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Effects of age and cortical infarction on EEG dynamic changes associated with spike wave discharges in F344 rats

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ABSTRACT

Rodent models of absence seizures are used to investigate the network properties and regulatory mechanisms of the seizure's generalized spike and wave discharge (SWD). As rats age, SWDs occur more frequently, suggesting aging-related changes in the regulation of the corticothalamic mechanisms generating the SWD. We hypothesized that brain resetting mechanisms – how the brain "resets" itself to a more normal functional state following a transient period of abnormal function, e.g., a SWD – are impaired in aged animals and that brain infarction would further affect these resetting mechanisms. The main objective of this study was to determine the effects of aging, infarction, and their potential interaction on the resetting of EEG dynamics assessed by quantitative EEG (qEEG) measures of linear (signal energy measured by amplitude variation; signal frequency measured by mean zero-crossings) and nonlinear (signal complexity measured by the pattern match regularity statistic and the short-term maximum Lyapunov exponent) brain EEG dynamics in 4- and 20-month-old F344 rats with and without brain infarction. The main findings of the study were: 1) dynamic resetting of both linear and nonlinear EEG characteristics occurred following SWDs; 2) animal age significantly affected the degree of dynamic resetting in all four qEEG measures: SWDs in older rats exhibited a lower degree of dynamic resetting; 3) infarction significantly affected the degree of dynamic resetting only in terms of EEG signal complexity: SWDs in infarcted rats exhibited a lower degree of dynamic resetting; and 4) in all four qEEG measures, there was no significant interaction effect between age and infarction on dynamic resetting. We conclude that recovery of the brain to its interictal state following SWDs was better in young adult animals compared with aged animals, and to a lesser degree, in age-matched controls compared with infarction-injured animal groups, suggesting possible effects of brain resetting mechanisms and/or the disruption of the epileptogenic network that triggers SWDs.

Introduction

Numerous studies have used rodent models of absence (petit mal) seizures to investigate the network properties and regulatory mechanisms of the seizure's ictal discharge. The defining EEG ictal event in these models is a 7–12 Hz generalized spike and wave discharge (SWD), characterized by abrupt onset, variable duration (seconds to minutes), and abrupt termination. SWDs typically occur during passive wakefulness and light sleep and at transitions of sleep states (Coenen et al., 1992; Willoughby and Mackenzie, 1992). Episodes of SWDs are characterized primarily by behavioral arrest and decreased responsiveness of the animal, with or without additional behavioral features (Buzsáki et al., 1990a, 1990b; Coenen et al., 1992; Vergnes et al., 1982; Willoughby and Mackenzie, 1992).

Two categories of animal models involving generalized SWDs exist: acquired SWD models and spontaneous SWD models. Acquired SWD models employ chemical agents to provoke SWDs or decrease the threshold for their expression. Alternatively, spontaneous SWD models utilize inherited factors leading to the development of spontaneous SWD activity, which more closely resembles spontaneous human epilepsy (Coenen et al., 1992). In the latter category of studies, WAG/Rij rats (Wistar Albino Glaxo strain, bred in Rijswijk, Netherlands) and genetic absence epilepsy rats from Strasbourg (GAERS) are commonly used because of the selective inbreeding that

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increases their predisposition to express generalized SWDs. While these particular rat strains appear most often in SWD studies, SWDs occur in many common inbred and outbred laboratory rat strains, which are not well recognized for SWD expression (Kelly, 2004). Inbred Wistar-unrelated strains that display generalized SWDs include Fischer 344 (F344), Brown Norway, and dark agouti (Coenen et al., 1992; van Luijtelaar and Coenen, 1986; Willoughby and Mackenzie, 1992). Outbred strains include Sprague-Dawley, Wistar, and Long-Evans (Buzsáki et al., 1990a, 1990b; Semba et al., 1980; Vergnes et al., 1982; Willoughby and Mackenzie, 1992).

Previous studies in rats have demonstrated that SWDs occur more frequently as the animals age (Buzsáki et al., 1990a, 1990b; Coenen and van Luijtelaar, 1987; Vergnes et al., 1986; Willoughby and Mackenzie, 1992), up to hundreds of times a day in aged animals (van Luijtelaar et al., 1995). The cause of this increased rate of SWDs over an animal's lifespan is not known but suggests aging-related changes in the regulation of the corticothalamic mechanisms generating the SWD (Buzsáki, 1991). Based on the convergence of three lines of experimental evidence: 1) a nonlinear measure of brain dynamics suggests that age-related changes in SWDs may be associated with resetting mechanisms of brain dynamics (Nair et al., 2008); 2) a cortical focus drives widespread corticothalamic networks during SWDs (Meeren et al., 2002; Polack et al., 2007); and 3) decreased incidence (Kelly et al., 2006; Kharlamov et al., 2003) and shorter duration (Kelly et al., 2006) of SWDs occurs in infarcted young rats compared to age-matched controls, we sought to determine whether the pairing of advanced age and cortical infarction in rats would alter the form of resetting mechanisms of brain dynamics previously reported for aged animals (Nair et al., 2008).

Brain dynamics, the neurophysiological changes of brain that occur in time and in space, can be assessed by the changes of quantitative EEG (qEEG) properties such as signal energy, frequency, or complexity. These qEEG measures can be applied to the study of resetting mechanisms of brain dynamics, i.e., how the brain "resets" itself to a more normal functional state following a transient period of abnormal function, e.g., a SWD. Resetting related to SWD occurrence can be evaluated by comparing qEEG measurements in the periods before (preictal) to those after (postictal) the SWD. In this study, we estimated: 1) signal energy using amplitude variation (AV); 2) signal frequency using mean zero-crossings per second (ZC); and 3) complexity using the nonlinear statistical measures Pattern Match Regularity Statistic (PMRS) (Kelly et al., 2010; Shiau, 2001; Shiau et al., 2004; 2010) and the Short-Term Maximum Lyapunov Exponent (STLmax) (Iasemidis et al., 1990; Iasemidis and Sackellares, 1996). Signal energy and frequency are conventional qEEG measures that are related to power spectrum (Fast Fourier Transform) analysis. Signal complexity measures are related to nonlinear dynamic statistics, which primarily characterize how ordered a time series is based on the temporal structure of the signal.

We applied these qEEG measures in this study to determine whether any or all of the measures could provide insight to specific brain (EEG) dynamics that are influential in brain resetting following SWDs. Based on the known increased frequency of SWDs in aged animals, we hypothesized that brain resetting mechanisms were impaired in aged animals and that photothrombotic cortical infarction (Kelly et al., 2001; Kharlamov et al., 2003) would further affect these resetting mechanisms. The main goals of this study were to determine the effects of aging, infarction, and their potential interaction on the resetting of EEG dynamics as measured by AV, ZC, PMRS, and STLmax.

Materials and methods

Animals

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of the Allegheny-Singer Research Institute and were carried out according to NIH guidelines and regulations. Animals were housed individually, maintained in a 12 h light/12 h dark cycle environment with controlled temperature (23 ± 2 °C), and provided food and water ad libitum. Sixteen F344 rats were divided into four groups for evaluation: 1) 4-month-old ($n = 4$); 2) 4-month-old with infarction ($n = 4$); 3) 20-month-old ($n = 4$); and 4) 20-month-old with infarction ($n = 4$).

Photothrombosis

Photothrombosis was performed according to Watson et al. (1985) with modifications (Kelly, 2006). Rats were anesthetized with ketamine and xylazine (9:1) and placed in a stereotaxic frame. A midline scalp incision was made and the scalp was retracted laterally. Rose bengal (20 mg/kg; Sigma) was injected through a catheter into the left femoral vein over 2 min, as the brain was stimulated through intact skull for 10 min by an argon laser-activated light beam (Lexel model 75, class IV, 514.5 nm, 15 amp power supply, 150 mW output). The incident beam was ~5 mm wide and focused 1.8 mm posterior to the bregma and 2.8 mm lateral to the midline corresponding to the area of the left sensorimotor cortex (Fig. 1A), creating ~25 mm^3 cortical infarcts in both 4- and 20-month-old animals (Fig. 1B). The skull was cooled by continuous airflow from a fan to prevent heat-mediated tissue injury. Body temperature was monitored and maintained at 37 °C using a thermo-regulated pad. After stimulation, the catheter was removed and incisions were sutured. Animals received a subcutaneous injection (5 ml) of lactated Ringer's solution immediately after surgery and each day postoperatively until adequate hydration and nutrition were reestablished.

Electrode placement

Electrodes were placed at least 1 week after animal lesioning. Six screws were placed in the skull and used as recording electrodes; two additional screws were placed laterally for anchoring the headset (Fig. 1C). The abbreviations (F3, C3, and P3) refer to skull screw electrodes that are placed on the left frontal, central, and parietal regions of the animal's brain, respectively; F4, C4, and P4 refer to the areas on the right. An exposed end of an insulated stainless steel wire was wrapped tightly around each recording screw; the other end was soldered into a pin contact. The six pin contacts were aligned in a plastic connector, which was secured to the skull with dental acrylic. The scalp was sutured and the animal recovered from anesthesia in a temperature-regulated chamber before return to the vivarium.

Video-EEG Recordings

Video-EEG recordings were obtained by serial connections from the animal's headset cable to the commutator of the recording chamber (Dragonfly) and the input box of a Stellate Systems EEG recording system. EEG amplifier outputs were cabled to a Compaq computer and processed by software (Harmonie, Stellate Systems) for EEG display. Video recordings were input to the computer and merged with EEG traces by compatible software (Diva, Stellate Systems). Eight EEG channels were generated for each animal (F3–C3, C3–P3, F3–P3, F4–C4, C4–P4, F4–P4, C3–C4, and P3–P4), sampling electrocerebral activity from both hemispheres; an "F3–C3" designation corresponds to the EEG channel produced by the output of one differential amplifier with inputs from the F3 and C3 electrodes. Because the F4 electrode was used as a linked common reference electrode for each set of four simultaneously recorded animals – the Stellate recording system utilized a single common reference – derivations including the F4 electrode (i.e., F4–C4, F4–P4) were excluded from analysis. Video-EEG data were stored on computer hard drives during acquisition and transferred to external hard drives for offline analysis.