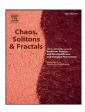
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Detrended fluctuation analysis of compound action potentials re-corded in the cutaneous nerves of diabetic rats



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ABSTRACT

The electrophysiological alterations in nerves due to diabetes are classically studied in relation to their instantaneous frequency, conduction velocity and amplitude. However, analysis of amplitude variability may reflect the occurrence of feedback loop mechanisms that adjust the output as a function of its previous activity could indicate fractal dynamics. We assume that a peripheral neuropathy, such as that evoked by diabetes, the inability to maintain a steady flow of sensory information is reflected as a breakdown of the long range power-law correlation of CAP area fluctuation from cutaneous nerves. To test this, we first explored in normal rats whether fluctuations in the trial-to-trial CAP area showed a self-similar behavior or fractal structure by means of detrended fluctuations analysis (DFA), and Poincare plots. In addition, we determine whether such CAP fluctuations varied by diabetes induction. Results showed that CAP area fluctuation of SU nerves evoked in normal rats present a long term correlation and self-similar organization (fractal behavior) from trial to trial stimulation as evidenced by DFA of CAP areas. However, CAPs recorded in diabetic nerves exhibited significant reductions in area, larger duration and increased area variability and different Poincare plots than control nerves. The Hurst exponent value determined with the DFA method from a series of 2000 CAPs evoked in diabetic SU nerves was smaller than in control nerves. It is proposed that in cutaneous nerves of normal rats variability of the CAP area present a long term correlation and self-similar organization (fractal behavior), and reflect the ability to maintain a steady flow of sensory information through cutaneous nerves. Nevertheless, this is not the case for sural nerves of diabetic rats which is reflected as a breakdown of the long range power-law correlation of CAP area fluctuation. Nonlinear time series analysis of CAP area fluctuations is a valuable new

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insight tool that can be used for the study of alterations in transmission of sensory information in humans suffering diabetes or under other demyelination diseases.

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1. Introduction

Diabetes mellitus is one of the most devastating diseases nowadays and the incidence is increasing substantially worldwide [1]. Because exists a close link between diabetes mellitus and cardiovascular disease it is one of the principal causes of death in the world [2]. This disease is characterized by a persistent or chronic high concentration of glucose in blood (hyperglycemia) and it is associated to a dysfunction of pancreatic β cells to produce insulin (principal regulator of glucose consumption by body cells) [3].

Peripheral nervous system (PNS) disorders are the most frequent long-term complications of Diabetes mellitus diseases [3,4]. Large-diameter myelinated sensory axons (mainly $A\alpha$ and $A\beta$ fibers) are the most affected fibers in nerves by such diseases [4]. In long-term diabetic peripheral neuropathies (DPN), the peripheral axons show a separation of their myelin sheet and a segmental demyelination process eventually occurs [5]. These changes provoke sensory deficits, such as reduced vibration and tactile perception [6], and the presence of unmyelinated sensory nerve fibers may result in neuropathic pain and dysesthesias [5,7]. The time course of electrophysiological alterations affecting the PNS of diabetic rats has been well established [4]. Deficits in motor and sensory nerve conduction velocity begin to be detected within weeks after diabetes onset [8], increased at 2-3 months and remain relatively stable thereafter [9–13]. A chronic exposure to experimental diabetes (more than 2 months after induction), biochemical and vascular changes in the microenvironment of nerves produced axonal degeneration and loss of myelinated fibers, leading to a reduction in the propagation of action potentials [14,15]. However, at the early stages of diabetes, deficits in conduction velocity of action potentials in peripheral sensory nerves do not match with axonal loss, fascicular area or myelinated fiber density [16,17]. These deficits are associated with changes in the intrinsic properties of ionic channels in the axon membrane [18-22]. The evidence concerning electrophysiological alterations in nerves due to diabetes is classically analyzed in relation to the instantaneous frequency, conduction velocity and amplitude of the compound action potential (CAP) [14,15,23]. It is considered that CAP responses are the sum of propagated action potentials in a population of individual axons contained in nerves [8]. In this study, we use detrended fluctuation analysis (DFA) with the Hurts exponent in combination with a Poincare analysis to determine the fluctuations in area of a series of consecutive CAPs evoked in the sural nerve of diabetic and normal rats, because it allows the determination of the fractal organization which may reflect the occurrence of feedback loop mechanisms that adjust the output as a function of previous activity [24]. Mathematical analysis of physical systems have shown long range power-law correlations in apparently random fluctuations which indicate that processes at particular temporal scales are linked to those at other scales (scale-invariance) [24]. This selfsimilar behavior and long term correlation of signals are features of fractal dynamics [25]. Fractal concept is associated with irregular non-Euclidean objects that display selfsimilarities at all scales. Examples of fractal structures as varied as the distribution of stars in the universe, the alveolar lung branching, diffuse boundary of a cloud, price fluctuations in the stock market, etc. However, for real world data upper and lower bounds need to be applied in order to see such scale-invariant behavior. The fractal tools today are irreplaceable elements in the work of physicists, chemists, biologists, physiologists, economists, etc. DFA has been applied in an extended variety of themes such as social phenomena like the evolution of language [26], inheritance patterns [27], and in human cultures [28]. In addition it has been amply used to study the kinetics of ion transport across neuronal membranes [29]. H reflex [30]. spontaneous cord dorsum potentials [31] and in cerebral and cerebellar activity [32]. In addition, fractal analysis of diseases and aging demonstrate the occurrence of impairment in the structural dynamics of organisms [33]. We assume that a peripheral neuropathy, such as that evoked by diabetes, the inability to maintain a steady flow of sensory information is reflected as a breakdown of the long range power-law correlation of CAP area fluctuation in cutaneous nerves. To test such hypothesis, this study was aimed to explore whether fluctuations in the trial-to-trial CAP area show a self-similar behavior or fractal structure by means of a DFA and determinate whether such CAP area fluctuations and long term correlations changed during a four week period of diabetes induction in the rat.

2. Materials and methods

2.1. Animals and diabetes induction

Male Wistar rats weighing 240 ± 10 g (6 weeks old) provided by the Animal House Facility of our institution were used. Animals were kept during two weeks in clear-sided acrylic cages under the following environmental conditions: Controlled room temperature ($22 \pm 2^{\circ}$ C); light and darkness cycles of 12 h; minimal noise and handling. During this period, the animals were fed with rodent diet lab pellets (Nutritional International, Brent Wood, MO, USA) and water ad libitum. After the adaptation period, the rats were randomly assigned to the following groups: control group (C): 10 rats received an injection of the citrate buffer solution. Diabetic group (D): 23 rats received an intraperitoneal injection of 60 mg/kg body weight of Streptozotocin (STZ; Sigma Chemical Co. St Louis, USA) diluted in sterile citrate buffer (0.1 M, pH 4.5). STZ is a chemical

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