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Research Paper

Impairment of frequency-specific responses associated with altered electrical activity patterns in auditory thalamus following focal and general demyelination

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ABSTRACT

Multiple sclerosis is characterized by intermingled episodes of de- and remyelination and the occurrence of white- and grey-matter damage. To mimic the randomly distributed pathophysiological brain lesions observed in MS, we assessed the impact of focal white and grey matter demyelination on thalamic function by directing targeted lysolecithin-induced lesions to the capsula interna (CI), the auditory cortex (A1), or the ventral medial geniculate nucleus (vMGN) in mice. Pathophysiological consequences were compared with those of cuprizone treatment at different stages of demyelination and remyelination. Combining single unit recordings and auditory stimulation in freely behaving mice revealed changes in auditory response profile and electrical activity pattern in the thalamus, depending on the region of the initial insult and the state of remyelination. Cuprizone-induced general demyelination significantly diminished vMGN neuronal activity and frequency-specific responses. Targeted lysolecithin-induced lesions directed either to A1 or to vMGN revealed a permanent impairment of frequency-specific responses, an increase in latency of auditory responses and a reduction in occurrence of burst firing in vMGN neurons. These findings indicate that demyelination of grey matter areas in the thalamocortical system permanently affects vMGN frequency specificity and the prevalence of bursting in the auditory thalamus.

1. Introduction

Multiple sclerosis (MS) has traditionally been considered as an immune-mediated, inflammatory and neurodegenerative disease of the human central nervous system (Hannoun et al., 2012; Trapp and Nave, 2008), which is characterized by intermingled episodes of de- and remyelination and the occurrence of white- and grey-matter damage (Calabrese, 2011). Numerous studies have reported on the effects of grey matter lesions in MS, which include neuronal degeneration, neuronal loss, and axonal transection (Geurts et al., 2012). One of the systems which recently was reported to be involved in MS pathophysiology is the thalamocortical (TC) system (Crandall et al., 2015; Groh et al., 2014; Suga, 2012). During physiological states of alertness, the TC systems provides the neuronal substrate to faithfully process and convey sensory information from the periphery to the cortex and vice versa in a modality-specific manner (Steriade et al., 1993). Undisturbed reciprocal thalamo-cortical and cortico-thalamic interactions are crucial for operating essential brain functions, including somato-sensory sensation, cognition and locomotion (Guido and Weyand, 1995; Steriade et al., 1993). In MS pathophysiology the thalamus has gained

Abbreviations: A1, Primary Auditory Cortex; CI, Capsula Interna; DAB, Diamino-3,3′-benzidine; GFAP, Glial fibrillary acid protein; i.p., intraperitoneal; MS, Multiple Sclerosis; n-RNs, non Responsive Neurons; PBS, Phospate Buffer Saline; PFA, Paraformaldehyde; PLP, Proteo-lipid protein; RNs, Responsive Neurons; TC, Thalamocortical; vMGN, Ventral Medio-geniculate nucleus

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recent interest because a reduced thalamic volume and a severely impaired thalamic network connectivity has been identified as disease marker in MS patients (Benedict et al., 2013; Geurts and Barkhof, 2008) (Batista et al., 2012; Houtchens et al., 2007). A correlation between early atrophy of the thalamus (as well as its interconnected regions) and the occurrence of cognitive disabilities has been suggested as a strong predictor for cognitive deficits in MS patients (Batista et al., 2012; D'Ambrosio et al., 2017; Houtchens et al., 2007; Koenig et al., 2018). Although fewer studies addressed the thalamic pathology at the cellular level in humans, histopathological and immunohistochemical post-mortem studies revealed an extensive neuronal loss in the thalamus of patients with different MS subtypes (Geurts et al., 2006) thus supporting considering the occurrence of thalamic alterations as common hallmark of MS at any stage of the disease (Deppe et al., 2016).

In our study, we focused on the topographically organized auditory thalamocortical system as model, in which TC neurons in the ventral part of medial geniculate nucleus (vMGN) relay auditory inputs from the inferior colliculus to the auditory cortex (A1). These projections are organized in a tonotopic manner and together with corticofugal connections (Mellott et al., 2014) are thought to process auditory signals with high spatial and temporal precision, thereby controlling selective attention and receptive field properties in the thalamus (Crandall et al., 2015). Recently, we found that demyelinating insults in the grey matter of A1 permanently disrupted the frequency-specific responses in this cortical area leading to the inability to induce tone-frequency-dependent conditioned behaviors and speaking for an altered network function (Cerina et al., 2018; Cerina et al., 2017). However, consequences for thalamic sensory processing, characteristic activity modes and network function have not yet been addressed. By taking advantage of the cuprizone model, we investigated here the functional consequences of extensive de- and remyelination in the TC system in which the establishment and maintenance of sensory maps depends on precisely localized and timed afferent inputs. Cuprizone is a copper chelator which, following oral administered to mice for several weeks, induces mature oligodendrocytes' death and consequent general demyelination in the brain (Matsushima and Morell, 2001; Skripuletz et al., 2011). Removal of this compound from the diet allows spontaneous remyelination (Matsushima and Morell, 2001; Skripuletz et al., 2011). However, the events triggered by cuprizone induce a general, massive myelin loss. Therefore, we tried to assess the impact of focal white or grey matter demyelination on thalamic function. In an attempt to mimic the randomly distributed pathophysiological brain lesions observed in MS, we used the lysolecithin model of demyelination (Muto et al., 2015; Sahin et al., 2015). The latter is a toxic compound targeting mature oligodendrocyte leading to full demyelination of the injected area already few days after the injection. Similarly to cuprizone, once the compound is metabolized, spontaneous remyelination occurs (Cerina et al., 2017; Hall, 1972; Pavelko et al., 1998). Targeted lysolecithin-induced lesions were directed to the capsula interna (CI), the A1 or the vMGN in mice, and pathophysiological consequences were compared with those of cuprizone treatment at different stages of de- and remyelination (Muto et al., 2015; Sahin et al., 2015). Single unit recordings were obtained from the vMGN in freely behaving mice and showed a significantly diminished neuronal activity upon demyelination (in both models), as well as impaired frequency-specific auditory responses with increased latencies (Bénardais et al., 2014; Wegener et al., 2015; Bénardais et al., 2014; Wegener et al., 2015; Bénardais et al., 2014; Wegener et al., 2015; Bénardais et al., 2014; Wegener et al., 2015). Targeted lysolecithin-induced lesions directed either to A1 or to vMGN revealed a permanent impairment of frequency-specific responses, an increase in latency of auditory responses and a reduced occurrence of burst firing in vMGN neurons. Taken together, our results provide essential building blocks to understand the sensory processing in the thalamus, to determine the role of regional lesions in pathophysiological hallmarks of MS, and thereby further establishing the study of the TC system as a preclinical MS model (Alusi et al., 2001).

2. Materials and methods

2.1. Animals and experimental outline

All experiments were carried out in accordance with the 2010/63/EU of the European Parliament and of the Council of 22 September 2010 and have been approved by the local authorities (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen; approval ID: 87-51.04.2010.A331 and 84-02.04.2015.A585). Animals were singly caged, kept in a 12-h light/dark cycle, and food and water were available *ad libitum* and we followed the ARRIVE guidelines (Kilkenny et al., 2010) in order to minimize the number of animals used and to avoid their stress and suffering. All data and materials described in the following are available.

2.1.1. General de- and remyelination - cuprizone treatment

The experiments were conducted with 2–3 months old C57BL/6J female mice. 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 to achieve complete demyelination (n = 9). A second group, matched for age and sex, served as control (n = 8). Cessation of cuprizone administration favors spontaneous remyelination (Skripuletz et al., 2008), therefore, we tested two other groups, 7 (n = 11) and 25 (n = 9) days after re-introduction of normal food (early remyelination and late remyelination in the text; see Supplementary Fig. 1a for the schematic experimental outline).

2.1.2. Focal de- and remyelination - lysolecithin injections

The experiments were conducted with 2–3 months old C57BL/6J female mice. Deep anesthesia was induced with isoflurane (3% in O_2; Abbot GmbH & Co. KG, Wiesbaden, Germany), maintained with intraperitoneal (i.p.) injection of pentobarbital (50 mg/kg). If the animals reacted to a tail or paw pinch, an additional dose (10–15% of the initial dose) was given in order to deepen the anesthesia. All pressure points were covered with 2% xylocaine gel (Astra Zeneca GmbH, Wedel, Germany) and 2% xylocain was injected to the tissue to be incised. Corneae were protected with a dexpanthenol-containing gel (Bepanthen®, Bayer, Leverkusen, Germany). 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, thus one hemisphere served as control. By means of a Hamilton syringe, lysolecithin (1 μl; at a speed of 3 nl/s) was injected 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), in the capsula interna (CI; anteroposterior, −0.94 mm; lateral, 2.10 mm; dorsoventral, 2.5 mm (Paxinos and Franklin, 2001) and in the ventral part of the medial geniculate nucleus (vMGN; anteroposterior, −3.16 mm; lateral, 2.20 mm; dorsoventral, 2.90 mm). Animals were tested at 7 days post-injection of lysolecithin (number of animals per group: A1 = 9; CI = 8; vMGN = 10) when the demyelination effect was maximal and then at 14 (number of animals per group: A1 = 7; CI = 7; vMGN = 6) and 28 (number of animals per group: A1 = 8; CI = 7; vMGN = 9) days to test for remyelination (Pavelko et al., 1998). Animals matching for age, gender and experimental time point were injected with saline and served as controls as well as animals which were never injected (naïve in the text; number of animals per group: A1 = 9; CI = 6 vMGN = 8). After completion of all experiments, all injection sites/locations were verified by histochemical staining. Forty-five days after lysolecithin injection, we tested another group of animals as an internal control for full remyelination (n = 6 for all groups; see Supplementary Fig. 1b for the schematic experimental outline).