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Modeling early-onset post-ischemic seizures in aging mice

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ABSTRACT

Stroke is the leading cause of seizures and epilepsy in the aged population, with post-stroke seizures being a poor prognostic factor. The pathological processes underlying post-stroke seizures are not well understood and studies of these seizures in aging/aged animals remain scarce. Therefore, our primary objective was to model post-stroke seizures in aging mice (C57 black strain, 16–20 months-old), with a focus on early-onset, convulsive seizures that occur within 24-hours of brain ischemia. We utilized a middle cerebral artery occlusion model and examined seizure activity and brain injury using combined behavioral and electroencephalographic monitoring and histological assessments. Aging mice exhibited vigorous convulsive seizures within hours of the middle cerebral artery occlusion. These seizures manifested with jumping, rapid running, barrel-rolling and/or falling all in the absence of hippocampal–cortical electrographic discharges. Seizure development was closely associated with severe brain injury and acute mortality. Anticonvulsive treatments after seizure occurrence offered temporary seizure control but failed to improve animal survival. A separate cohort of adult mice (6–8 months-old) exhibited analogous early-onset convulsive seizures following the middle cerebral artery occlusion but had better survival outcomes following anticonvulsive treatment. Collectively, our data suggest that early-onset convulsive seizures are a result of severe brain ischemia in aging animals.

1. Introduction

Stroke is the most common cause of seizures in the elderly population, with reported incidences ranging from 3–10% of all stroke cases (Bladin and Bornstein, 2009; Brodie et al., 2009; Menon and Shorvon, 2009; Chen et al., 2010; Balami et al., 2011; Gilad, 2012; Guekht and Bornstein, 2012; Procaccianti et al., 2012; Guth et al., 2014). Post-stroke seizures are associated with higher mortality rates, prolonged hospitalization, and increased incidences of long-term disability (Waterhouse et al., 1998; Vespa et al., 2003; Szaflarski et al., 2008; Burneo et al., 2010; Huang et al., 2014). These consequences contribute significantly to the socioeconomic burden of stroke, already the greatest amongst all disease in North America, in addition to the massive personal burden felt by patients and their families (Smurawska et al., 1994; Zorowitz et al., 2009; Publication Health Agency of Canada., 2011; Mozaffarian et al., 2015). Specific guidelines for the definitive treatment of post-stroke seizures have yet to be established but may require a deeper understanding of seizure pathogenesis (Gilad, 2012; Guekht and Bornstein, 2012; Procaccianti et al., 2012; Kulhari et al., 2014; Sykes et al., 2014).

Early-onset seizures are largely observed within 24 h and are a medical emergency as life-threatening status epilepticus may follow (Waterhouse et al., 1998; Waterhouse and DeLorenzo, 2001). Early-onset seizures can manifest as generalized tonic–clonic convulsions, or non-convulsive seizures which require EEG monitoring for diagnosis (Silverman et al., 2002; Jordan, 2004; Claassen et al., 2007; Chung, 2014). Stroke severity and the degree of cortical involvement have been recognized as risk factors for early-onset seizure development following ischemic stroke (Bladin and Bornstein, 2009; Brodie et al., 2009; Menon and Shorvon, 2009; Balami et al., 2011; Chen et al., 2010; Gilad, 2012; Guekht and Bornstein, 2012; Procaccianti et al., 2012; Chung, 2014). However, the regional initiation and progression of early-onset seizures are often difficult to assess clinically in patients with severe brain ischemia. Therefore, it may be of more practical value to study these phenomena in a viable animal model.

Previous studies have characterized early-onset, non-convulsive seizures (NCS) in adult rats following a middle cerebral artery occlusion (MCAO; Hartings et al., 2003; Williams et al., 2004, 2006; Karhunen et al., 2006; Lu et al., 2009; Cuomo et al., 2013). These NCS were

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generally observed a few hours following the MCAO with matching cortical EEG discharges. A link between development of these NCS and brain injury was established as anticonvulsant treatment reduced both ischemic injury and acute mortality (Williams et al., 2004, 2006; Cuomo et al., 2013). Other studies investigated early-onset, convulsive seizures (CS) in adult animals following brain ischemia (Reglodi et al., 2000; Wang et al., 2001; Shabanzadeh et al., 2005; El-Hayek et al., 2011a). However, inconsistencies in the EEG discharges corresponding to these CS demonstrate a need for further examination. These prior studies also utilized adult animals exclusively, overlooking aging as the most important non-modifiable risk factor for stroke and its link to greater brain damage and poor outcomes (Mozaffarian et al., 2015). While late-onset seizures have been documented in aged rats following MCAO and photothrombotic ischemia (Kelly, 2006; Karhunen et al., 2007; Kelly et al., 2001, 2011), very little information remains available on the behavioral and EEG characteristics of early-onset, post-ischemic seizures in aging/aged animals. Therefore, for our study, we aimed to develop a viable model of early-onset, post-MCAO seizures (CS, NCS) in aging mice in order to characterize the prognostic role of these seizures and the pathophysiological processes underlying their development.

2. Material and methods

2.1. Animals

Male C57 black mice (C57BL/6; Charles River, Senneville St-Constant, Quebec, Canada) were used. C57 mice that are ≥24-months-old may correspond to a human age of approximately ≥70 years (Flurkey et al., 2007). However, aged C57 mice often encounter many health-related complications such as skin lesions, ear infections and tumors (Flurkey et al., 2007). Therefore we chose to conduct our MCAO experiments in 16–20 month-old C57 mice in order to minimize confounding health complications while effectively modeling brain ischemia in aging animals. Overall, 89 aging mice were used: 67 animals underwent a MCAO, 15 underwent a sham surgery or occlusion of the common carotid artery alone (n = 7 or 8), and 7 died during the course of surgery/anesthesia due to either respiratory suppression or bleeding. A separate cohort of adult C57 black mice (male, 6–8 months-old, n = 38) were used to compare the effects of age on early-onset seizures.

The animals were housed in a vivarium that was maintained at 22–23 °C with a 12-hour light on/off cycle. Food and water were available ad libitum. All experiments detailed were reviewed and approved by the animal care committee of the University Health Network in accordance with the Canadian Guidelines for Animal Care. In line with the guidelines, animals with severe CS were treated with clinically appropriate anticonvulsants. Mandatory euthanization was conducted if animals exhibited severe, recurrent CS inadequately suppressed by anticonvulsant treatments and/or presented in poor physical condition such as with persistent immobility, lack of eating and drinking, irresponsiveness to touch, loss of the righting reflex, and/or a substantial reduction in body weight (≥20% of baseline level). In our study, acute mortality was defined as spontaneous death or mandatory euthanization within 48 h post-ischemia.

2.2. Intracranial EEG recordings

Electrode construction, implantation and EEG recordings were conducted as previously described (Wu et al., 2008; Wais et al., 2009; El-Hayek et al., 2011a,b; Jeffrey et al., 2014). All electrodes were constructed using polyamide-insulated stainless steel wires (outside diameter of 200 μm for monopolar electrodes and 125 μm for twisted bipolar electrodes; Plastics One, Ranoake, VA, USA). Monopolar electrodes were pre-assembled in an array before implantation (Wu et al., 2008). The tips of the twisted bipolar wires were separated by approximately 100 μm (Jeffrey et al., 2014). EEG recordings were made using a dual-channel AC microelectrode amplifier with extended headstages (model 1800, AM Systems, Carlsborg, WA, USA). Signals were collected in a frequency bandwidth of 0.1–1000 Hz, amplified 1000 times and then digitized at ≥5 KHz (Digidata 1300, Molecular Devices; Sunnyvale, CA, USA). Data acquisition, storage, and analysis were conducted using pClamp software (version 9 or 10; Molecular Devices).

Monopolar recordings were conducted in the majority of animals since electrode implantation involved a shorter surgery with minimal perioperative complications, a particularly important consideration in more susceptible aging animals (Wu et al., 2008). EEG activity recorded with monopolar electrodes represented the signal difference between the recording and reference electrodes. Therefore, this method was more sensitive to movement artifacts and other remote signals. Twisted bipolar electrodes were used for local differential recordings in some animals but the relatively long surgery for implantation was associated with greater perioperative complications including mortality. EEG activity in these local differential recordings represented the signal difference between the tips of the two twisted electrodes. Therefore, this method was less susceptible to other artifacts or remote signals, and was preferable for sampling the local circuitry activity.

For monopolar recordings, electrodes were implanted bilaterally into the hippocampal CA1 (bregma −2.3 mm, lateral 2.0 mm and depth 2.0 mm) and parietal cortex (bregma −0.6 mm, lateral 1.5 mm and depth 1 mm; Franklin and Paxinos, 1997) or unilaterally into the hippocampal CA3 (bregma: −2.6 mm, lateral 2.5 mm and depth 3.0 mm) and parietal cortex. For local differential recordings, twisted bipolar electrodes were implanted bilaterally into the hippocampal CA3 or into CA3 and the parietal cortex. The locations of the implanted electrodes were verified histologically (Fig. 3C, Supplemental Fig. 1).

Most EEG recordings were performed in free-moving animals. During some EEG recordings, animals were placed in a mouse restrainer (Type C or D, Canadawide Scientific, Ottawa, Canada) to limit the amount of CS-related movement artifacts. Although the restrainers inhibited vigorous convulsive behavior such as jumping and rapid running, other characteristic seizure activity such as barrel-rolling, rapid limb movements, and tail erections remained observable and analogous to those seen in their unrestrained counterparts.

To quantify EEG changes over time, we used the root mean square (RMS) of the EEG signals as this has been shown to be a sensitive measure of ischemic EEG suppression in adult C57 black mice following hypoxia–ischemia (El-Hayek et al., 2011b). Others have used EEG RMS to assess brain activities in a rat model of post hemorrhagic seizures (Klahr et al., 2015). RMS calculations were made from 30-sec EEG segments collected while animals were immobile as these data segments were minimally contaminated by movement-related artifacts. Power spectra were then generated with 50% window overlap at a spectral resolution of 0.3 Hz. The RMS of the EEG power spectrum was automatically calculated in pClamp.

2.3. Middle cerebral artery occlusion (MCAO)

Animals were anesthetized with 2% isoflurane. During surgery, animal rectal temperatures were maintained between 35.5–36.5 °C via an automatic heating device (DC temperature controller, FHC Inc., Bowdoin, ME, USA). Permanent and reversible MCAO, referred to as pMCAO and rMCAO in the following text, were conducted via intra-luminal suture insertion (Durukan and Tatlisumak, 2007; Hossmann, 2008; Howells et al., 2010; Liu and McCullough, 2011). Each surgery lasted 20–40 min. The protocol for electrocoagulation of the MCA (MCAO-e) was modified from previous studies in adult mice (Liu et al., 2005; Cipriani et al., 2011; Moyanova et al., 2011; Wang et al., 2011).

For the pMCAO, a silicon-coated fine suture (#6, Doccol Corporation, Redlands, CA, USA) was inserted into the common carotid artery and advanced through the internal carotid artery with its tip 7–8 mm distal to the carotid bifurcation (the Koizumi's method; see Durukan and Tatlisumak, 2007). The common carotid artery and the inserted suture were then permanently ligated.