Neurobiology of Disease 64 (2014) 98–106

Contents lists available at ScienceDirect

Neurobiology of Disease

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

Impaired cognitive ability and anxiety-like behavior following acute seizures in the Theiler's virus model of temporal lobe epilepsy ☆

Anthony D. Umpierre [a], Gregory J. Remigio [a], E. Jill Dahle [b], Kate Bradford [b], Anitha B. Alex [c], Misty D. Smith [b,c], Peter J. West [a,b,c], H. Steve White [a,b,c], Karen S. Wilcox [a,b,c,*]

[a] Interdepartmental Program in Neuroscience, University of Utah, 1401 MREB, 20 North 1900 East, Salt Lake City, UT 84132
[b] Department of Pharmacology and Toxicology, University of Utah
[c] Anticonvulsant Drug Development Program, University of Utah, 417 Wakara Way, Suite 3211, Salt Lake City, UT 84108, USA

ARTICLE INFO

Article history:
Received 6 November 2013
Revised 13 December 2013
Accepted 31 December 2013
Available online 9 January 2014

Keywords:
Temporal lobe epilepsy
Cognitive impairment
Novel object place recognition
Viral encephalitis
Acute seizures

ABSTRACT

Viral infection of the CNS can result in encephalitis and acute seizures, increasing the risk for later-life epilepsy. We have previously characterized a novel animal model of temporal lobe epilepsy that recapitulates key sequela in the development of epilepsy following viral infection. C57BL/6 J mice inoculated with the Daniel's strain of Theiler's Murine Encephalomyelitis Virus (TMEV; 3×10^5 PFU, i.c.) display acute limbic seizures that secondarily generalize. A majority of acutely seized animals develop spontaneous seizures weeks to months later. As part of our investigation, we sought to assess behavioral comorbidity following TMEV inoculation. Anxiety, depression, cognitive impairment, and certain psychoses are diagnosed in persons with epilepsy at rates far more frequent than in the general population. We used a battery of behavioral tests to assess anxiety, depression, cognitive impairment, and general health in acutely seized animals inoculated with TMEV and compared behavioral outcomes against age-matched controls receiving a sham injection. We determined that TMEV-seized animals are less likely to move through the exposed center of an open field and are less likely to enter into the lighted half of a light/dark box; both behaviors may be indicative of anxiety-like behavior. TMEV-seized animals also display early and persistent reductions in novel object exploration during novel object place tasks and do not improve in their ability to find a hidden escape platform in Morris water maze testing, indicative of impairment in episodic and spatial memory, respectively. Cresyl violet staining at 35 and 250 days after injection reveals bilateral reductions in hippocampal area, with extensive sclerosis of CA1 evident bilaterally along the rostral–caudal axis. Early and persistent behavioral changes in the TMEV model provide surrogate markers for assessing disease progression as well as endpoints in screening for the efficacy of novel compounds to manage both seizure burden and comorbid conditions.

Introduction

While most epilepsy etiology is idiopathic in nature, a growing literature describes the prevalence of acquired epilepsy following viral infection. Viral infections of the CNS can result in encephalitis, which has the ability to provoke early acute seizures, increasing the risk for unprovoked, later-life seizures 22-fold (Michael and Solomon, 2012; Misra et al., 2008a). In the United States, encephalitis leads to as many new cases of acquired epilepsy as head trauma (Misra et al., 2008a).

Our group has previously characterized the first infection-based animal model of epilepsy, closely recapitulating temporal lobe epilepsy (TLE). Intracortical injection of Theiler's Murine Encephalomyelitis Virus (TMEV) into C57BL/6 J mice leads to acute encephalitic seizures from 3 to 10 days post injection (DPI) (Libbey et al., 2008; Stewart et al., 2010). TMEV antigens are present bilaterally in limbic and temporal areas during acute infection, including hippocampus (notably CA1 and CA2), periventricular thalamic nuclei, septal nuclei, and piriform, parietal, and entorhinal cortices (Stewart et al., 2009), but are virtually undetectable by 14 DPI (Kirkman et al., 2010; Libbey et al., 2011). During the acute, active infection period, neuronal death is observed preferentially among CA1 and CA2 neurons of the hippocampus (Stewart et al., 2009). After acute infection and viral clearance, a latent period of weeks to months precedes infrequent spontaneous seizures (approximately 2 seizures/animal/week) in a majority of animals seized during acute infection (Stewart et al., 2010). The TMEV model reflects periods of human epilepsy development following viral infection, and as such, this model may serve as a useful platform in the development and screening of disease modifying therapeutics administered during periods of acute infection.

TLE is the most common form of focal epilepsy and often the most refractory to currently available anti-seizure drugs; like many forms of

☆ Funding: NIH RO1 NS065434 (HSW and KSW) NIH T32 NS076067 (ADU and GJR) NIH HHSN 271201100029C (HSW)
* Corresponding author at: Department of Pharmacology and Toxicology, University of Utah, 417 Wakara Way, Suite 3211, Salt Lake City, UT 84108, USA.
E-mail address: karen.wilcox@hsc.utah.edu (K.S. Wilcox).
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nbd.2013.12.015

epilepsy, it often persists with comorbidity. Patients with epilepsy suffer from comorbid psychiatric conditions at rates considerably higher than the general population (Brooks-Kayal et al., 2013), with some of the strongest associations appearing in focal refractory patient populations (Adams et al., 2008; Dalmagro et al., 2012). A recent meta-analysis found that approximately 23% of people with epilepsy currently experience depression or have within the past year (Fiest et al., 2013). Often, depressive episodes in persons with epilepsy are associated with anxiety symptoms or an anxiety disorder (Kanner, 2012; Kanner, 2013), but depressive episodes may also manifest independently. Apart from anxiety and mood disorders, cognitive impairment is a particularly prevalent focus in TLE research and is often identified by patients with epilepsy as one of its most debilitating complications (Bell et al., 2011; Fisher, 2000; Murphy, 2013). Cellular and molecular insight is needed to better define relationships between behavioral changes and the neuropathology often observed in epilepsy. In the case of TLE, special emphasis has been placed on hippocampal pathology like sclerosis, mossy fiber sprouting, granule cell dispersion, and gray matter loss (Kandratavicius et al., 2013; Thom et al., 2012; Winston et al., 2013).

In the present investigation, and in keeping with the recent International League Against Epilepsy/American Epilepsy Society workshop on comorbidities (Brooks-Kayal et al., 2013), we have assessed the general health, depression-like and anxiety-like behaviors, cognitive impairment, and hippocampal pathology associated with the TMEV model of infection-induced TLE. We report that TMEV-infected animals that initially exhibit acute encephalitic seizures experience early deficits in motor coordination concomitant with significant weight loss. Certain measures of anxiety-like behavior appear elevated in TMEV animals, but do not co-occur with depression-like symptoms. Finally, early cognitive impairment can be detected in parallel with severe hippocampal sclerosis and hippocampal area reduction.

Methods

Animals

Four- to five-week-old male C57BL/6 J (B6) mice (14–20 g; Jackson Laboratory, Bar Harbor, ME, U.S.A.) were used in this study. Animals were group housed in a temperature- and light-controlled (12 h on/12 h off) environment and permitted access to food and water ad libitum throughout the study. All animal care and experimental manipulations were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the University of Utah Institutional Animal Care and Use Committee (IACUC).

TMEV infection and acute period monitoring

Under isoflurane anesthesia, mice were injected intracortically (i.c.) in the right posterior parietal cortex with either 20 μl of phosphate-buffered saline (PBS; sham injection; $N = 30$) or 20 μl of PBS containing 3×10^5 plaque forming units (PFUs) of the Daniel's strain of TMEV ($N = 45$) as previously described (Libbey et al., 2008; Stewart et al., 2009). Following i.c. injections, mice were monitored once daily from 3 to 8 DPI between the hours of 9 am and 11 am for handling-induced behavioral seizures and changes in body weight (Libbey et al., 2008). Behavioral seizures were scored according to the Racine scale (Racine, 1972), and any TMEV-injected B6 mouse that displayed at least one behavioral seizure during the observation periods was classified as a "TMEV-seized" animal. TMEV-injected mice that did not display a seizure ($N = 10/45$) during the limited observation periods were excluded from study.

Cohort selection and behavioral testing

Following the acute period, control ($N = 30$) and TMEV-seized ($N = 35$) animals were separated into three age-matched cohorts (PBS, $N = 10$; TMEV, $N = 10$ to 12) for use in behavioral tests. Most behavioral tests were conducted once within two epochs (10–60 DPI and > 60 DPI). With exception to the rotarod and novel object place recognition tasks, no cohort performed the same behavioral task twice to preserve novelty and mitigate stress. Animals were allowed 1 h to acclimate to the testing room on any day experiments were conducted. All tests were conducted during the light cycle. Arenas were cleaned between animals and phases (novel object place recognition) with a 4% Spartan HDQ-basic solution.

Tests of motor performance

Open field test (OFT)

Mice were tested for locomotive behaviors by singly placing an animal in a 40 L × 40 W × 30H cm open field under ambient room light for 30 min. Fusion software (OmniTech Electronics, Columbus, OH, USA) scored animals for aspects of horizontal and vertical movement as well as stereotypy.

Rotarod test

We tested mice for motor coordination by assessing their ability to maintain balance on a knurled rod (2.5 cm diameter) rotating at 6 rpm. Mice were manually timed for latency to fall off the rotarod during each of three successive 1 min trials separated by 15 s. Individual latencies were averaged over three trials and computed into group averages.

Tests of anxiety-like and depression-like behavior

Open field zones (OFZ)

Open field data was analyzed for duration and movement within the periphery of the apparatus (5 cm from the edge of all walls) or the center (30 L × 30 W cm) using Fusion software (OmniTech Electronics).

Light/dark box test (LD)

Employed as an ethological model of anxiety-like behavior, LD testing assessed innate aversion to a white, lighted region (Bourin and Hascoët, 2003). Fusion software (OmniTech Electronics) determined the percentage of time an animal spent in the lighted region of an open field (20 L × 40 W cm; 425 lux) or the dark, enclosed region (20 L × 40 W × 14H cm) separated by a small opening (10 L cm × 2.5 W cm) during a 10 m trial.

Saccharin preference test

Mice were singly housed in wire top cages and allowed to acclimate for 24 h. Volumes consumed of 1.6 mM saccharin (Reed et al., 2004) or tap water solutions were determined by the mass of solution remaining in separate bottles originally containing 200 g of solution (densities: 0.998 ± 0.003 g/ml tap water; 0.997 ± 0.002 g/ml 1.6 mM saccharin) after a 48 h period. Saccharin preference (%) was calculated as the volume of saccharin solution consumed divided by the total volume of fluid consumed per animal, multiplied by 100. Averages were obtained by group, and anhedonia was defined as a saccharin preference that did not significantly differ from 50% preference.

Tests of cognitive impairment

Novel object place recognition (NOPR)

Animals were habituated to the clear, plexiglass NOPR arena (40 L cm × 40 W cm × 60H cm) two consecutive days prior to testing for 15 min/d. Spatial cues of distinct shape remained 15 cm from each wall throughout habituation and experimental trials. Experimentation, conducted on day 3, encompassed three distinct phases. During phase 1, the familiarization phase, animals were exposed to two identical objects placed in two of three possible object locations over a 15 min span. Phase 2, the delay phase, involved sequestering the animal within a