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# FUNDAMENTAL INTERSTRAIN DIFFERENCES IN CORTICAL ACTIVITY BETWEEN WISTAR AND SPRAGUE–DAWLEY RATS DURING GLOBAL ISCHEMIA

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**Abstract—Four-vessel occlusion (4VO), a frequently used model of global cerebral ischemia in rats, results in a dysfunction in wide brain areas, including the cerebral cortex and hippocampus. However, there are pronounced differences in response to global ischemia between the laboratory rat strains used in these studies. In the present work, the immediate acute effects of 4VO-induced global ischemia on the spontaneous electrocorticogram (ECoG) signals were analyzed in Wistar and Sprague–Dawley rats. The ECoG was isoelectric during the 10 min of global cerebral ischemia in Wistar rats and the first burst (FB) was seen 10–13 min after the start of reperfusion. In Sprague–Dawley rats, the FB was detected immediately after the start of 4VO or a few seconds later. The burst suppression ratio (BSR) in Wistar rats decreased to 45% in 5 min after FB, and after 25 min it was approximately 40%. In Sprague–Dawley rats, the BSR was 55% immediately after the FB and it decreased steeply to reach 0% by 10 min. There was also a significant difference between the two strains in the frequency composition of the ECoG pattern. The power spectral densities of the two strains differed virtually throughout the post-ischemic state. The histological results (Evans Blue, Cresyl Violet and Fluoro Jade C stainings) supplemented the electrophysiological data: the neuronal damage in the CA1 pyramids in Wistar rats was severe, whereas in the Sprague–Dawley animals it was only partial. These observations clearly demonstrate that the use of different rat strains (e.g. Wistar vs. Sprague–Dawley) can be a source of considerable variability in the results of acute experiments on global ischemia and it is important that the laboratory rats used in such experiments should be carefully chosen.** 

**Key words: four-vessel occlusion, electrocorticogram, burst suppression ratio, global ischemia, Wistar strain, Sprague–Dawley strain.**

## INTRODUCTION

A physiological, uninterrupted blood supply is essential for the appropriate functioning of the central nervous system (Heiss et al., 1977). Depending on the variety and severity of a circulatory failure which leads to brain ischemia, damage may occur in several ways (Hossmann, 2008). Accordingly, different experimental models of ischemia are required which correspond to the various clinical pathophysiological situations (Perel et al., 2007). Application of an appropriate model is essential in the investigations of ischemic pathophysiological conditions and for the evaluation of treatment strategies for patients with cerebrovascular diseases (Khaja and Grotta, 2007; Sacco et al., 2007).

Many independent research groups have reported significant discrepancies between the data recorded in human pathophysiological states and the data obtained from animal experiments. It is clear that the most important step in designing an animal experiment is the selection of a suitable model and the method for the induction of ischemia. In this respect, it should be borne in mind that there is a considerable diversity in cerebral vascular architecture in the different species, and there are also interstrain and even intrastrain anatomical differences in the vasculature of mice (Barone et al., 1993) and rats (Oliff et al., 1995a,b, 1996). As merely one example, there are six different variations in the anatomy of the arterial circle of rats (Brown, 1966). In consequence of such anatomical differences, it has been found that the specific features of the different rat and mouse strains (Ginsberg and Busto, 1989; Barone et al., 1992) supplied by the different vendors (Marosi et al., 2006) are the determining factors most strongly influencing the outcome of global or focal cerebral ischemia (Barone et al., 1993). To the best of our knowledge, there are no published comparative morphological and electrophysiological data dealing with the ischemic interstrain differences between Wistar and Sprague–Dawley rats.

The primary aim of the present study was to investigate whether any difference in cerebral cortical activity and the consequent histological changes can be

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*Abbreviations:* 4VO, four-vessel occlusion; BSR, burst suppression ratio; CBF, cerebral blood flow; CCAs, common carotid arteries; EEG, electroencephalogram; FB, first burst; PBS, phosphate-buffered saline; ECoG, electrocorticogram.

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observed following global cerebral ischemia in Wistar and Sprague–Dawley rats supplied by the same vendor (Charles River Laboratories). A further goal was to emphasize that such differences can greatly influence the results of experiments and even the clinical application of neuroprotective strategies.

## MATERIALS AND METHODS

### Animals and housing conditions

Male Wistar and Sprague–Dawley rats (250–300 g) supplied by the same vendor (Charles River Laboratories, $N = 28$) were housed individually in standard plastic cages, with free access to food and water. The animal house was light-controlled in 12 h cycles and the animals were kept under conditions of constant room temperature (22 ± 1 °C) and humidity. Every effort was made to minimize the number of animals used and their suffering. The principles of animal care (NIH Publication No. 85-23) and the protocol for animal care approved by both the Hungarian Health Committee (1998) and the European Communities Council Directive (86/609/EEC) were strictly followed.

### Preparation of the transient global cerebral ischemia model (4VO)

From the total of 28 animals, the data on 14 (out of 17) Wistar and 11 (out of 12) Sprague–Dawley rats could be used in this study. The four-vessel occlusion (4VO) procedure was based on the method of Pulsinelli and Brierley (1979) as adopted by our laboratory (Sas et al., 2008; Gellert et al., 2011). Under sodium pentobarbital anesthesia (60 mg/kg, i.p.), the atlas bone was exposed and both vertebral arteries were electrocauterized through the alar foramina located on the lateral surface of the atlas by alternate cooling and drilling, in order to protect the brainstem from heat damage, which can lead to a respiratory malfunction. The exposure and cauterization of the vertebral arteries was judged to be successful if both the proximal and the distal stump emerged. Twenty four hours later, under sodium pentobarbital anesthesia (60 mg/kg, i.p.), the common carotid arteries (CCAs) were blunt-dissected free and the animal was fixed stereotaxically. After preparation of the skull for the extradural spontaneous electrocorticogram (ECoG) baseline recording, the CCAs were clamped with non-traumatic aneurysm clips (Aesculap, B. Braun Medical Ltd., Hungary) for 10 min. Twelve Wistar rats destined for histology did not receive pentobarbital, but slight ether anesthesia during the 4VO (as is common for this strain in 4VO ischemia; Sas et al., 2008). In order to rule out the putative neuroprotective effects of pentobarbital anesthesia in our 4VO model, 3 Wistar rats underwent pentobarbital anesthesia during 4VO, too. The carotid artery blood flow was recommenced by releasing the clips following 10 min of global cerebral ischemia. Body temperature was monitored and maintained at 37 °C by means of an automatic heat controller placed in the stereotaxic stand (Supertech TMP-5a, Hungary). In order to reduce the number of experimental animals and their suffering in this study, the sham-operated group was omitted and a control group was used for comparison, as in our previous study (Sas et al., 2008), when a 4VO-induced hippocampal injury was studied with Fluoro Jade C and anti-neuronal nuclei (NeuN) labeling; it was found that sham surgery caused no tissue damage, as assessed by Fluoro Jade C staining on hippocampal slices (data not shown).

### Electrophysiology

*Burst suppression ratio analysis.* For electrophysiological recordings, 4 Wistar and 4 Sprague–Dawley rats were used. The intermittent cortical activity which could be observed after ischemic insults was quantified by estimating the burst suppression ratio (BSR), defined as the percentage of time spent in suppression (Rampil and Laster, 1992). We applied the method of Vijn and Sneyd (1998) for burst identification. The resolution in the BSR estimation was 10 s. In order to increase the sensitivity of BSR extraction, the threshold amplitude was adjusted on every channel used individually so as to exclude differences originating from the experimental conditions. The absolute value of a 5-min baseline period for each channel was used to determine BSR repeatedly with a decreasing voltage threshold and 200 ms for the minimum allowed BS duration. The threshold with a BSR value of $< 5\%$ (20–25 μV) was used to monitor the changes in BSR after the first burst (FB) following the 10-min global cerebral ischemia. Analysis was carried out with custom-written routines in MATLAB (MathWorks, Natick, USA).

*Threshold-crossing event detection.* The post-ischemic burst period is characterized by a higher rate of firing. The threshold for the quantification of threshold-crossing events was defined with the BSR-analyzing algorithm. The resolution of the BSR estimation was 10 s. Raw data were filtered to 1.5–50 Hz. The absolute value of ECoG 30–35 min after the FB was used to determine BSR repeatedly with an increasing voltage threshold, and one with a BSR $> 98\%$ was set for spike detection. The numbers of threshold-crossing spikes were determined in every 5-min period during 30 min after the FB and divided by the number of spikes counted between 30 and 35 min after the FB. Analysis was carried out with the event detection function of Stimfit 0.10 (http://www.stimfit.org/; courtesy of C. Schmidt-Hieber, University College London, London, UK; and P. Jonas, Physiological Institute, University of Freiburg, Freiburg, Germany).

*Spectral density estimation.* For the conversion of ECoG data from time domain to frequency domain and for computation of the *discrete Fourier transform* and its squared magnitude, Welch's method was used to calculate periodograms to estimate the power of ECoG at different frequencies (Alkan and Kiymik, 2006). Measurements of ECoG were made in every first 30-s period of every 5 min after the FB. The segment length