis (MS). Demyelination in the central nervous system appears in the form of lesions affecting both white and gray matter structures. The functional consequences of demyelination on neuronal network and brain function are not well understood. Current therapeutic strategies for ameliorating the course of such diseases usually focus on promoting remyelination, but the effectiveness of these approaches strongly depends on the timing in relation to the disease state. In this study, we sought to characterize the time course of sensory and behavioral alterations induced by de- and remyelination to establish a rational for the use of remyelination strategies. By taking advantage of animal models of general and focal demyelination, we tested the consequences of myelin loss on the functionality of the auditory thalamocortical system: a well-studied neuronal network consisting of both white and gray matter regions. We found that general demyelination was associated with a permanent loss of the tonotopic cortical organization *in vivo*, and the inability to induce tone-frequency-dependent conditioned behaviors, a status persisting after remyelination. Targeted, focal lysolecithin-induced lesions in the white matter fiber tract, but not in the gray matter regions of cortex, were fully reversible at the morphological, functional and behavioral level. These findings indicate that remyelination of white and gray matter lesions have a different functional regeneration potential, with the white matter being able to regain full functionality while cortical gray matter lesions suffer from permanently altered network function. Therefore therapeutic interventions aiming for remyelination have to consider both region- and time-dependent strategies.

1. Introduction

Processes influencing axonal myelination, including myelin loss and gain, are well known physiological events. Severe pathophysiological alterations of the degree of myelination are peculiar hallmarks of a number of neurodegenerative diseases, including multiple sclerosis (MS; Meuth et al., 2010). In MS patients, intermingled episodes of de- and remyelination are associated with the occurrence of gray and white matter lesions in brain and spinal cord. Furthermore, converging evidence relates the occurrence of lesions to the onset or worsening of disease symptoms (Franklin et al., 2012). Myelin plays multiple physiological roles and it is intuitive that its loss may have severe consequences; after all, myelin integrity is important for neuronal and axonal survival, as well as faithful transmission of information in a given network of the central nervous system (CNS; Yates, 2014; Nave and Werner, 2014). In line with white matter damage, MS patients often are

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diagnosed with altered conduction latencies in the CNS (Kim et al., 2013) as indicated by delayed evoked auditory, sensory, motor and visual potentials (Markianos et al., 2009; Matas et al., 2010; Niklas et al., 2009).

Physicians and researchers are now developing new therapeutic strategies to ameliorate disease symptoms by promoting remyelination. This attempt is fueled by recent evidence demonstrating that myelin is required for learning motor tasks and similarly how the acquisition of new information promotes myelin synthesis, both in humans and rodents (Franklin and Gallo, 2014; McKenzie et al., 2014). Indeed, this approach has already been adopted in many clinical trials, but positive results have not been observed in all patients (Bhatt et al., 2014). Therefore strategies to identify eligible patients with highest possible benefit are urgently needed. In this context, it is important to note that numerous MS patients also report severe learning and cognitive dysfunctions in addition to locomotor impairment (Hulst et al., 2013; Manrique-Hoyos et al., 2012), and none of these symptoms can be fully explained by massive or local myelin loss alone. New techniques used for MS diagnosis point to many gray matter structures being severely damaged in MS patients (Hulst and Geurts, 2011). In particular, the thalamocortical system seems to be susceptible as cortical atrophy (Deppe et al., 2014; Minagar et al., 2013) and thalamic lesions as well as lesion-independent degeneration are often observed very early in the disease course. In the light of such evidence and the obvious complexity of MS pathophysiology, the aim of our study was to investigate functional consequences of various demyelination strategies and then to allow endogenous remyelination. By taking into consideration the diversity (whether white or gray matter) and the timing of the lesions we tried to establish a rational for optimal remyelinating intervention times. In this way we tried to answer the following questions: (i) Is promoting remyelination a beneficial strategy? And, if so, (ii) does the success of promoting remyelination depend on the localization of the lesion? (iii) Does the time of intervention/remyelination affect the course of the disease?

In order to answer these questions, we needed to isolate the demyelinating events from other MS hallmarks, an attempt which was possible only by performing a translational study in animal models. We choose to use (i) the cuprizone model of general de- and remyelination to determine the consequences of massive myelin loss and re-growth and (ii) the lysolecithin model of focal demyelination which allowed us to target locally restricted white or gray matter lesions. We combined electrophysiology *in vivo* with behavioral assays in freely behaving animals to investigate the thalamocortical system which, besides being the "hot spot" for gray matter lesions occurring in MS patients, has the advantage of being a highly interconnected network featuring an extensive white fiber tract (the internal capsule).

2. Materials and methods

2.1. Animals and experimental design

All animal work was performed in accordance with the 2010/63/EU of the European Parliament and of the Council of 22 September 2010 and has been approved by the local authorities (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen; approval ID: 87-51.04.2010.A331). All efforts were made to minimize the number of animals used and to avoid their stress and suffering strictly following the ARRIVE guidelines (Kilkenny et al., 2010). C57BL6 mice were used for all the experiments and were singly caged, kept in a 12-h light/dark cycle, and food and water were available *ad libitum*.

2.1.1. Cuprizone treatment

C57BL6 mice were used for all experiments. The animals were 2–3 months old at the beginning of the experiments. Experimental toxic demyelination was induced by feeding mice a diet containing 0.2% cuprizone (bis-cyclohexanone oxaldihydrazone, Sigma-Aldrich Inc., Hamburg, Germany) mixed into a ground standard rodent chow (Skripuletz et al., 2011). The cuprizone diet was maintained for 5–6 weeks. A second group, matched for age and sex, served as control. Interruption of cuprizone administration favors spontaneous remyelination (Skripuletz et al., 2008), therefore we tested two other groups, 7 and 25 days after re-introduction of normal food (7-day remyelination and 25-day remyelination in the text). In order to assess long term effects, an additional group of animals was tested 45 days after stopping the treatment.

2.1.2. Lysolecithin injections

Anesthesia was induced with isoflurane (3% in O_2; Abbot GmbH & Co. KG, Wiesbaden, Germany), maintained with i.p. injection of pentobarbital (50 mg/kg, Narcoren, Merial GmbH, Germany), and additional doses were given if necessary (10–15% of the initial dose). All pressure points were covered with 2% xylocaine gel (Astra Zeneca GmbH, Wedel, Germany) and tissue to be incised was injected with 2% xylocaine solution. Corneas were protected with a dexpanthenol-containing gel (Bepanthen®, Bayer, Leverkusen, Germany). When animals were already anesthetized, before beginning with the surgery, they received an additional injection of carprofen (Rymadil, 5 mg/kg) in order to relieve post-operatory pain. The head was mounted in a stereotaxic apparatus (ASI Instruments, Inc., Warren, MI, USA) via ear bars, and the levels of bregma and lambda were equalized. Craniotomies were performed unilaterally (left hemisphere), thus one hemisphere served as control. The dura mater was removed and then, by means of a Hamilton syringe, lysolecithin (2 µl; at a speed of 10 nl/s) was injected either in layer 4 of the primary auditory cortex (A1): anteroposterior, −2.18 mm; lateral, 4.2 mm from bregma; and dorsoventral, 1 mm from the brain surface; or in the internal capsule (IC): anteroposterior, −0.94 mm; lateral, 2.10 mm; dorsoventral, 2.5 mm (Paxinos and Franklin, 2001). The health status of the animals, e.g. healing of the cranial wound and exploratory activity, was checked daily for 7 days after surgery. Then only after full recovery, animals were tested at 7 days after lysolecithin injection when the demyelination effect was maximal and then at 14 and 28 days to test for remyelination (Hall, 1972; Pavelko et al., 1998). Animals matched for age, gender and experimental time point were injected with vehicle solution and served as controls. All injection sites/locations were verified by histochemical staining after the recordings.

2.2. Tissue preparation and immunohistochemistry

Immunohistochemistry was performed on a group of six animals for each time point for the general demyelination and on a group of three animals for the focal demyelination model. Briefly, mice were deeply anesthetized using Foren (isofluran, 1-chloro-2,2,2 trifluoroethyldifluoromethylether; 5% in O_2) and then perfused with 4% paraformaldehyde (PFA) in phosphate buffer via the left cardiac ventricle as previously described (Skripuletz et al., 2008). Brains were removed, postfixed in 4% PFA and paraffin embedded. For light microscopy, 7-µm serial paraffin sections were cut and dried at 37 °C overnight, as described before (Skripuletz et al., 2013). Paraffin embedded sections were dewaxed, rehydrated, and microwaved for 5 min in 10 mM citrate buffer (pH 6.0). Sections were quenched with H_2O_2, blocked for 1 h in phosphate-buffered solution (PBS) containing 3% normal goat serum, 0.1% Triton X-100, and then incubated overnight with the primary antibody. The following primary antibodies were