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Susceptibility to hippocampal kindling seizures is increased in aging C57 black mice

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ABSTRACT

The incidence of seizures increases with old age. Stroke, dementia and brain tumors are recognized risk factors for new-onset seizures in the aging populations and the incidence of these conditions also increased with age. Whether aging is associated with higher seizure susceptibility in the absence of the above pathologies remains unclear. We used classic kindling to explore this issue as the kindling model is highly reproducible and allows close monitoring of electrographic and motor seizure activities in individual animals. We kindled male young and aging mice (C57BL/6 strain, 2–3 and 18–22 months of age) via daily hippocampal CA3 stimulation and monitored seizure activity via video and electroencephalographic recordings. The aging mice needed fewer stimuli to evoke stage-5 motor seizures and exhibited longer hippocampal afterdischarges and more frequent hippocampal spikes relative to the young mice, but afterdischarge thresholds and cumulative afterdischarge durations to stage 5 motor seizures were not different between the two age groups. While hippocampal injury and structural alterations at cellular and micro-circuitry levels remain to be examined in the kindled mice, our present observations suggest that susceptibility to hippocampal CA3 kindling seizures is increased with aging in male C57 black mice.

Introduction

Old age is associated with high incidence of seizures and epilepsy, and temporal lobe epilepsy is the most common type of seizure disorder in aging/aged populations. While stroke, dementia and brain tumors are recognized risk factors, the etiology is unknown for many aging/aged individuals with new-onset epilepsy (Hauser, 1992; Jetter and Cavazos, 2008; Brodie et al., 2009; Ferlazzo et al., 2016). This raises an intriguing question as to whether intrinsic processes within the aging brain promote seizures/epilepsy susceptibility (Baram, 2012).

Experimental investigation using animal models may help explore this issue. However, studies that compare seizure susceptibility between young and aging/aged animals remain scarce (de Toledo-Morrell et al., 1984; Fanelli and McNamara, 1986; Leppik et al., 2006; Kelly 2010; Hattiangady et al., 2011).

The hippocampus is known to undergo structural and functional alterations during aging (Burke and Barnes, 2010; Bartsch and Wulff, 2015). These include a loss of subgroups of hippocampal GABAergic interneurons (Shetty and Turner, 1998; Cadacio et al., 2003; Vela et al., 2003; Shi et al., 2004; Stanley and Shetty, 2004; Potier et al., 2006; Kuruba et al., 2011; Smith et al., 2000; Stanley et al., 2012; Spiegel et al., 2013) and an increase in hyperactive or hyperexcitable responses of hippocampal CA3 neurons in aging/aged animals (Vreugdenhil and Toescu, 2005; Wilson et al., 2005; Patrylo et al., 2007; Kanak et al., 2011; Lu et al., 2011; El-Hayek et al., 2013; Spiegel et al., 2013; Moradi-Chameh et al., 2014; Simkin et al., 2015; Villanueva-Castillo et al., 2017). In light of these findings and classic kindling as a widely used model of temporal lobe epilepsy (see reviews by Morimoto et al., 2004; Bertram, 2007;

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Sharma et al., 2007; Coppola and Moshé, 2012; Gilby and O'Brien, 2013; Chauvette et al., 2016; Gorter et al., 2016; Löscher, 2017), we explored whether susceptibility to hippocampal CA3 kindling seizures is different between young and aging C57 black mice.

Experimental procedures

Animals

Male C57 black mice (C57BL/6N) were obtained from Charles River Laboratory (Senneville, Quebec, Canada). Experiments were carried out on mice of 2–3 and 18-22 months of age. C57 black mice have a maximum lifespan up to 36–39 months (Flurkey et al., 2007; Harrison et al., 2009) but mice of ≥24 months of age often encounter health-related complications including skin lesions, ear infections, and tumors (Flurkey et al., 2007). Therefore we chose to conduct our experiments in mice of 18-22 months of age in an attempt to model epileptogenic processes in aging while minimizing the health-related complications that are common in aged mice. We refer to mice of 2–3 and 18–22 months of age as "young" and "aging" respectively for simplicity.

All mice were housed in a vivarium that was maintained between 22–23 °C with a 12-h light on/off cycle (light-on stating at 6:00 am). Food and water were available ad libitum. Hippocampal electrical stimulations and EEG/video recordings were conducted between 10 am and 5 pm. All experimental procedures described below were reviewed and approved by the Animal Care Committee of the University Health Network, in accordance with the Guidelines of the Canadian Council on Animal Care.

Electrode implantation

Surgeries were similarly performed as previously described (Wu et al., 2008; Jeffrey et al., 2014). The animal was anaesthetized with isofluorane and placed in a stereotaxic frame. After a skin incision to expose the skull surface, three small holes (~0.5 mm in diameter) were drilled through the skull. Electrodes were inserted into the brain using micromanipulators and then glued onto the skull (Wu et al., 2008). Twisted bipolar electrodes (tips of ~100 μm apart) were placed into bilateral hippocampal CA3 areas (bregma: −2.5 mm, lateral 3.0 mm and depth 2.5 mm; Franklin and Paxinos, 1997). A reference electrode was positioned at a frontal area (bregma +1.0 mm, lateral 1.0 mm and depth 0.5 mm). All electrodes were made of polyamide-insulated stainless steel wires (outer diameter 200 μm; Plastics One, Ranoake, VA, USA). Implanted animals were allowed to recover for ≥1 week prior to further experimentation. The locations of implanted electrodes were verified by behavioral state-dependent hippocampal EEG activities and/or by later histological assessments.

Hippocampal CA3 kindling

The animal was placed in a large bowl-shaped glass container for video/EEG monitoring. Unilateral CA3 kindling was conducted using a standard protocol (Albright and Burnham, 1980; Reddy and Rogawski, 2010; Jeffrey et al., 2014). Constant current pulses with monophasic square waveforms, pulse duration of 0.5 ms and current intensities of 10–100 μA were generated by a Grass stimulator and delivered through an isolation unit (model S88, Grass Medical Instruments, Warwick RI, USA). Initially, an ascending series was performed to determine afterdischarges (AD) threshold for individual animals. In the ascending series, a train of current pulses (60 Hz for 2 s) with incremental intensities (10 μA per step) were applied every 30 minutes. The lowest stimulation intensity by which an AD event of ≥5 s was elicited was considered the AD threshold. The animal was then stimulated at 125% of the AD threshold daily for several weeks. The animal was considered kindled when stage 5 motor seizures (see below) were elicited over three consecutive days (Fanelli and McNamara, 1986; Reddy et al., 2010; Jeffrey et al., 2014). An ascending series was similarly conducted in each kindled animal.

EEG recordings and data analysis

Local differential recordings via twisted bipolar electrodes were used in most of experiments as this recording mode detects signal differences between adjacent electrode tips hence being more effective than mono-polar recordings in sampling local signals and reducing artifacts (Jeffrey et al., 2014; Wu et al., 2015). Monopolar recordings that detect signal differences between recording and reference electrodes were used if local differential recordings were unsuccessful. EEG signals were collected using a two-channel microelectrode AC amplifier (model 1800, A-M systems, Carlsborg, WA, USA). The input frequency band of the amplifier was set in the range of 0.1–1000 Hz and amplification gain at 1000x. The output signals of the amplifier were digitized at 5000 Hz (Digidata 1440A, Molecular Devices; Sunnyvale, CA, USA). Data acquisition, storage and analyses were done using PClamp software (Version 10; Molecular Devices). In some experiments, a single-channel microelectrode AC amplifier (model 3000, A-M systems) was used to capture ipsilateral (in reference to the unilateral kindling site) AD and TTL-gated switches were used to switch between recording and stimulating modes. When the input frequency band of this amplifier was set in the range of 10–1000 Hz, switching artifacts were usually ≤3 s which masked the early component of evoked AD.

Evoked AD events were recognized as repetitive single spike and poly-spike events with large amplitudes and durations of ≥5 s. AD durations were determined from the end of kindling stimulation to the time point at which AD signals were less than 4 times of standard deviation of pre-stimulation background signals. If needed, original signals were treated with a band filter (1–500 Hz, Bessel) to diminish slow drifts and artifact contaminations prior to the measurements. Contralateral (in reference to the unilateral kindling site) AD events were measured for all animals, and ipsilateral AD were captured and measured from some animals. The contralateral AD durations that were required to reach the first stage 2–5 motor seizure in individual animals were measured and summed, and data were presented as cumulative AD durations to seizure stage (Peterson et al., 1981; Löscher et al., 1998).

Spontaneous or non-evoked hippocampal EEG signals were recorded from individual animals during baseline monitoring and after observation of five consecutive stage 4–5 motor seizures. Large irregular activities that occurred during immobile/sleep behaviors (Buzsáki et al., 2003) and type-2 theta rhythm that corresponded to periods of wake immobility (Sainsbury, 1998) were analyzed. Spectral analysis was used to determine the main frequencies of these activities. Spectral plots (rectangular function, 50% window overlap and spectral resolution 0.3 Hz, PClamp software) were generated from 60-s or 15-s data segments that encompassed the large irregular activities and type-2 theta rhythm. Three spectral plots were averaged for baseline or post-kindling measures in individual animals.

Incidences of aberrant hippocampal EEG spikes were measured from accumulative 20–80 min EEG segments corresponding to immobility/sleep behaviors but excluding the periods with the type-2 theta rhythm (Leung, 1990; Lang et al., 2014). These spikes were recognized with large peak amplitudes (≥8 times of standard deviation of background signals), simple/complex waveforms and durations of 30–150 ms. The event detection function (threshold search method) of PClamp software was used to automatically detect spikes, and detected events were then visually inspected and false events were rejected (El-Hayek et al, 2013; Lang et al.,