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Lynn Carr, Sylvia M. Bardet, Malak Soueid, Delia Arnaud-Cormos, Philippe Lévêque, et al.. Calcium-independent disruption of microtubule growth following nanosecond pulsed electric field exposure in U87 human glioblastoma cells. BIOEM, BEMS and EBEAM, 2015, Pacific Grove, United States. hal-02469304

HAL Id: hal-02469304

<https://hal.science/hal-02469304>

Submitted on 14 Feb 2020

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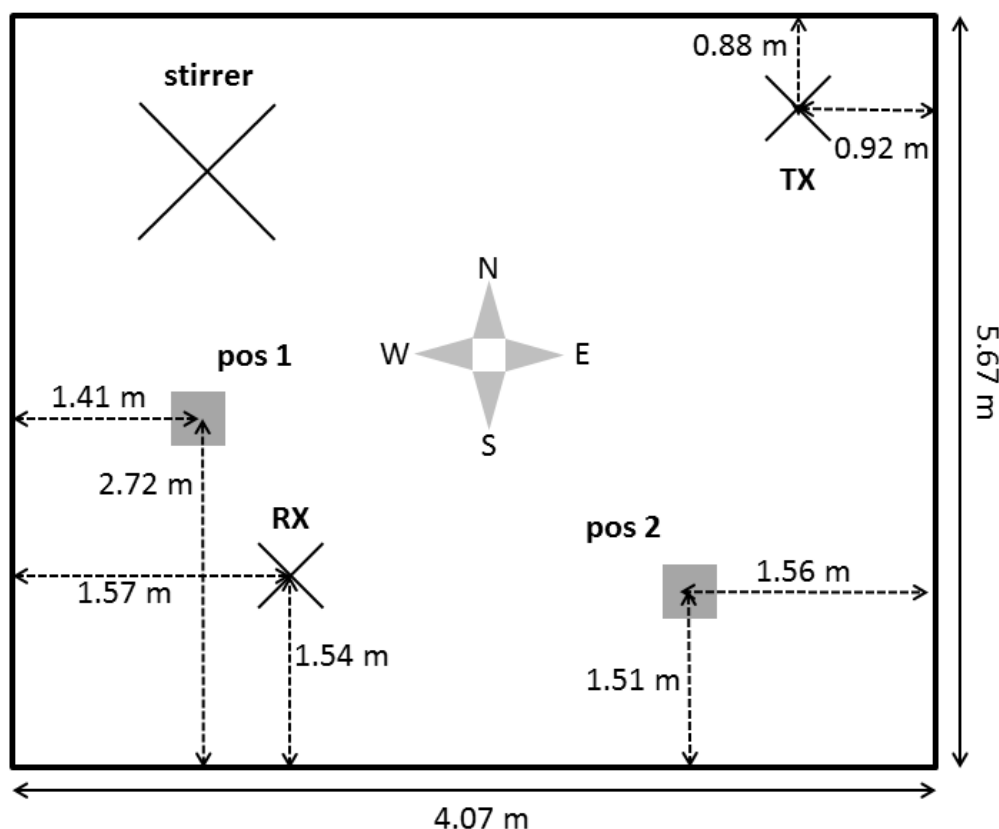


Figure 1. Top view of the measurement set-up in the reverberation chamber (not to scale). The measurement positions (pos 1 and 2) are indicated by grey squares.

PA-91 [19:00]

STUDENT PAPER

Calcium-independent disruption of microtubule growth following nanosecond pulsed electric field exposure in U87 human glioblastoma cells

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Keywords: *In vitro*, Pulsed, Work in Progress

Nanosecond pulsed electric field (nsPEF) exposure causes apoptosis in cancer cells via a currently unknown mechanism. We used live cell imaging to show that 100, 10 ns, 15 kV/cm pulses, applied at 10 Hz to U87 EB3-GFP glioblastoma cells, results in calcium-independent disruption of microtubule growth. Microtubule depolymerization is a key event in apoptosis execution, making the effect we report on the microtubule network a candidate for the mechanism behind nsPEF induced apoptosis.

Introduction

High powered, nanosecond duration pulsed electric fields (nsPEF) have been proposed as a minimal side-effect, electrical cancer therapy that is unlikely to result in resistance. In vitro and in vivo studies have demonstrated that nsPEF are able to induce apoptotic death in cancerous cells and reduce tumor size [1,2]. However, the mechanism of how nsPEF triggers apoptosis remains unknown.

Microtubules form part of the cell cytoskeleton and their depolymerization is an intrinsic, early event in normally occurring apoptosis[3]. Electric fields have been shown to disrupt microtubule polymerization[4,5]. Given this, it is therefore possible that nsPEF cause microtubule depolymerization which initiates apoptosis.

In this study we follow the microtubule plus end tracking protein EB3-GFP with live cell imaging to visualize microtubule growth dynamics in U87 glioblastoma cells. We demonstrate that 100, 10ns pulses delivered at a frequency of 10 Hz cause a rapid disruption of microtubule growth. We also show that this effect is not dependent on influx or release of calcium, a

known microtubule destabilizer.

Methods

U87 cells were transfected using LentiBrite™ EB3-GFP Lentiviral Biosensor (Merck Millipore) and observed by epifluorescence using a Leica DMI6000 microscope with a 100x objective. Fluorescent excitation was provided by a Spectra 7 light engine (Lumencor). Emitted light was filtered and captured on an electron-multiplying charge-coupled device camera (EMCCD Evolve 512, Roper) with 512 x 512 pixels. Changes in intracellular calcium were measured in U87 cells loaded with 1.25 μM Fura Red AM (Life Technologies). Standard HBSS containing either 1.8 mM of calcium or 4 mM EGTA (calcium free) were used as imaging buffers. Intracellular calcium stores were depleted by incubating cells for 30 minutes in calcium free imaging buffer containing 1 μM thapsigargin.

Images were analyzed using Image Analyst MKII (Image Analyst Software). After background subtraction, EB3 fluorescence intensity was measured in a region of interest drawn within the cell proximal to the microtubule organization center.

100, 10 ns, 15 kV/cm pulses were applied to cells at a frequency of 10 Hz using an nsPEF generator with 50Ω output impedance. A high-voltage measurement device (tap-off) connected to a digital phosphor oscilloscope was used to visualize the time-domain measurements of the pulse. Pulses were applied by positioning an electrode delivery system with a micromanipulator (Sutter MP285) comprising of two steel electrodes, separated by a gap of 1.2 mm and with a 50Ω impedance in parallel.

Results

The effect of nsPEF on cellular microtubule dynamics was studied using U87 cells stably expressing EB3-GFP. Disruption of microtubule growth, shown by a decrease in EB3 fluorescence, was observed following the application of 100, 10 ns pulses at 10 Hz (figure 1). As soon as 3 minutes after pulse application, a statistically significant decrease in EB3 fluorescence was observed in nsPEF pulsed samples, as compared to non-pulsed controls ($t = 2.64$, $p = 0.02$, $n = 11$).

Measuring calcium changes in pulsed U87 cells loaded with Fura Red AM showed a 50% decrease in fluorescence by the end of the 10 second pulse period (decrease in Fura Red AM fluorescence corresponds to an increase in intracellular calcium concentration). The increase in intracellular calcium was due to both an influx of calcium from the imaging buffer and release from internal stores. As calcium is known to depolymerize microtubule we sought to identify if it was the source of the observed microtubule growth disruption. U87 EB3-GFP cells were pulsed in either a calcium free imaging buffer, containing the calcium chelator EGTA, or in the same calcium free imaging buffer with their internal calcium stores depleted by thapsigargin treatment. Under both conditions a post-pulse decrease in microtubule growth, similar to that seen with calcium, was observed (figure 2). 5.5 minutes after pulse application there was no significant difference in relative EB3 fluorescence between any of the pulsed cell conditions. All three pulsed conditions were significantly different to the non-pulsed control; 1 way analysis of variance with Bonferroni test $f(5,27) = 17.93$, $p = 4.15 \times 10^{-7}$. Absence of post-pulse increases in intracellular calcium concentration, in both calcium free conditions, was confirmed by Fura Red AM imaging.

Discussion and Conclusion

In this study we show a calcium-independent disruption of the cellular microtubule network following nsPEF exposure.

Microtubule depolymerization has previously been reported in nsPEF treated cells with the authors attributing it to an influx of extracellular calcium[6]. Our results, however, using a different cell type and pulse regime, suggest an alternative mechanism, such as a direct destabilizing action of nsPEF on microtubules or modulation of one of the many microtubule-associated proteins that are linked to microtubule stability. Experiments carried out on cell-free polymerized microtubules have shown that these biological nanostructures are sensitive to MV/m static electric fields⁵ and theoretical simulations suggest microtubules possess electromechanical properties in the microwave range[7].

Given the essential role of microtubules in apoptosis execution we propose that microtubule depolymerization could be an important factor in nsPEF-induced apoptosis. Identifying the mechanism behind the depolymerization remains the focus of our future work.

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Figures

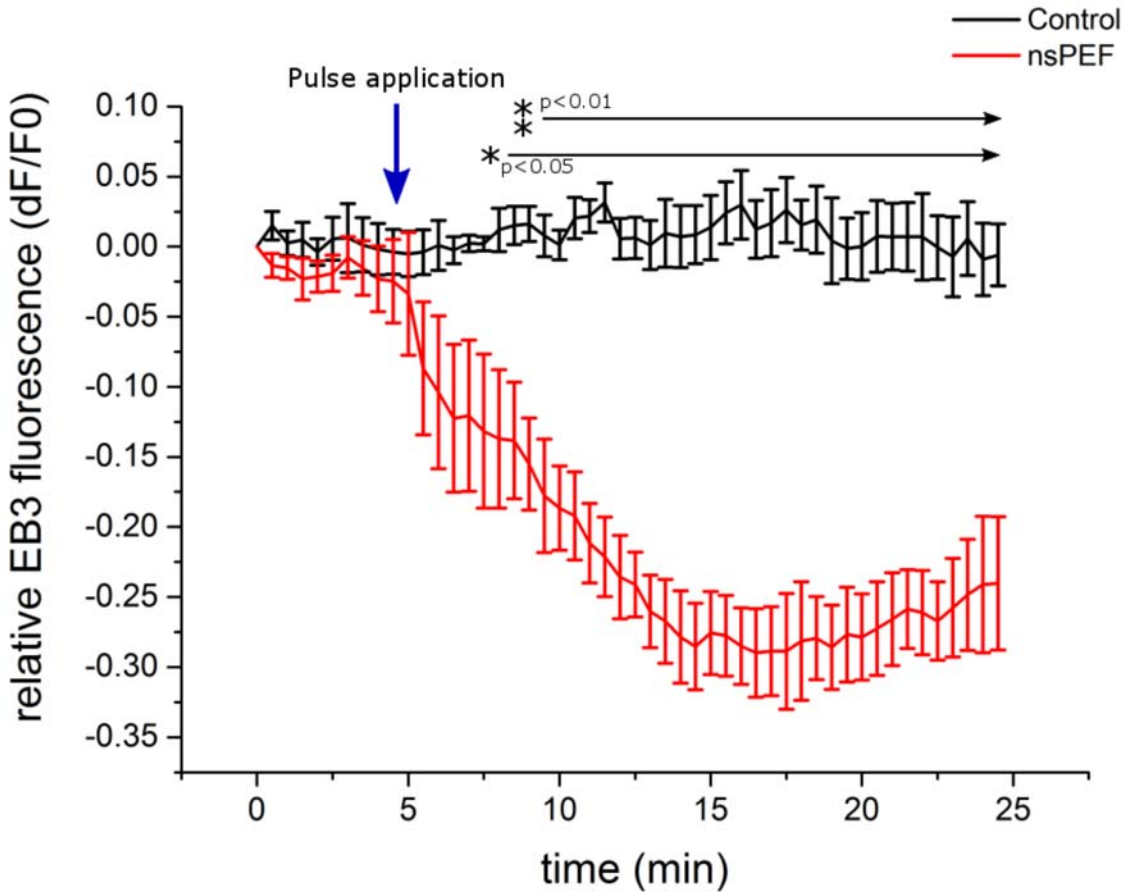


Figure 1. Disruption of microtubule growth by nsPEF treatment. 100, 10 ns pulses delivered at a frequency of 10 Hz were applied to U87-EB3-GFP glioblastoma cells. For control experiments electrodes were placed but no pulse was administered. Cells were bathed in imaging solution containing 1.8 mM Ca²⁺. Control and nsPEF treated cells showed significantly different responses in microtubule growth as measured by EB3 fluorescence: * p < 0.05, ** p < 0.01 (2 sample t-test).

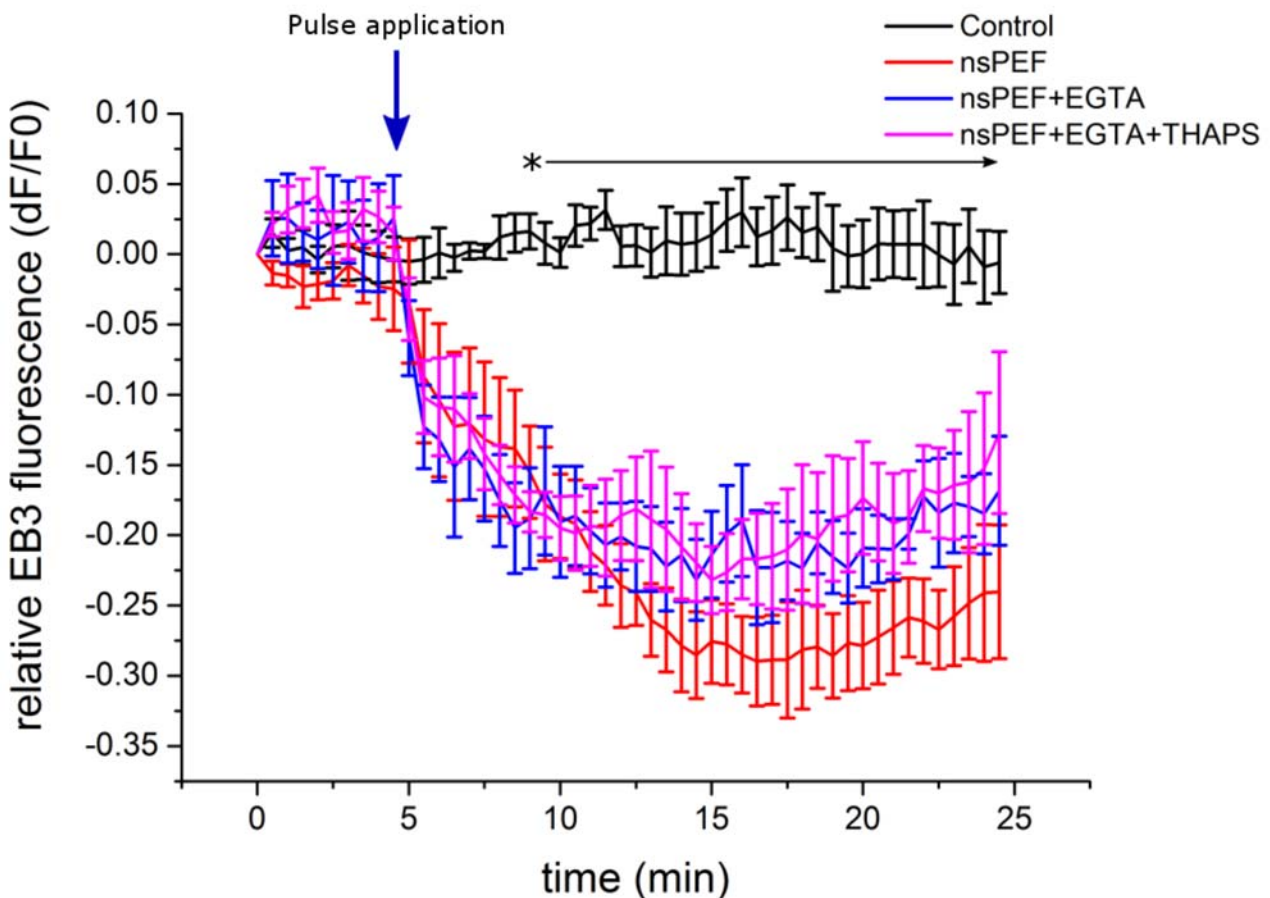


Figure 2. nsPEF induced disruption of microtubule growth is independent of calcium. 100, 10 ns pulses delivered at a frequency of 10 Hz were applied to U87-EB3-GFP glioblastoma cells. nsPEF condition: cells pulsed in imaging solution containing 1.8 mM Ca²⁺; nsPEF + EGTA condition: cells pulsed in calcium free imaging solution containing 4 mM of the calcium chelator EGTA; nsPEF + EGTA + THAPS condition: cells pulsed in calcium free imaging solution with internal calcium stores depleted by thapsigargin. For control experiments electrodes were placed but no pulse was administered. * indicates a significant difference between pulsed conditions and control and no significant difference between the different pulsed conditions $f(5,27) = 17.93$, $p = 4.15 \times 10^{-7}$ (1 way analysis of variance using a Bonferroni test).

PA-93 [19:00]

A questionnaire-based survey of a French group self-reporting Idiopathic environmental intolerance attributed to electromagnetic fields (IEI-EMF)

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Keywords: Clinical (diagnostics), RF/Microwaves, Work in Progress

In France, a debate around electro-hypersensitivity is growing these last decades. Our objective was to analyze the reported symptoms and the incriminate sources that might induce these symptoms by a questionnaire-based study. From April to November of 2014, Fifty four auto diagnosed EHS across France respond to our questionnaire. Thirty belonged to different French self-help groups or associations and 22 were not attached to any groups. We had a return rate of 61%.

Introduction:

Increasing use of electric power and wireless telecommunications, leads, obviously, to greater human exposure to electromagnetic field. Some individuals experience adverse effects while using or being in the vicinity of devices or equipments emitting electric, magnetic or electromagnetic fields. This sensitivity to electromagnetic fields is known as electromagnetic hypersensitivity (EHS).

Objective:

The aim of our study was to analyze the subjective reported experiences of French people who describe themselves as suffering from EHS as there have been no previous studies about EHS symptoms and treatments in France.

Population: