Neuroscience 346 (2017) 409–422

CHRONIC DEMYELINATION-INDUCED SEIZURES

ANDREW S. LAPATO, [a,c†] **JENNY I. SZU,** [b†] **JONATHAN P. C. HASSELMANN,** [a] **ANNA J. KHALAJ,** [a] **DEVIN K. BINDER** [a,b,c] **AND SEEMA K. TIWARI-WOODRUFF** [a,b,c*]

[a] *Division of Biomedical Sciences, School of Medicine, University of California Riverside, Riverside, CA 92521, USA*

[b] *Neuroscience Graduate Program, University of California Riverside, Riverside, CA 92521, USA*

[c] *Center for Glial-Neuronal Interactions, University of California Riverside, Riverside, CA 92521, USA*

Abstract—Multiple sclerosis (MS) patients are three to six times more likely to develop epilepsy compared to the rest of the population. Seizures are more common in patients with early onset or progressive forms of the disease and prognosticate rapid progression to disability and death. Gray matter atrophy, hippocampal lesions, interneuron loss, and elevated juxtacortical lesion burden have been identified in MS patients with seizures; however, translational studies aimed at elucidating the pathophysiological processes underlying MS epileptogenesis are limited. Here, we report that cuprizone-mediated chronically demyelinated (9–12 weeks) mice exhibit marked changes to dorsal hippocampal electroencephalography (EEG) and evidence of overt seizure activity. Immunohistochemical (IHC) analyses within the hippocampal CA1 region revealed extensive demyelination, loss of parvalbumin (PV^+) interneurons, widespread gliosis, and changes in aquaporin-4 (AQP4) expression. Our results suggest that chronically demyelinated mice are a valuable model with which we may begin to understand the mechanisms underlying demyelination-induced seizures.

Key words: cuprizone, multiple sclerosis, axon damage, parvalbumin, electroencephalography, hippocampus.

INTRODUCTION

Multiple sclerosis (MS) is an autoimmune demyelinating disorder of the central nervous system (CNS) that affects roughly 2.3 million people worldwide (Browne et al., 2014). In the United States, MS prevalence is estimated at 40–177 people per 100,000 (Evans et al., 2013) and varies with sex, ethnicity, and distance from the equator (Ropper et al., 2014). While MS clinical presentation is multifarious, epidemiological studies show that MS patients are three to six times more likely to develop epileptic seizures than the overall population, with incidence rising with MS duration (Engelsen and Grønning, 1997; Poser and Brinar, 2003; Lund et al., 2014; Marrie et al., 2015). Seizures in MS may signal disease onset or relapse in a subset of patients (Allen et al., 2013; Uribe-San-Martin et al., 2014) and are associated with diminished cognitive function (Calabrese et al., 2012), fulminant disease course, and accelerated time to disability (Martinez-Juarez et al., 2009; Nicholas et al., 2016). However, despite the increased occurrence of seizures among MS patients, little research exists probing their pathogenesis.

Electroencephalography (EEG) recordings from MS patients have revealed aberrant low-amplitude cortical alpha waves and the appearance of delta waves in awake patients (Babiloni et al., 2016), while MS with seizures (MS + S) patients also exhibit waking theta frequencies and paroxysmal discharges (Ghezzi et al., 1990). Magnetic resonance imaging (MRI) of MS + S patients demonstrates abundant temporal leukocortical lesions and hippocampal accumulation of demyelinating foci (Kutzelnigg et al., 2005; Martinez-Lapiscina et al., 2013; Cawley et al., 2015; Calabrese et al., 2016) in addition to findings common in MS, such as global cortical thinning and ventricular hypertrophy (Zivadinov et al., 2016).

Examining postmortem MS + S entorhinal cortex, Nicholas *et al.* reported augmented middle temporal gyrus thinning relative to other cortical regions and layer IV & VI GABAergic interneuron loss not explained by leukocyte infiltration or mitochondrial dysfunction (Nicholas et al., 2016). This is analogous to findings from epileptic hippocampi, which show loss of pyramidal neurons and GABAergic interneurons throughout the hippocampal formation (de Lanerolle et al., 2012; Huusko et al., 2015). Interestingly, MS + S cortical atrophy and neuron loss is localized to structures associated with mesial temporal lobe epilepsy (MTLE) (Calabrese et al., 2012; Nicholas et al., 2016), suggesting convergent pathology between MTLE and MS + S. However, while

*Correspondence to, S.K. Tiwari-Woodruff: Division of Biomedical Sciences, School of Medicine, University of California Riverside, Room 205, 311 School of Medicine Research Building, 900 University Ave, Riverside, CA 92521, USA.
E-mail addresses: seema.tiwari-woodruff@medsch.ucr.edu, seema-tiwari-woodruff@axonremyelination.org (S. K. Tiwari-Woodruff).
† ASL and JIS contributed equally to the preparation of this manuscript and should be considered first co-authors.

Abbreviations: AQP4, aquaporin-4; CC, corpus callosum; CD, cluster of differentiation; CNS, central nervous system; EEG, electroencephalography; GFAP, glial fibrillary acidic protein; IHC, immunohistochemistry; IL, interleukin; MRI, magnetic resonance imaging; MS, multiple sclerosis; MTLE, mesial temporal lobe epilepsy; PV^+, parvalbumin; ROI, region of interest; SO, stratum oriens; vEEG, video-EEG; YFP, yellow fluorescent protein.

http://dx.doi.org/10.1016/j.neuroscience.2017.01.035
0306-4522/

the development of seizures secondary to MS has been probed by a limited number of EEG, MRI, and post-mortem histological studies, little is known regarding its etiology or neuropathology. Furthermore, translational research examining this phenomenon and its origins is restricted to a single study (Hoffmann et al., 2008).

Although several well-established mouse models of MS are available (Merrill, 2009; McCarthy et al., 2012), the cuprizone (bis-cyclohexanone-oxalyldihydrazone; CPZ) diet model was utilized by this study to probe seizures secondary to demyelination. CPZ symptomology is highly reproducible and mirrors clinical features of progressive MS (Lucchinetti et al., 2000; Matsushima and Morell, 2006; Praet et al., 2014). CPZ neuropathology includes axon damage (Kim et al., 2010; Manrique-Hoyos et al., 2012), mitochondrial stress (Praet et al., 2014; Mahad et al., 2015), motor deficits primarily relegated to gait ataxias (Franco-Pons et al., 2007), and easily identifiable tonic–clonic seizures (Hoffmann et al., 2008; Praet et al., 2014). Early reports examining CPZ intoxication noted convulsions in Swiss mice fed $\geqslant$0.3% CPZ for $\geqslant$7 weeks, but did not investigate their pathogenesis (Kesterson and Carlton, 1970, 1983). To our knowledge, only one group has characterized seizures induced by the lower 0.2% concentration of CPZ currently used to model MS (Hoffmann et al., 2008). This group observed unusual spiking in cortical EEG recordings from chronically ($\geqslant$9 weeks) CPZ demyelinated C57BL/6 mice and hippocampal neuron degeneration, but did not identify vulnerable populations or address glial involvement.

To elucidate specific cellular involvement, our group examined CA1 electrophysiology and histopathology in mice subjected to chronic CPZ-induced demyelination. The hippocampal formation frequently exhibits profound changes in MS (Geurts et al., 2007) and MTLE, and has been implicated as a focus of seizure initiation (de Lanerolle et al., 2012) and maintenance (Ellender et al., 2014; Toyoda et al., 2015). These changes include loss of inhibitory interneuron populations, degeneration of CA1 principal neurons, and derangement of dentate gyrus projections into CA regions (de Lanerolle et al., 2012; Liu et al., 2014). For these reasons, we probed CA1 neuronal pathology, including parvalbumin $(PV)^+$ inhibitory interneurons due to this population's vulnerability in demyelination and seizure models (Schwaller et al., 2004; Rossi et al., 2012; Liu et al., 2014).

Glial changes were also assessed, since CPZ induces astrogliosis and phagocyte infiltration at intervals shorter than the chronic model utilized in the present study (Norkute et al., 2009; Skripuletz et al., 2013; Gudi et al., 2014; Praet et al., 2014; Clarner et al., 2015). In addition, both human postmortem tissue and animal models of seizure show reactive astrocytes, which exhibit increased expression of the intermediate filament glial fibrillary acidic protein (GFAP) expression and alterations to channel proteins, including the water channel aquaporin-4 (AQP4) and the inward rectifier potassium channel Kir4.1, both of which are central to epileptogenesis (Oberheim et al., 2008; Anderson and Rodriguez, 2011; Binder et al., 2012; de Lanerolle et al., 2012; Rodriguez-Cruces and Concha, 2015; Nwaobi et al., 2016).

In this study, we characterized EEG waveforms recorded from within the dorsal hippocampus of chronically demyelinated mice with seizures. Our findings indicate that theta and beta frequency power differs with duration of CPZ administration. Additionally, we describe CA1 neuronal and glial histopathology, including infiltration of microglia/macrophages, astrogliosis, changes to AQP4 expression with CPZ duration, pyramidal layer atrophy, and loss of PV^+ interneurons. These results lay a solid foundation for the study of seizure development secondary to demyelination and highlight the necessity for additional research into the pathogenesis of this phenomenon.

EXPERIMENTAL PROCEDURES

Animals

B6.Cg-Tg(Thy1-YFP)16Jrs/J mice backcrossed to wild-type C57BL/6 mice for more than five generations (hereafter referred to as Thy1-YFP mice) were obtained from the Jackson Laboratory (Bar Harbor, ME) and maintained at the University of California Riverside (UCR) animal facility. Originally described by Feng et al. (2000), Thy1-YFP mice express yellow fluorescent protein (YFP) under the control of modified mouse *Thy1* regulatory elements at high levels, resulting in YFP expression in axons, dendrites, and soma of sensory, motor, and some central neurons with no detectable expression in other cell types. Thy1-YFP mice are healthy and have no distinguishable phenotype aside from neuronal YFP expression. All procedures and experiments were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at UCR.

Cuprizone treatment

Eight-week-old male Thy1-YFP mice were assigned to groups that received standard chow (normal; n = 10) or 0.2% cuprizone (CPZ)-milled chow (Harlan Teklad, Madison, WI) as detailed in Crawford et al. (Crawford et al., 2009) for 9 or 12 weeks—hereafter referred to as 9-wk CPZ (n = 10) and 12-wk CPZ (n = 9), respectively. Normal mice received standard chow (Picolab, St. Louis, MO) following the same timeline. All groups had access to food and water *ad libidum* and were housed on a 12-h light/dark cycle under pathogen-free conditions at the UCR animal facility.

EEG probe preparation

A 3-channel twisted bipolar stainless steel electrode was used for all EEG experiments (Plastics One, Roanoke, VA). The bipolar wires were cut to 2 mm in length for intrahippocampal recordings. Similarly, an epidural ground electrode was cut to 0.5 mm and placed during stereotactic surgery (Paxinos and Franklin, 2004). Approximately 0.5 mm of the insulating coat was removed at the distal tip of the bipolar recording electrode using a scalpel to ensure high-fidelity EEG recordings. Implants were sterilized with 70% ethanol and the ground position