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COGNITIVE IMPAIRMENTS AND NEURONAL INJURY IN DIFFERENT BRAIN REGIONS OF A GENETIC RAT MODEL OF ABSENCE EPILEPSY

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Abstract—Growing numbers of evidence indicate that cognitive impairments are part of clinical profile of childhood absence epilepsy. Little is known on neuropathological changes accompanied by cognitive deficits in absence epilepsy. The aim of the present study was to investigate age-dependent neuropathological changes accompanied by learning and memory impairments in Wistar Albino Glaxo from Rijswijk (WAG/Rij) rat model of absence epilepsy. Experimental groups were divided into four groups of six rats of both WAG/Rij and Wistar strains with 2 and 6 months of age. The learning and memory performances were assessed using passive avoidance paradigm and neuropathological alterations were investigated by the evaluation of the number of dark neurons and apoptotic cells as well as the expression of caspase-3 in the neocortex, the hippocampus, and different regions of the thalamus. Results revealed a decline in learning and spatial memory of 6-month-old WAG/Rij rats compared to age-matched Wistar rats as well as 2-month-old WAG/Rij and Wistar rats. The mean number of dark neurons was significantly higher in the hippocampal CA1 and CA3 areas as well as in the laterodorsal, centromedial, and reticular thalamic nuclei and the somatosensory cortex of 6-month-old WAG/Rij rats. In addition, a higher number of apoptotic cells as well as a higher expression of caspase-3 was observed in the hippocampal CA1 and CA3 regions, the laterodorsal thalamic nucleus, and the somatosensory cortex of 6-month-old WAG/Rij rats compared to other animal groups. These results indicate significant enhancement of neuronal damage and cell death accompanied by memory deficits after seizure attacks in a rat model of absence epilepsy. Seizure-induced neuronal injury and death may underlie cognitive impairments in absence epilepsy.

Key words: thalamo-cortical networks, epileptogenesis, neocortex, memory, apoptosis.

INTRODUCTION

Childhood absence epilepsy (CAE) accounts for approximately 8% of epileptic patients among school-aged children (Pavone et al., 2001). CAE is characterized by the occurrence of generalized 3-Hz spike-wave discharges (SWDs) on electroencephalographic recordings and concomitant with frequent behavioral arrest in an otherwise neurologically normal child (Danober et al., 1998; Crunelli and Leresche, 2002). Although CAE previously was considered as a benign syndrome, significant cognitive and behavioral disabilities have been reported in many of these children (Wirrell et al., 1997; Masur et al., 2013). The high rates of sustained and divided attentional problems (Caplan et al., 2008; D'Agati et al., 2012) with the impact on academic achievements (Masur et al., 2013) and general cognition (Pavone et al., 2001) as well as difficulties in verbal memory (Henkin et al., 2005), non-verbal and short-term verbal memories (Pothion et al.,2004; Bhise et al., 2010), visuospatial skills (Pavone et al., 2001), language abilities (Vanasse et al., 2005), and psychosocial functioning (Conant et al., 2010) have been reported in patients with CAE. Using surface-based morphometry, it has been shown that the average intellectual functioning of children with CAE reflects different plasticity and reorganization in the brain regions associated with cognitive functioning; probably due to neuropathological changes (Tosun et al., 2011). In spite of several studies on neurocognitive deficits in CAE, the precise mechanisms of cognitive and behavioral impairments are unclear (Masur et al., 2013).

The strain of genetic absence epilepsy rats from Wistar Albino Glaxo from Rijswijk (WAG/Rij) provides a validated genetic model of human generalized idiopathic absence epilepsy (Coenen and Van Luijtelaar, 2003; Sarkisova and Van Luijtelaar, 2011). This model is particularly suited for evaluation of age-related changes of learning and memory and behavior alterations (Tolmacheva et al., 2012; Karson et al., 2012). A decline

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Abbreviations: ANOVA, analysis of variance; CAE, childhood absence epilepsy; CM, centromedian thalamic; DAB, 3,3′-diaminobenzidine; DG, dentate gyrus; ECoG, electrocorticencephalogram; LD, laterodorsal thalamic; NGS, normal goat serum; PBS, phosphate-buffered saline; RT, reticular thalamic; SC, somatosensory cortex; STL, step-through latency; SWDs, spike-wave discharges; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling; WAG/Rij, Wistar Albino Glaxo from Rijswijk.

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in emotional and spatial memory of WAG/Rij rats compared to age-matched Wistar rats has been reported (Karson et al., 2012). In agreement with clinical findings in CAE, cognitive (decreased emotional and spatial memory as well as long-term memory) and behavioral (depression-like behavior and difficulties in maternal behavior) disturbances have been reported in WAG/Rij rats (Karson et al., 2012; Bazyan and van Luijtelaar, 2013). Cortical and thalamic networks are critically involved in the production of SWDs and seizure attacks in WAG/Rij rats (Steriade, 2005) as well as in the regulation of behavior and cognition (Merzenich and Sameshima, 1993; van Groen et al., 2002). Abnormalities of the cortico-thalamic circuit play a crucial role in the pathophysiology of different neuropsychologic disorders (Tekin and Cummings, 2002).

Brain damage as a consequence of epilepsy has been suggested in the medieval literatures (Gorji and Khaleghi Ghadiri, 2001). Clinical investigations and experimental studies have demonstrated that certain types of seizures are able to cause neuronal injury and cell death, and this may in turn lead to cognitive dysfunction and behavioral deficits (for review see Henshall and Simon, 2005). Our previous study revealed that occurrence of SWDs in WAG/Rij rats is accompanied by changes of synaptic architecture in the neocortex (Karimzadeh et al., 2013a). Studies in genetic absence epilepsy rats from Strasbourg (another absence epilepsy model) have revealed alterations in the membrane and intracellular proteins (such as ATP synthase subunits, myelin basic protein and macrophage migration inhibitory factor), metabolic activities, and cerebral blood flow in different brain regions (Nehlig et al., 1996, 1998; Melo et al., 2006; Danis et al., 2011). These metabolic and molecular changes may have functional and structural effects on thalamocortical loop and limbic system and lead to neuronal injury (Danis et al., 2011; Vakilzadeh et al., 2014). The aim of the present study was to evaluate the cognitive function as well as its concomitant histopathological alterations in different brain regions, including the somatosensory cortex (SC), the laterodorsal thalamic nucleus (LD), centromedian thalamic nucleus (CM), and reticular thalamic nucleus (RT) as well as the hippocampal CA1, CA3 areas and dentate gyrus (DG) in WAG/Rij rats.

EXPERIMENTAL PROCEDURES

Animals

All animal experiments were confirmed by the ethic committee of Shefa Neuroscience Research Center. Male WAG/Rij and Wistar rats were kept under environmentally controlled conditions in a room with reversed light–dark cycle (light on from 7:00 p.m. to 7:00 a.m.). Rats were given ad libitum access to food and water. Experiments were divided into four groups of both WAG/Rij and Wistar strains with 2 and 6 months of age ($n = 6$ for each group).

To investigate a strain per age interaction with the development of the SWDs, two groups of WAG/Rij rats with different ages were used; 2- and 6-month-old. WAG/Rij rats of 2 months of age did not express SWDs and considered as pre-symptomatic group. At an age of 6 months, the WAG/Rij rats exhibited SWDs (symptomatic group; van Rijn et al., 2010). Two- and six-month-old Wister rats were used as non-epileptic animals from another strain.

Electrophysiological recordings

Electrocorticencephalogram (ECoG) recordings were done by silver electrodes that implanted stereotaxically on the dura mater of the left and right somatosensory cortices under intraperitoneal chloral hydrate anesthesia (350 mg/kg; Sigma–Aldrich, Munich, Germany). Silver electrodes were connected to an amplifier (EXT-02 F; NPI, Germany) and stored by a digital oscilloscope. ECoG recordings were performed 3–6 h to monitor SWDs occurrence. ECoG was recorded under sedated state by neuroleptic anesthesia (fentanyl 0.033 mg/kg body weight per hour, i.p.; Janssen-Cilag, France) repeated every 20–30 min. Six-month-old WAG/Rij rats exhibited SWDs in their ECoG (recorded for at least 3 h) were chosen as the epileptic rats. ECoG was monitored in 2-month-old WAG/Rij rats and in 2- and 6-month-old Wistar rats for 6 h to rule out occurrence of SWDs (Karimzadeh et al., 2013a). Behavioral procedures were performed after recordings of ECoG in different rat groups.

Behavioral procedure

Passive avoidance test was performed in a closed, quiet and temperature and light-controlled room. Rats were tested in a step-through type passive avoidance apparatus. The apparatus (measuring 25 cm × 15 cm × 15 cm) consisted of a light and a dark compartment separated by a guillotine door. Passive avoidance test consisted of three trials:

1. Pre-acquisition or adaptation trial (training trial): On the first day, rats were placed individually in the light compartment and allowed to explore this compartment. The door between the two compartments was opened after 10 s. During this trial, the animal could freely move into the dark compartment. The primary latency of the animals entering the dark compartment was recorded as a control of visual ability and locomotor activity (Crawley, 1999). Any animal failing to cross from the light to the dark compartment within 60 s was discarded from the experiments. Between two training sessions, compartments were cleaned to remove olfactory cues.
2. Acquisition trial: The acquisition trial was conducted 30 min after the pre-acquisition trial. Rats were placed in the light compartment. After 10 s, the door separating two compartments was opened. After the rat completely entered the dark compartment (four paws in), the door immediately closed, and an electric foot-shock (1 mA) was delivered for 1 s via the grid floor. After 20 s, rats were removed from the dark compartment and returned to their home cages. This training stage was repeated until the animal remained in the light compartment for 300 s. Both compartments were cleaned to remove olfactory cues between training sessions.