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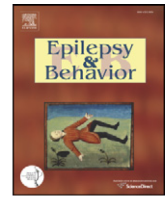

Activation and involvement of the lateral–posterior nucleus of the thalamus after a single generalized tonic–clonic seizure

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ABSTRACT

The lateral–posterior thalamic nuclei (LP) have been shown to play an important role in controlling epileptic activity. In addition, thalamic atrophy and neuronal loss have been observed in epilepsy. The objective of this study was to investigate whether lateral–posterior neuronal activation may be observed shortly after a single generalized seizure in rats submitted to the pilocarpine model of epilepsy. The results showed an increased lateral–posterior activation as soon as the seizure occurred, suggesting that neuronal loss in the thalamus is not only the consequence of chronic epilepsy.

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1. Introduction

Epilepsy is the most common serious neurological condition, and its relationship with specific cerebral structures is crucial, particularly the thalamic complex. As evidence for this, an experimental study investigating the interictal cerebral metabolic rate by 14C-2DG autoradiography in a chronic model of epilepsy clearly demonstrated an increase in glucose utilization by several brain regions [1]. The most relevant finding was a consistent rise of the metabolic rate in the lateral–posterior thalamic nuclei (LP), suggesting that the LP may be involved in the cerebral circuitry controlling epileptic activity during interictal intervals [1]. Then, the contribution and the participation of LP on spontaneous recurrent seizure (SRS) activity in the same epilepsy model were demonstrated [2]. Briefly, it was shown that bilateral lateral–posterior lesions by ibotenic acid in rats with epilepsy resulted in a five-time increase in seizure frequency, suggesting that the lateral–posterior thalamic nucleus is one of the most important thalamic nuclei involved in the inhibition of spreading mechanisms [2]. Along these lines, these data clearly demonstrate the possible role of the LP in the inhibitory control of excessive activation during a seizure. Following this line of reasoning, it is pertinent to think that the integrity of this nucleus may be critical for decreasing the probability of sudden unexpected death in epilepsy (SUDEP) occurrence, since high seizure frequency is one of the main risk factors for SUDEP [3].

The lateral–posterior nucleus is important not only for the regulation of brain hyperexcitation but also for the control of cardiac autonomic function. The thalamus receives inputs from the insular cortex and amygdala, which are interconnected with each other and with autonomic nuclei of the brain stem and spinal cord. Additionally, its projections to the hypothalamus, periaqueductal gray, and nucleus ambiguus give rise to parallel pathways that influence both parasympathetic and sympathetic outflows associated with cardiac and respiratory control [4]. Previous findings in the literature have associated abnormal activity of thalamic nuclei with nocturnal apneic episodes [5] as well as with cardiac arrhythmias [6] and altered heart rate variability [7]. Recent studies performed in our laboratory have found transient alterations in heart rate and heart rate variability (such as decreased RR intervals, SDNN, and RMSSD) in LP-injured rats free from epilepsy [8]. Animals submitted to the pilocarpine model of epilepsy also showed decreased RR intervals, and RMSSD and increased LF% [8]. It has been recognized that seizures can be associated with heart rate changes and ECG abnormalities [9], and cardiac arrhythmias associated with respiratory changes may also be an important cause or factor for the occurrence of SUDEP [10]. In our previous study [8], morphological analyses of the lateral–posterior nucleus in rats with epilepsy were also undertaken and showed a 62.05% reduction in the immunohistochemistry for Neu-N (100% being the staining quantified in the control group). There were no significant differences for Nissl staining, although qualitative differences in the

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shape of the stained cells were observed between the group with epilepsy and the control group [8].

However, a question that has not yet been addressed is whether alterations in the lateral–posterior nucleus may be observed in the early stages of epileptogenesis (e.g., after the first seizure) or whether lateral–posterior abnormalities require longer periods to be observed (e.g., after the occurrence of many seizures). Therefore, the objective of the present study was to investigate whether the lateral–posterior neuronal activation may be observed shortly after a single generalized tonic–clonic seizure in rats submitted to the pilocarpine model of epilepsy.

2. Methods

Adult male Wistar rats (n = 15) were divided randomly into three groups. The first group was composed of rats that received pilocarpine (n = 05). *Status epilepticus* (SE) was induced according to the procedure described previously [11,12]. In brief, 30 min after methylscopolamine injection (1 mg/kg sc, Sigma, USA, used to reduce the peripheral effects of pilocarpine), pilocarpine (450 mg/kg ip, Sigma, USA) was administered to rats. Only rats that displayed a tonic–clonic seizure (classified according to the Racine's seizure index [13]) were included in this study and perfused as soon as this tonic–clonic seizure occurred. The second group was composed of rats that were placed in CO_2 chamber and then perfused as soon as unconscious (n = 05). This group is extremely important because the lateral–posterior nucleus can be activated because of apnea associated with a seizure, and hence, this group controls for the apnea factor. The third group was composed of control rats. These rats were injected with saline solution and perfused after 30 min (n = 05). After the perfusion process, the brains were fixed, sliced 40 μm in vibratome (Leica®), and processed for c-Fos immunohistochemistry. The procedures involving the animals and their care at the Experimental Neurology Laboratory of the Federal University of São Paulo (CEP Protocol 0401/11) conformed with the institution's guidelines, which comply with the International Ethical Guidelines for Biomedical Research [14].

A Nikon E600FN microscope equipped with a digital camera was used for image digitalization. Quantification of c-fos immunolabeled cells was performed using the ImageJ software (National Institutes of Health, Bethesda, MD, USA) by an individual blinded to the treatments. Following background subtraction, the threshold was determined so that all labeled nuclei could be recognized. Quantitative analysis was performed on three sections from every animal (the mean count for each animal was used in the statistical analysis). The appropriate areas were identified according to the atlas of Paxinos and Watson [15]. Cell densities were obtained by dividing the total number of positive cell nuclei by the area of the region examined at 20× magnification. The amount of c-fos immunolabeled cells (expressed as the average number of cells per mm^2) was compared between groups by means of a Kruskal–Wallis one-way analysis of variance (with a Mann Whitney *U* test for individual comparisons).

3. Results

Fig. 1 shows representative images of c-Fos expression from control, CO_2 chamber, and experimental rats. Qualitative analysis in c-Fos expression showed that the lateral–posterior nucleus is not active in the control condition. On the other hand, the generalized seizure induced by pilocarpine injection activated the lateral–posterior nucleus after a few minutes from the tonic–clonic seizure initiation. The apnea caused by the CO_2 chamber did not induce increased activation in this nucleus, indicating that the activation of lateral–posterior neurons was caused by the seizure per se (see the Discussion section for details). Fig. 2 shows box plots representing the quantification of c-fos expression for each group. The Kruskal–

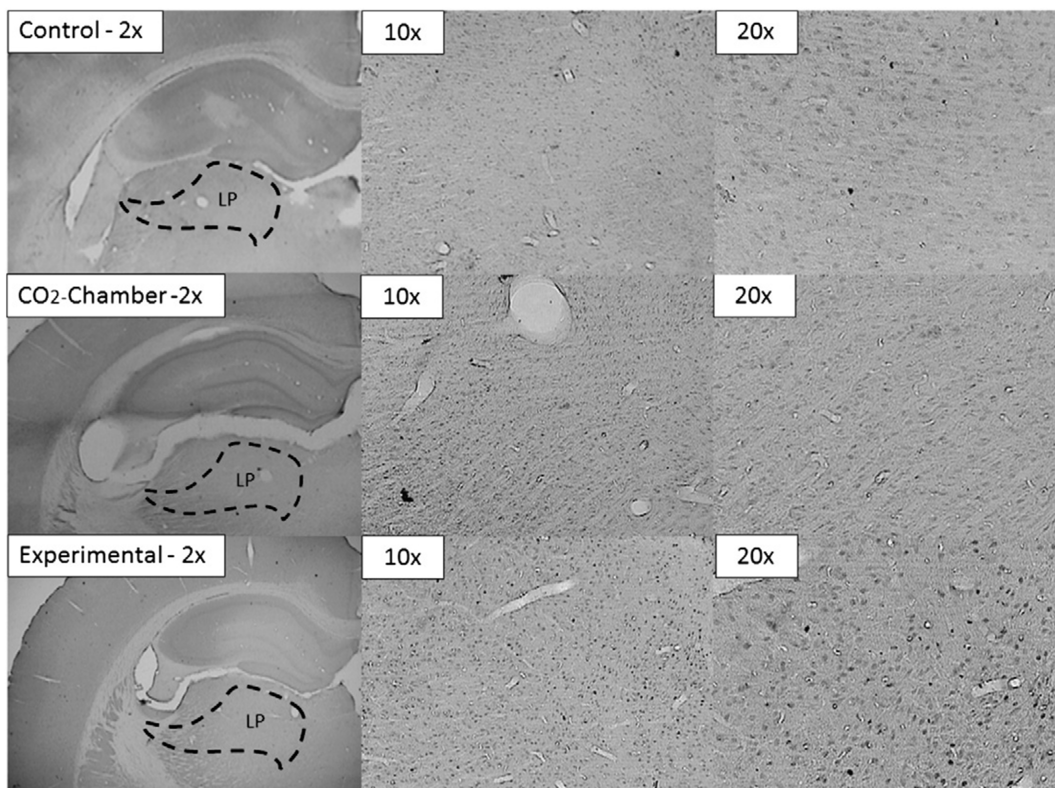

Fig. 1. Representative digital images (coronal sections) of c-Fos immunolabeled neurons in the lateral–posterior nucleus of the thalamus (LP) for the control (upper panels), CO_2 chamber (middle panels), and pilocarpine (bottom panels) groups. Note the increased c-Fos expression for the experimental group (pilocarpine-induced seizure, bottom images) as compared with the control and CO_2 chamber groups.

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