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Hippocampal excitability is increased in aged mice

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ABSTRACT

Aging is known to be associated with a high risk of developing seizure disorders. Currently, the mechanisms underlying this increased seizure susceptibility are not fully understood. Several previous studies have shown a loss of subgroups of GABAergic inhibitory interneurons in the hippocampus of aged rodents, yet the network excitability intrinsic to the aged hippocampus remains to be elucidated. The aim of this study is to examine age-dependent changes of hippocampal network activities in young adult (3–5 months), aging (16–18 months), and aged (24–28 months) mice. We conducted intracranial electroencephalographic (EEG) recordings in free-moving animals and extracellular recordings in hippocampal slices in vitro. EEG recordings revealed frequent spikes in aging and aged mice but only occasionally in young adults. These EEG spikes were suppressed following diazepam administration. Spontaneous field potentials with large amplitudes were frequently observed in hippocampal slices of aged mice but rarely in slices from young adults. These spontaneous field potentials originated from the CA3 area and their generation was dependent upon the excitatory glutamatergic activity. We therefore postulate that hippocampal network excitability is increased in aged mice and that such hyperactivity may be relevant to the increased seizure susceptibility observed in aged subjects.

Introduction

Aging is known to be associated with structural and functional alterations in the rodent hippocampus (Burke and Barnes, 2006; Wilson et al., 2006), particularly a loss of subgroups of GABAergic inhibitory interneurons (Cadacio et al., 2003; Kuruba et al., 2011; Potier et al., 2006; Shetty and Turner, 1998; Shi et al., 2004; Stanley and Shetty, 2004; Stanley et al., 2012; Vela et al., 2003). Reduced inhibition as a consequence of hippocampal inhibitory interneuron loss is predicted to increase neuronal excitability. Several studies have highlighted aging-related changes of hippocampal network activities. For example, Wilson et al. (2005) compared the spatial firing characteristics of hippocampal place cells (a group of pyramidal neurons) in young and aged memory-impaired rats. Their data show that aged CA3 place cells have higher firing rates but less plasticity changes compared to young CA3 place cells. Others (Vreugdenhil and Toescu, 2005; Lu et al., 2011) have examined CA3 gamma oscillations (20–80 Hz) in mouse hippocampal slices. These studies show that induced gamma oscillations are decreased in aged relative to young mice, and that the decreased gamma oscillations are associated with reduced activity in CA3 inhibitory interneurons. Additionally, Patrylo et al. (2007) examined stimulation-induced field potentials in rat hippocampal slices. Their data show that a 5 Hz stimulation of the dentate gyrus (DG) can induce repetitive bursting potentials in the CA3 area of aged rats but not young rats. The induction of these bursting potentials is thought to involve attenuated GABAergic inhibition in the aged DG–CA3 circuitry.

Collectively, the above studies suggest that aged hippocampal neurons may be hyperexcitable; however, population activities intrinsic to the aged hippocampal circuitry remain to be thoroughly examined. Therefore this study attempted to examine hippocampal network activity in aged mice. Specifically, we conducted intracranial electroencephalographic (EEG) recordings in young adult, aging and aged mice and screened for evidence of hyperexcitable EEG events. In addition, we carried out extracellular recordings in hippocampal slices to explore age-dependent changes in the excitability of the isolated hippocampal circuitry.

Materials and methods

Animals

C57 black mice (C57BL/6N, Charles River Laboratory, Quebec, Canada) aged 3–5 months, 16–18 months, and 24–28 months were used. We refer to these animals as “young”, “aging” and “aged” as their ages are thought to correlate to human ages of 20–30, 51–56, and 69–73 years, respectively (Flurkey et al., 2007). Animals were housed in a vivarium maintained at 22–23 °C with a 12-h light on/off cycle. Food and water were available ad libitum. All experimental procedures were reviewed

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and approved by local Animal Care Committees in accordance with the guidelines established by the Canadian Council on Animal Care.

Intracranial EEG recordings in free-moving mice

Surgical implantation of EEG electrodes, EEG recordings and data analysis were done as we previously described (El-Hayek et al., 2011a, 2011b; Wais et al., 2009; Wu et al., 2008). Mice were anesthetized under isoflurane anesthesia (2%) and secured in a stereotaxic frame. A skin incision was made, and three small holes drilled through the skull. A pre-constructed electrode assembly was inserted into the brain and secured in place with a cyanoacrylate-based glue (Insta-cure+, BSI Adhesives, Atascadero, CA, USA). The electrodes were made with polyimide-insulated stainless steel wires (outside diameter 0.125 mm, Plastics One, Roanoke, VA, USA), and implanted into the right hippocampal CA1 area (bregma −2.3 mm, lateral 2.0 mm and depth 2.0 mm; Franklin and Paxinos, 1997) and the left parietal cortex (bregma −0.6 mm, lateral 1.5 mm and depth 1 mm). A reference electrode was placed into the right frontal lobe area (bregma +1.2 mm, lateral 2.0 mm, depth 0.5 mm). The locations of implanted electrodes in the desired hippocampal and/or cortical areas were verified by region-specific and behavioral state-dependent EEG signals and/or by later histological assessments in cresyl violet-stained brain sections (30–50 μm thickness, n = 45 mice; Fig. 3C) (El-Hayek et al., 2011a; Wais et al., 2009; Wu et al., 2008).

Extracellular amplifiers with extended head-stages (Model-300 or Model 1800, AM Systems Inc., Carlsborg, WA, USA) were used. EEG signals were recorded in a frequency band of 0.05–5000 Hz and amplified 1000 times before digitization (digitization rates of 60 kHz, Digidata, 1300, Molecular Devices, Unit City, CA, USA). Data acquisition, storage and analyses were done using pCLAMP software (version 10, Molecular Devices).

Individual animals were recorded after ≥7 days of recovery from surgery/electrode implantation. All EEG recordings were performed in daylight or light-on periods (10 am to 5 pm) while animals stayed in their home cage or a large glass container. Because EEG spikes were observed primarily while animals exhibited immobility and/or sleep, spike quantification was carried out on EEG segments collected during these behavioral states. Two quantification approaches were utilized. Firstly, an experimenter blind to animal age was instructed to identify intermittent, large-amplitude EEG spikes (≥2 times the amplitude of background EEG signals). Secondly, EEG spikes were detected utilizing the event detection function (threshold search method) of the pClamp software, a similar approach as described by Antonucci et al. (2012). Events exhibiting amplitudes greater than 6 standard deviations (SD) above background EEG signals were automatically detected. The detected spikes were then visually inspected and false events were rejected. Spike detection was performed on EEG segments that were continuously recorded for ≥10 min. The total lengths of EEG segments analyzed per animal were 66.2 ± 8.4 min, 56.7 ± 8.2 min and 66.2 ± 11.9 min for young, aging and aged mice (n = 24, 35 and 22). The SD values of background hippocampal EEG signals, calculated from 5-min segments in individual animals, were 0.20 ± 0.03 mV, 0.15 ± 0.01 mV and 0.16 ± 0.01 mV for young, aging and aged mice. There were no significant group differences in the EEG segment lengths or the SD values analyzed (P = 0.410 or P = 0.12, Kruskal–Wallis One Way Analysis of Variance on ranks).

In addition to EEG spikes, we also measured the incidences and amplitudes of hippocampal EEG sharp waves (SPWs; Buzsáki, 1986; Buzsáki et al, 2003; Chrobak et al, 2000; Leung and Shen, 2004) using the event detection approach. Compared to EEG spikes, the SPWs displayed lower amplitudes and more variable waveforms due to the influence of background signals. Thus, event detection was carried out in relatively short EEG segments with quantifiable SPWs (3–5 min per animal). The detected SPWs were then visually inspected. Only the SPWs with mono-phasic waveforms and amplitudes 30–50% above background signals were selected. Because such SPW detection was limited, we only examined the SPWs in spike-exhibiting aging/aged mice in an attempt to compare these two types of hippocampal EEG events in same animals.

Extracellular recordings in hippocampal slices

The procedures for preparing hippocampal slices were modified from those used in our previous studies (Wais et al., 2009; Wu et al., 2005b, 2009; Zhang et al., 2008). Briefly, we anesthetized the animal with an intra-peritoneal injection of sodium pentobarbital (70 mg/kg, Somnotol, WTC Pharmaceuticals, Cambridge, Ontario, Canada), and conducted a trans-cardiac infusion with cold (4 °C), low-Na^+/Ca^{2+} artificial cerebrospinal fluid (ACSF) before decapitation (Moyer and Brown, 1998). The brain was quickly dissected out, hemi-sectioned, and maintained in ice-cold, oxygenated low-Na^+/Ca^{2+} ACSF for 3–4 min before further dissection. We then removed brainstem tissue to extend the curved hippocampus, and glued the remaining brain tissue onto a block with the longitudinal axis of the hippocampus perpendicular to the horizontal cutting plane. Transverse hippocampal slices of 0.5 mm thickness were obtained via a Leica vibratome (VT1200) in ice-cold, low-Na^+/Ca^{2+} ACSF. After sectioning, the slices were stabilized in standard ACSF at 35 °C for 30 min (Bischofberger et al., 2006). During the stabilizing period, 2 mM kynurenic acid was included in the ACSF to reduce the activities of the ionotropic glutamate receptors (Stone, 2001). After the 30-min stabilization, slices were rinsed twice and then maintained in standard ACSF at 22 °C for at 1–6 h before recording. The low-Na^+/Ca^{2+} ACSF contained (in mM) the following: NaCl 50, choline chloride 80, KCl 3.5, NaH_2PO_4 2, $CaCl_2$ 0.5, $MgCl_2$ 7, glucose 20 and HEPES 5 (pH adjusted to 7.4). The standard ACSF contained (in mM) the following: NaCl 125, KCl 3.5, NaH_2PO_4 1.25, $NaHCO_3$ 25, $CaCl_2$ 2, $MgSO_4$ 1.3 and glucose 10 (pH of 7.4 when aerated with 95%O_2–5%CO_2).

Extracellular recordings were conducted in a submerged chamber at 35 °C. Under our recording conditions, the ACSF was perfused at a high rate (15 ml/min), with both the top and bottom surfaces of the slice exposed to the perfusate (Wu et al., 2002, 2005a, 2005b). In addition, humidified gas of 95%O_2–5%CO_2 was allowed to pass over the perfusate to increase local oxygen tension. We (Wu et al., 2002, 2005a, 2005b; Zhang et al., 2008) and others (Hájos and Mody, 2009; Hájos et al., 2009) have shown that a fast, top and bottom perfusion of the slice is important for maintaining in vitro spontaneous population activities under submerged conditions.

Extracellular electrodes were made with thin-wall glass tubes (1.5 mm outside diameter, World Precision Instruments, Sarasota, Florida, USA) and filled with a solution containing 150 mM NaCl and 2 mM HEPES (pH 7.4). The resistance of these electrodes was 1–2 MΩ. Signals were recorded using a dual channel amplifier (700A or 700B, Molecular Devices). Data acquisition, storage and analysis were done using the PCLAMP package.

A bipolar electrode, made of polyimide-insulated stainless steel wires (outside diameter 0.125 mm, Plastics One, Roanoka, VA, USA), was used for afferent stimulation. Constant current pulses (0.1 ms duration, 20–150 μA) were generated by a Grass stimulator (S88) and delivered through an isolation unit every 30 s. To examine the DG population spikes and the CA3 field EPSPs, we stimulated the DG molecular layer at the maximal intensity (current pulses of 150 μA) and recorded simultaneously from the DG somatic and the CA3 apical dendritic (stratum radiatum) layers. To examine the CA3 population spikes and the CA1 field EPSPs, we stimulated the CA3 stratum oriens area and recorded from the CA3 somatic and the CA1 apical dendritic layers. In some experiments, the later recording was made from the CA1 somatic layer or the subicular area to monitor population spikes.