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Alterations in hippocampal and cortical densities of functionally different interneurons in rat models of absence epilepsy

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ABSTRACT

Recent data from absence epileptic patients and animal models provide evidence for significant impairments of attention, memory, and psychosocial functioning. Here, we outline aspects of the electrophysiological and structural background of these dysfunctions by investigating changes in hippocampal and cortical GABAergic inhibitory interneurons in two genetically absence epileptic rat strains: the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and the Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats. Using simultaneously recorded field potentials from the primary somatosensory cortex (S1 cortex, seizure focus) and the hippocampal hilus, we demonstrated that typical frequencies of spike-wave discharges (SWDs; 7–8 Hz, GAERS; 7–9 Hz, WAG/Rij) and their harmonics appeared and their EEG spectral power markedly increased on recordings not only from the S1 cortex, but also from the hilus in both GAERS and WAG/Rij rats during SWDs. Moreover, we observed an increased synchronization between S1 cortex and hilus at 7–8 Hz (GAERS) and 7–9 Hz (WAG/Rij) and at their harmonics when SWDs occurred in the S1 cortex in both rat strains. In addition, using immunohistochemistry we demonstrated changes in the densities of perisomatic (parvalbumin-immunopositive, PV+) and interneuron-selective (calretinin-immunopositive, CR+) GABAergic inhibitory interneuron somata. Specifically, GAERS and WAG/Rij rats displayed lower densities of PV-immunopositivity in the hippocampal hilus compared to non-epileptic control (NEC) and normal Wistar rats. GAERS and WAG/Rij rats also show a marked reduction in the density of CR + interneurons in the same region in comparison with NEC rats. Data from the S1 cortex reveals bidirectional differences in PV + density, with GAERS displaying a significant increase, whereas WAG/Rij a reduction compared to control rat strains. Our results suggest an enhanced synchronization and functional connections between the hippocampus and S1 cortex as well as thalamocortical activities during SWDs and a functional alteration of inhibitory mechanisms in the hippocampus and S1 cortex of two genetic models of absence epilepsy, presumably in relation with increased neuronal activity and seizure-induced neuronal injury.

1. Introduction

Human absence epilepsy is classically regarded as a neurological disorder with no apparent structural alterations and a relatively benign prognosis (Livingstone et al., 1965; Niedermeyer, 1996; Danober et al., 1998). Absence seizures in humans are characterized by generalized, synchronous and symmetrical 3–4 Hz spike-wave discharges (SWDs) on electroencephalographic (EEG) recordings concomitant with brief periods of behavioral arrest and impaired consciousness (Depaulis and van Luijtelaar, 2006).

More recently, a growing body of evidence suggests a set of neuropsychological dysfunctions to belong in the clinical profile of absence epileptic syndromes. Research focusing on childhood absence epilepsy indicates, that affected children often display impairments in attention (Caplan et al., 2008), verbal memory (Henkin et al., 2005), general cognition, visuospatial skills (Pavone et al., 2001), and language abilities (Vanasse et al., 2005). Comparable results were provided by investigations of genetic models of absence epilepsy, such as Genetic Absence Epilepsy Rats from Strasbourg (GAERS, Marescaux and Vergnes, 1995; Depaulis and van Luijtelaar, 2006) and Wistar Albino Glaxo/Rijswijk (WAG/Rij, Coenen and van Luijtelaar, 2003) rats. Both strains are considered valid models of human absence epilepsy, displaying abrupt and transient behavioral arrests accompanied by generalized SWDs on the EEG, and similar pharmacological reactivity to

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the human disorder (Depaulis et al., 2016; Renier and Coenen, 2000). In a recent account, Marques-Carneiro et al. (2016) identified delayed spatial learning and less efficient working memory performance in GAERS rats engaged in the Morris water maze task. Further evidence is available from studies based on the WAG/Rij strain. Karson et al., 2012 employed extensive behavioral testing to assess learning and memory performance of 5 and 13-month-old WAG/Rij rats compared to age-matched Wistar controls. Results revealed WAG/Rij rats to perform significantly worse in passive avoidance and the Morris water maze compared to their controls at 13 months but not at 5 months of age, suggesting an age-dependent decline in learning and memory performance.

As traditional accounts of absence epilepsy dismissed the idea of underlying structural changes, studies aiming at the investigation of neuropathology in animal models of the disorder are scarce. Based on unbiased stereology, Sabers et al. (1996) reported no difference in the total number of neurons in the reticular thalamic nucleus and neocortex of GAERS and normal control rats, both of these brain areas crucially involved in the generation and maintenance of absence seizure activity. In a more recent study, Jafarian et al. (2015) subjected WAG/Rij rats to passive avoidance testing, and found a decline in learning and memory performance of 6-month-old WAG/Rij versus age-matched controls, 2-month-old WAG/Rij and 2-month-old control rats. Histopathological assessments carried out on the same animals revealed an increased number of degenerating neurons in the hippocampal CA1 and CA3 subfields, somatosensory cortex, and laterodorsal, centromedial and reticular thalamic nuclei of 6-month-old WAG/Rij rats. A higher number of apoptotic cells in these brain regions may hint at the possibility, that seizure-induced injury and cell death may play a part in explaining cognitive impairments in absence epilepsy.

Little attention was paid to inhibitory interneurons (INs) in comparison, which are precise determinants of virtually all circuit functions by providing inhibitory synaptic input via the release of the neurotransmitter GABA to various cellular compartments of principal cells (Klausberger and Somogyi, 2008). Different types of INs selectively target spatially restricted domains of principal cells evoking temporally organized inhibitory postsynaptic potentials, which makes these cells extremely effective in the regulation of single cell excitability and setting the temporal window for synaptic excitation. (Reviews are provided by Pelkey et al., 2017 and Freund and Buzsáki, 1996) Thus, INs can shape the timing of afferent and efferent information flow by GABAergic gating, synchronizing the activity of large assemblies of principal cells. In addition, as axons of some INs form long-range projections reaching distant cortical or subcortical targets, they can harness distributed circuits to facilitate oscillatory activity across a broad range of frequency domains (Pelkey et al., 2017). Hippocampal oscillations can be associated with specific behavioral states and are thought to be crucial for learning and memory formation (Buzsáki, 1996). Neurons expressing the Ca^{2+}-binding protein parvalbumin (PV) comprise a well-characterized subset of INs across multiple species including axo-axonic and basket cells, which provide perisomatic GABAergic innervation to hippocampal neurons. Fast-spiking PV-immunopositive basket cell (PVBC) activity was demonstrated to be required and sufficient for generating gamma-frequency oscillation in vivo, which is associated with memory and spatial navigation (Cardin et al., 2009; Sohal et al., 2009). PVBCs are very active during sharp wave-ripples as well, which are thought to represent consolidation of waking memories during sleep (Klausberger et al., 2003, 2005). Furthermore, as Cobb et al. (1995) showed, a single PVBC can phase-lock the firing of CA1 pyramidal cells at theta-frequencies, an oscillatory pattern apparent during exploration and REM sleep. Interneurons expressing another Ca^{2+}-binding protein, calretinin (CR), on the other hand were shown to be interneuron-selective, contributing to the regulation of hippocampal and cortical activity by indirectly participating in GABAergic gating: they terminate on INs which in turn target dendritic compartments of principal cells (Gulyás et al., 1996; Meskenaite, 1997; Gonchar and Burkhalter, 1999; Cauli et al., 2014).

Both PV- and CR-expressing interneurons have been examined in various epilepsy models and surgically extracted tissue samples of human epileptic patients. A reduced number of PV-expressing perisomatic inhibitory interneurons was reported by multiple studies in cortical areas of the WAG/Rij rat model of absence epilepsy (Van de Bovenkamp-Janssen et al., 2004; Van Luijtelaar and Sitnikova, 2004). In epileptic human hippocampal samples, the number of PV-immunolabeled somata was similarly reduced (Andrioli et al., 2007), however, the perisomatic innervation of principal cells was found to be preserved by ultrastructural analysis (Wittner et al., 2001). CR-containing interneurons on the other hand have been found to be extremely vulnerable to excitotoxic insults in multiple species and across different conditions such as ischemia in rats (Freund and Maglóczky, 1993), as well as human temporal lobe epilepsy (TLE; Tóth and Maglóczky, 2016). With the loss of interneuron-selective cells, glutamatergic synaptic input to principal cell dendrites may undergo excessive potentiation due to the deficient regulation of dendritic electrogenesis or abnormal NMDA-receptor activation (Miles et al., 1996). Together with preserved perisomatic inhibitory input inducing the same principal neurons to fire in synchrony, an epileptogenic neural network may develop with a high propensity to produce recurrent seizures.

The hippocampus plays a key role in learning, memory, and spatial navigation (Buzsáki, 1989; Moser et al., 2008; Girardeau et al., 2009; Buzsáki and Moser, 2013), which functions have been repeatedly shown to be impaired in animal models and human patients with absence epilepsy. The dentate gyrus and its hilar region receive, transform and transmit excitatory input towards the principal neurons of the cornu Ammonis. Previous evidence indicates, that interneurons of the hilus show specific vulnerability to epileptic seizures in both animal models (Freund and Maglóczky, 1993; Long et al., 2011; Marx et al., 2013; Hofmann et al., 2016) and human patients (for reviews, see Tóth and Maglóczky, 2016; Wittner and Maglóczky, 2017). The primary somatosensory cortex (S1 cortex) was targeted due to its well described role in seizure generation in rat models of absence epilepsy (Meeren et al., 2002; Sitnikova and van Luijtelaar, 2004). Although the lack of SWDs in the hippocampi of absence epileptic rats had been demonstrated (Vergnes et al., 1990), there are multiple sources of evidence suggesting the involvement of limbic brain regions in absence epilepsy (Richards et al., 2000; Nersesyan et al., 2004; Onat et al., 2013). To shed more light on this putative coupling, we suggest a simultaneous EEG recording procedure with finer-grained analysis compared to earlier investigations. The present study aims at delivering novel evidence of neuropathological changes affecting perisomatic and interneuron-selective interneurons in the hippocampus and S1 cortex of both the GAERS and WAG/Rij rats. Thus, we used simultaneous EEG recordings from the S1 cortex and hilus to show changes in power of the frequency bands (relative proportion of different frequencies in recorded field potentials) and to investigate putative alterations in synchronization level between field potentials of these brain areas in both GAERS rats and WAG/Rij rats. In addition, using immunohistochemistry we investigated changes in the densities of perisomatic (parvalbumin-immunopositive, PV+) and interneuron-selective (calretinin-immunopositive, CR+) GABAergic inhibitory interneuron somata. We hypothesize, that absence epileptic seizure-related overexcitation may damage GABAergic interneurons in hippocampal and neocortical areas.

2. Material and methods

2.1. Animals

Animal experiments were performed according to the guidelines of the Hungarian Act of Animal Care and Experimentation (1998, XXVIII, section 243) and in accordance with directive 2010/63/EU of the European Parliament and Council. The experimental design was approved by the Animal Care and Experimentation Committees of the