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Research Report

Retrosplenial granular b cortex in normal and epileptic rats: A stereological study

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ABSTRACT

Human temporal lobe epilepsy and experimental models of this disease are associated with loss of neurons and other structural alterations in several limbic brain structures including the hippocampal formation and adjacent parahippocampal cortical areas. The goal of this study was to test the hypothesis that seizure activity can produce damage to the retrosplenial granular b cortex (Rgb) which is known to be strongly connected with other limbic structures implicated in epilepsy. To test this hypothesis, we estimated, using stereological methods, the volumes and total neuronal numbers in Rgb cortex of rats that had experienced prolonged status epilepticus induced by pilocarpine (350 mg/kg), rats treated with six electroshock seizures (the first five seizures were spaced by 24-h intervals, whilst the last two were only 2 h apart), and control rats. Adult male Wistar rats were used in this experiment. Status epilepticus produced significant loss of neurons in Rgb cortical layers IV (22%) and V (44%), which was accompanied by volume reductions in layers I (17%), IV (11%), V (18%) and VI (24%). In electroshock-treated rats, the volume of Rgb cortical layer VI was reduced by 17% and the number of neurons estimated in layer V was smaller by 16% relative to control rats. Thus, the finding that status epilepticus and administration of brief generalized seizures both lead to degenerative morphological alterations in Rgb cortex provides the first experimental support for the hypothesis that this cortical area can be involved in seizure activity, as suggested by its anatomical connections.

1. Introduction

Temporal lobe epilepsy (TLE) is often associated with progressive loss of neurons in the hippocampus proper, dentate gyrus, subicular and entorhinal cortices, which may lead to permanent cognitive impairments (Dawodu and Thom, 2005; Du et al., 1993; Engel, 1996; Fisher et al., 1998; Houser, 1999; Stafstrom, 2005). Neuronal death in TLE has been ascribed to sustained hyperactivity of brain circuits involved in either genesis or propagation of seizures, or both (McNamara, 1999; Meldrum, 1991; Pitkänen and Sutula, 2002; Salmenperä et al., 1998). This assumption is supported by animal studies in which prolonged seizures, induced by either chemoconvulsants, such as pilocarpine and kainic acid, or continuous electrical stimulation, produce a similar pattern of hippocampal neurodegeneration (Ben-Ari et al., 1980; Buckmaster and Dudek, 1997; Sloviter, 1987; Sloviter et al., 2003; Turski et al., 1983). It is further supported by evidence that, whereas single or widely spaced brief seizures do not cause considerable brain damage (Gombos et al., 1999; Vaidya et al., 1999), they certainly do so when administered at shorter intervals, particularly with respect to several seizure-vulnerable neuronal populations,

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such as hilar cells of the dentate gyrus and neurons of the entorhinal cortex (Cardoso et al., 2008; Cavazos and Sutula, 1990; Lukoyanov et al., 2004; Sutula et al., 1994).

The retrosplenial granular b cortex (Rgb) of the rat occupies the anterodorsal part of the retrosplenial granular area and lies ventral to the retrosplenial dysgranular cortex (Rdg) and caudal to the anterior cingulate cortex (Fig. 1; Vogt and Peters, 1981; Zilles and Wree, 1995). It is strongly interconnected with several other brain regions that are thought to be involved in seizure activity. More specifically, Rgb projects heavily to and receives afferent input from the hippocampal formation (Miyashita and Rockland, 2007; Van Groen and Wyss, 1990a, 1992b, 2003; Vogt and Miller, 1983; Wyss and Van Groen, 1992), mainly via the subicular complex (Finch et al., 1984; Meibach and Siegel, 1977; Van Groen and Wyss, 1990b, 2003; Wyss and Van Groen, 1992). In addition, Rgb is reciprocally connected with the anteroventral and anterodorsal thalamic nuclei (Shibata, 1998; Sripanidkulchai and Wyss, 1986; Van Groen and Wyss, 1992a), which are known to be specifically recruited in the propagation of limbic seizures within the Papez circuit (Dubé et al., 1998; Mirski et al., 2003; Mraovitch and Calando, 1999; Sherman et al., 1997). That Rgb area can be involved in epilepsy is supported by the results of a recent study of TLE patients with hippocampal sclerosis, which showed that hippocampal atrophy significantly correlates with loss of cortical gray matter in the retrosplenial cortex (Düzel et al., 2006). Furthermore, this possibility is also consistent with evidence from experimental studies in rats that generalized seizures produce a marked increase in blood oxygen level-dependent signal intensity in Rgb (Brevard et al., 2006) and that status epilepticus (SE) is associated with atrophic changes in the dendrites of Rgb pyramidal neurons (Ampuero et al., 2007). However, too few data are available at present to make conclusive statements relative to the implication of Rgb in epilepsy.

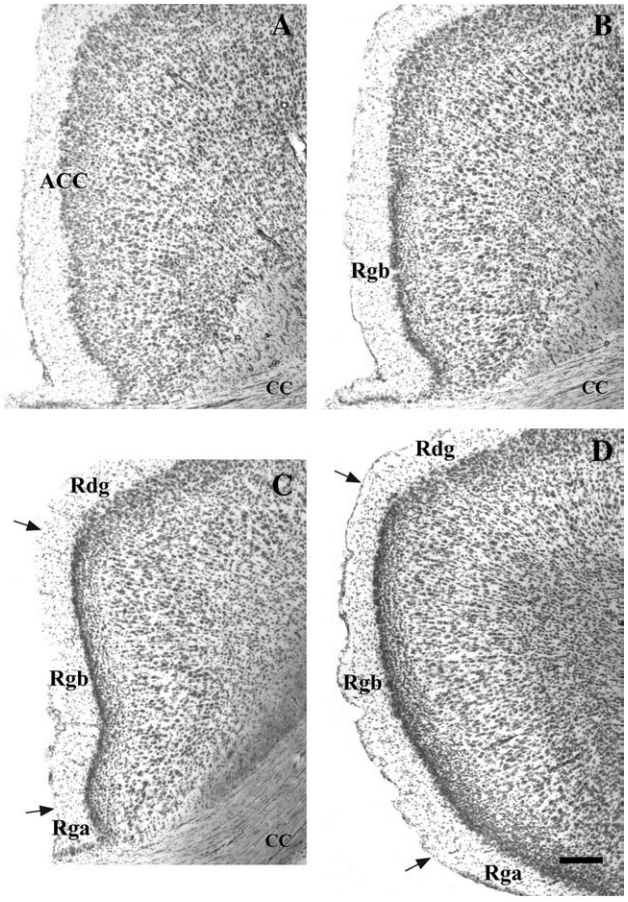

Fig. 1 – Photomicrographs of Nissl-stained coronal sections cut at the caudalmost level of the anterior cingulate cortex (A), and rostral (B), mid-rostrocaudal (C), and caudal (D) levels of the retrosplenial granular b (Rgb) cortex of a control rat. Note the high cell packing density in Rgb layers II/III (B) that distinguishes this retrosplenial area from the adjacent anterior cingulate cortex (ACC) in which layer II/III neurons are much more loosely packed (A). Rgb cortex borders dorsally the retrosplenial dysgranular (Rdg) cortex and joins caudally and ventrally the retrosplenial granular a (Rga) cortex, as indicated by arrows in C and D. CC, corpus callosum. Scale bar = 150 μm.

Whereas it is well documented that seizure activity can lead to degeneration of hippocampal and entorhinal neurons, the issue of what actually happens following seizures to neurons located in Rgb cortex has not yet been addressed. We hypothesized that, if Rgb cortex is indeed involved in epileptiform activity, as suggested by its strong connections with brain regions implicated in epilepsy, then, at least part of its neurons should be injured by the seizures and probably lost. To test this hypothesis, we estimated the total neuronal numbers in all Rgb cortical layers in control rats and compared them to the numbers found in rats that had experienced prolonged status epilepticus induced by pilocarpine. The volumes of the respective cortical layers were also estimated and compared between the groups. Furthermore, because the degenerative changes in the brain of pilocarpine-treated animals might be related to neurotoxic effects of this drug rather than to the seizures it induces, we also analyzed the morphology of Rgb cortex in rats following repeated administration of brief generalized seizures elicited by electroconvulsive shock (ECS). In doing so, we applied the treatment protocol that has been previously shown to produce small, but significant loss of seizure-vulnerable entorhinal and hilar neurons (Cardoso et al., 2008; Lukoyanov et al., 2004).

2 Results

2.1 Behavioral monitoring

From 8 rats treated with pilocarpine, 1 animal did not show any seizure-like activity and, therefore, was excluded from the study. Remaining 7 rats developed SE, but one of them died approximately 4 h after the treatment. After recovery, the 6 rats that survived in SE group went through a latent phase, during which they showed asymptomatic (seizure-free) behavior. Following this salient period lasting 2–3 weeks, spontaneous motor seizures of stage 3 or greater on the Racine scale (Racine, 1972) were repeatedly observed in all rats of this group. No behavioral alterations were detected in animals from the ECS-treated and control groups.