Journal of Neuroimmunology 264 (2013) 48–53

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

An electrophysiologic approach to quantify impaired synaptic transmission and plasticity in experimental autoimmune encephalomyelitis

Nora Prochnow [a], Ralf Gold [b], Aiden Haghikia [b,*]

[a] Department of Neuroanatomy and Molecular Brain Research, Ruhr-University Bochum, Germany
[b] Department of Neurology, Ruhr-University Bochum, Germany

ARTICLE INFO

Article history:
Received 15 June 2013
Received in revised form 8 September 2013
Accepted 12 September 2013

Keywords:
EAE
Cortical dysfunction
Synaptic transmission

ABSTRACT

Despite its various limitations, for many decades the experimental autoimmune encephalomyelitis (EAE) has been indispensable for understanding the pathology of multiple sclerosis (MS) and for establishing widely used MS therapeutics.

We tested whether synaptic plasticity is a suitable measure for EAE and whether it can detect detrimental effects on supra-spinal structures that are too subtle to be captured by the motor score. Our data show functional synaptic deficits in the EAE that were beyond the measurable EAE score: long-term depression responses were strongly weakened in superior colliculus and cerebellum resulting from impaired postsynaptic transmission. In addition to further insight into neuronal deficits associated with the autoimmune disease, quantification of synaptic transmission may serve as a complementary method of EAE evaluation.

1. Introduction

Efforts to investigate multiple sclerosis (MS), alike all disorders that primarily affect the central nervous system (CNS) are naturally hampered as the living brain and any dynamic process within the brain is unapproachable for direct assessment. Therefore, most of what is known on MS pathogenesis is derived either histomorphologically from biopsy and post-mortem tissue of MS patients, or experimentally from the animal model of MS, the experimental autoimmune encephalomyelitis (EAE). Without extensive histopathological studies many immunological (Lucchinetti et al., 2011) and neurodegenerative (Lassmann et al., 2012) aspects of MS would not have been discovered, and one of the most effective drugs in use for MS, natalizumab, is a prominent example for a so called bench-to-bedside, and its deployment would have not been possible without the EAE (Yednock et al., 1992).

However important these approaches have been and still are for understanding MS and drug development, both have their limitations; morphologic studies capture snapshots of complex dynamic processes and the EAE in many aspects only partially reflects human disease (Gold et al., 2006; Wekerle et al., 2012). Another limitation of the EAE is the low sensitivity of its clinical readout, the EAE score that solely allows the quantification of gross motor deficits caused by spinal cord inflammatory injury. Other deficits more subtle than paralysis originating from supra-spinal CNS regions, i.e. cortical and deep gray matter structures are hardly assessable by available EAE scores. These MS associated deficits include optic neuritis, but also higher cognitive functions such as memory and emotional processing. Interestingly, newer data indicate that these MS associated supra-spinal deficits do not necessarily result directly from inflammatory demyelinating lesions, but may as well occur early on in normal appearing brain regions, for instance as a consequence of disturbed neuronal connectivity or altered synaptic transmission (Passamonti et al., 2009; Deloire et al., 2011; Tomassini et al., 2012).

The rationale for our study was to evaluate i) whether in acute EAE – independent of demyelinating lesions – measurable disturbances of synaptic transmission and plasticity occur in supra-spinal CNS regions that are often affected in MS patients; and ii) to test the potential of synaptic plasticity as a more reproducible readout, in addition to the EAE motor score.

2. Materials and methods

2.1. EAE

Animal experiments were approved by responsible authorities in Northrhine-Westfalia, Germany. For EAE wildtype C57BL/6 mice were anesthetized and immunized subcutaneously with 100 μg myelin oligodendrocyte glycoprotein (MOG) 35–55 peptide in PBS, emulsified in an equal volume of complete Freund's adjuvant (CFA) containing mycobacterium tuberculosis H37RA (Difco, Detroit, MI, USA, 1 mg/ml). Pertussis toxin (List Biological Laboratories, Campbell, CA, USA; 200 ng

* Corresponding author at: Department of Neurology, Ruhr-University Bochum, Gudrunstr. 56, 44791 Bochum, Germany.
E-mail address: aiden.haghikia@ruhr-uni-bochum.de (A. Haghikia).

0165-5728/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jneuroim.2013.09.012

i.p.) was given on days 0 and 2. Animals were assessed daily in a blinded fashion using a 10 point scale score (EAE disease score) and sacrificed and assessed electrophysiologically as soon as paraplegia occurred according to EAE score 6.

2.2. Slice preparation and extracellular solutions

Mice were sacrificed by cervical dislocation and slices from respective brain structures used in our study were cut with a Leica VT1000 vibratome (Leica Microsystems, Wetzlar, Germany) in ice cold artificial cerebrospinal fluid (ACSF) containing in mM: 85 NaCl, 25 $NaHCO_3$, 2.5 KCl, 4 $MgSO_4$, 0.5 $CaCl_2$, 1.25 NaH_2PO_4, 25 glucose, and 7.5 sucrose and then incubated for at least 2 h prior to recording at room temperature for recovery. External solutions were continuously gassed with 95% O_2 and 5% CO_2 at room temperature. For intensified visualization of the tissue a Dodt gradient contrast filter (Luigs & Neumann, Ratingen, Germany) was used.

2.3. Electrode positions and input stimulus intensities

2.3.1. Hippocampus (350 μm horizontal slices)

Field excitatory postsynaptic potentials (fEPSPs) were recorded extracellularly by placing a metal recording microelectrode (impedances 0.5–0.8 M Ω) (TREC-SE, Multi Channel Systems, Reutlingen, Germany) into the stratum radiatum of the hippocampal CA1 region (Fig. 1A). A concentric SNEX1200 Wolfram electrode (Hugo Sachs Elektronik, Harvard Apparatus, March-Hugstetten, Germany) was placed into the Schaffer collateral fibers to stimulate inputs to hippocampal CA1 pyramidal neurons. fEPSP responses were driven by bipolar stimuli (50–600 μA) with a STG 1200 (Lohmann Research Equipment, Castrop-Rauxel, Germany). Before recording, input to output correlations were run to set the optimum stimulation intensity which was adjusted to 50% of the evoked maximal response amplitude. To elicit fEPSP response for long-term potentiation (LTP) recordings 5 Hz baseline stimulation was performed for 20 min in advance to the applied LTP protocol. LTP was evoked by using high frequency stimulation (HFS) with four trains of 10 shocks at 100 Hz every 1 s with doubled stimulus amplitude (Abraham and Huggett, 1997). Hippocampal LTD was elicited by application of low frequency stimulation (LFS) with 1 Hz for 15 min following 10 min of baseline recording. For paired pulse facilitation (PPF), two bipolar shocks of equal amplitude with an inter-stimulus-interval of 25 ms were applied to the afferent fibers (Clark et al., 1994).

2.3.2. Superior colliculus (350 μm coronal slices)

For afferent stimulation of the retinocollicular projection, a concentric electrode was placed into the optic tract over the lateral geniculate nucleus and nucleus of the optic tract. The metal recording microelectrode was placed onto the surface of the superficial gray layer of the superior colliculus (Fig. 1B). Stimulation and recording electrodes were kept at ≥2 mm apart from each other. Before recording, input to output correlations were run to set the optimum stimulation intensity which was adjusted to 75% of the evoked maximal response amplitude. Field potentials (FP) were measured before and after LTD induction via application of high intensity tetanic stimulation at doubled amplitude (~500 μA) and a frequency of 50 Hz for 20 s (Lo and Mize, 2002).

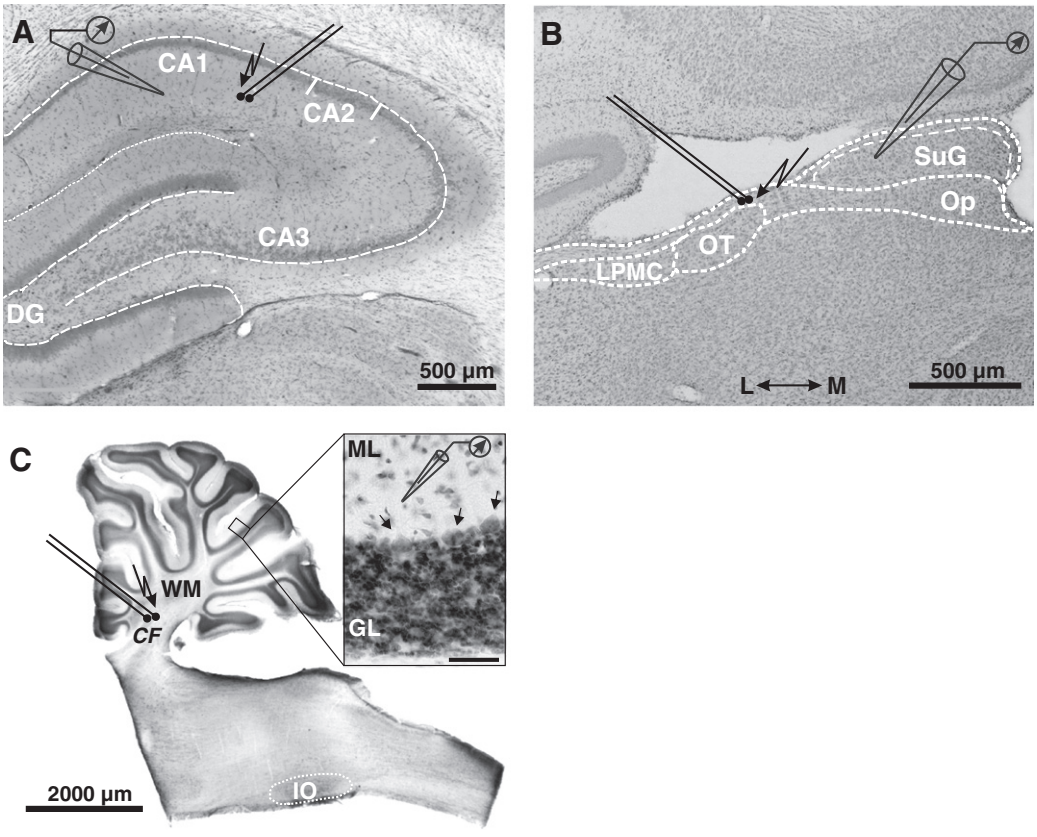

Fig. 1. A: Stimulus (thunderbolt arrow) and recording electrode (↗) positions for afferent stimulation of the Schaffer collateral–commissural pathway and for field excitatory postsynaptic potential response (fEPSP) recording in the hippocampal CA1 layer. Original electrode positions are demonstrated on a horizontal section of the hippocampus. B: Electrode positions for afferent stimulation of the optic tract for field potential (FP)/population spike recording in the superficial gray layer of the superior colliculus (SuG). Original positions are demonstrated on a coronal section (L = lateral, M = medial). LPMC = lateral posterior thalamic nucleus mediocaudal, OT = nucleus of the optic tract, Op = optic nerve layer. C: Electrode settings for afferent stimulation of the cerebellar climbing fibers (CF) for field potential (FP) recordings in the dendritic field of Purkinje-cells (indexed by arrows). Original electrode positions are demonstrated on a sagittal section. IO = inferior olive, WM = white matter, ML = molecular layer, GL = granular layer.