VILNIUS UNIVERSITY

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# LOW-DOSE IONISING RADIATION EFFECT ON THE ELECTROPHYSIOLOGICAL PROPERTIES OF *NITELLOPSIS OBTUSA* CELLS

Summary of doctoral dissertation

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VILNIAUS UNIVERSITETAS

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# MAŽŲ JONIZUOJANČIOSIOS SPINDULIUOTĖS DOZIŲ POVEIKIS MENTURDUMBLIŲ *NITELLOPSIS OBTUSA* LĄSTELIŲ ELEKTROFIZIOLOGINĖMS SAVYBĖMS

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**CONTENTS**

## ABBREVIATIONS

Al – Aluminium

AP – action potential

APW – control solution (Artificial Pond Water)

E – membrane potential

HTO – tritiated water

HEPES – 4-(2-hydroxyethyl) piperazine – 1 – ethanesulfonic acid

INPP – Ignalina Nuclear Power Plant

IR – ionizing radiation

MAPK – mitogen-activated protein kinase

OBT – Organically bound tritium

PM – plasma membrane

RIBE – radiation induced bystander effect

ROS – reactive oxygen species

RPC – Radiation Protection Centre

TRIS – tris (hydroxymethyl) aminomethane

$H^+$– ATPase - proton pump

Na, K, Cl, Ca, Al – chemical elements

$Na^+$, $K^+$, $Cl^-$, $Ca^{2+}$ $Al^{3+}$ – ions

## 1. INTRODUCTION

Ionizing radiation (IR) constantly affects all of nature. In addition to natural background radiation, the application of ionizing radiation in different areas of human activity and the subsequent formation of radioactive waste cause additional radioactive pollution, thus endangering the environment. Unfortunately, for a long time worldwide, the radiation protection standards established for humans were believed to provide adequate protection to all other species of the living environment (ICRP, 1998). However, this assumption was not supported by sufficient scientific evidence; thus, radioprotection of the environment should be demonstrated independently of that for humans to maintain biodiversity and sustainability (Delistraty, 2008). Therefore environmental radiation protection is of international concern and the regulatory agencies have only intensively worked on developing radiation protection regulations for non-human biota for the last decade (Garnier-Laplace et al., 2013). During this period, selected groups of reference plants and animals were used, and the dose rate limits for these groups were adopted (ICRP, 2008; Luksiene et al., 2013). However, the lack of scientific data about the effects of radiation on non-human species continues to be one of the problem delaying the establishment and implementation of sufficient radiation protection of the environment (Dallas et al., 2012). In particular, more assessments of the effects of low-dose radiation determined by higher natural background radiation, medical, occupational, and accidental exposure are required. The effects induced by low-dose radiation are not just a smaller response than those from high-dose radiation, and the dose-response relationship is not linear; therefore, the effects are often difficult to explain (Geras'kin et al., 2007; UNSCEAR, 2012). It has been estimated that IR at low-doses can be beneficial for living organisms via stimulation of growth, cell proliferation, activation of reparation mechanisms and other essential physiological processes (so called radiation hormesis) (Feinendegen, 2005). On the other hand, it was showed that low-dose ionizing radiation can influence the sensitivity of living organisms in response to other environmental factors (Burlakova, 1995). The other low-dose IR phenomenon is associated with induction of biological effects in cells that are not directly affected by IR as a result of signals transduction from close neighbouring irradiated cells (radiation induced bystander effect – RIBE) (Prise et al., 2003). Investigations of above mentioned phenomenon provide different and even contradictory results, leading to long-lasting

debates at the International Atomic Energy Agency (IAEA), the International Commission on Radiological Protection (ICRP) and other organizations. For example, there is no consensus whether RIBE is one of the adaptive mechanisms needed to protect an organism against development of radiation effects (Mothersill et al., 2005; 2006) or on the contrary it enhances the biological effectiveness of IR via propagation of radiation injury within the organism (Shemetun et al., 2007). Similar contra version occurs in the interpretation of low-dose IR ability to change the response of an organism to abiotic stress. Some researches argue that exposure to low doses of IR initiates an adaptive response and determines further higher resistance of the organism to the effects of other factors including high dose IR (Adelstein, 2001; Marples et al., 1997). Another statement is that weak IR can innate organism's resistance to environmental stress (Dmitriev et al., 2009) or enhance biological toxicity of contaminants (Daňová et al., 2010; Rety et al., 2010). Evaluation of these properties of low-dose IR is very important in the context of mixed anthropogenic pollution of the environment, where different multiple interactions between contaminants subsequently determine bioaccessibility, bioavailability and biotoxicity of consisting pollutants (Fairbrother et al., 2007). Aluminium (Al) is the third most abundant metal in the Earth's crust and is very common in the environment. Moreover, it content in biosphere rises due to technogenic pollution. Al can be found in all living organisms without defined physiological implication (Poschenrieder et al., 2008); meanwhile cancerogenic and neurotoxic effect of Al has been found in human and animals (Darbre et al., 2013; Kawahara et al., 2011; Martac et al., 2010). In plants Al inhibits root growth, photosynthesis, nutrient metabolism (Alvim et al., 2012; Gupta et al., 2013; Mossor-Pietraszewska, 2001; Panda et al., 2009; Rout et al., 2001; Silva et al., 2012). However Al biological behaviour in interaction with other environmental factors including IR is not well studied. Few studies evidenced modifying action of IR on Al biological toxicity. One investigation showed that combined effect of Al and weak γ radiation enhances expression of GFAP in rats brain more intensively than occurs due to separate effect of both Al and IR. (Nedzvetskii et al., 2001). Other investigations also showed synergetic interaction of Al and IR; it was found that Al and low-dose γ radiation alone reduced the clonogenic survival of HPV-G reporter cells from explants of salmon skin or gill reporters by approximately 50 %; however, the combination of both was much more toxic (Mothersill et al., 2014).

Nevertheless mechanisms of these effects as well as low dose IR induced phenomenon remains not clear and necessitates further full-scale investigations.

Electrophysiological investigations of the properties of a plant cell plasma membrane (PM) may be a useful technique for improving our understanding of signalling pathways and may help to describe the underlying processes of acclimation and adaptation in cells that are exposed to environmental stress, including IR. The PM of plant cells constitutes not only a barrier, but also a primary target for the action of exogenous stimuli that can alter the membrane potential (E) by directly acting on the membrane structure or by affecting the ion channels, involved in electrogenesis (Tekpli et al., 2013). Radiation-induced alterations in the supramolecular organisation of the membrane, surface charge, proteins and receptors activities play a significant role in the development of radiation injury; these changes could be considered when evaluating cell disturbances induced by various types and intensities of radiation, including the risk from low-dose radiation (Cramp et al., 1994; Somosy, 2000). The generation of electrical gradients across the PM is a fundamental aspect of signal transduction, and changes to these gradients or to ion flux are amongst the earliest cellular events within a plant in response to ionizing radiation exposure. Furthermore, plants can respond to the various stimuli in the extracellular environment by activating and propagating fast electrical signals, i.e., action potentials (APs), similar to those identified in animal nervous systems (Baluska et al., 2013). The AP is an important bioelectrochemical signal that induces several changes in plant physiological processes, including photosynthesis, elongation, growth, water uptake, respiration, phloem transport, and gene expression, as well as in long-distance actions within a plant. Thus, AP is capable of informing distant cells about local irritants, allowing them to generate an appropriate response (Sukhov et al., 2011). The shape, duration, amplitude, and excitation threshold of AP can differ according to the modality, nature, intensity, and other parameters of the external stimuli (Fromm et al., 2007). Such differences appear to be stimulus-specific; thus, APs can be used as biomarkers of environmental toxicity. Therefore, plants can be used as fast-reacting biosensors for environmental monitoring and for real - time detection of the effects of different pollutants (Volkov et al., 2006). The capability of high-dose ionizing radiation to induce AP and other electrophysiological changes in algae has been demonstrated previously (Röttinger et al., 1972). Algae have also been reported to be

highly sensitive to environmental contamination and could be used as bio-indicators in radiotoxicological studies (Adliene et al., 2006). The green algae *Nitellopsis obtusa* has been successfully used as indicator of water quality for radioecological monitoring in an aquatic ecosystem (Nedveckaite et al., 2007, 2011). In addition, these internodes appear to be an ideal material for many physiological observations and electrophysiological studies (Shimmen et al., 1994). The rapid depolarization of the membrane potential has been shown to correlate with electrophysiological responses and contaminant toxicity (Manusadzianas et al., 2002). These Charophytes generate APs in response to mechanical stimulation, injury, and similar factors or in response to direct electrical stimulation. The bioelectrical response of a Charophyte cell is rapid and highly sensitive to even very small exogenous stress factors, and it can be used to assess the toxicity and consequences of low-dose radiation exposure at the cellular level.

Tritium is an important source of IR in aquatic environments. This low-energy beta emitter with a physical half-life of 12.3 years can be formed by the action of cosmic rays on the atmosphere or can be released to the environment from nuclear reactors, accelerators, nuclear weapon manufacturing, and the radio-labelled materials that are utilised in medicine, research and industry. Interest in and concern about tritium has developed because of its ubiquity and mobility in environmental and biological systems because tritium can occur as water (tritiated water - HTO) and its behaviour is identical to that of hydrogen (Melintescu et al., 2011). After being incorporated into a cell, tritium can be partially transformed into organically bound tritium with a very different behaviour (Galeriu et al., 2013; Kim et al., 2013). As a source of ionizing radiation, HTO can damage an organism by directly ionizing cell molecules or indirectly via the production of reactive oxygen species (ROS). The biological effectiveness of tritium radiation is greater than that of γ or X rays (Little & Lambert, 2008); thus, this radiation occasionally tends to affect living organisms even at a very low dose and at levels below the predictable effective dose rate threshold. Accidental release of tritium, caused by human activity is likely to increase in the near future due to development of nuclear energy; thus it can expose aquatic ecosystems to higher tritium concentrations; therefore, extensive assessment of the potential radiological risks to the environment from accidental tritium exposure is increasingly necessary and requires an appropriate experimental database (Boyer et al., 2009). For example, the Fukushima nuclear accident

in 2011 caused a significant prolonged release of tritium into the Pacific Ocean and ground waters, thus endangering the aquatic environment. This also applies to Lithuania. Lithuania lies on the eastern coast of the Baltic Sea, which contains the highest level of radioactive contamination compared to that of other large reservoirs in the world, and tritium concentration here varies from 4.1 Bq $L^{-1}$ to 43 Bq $L^{-1}$. This region of high tritium concentration is dangerous due to the potential risk of tritium to be transfered through the biotic food chain to other sites in the country that currently have a low level of tritium (Bradshaw et al., 2013). Furthermore, during the operation of the Ignalina Nuclear Power Plant (INPP) in Lithuania, the highest tritium activity concentration in its cooling pond was reached: 24 Bq $L^{-1}$. Meanwhile, the activity concentration in the background water was 2-3 Bq $L^{-1}$; thus, approximately 20 Bq $L^{-1}$ originated from the INPP (Nedveckaite et al., 2011). In addition, the 2005-2012 evaluation of radionuclide transfer from the near-surface Maisiagala radioactive waste disposal facility to the biosphere showed, that tritium contaminated drinking and farming water, becoming the main source of local population contamination (Nedveckaite et al., 2013). The decommissioning of INPP and the plans to build a new nuclear power plant implies additional risk for the accidental release of tritium to the environment and require a comprehensive investigation of the risk to aquatic biota from tritium exposure (Jean-Baptiste et al., 2013).

### 1.1. Aim and tasks of the study

The aim of the present study was to investigate how exposure to low-dose ionizing radiation alters vital bioelectrical properties of intact Charophyte cells.

The tasks to accomplish the aim of the study were as follows:

1. To investigate the effect of low-dose tritium on:
   - resting potential and specific membrane resistance of *Nitellopsis obtusa* cells;
   - dynamics of action potentials in Characeaen *Nitellopsis obtusa* cells;
   - $Cl^-$ and $Ca^{2+}$ transport during the electrical excitation of *Nitellopsis obtusa*
2. To investigate the combined effect of tritium and aluminium on the activity of $K^+$, $Cl^-$ and $Ca^{2+}$ transport systems involved in *Characeaen* electrogenesis
3. To investigate the bioelectrical expression of low-dose ionizing radiation induced bystander effects in two adjoining internodal cells of *Nitellopsis obtusa*

## 1.2. Novelty and significance of the study

1. For the first time the analysis of the effect of low-dose tritium on the membrane bioelectrical properties of Characeaen cell, as a model plant system was accomplished.
2. For the first time the investigation of combined effect of tritium and aluminium on the membrane ion transport systems involved in electrogenesis of *Nitellopsis obtusa* cells was accomplished.
3. For the first time ionizing radiation induced bystander effects were investigated using analysis of electrophysiological characteristics of *Nitellopsis obtusa* cells.

Obtained results showed that electrophysiological investigations of charophyte cells could provide a useful tool in assessing the risk from low-dose ionizing radiation to a single plant cell *in vivo*. Due to responsive bioelectrical reactions of *Nitellopsis obtusa* to ionizing radiation, it could be used for practically considering as sensitive parameters for radioecological monitoring of the aquatic environment and assessment of radiation exposure to non-human biota

## 1.3. Statements to be defended

1. Low-dose tritium alters bioelectrical activity of *Nitellopsis obtusa* cells, therefore electrophysiologycal parameters of Charophyte could provide a useful endpoint in assessing the effect of low-dose ionizing radiation to a single plant cell *in vivo*
2. Bioelectrical response of *Nitellopsis obtusa* cells to aluminium exposure changed due to tritium addition, therefore, tritium could be considered as an important factor in aluminium phytotoxicity expression.
3. Electrophysiological investigations of *Nitellopsis obtusa* cells could provide a useful tool in studying mechanisms of radiation induced bystander phenomenon *in vivo.*

## 2. MATERIALS AND METHODS

**Model organism**. Starry stonewort *Nitellopsis obtusa* (Devs.) J. Gr. cells were used for all experiments. A giant cell that is isolated from a plant can be considered to be a single organism wich can maintain essential physiological functions for a long time while kept in APW (artificial pond water), and each cell is about 10 cm length and 1 mm in diameter of regular cylindrical shape; these characteristics make these giant algae cells useful for laboratory studies (Beilby et al., 2014). The *Nitellopsis obtusa* were collected in Siesartis Lake (Lithuania) and stored at room temperature in a glass aquarium containing tap water

**Chemicals and sources of ionising radiation.** The control solution in the experiments was APW containing 0.1 $M^{-3}$ KCl, 1.0 $M^{-3}$ NaCl, 0.1 $M^{-3}$ $CaCl_2$, and 2.5 $M^{-3}$ TRIS (tris (hydroxymethyl) aminomethane), adjusted to a 7.2 pH by HEPES (4-(2-hydroxyethyl) piperazine – 1 – ethanesulfonic acid) or HCl. All chemicals were of analytical grade and were purchased from Sigma-Aldrich. The HTO solution was prepared from basic APW supplemented with HTO (248.65 kBq $L^{-1}$ activity concentration, Isotrak, Braunschweig, Germany). The basic HTO solution was freshly diluted to the desired activity concentration for each experiment in the Radiation Protection Centre (RPC) in accordance with all radiation protection requirements for Lithuania. The HTO activity concentration was estimated by liquid scintillation (Quantilus 1220, Wallac Oy, Turku, Finland; detection limit: 30 mBq) after the addition of an Optiphase Hisafe 3 scintillation cocktail (Packard Instrument, Waltham, USA) at the RPC using the standard procedure described in LST ISO 9698:2006, which is identical to ISO 9698:1989. Aluminium solution was freshly prepared from the basic APW supplemented with $AlCl_3$ (pH 7.2).

In the experiments on the combined effect of aluminium and tritium, pH 7.2 of all solutions was maintained by HCl instead of HEPES to keep constant concentration of chloride.

Sealed source of ionizing radiation (radioactive strontium isotope Sr 90 with 74 kBq radioactivity) was used in the bystander experiments. The applied radioactivity on the investigated plant cell surface was evaluated using Geiger Muller counter (Phywe, Germany).

**Experimental procedures**. The experiments were performed at room temperature (20±1°C) under daylight conditions (500±10 Lx). The internodal cells were isolated from the neighbouring cells and branchlets. The internodes were kept in the buffered APW at least overnight.

Conventional intracellular methods of microelectrodes ant voltage clamp technique was applied to investigate the impact of tritium, combined exogenous exposure to aluminium and tritium on the bioelectrical properties of the plant cell plasma membrane and RIBE bioelectrical expression in adjoining internodal cells of *Nitellopsis obtusa.*

During the experiments, cells were placed in a plexiglass chamber and continuously bathed in a solution of APW or the test solution that flowed at a rate of approximately 1 ml $min^{-1}$. *Nitellopsis obtusa* is an aquatic plant, and the electrical measurements were performed under their native external condition, an aquatic solution. Reference electrode filled with 3M KCl in agar-agar jelly, was immersed into the experimental solution near the cell and the microelectrode (with 1μm tip diameter) filled with 3M KCl was inserted into the cell to measure electrical potential differences between outside and inside the intermodal cell. The parameters and specifications of the experimental techniques are thoroughly described in our previous studies (Kisnierienė et al., 2009; 2012). Measurements of electrical properties of the PM were started when membrane resting potential was constant for 30 min. The pCLAMP 10 software package was used for controlling the hardware of the data acquisition device, acquiring data, writing data to the computer memory and displaying the data in real time.

The experiments were performed in current-clamp and voltage-clamp modes. The current-clamp mode was used to record specific membrane resistance (R), membrane potential (E) and the AP. The E was measured as a voltage difference across the membrane. By injection a pre-threshold short (1-2 s.) square depolarizing current pulses (0.1 μA, that do not evoke the generation of action potential) through external Ag/AgCl electrodes, the magnitude of membrane potential deflection was used to calculate the cell membrane R under the formula (1) (Spiewla, 1995):

$$R=\frac{\Delta E}{I}\pi dl \quad (1),$$

where ΔE – membrane potential deflection, mV; I – applied current, μA; d – cell diameter, cm; l – cell length in the measurement chamber, cm.

The AP of the cells was evoked by external electrical stimulation (injection of 0.1 s duration, 1-5 μA $cm^{-2}$ square pulse of depolarizing current) between two pools of the chamber. The AP kinetics was analysed via plots of the function:

$$f(t) = \frac{\Delta E}{\Delta t} \quad (2)$$

that were obtained from the experimental data by differentiation of the E drop from the AP peak for 15 s (Opritov et al., 2002). The average absolute maximum value of ΔE/Δt was taken to evaluate the rate of fast repolarization of AP. The voltage-clamp mode was used to investigate the potassium, chloride and calcium ion transport systems. The clamped region of the cell in the compartment was 0.5 cm long. The current records and analyses were constructed from leak-subtracted data. For the investigation of the transient currents (I), the E of the cells was hold at -180 mV (the voltage value close to potassium equilibrium potential, when cell membrane conductance is determined mainly by $K^+$ transport and electrogenic contribution of metabolic component to membrane potential is negligible). The current-voltage characteristics (I-V) for $Cl^-$ and $Ca^{2+}$ were obtained by injecting rectangular depolarization current pulses (10 s) in 20 mV steps from the holding potential for the voltage interval from -140 mV to +60 mV applied every 5 min. Two distinct components of transient current curves occurs during these depolarizing changes: the first quick smaller current peak arising in ~100 ms after the onset of stimulation was considered as $Ca^{2+}$ current, whereas following larger and slower component represents $Cl^-$ current (Лапковская et al., 2010) Regarding the outwardly rectifying potassium current, voltage-clamp protocol comprised: holding potential - 180 mV; voltage interval for construction of I-V curves [ -270 mV ÷ +60 mV]; electrical stimulation conducted via injection of short (30 ms) rectangular hyperpolarisation or depolarisation current pulses every 10 mV step from the holding potential (Demidchik et al., 1997; Kisnieriene et al., 2012). The current-voltage curves plotted from the peak current values against the applied voltages. The value of the peak current when E was in the range from -60 mV ÷ -40 mV was used to evaluate the chloride and calcium transport system activity (Berestovsky et al., 2005). At this voltage interval the chord conductance (G) for $Cl^-$ and $Ca^{2+}$representing the dependence of the PM conductance at the peak current on the applied voltage was also evaluated. It was calculated from (3):

$$G_j = \frac{I_j}{\left(E - E_j\right)} \quad (3),$$

where $G_j$ – specific membrane conductance for *j* ion, μS $cm^{-2}$ , $I_j$ - peak *j* ion current to the area unit value, μA $cm^{-2}$, E - membrane potential value at which this peak current was recorded, mV; $E_j$ - the reversal potential for $I_j$, mV.

Reversal potential of $Cl^-$ and $Ca^{2+}$current was estimated from corresponding I-V curves (Khan et al., 2008)

Regarding the potassium transport the value of the peak of outward $K^+$ current at E = 60 mV has been evaluated (Horváth et al., 2002; Kisnierienė et al., 2009).

**Investigation of the effect of low-dose tritium on electrophysiological properties of *Nitellopsis obtusa* cells.** The experiment protocol included control conditions, i.e., the experiments began in APW, and the samples were subsequently exposed to tritium in the tritiated water form (HTO) with an activity concentrations of 7 kBq $L^{-1}$, 15 kBq $L^{-1}$, 30 kBq $L^{-1}$, 65 kBq $L^{-1}$ (the HTO activity concentrations is presented as the value above the background radiation level: tritium activity concentration in APW was 2.6 ± 0.4 Bq $L^{-1}$ that is close to the minimum detectable activity concentration of tritium (1.8 Bq $L^{-1}$ when counting time is 30 min). The examined cells were incubated for approximately half an hour after the exogenous application of HTO to provide sufficient time for cellular effects (Rety et al., 2010). Each HTO activity concentration was investigated in different cells group (each group contains 10 cells).

**Investigation of the combined effect of tritium and aluminium on ion transport systems of *Nitellopsis obtusa* cells.** During these investigations two cell groups were examined: first group was exposed to single 1 $10^{-3}$M Al + APW and the second was treated with mixture of 1 $10^{-3}$M Al, 15 kBq·$L^{-1}$ HTO and APW. The cells in both groups were incubated for half an hour after the exogenous application of Al or Al and HTO mixture to provide sufficient time for Al to penetrate into the cell (Zdenko Rengel & Reid, 1997). Both cell groups were examined in control conditions (APW) before administration of Al or mixture of Al and HTO.

**Investigation of the radiation induced bystander effect (RIBE) in two adjoining internodal cells of *Nitellopsis obtusa*.** During the investigation of RIBE electrophysiological changes in not irradiated cell caused by irradiation of neighbouring

adjoining cell has been evaluated. The experimental set-up on signal transmission between the two adjoining charophyte cells was adopted from Shimmen et al. electrophysiological investigations of wound signalling in Characeae *Chara corallina* (Shimmen, 2003). In these experiments two adjoining *Nitellopsis obtuse* internodal cells of similar length (~ 10 cm) and diameter (~ 0.06 cm) was placed in a plexiglass chamber as it is shown in figure 2.1

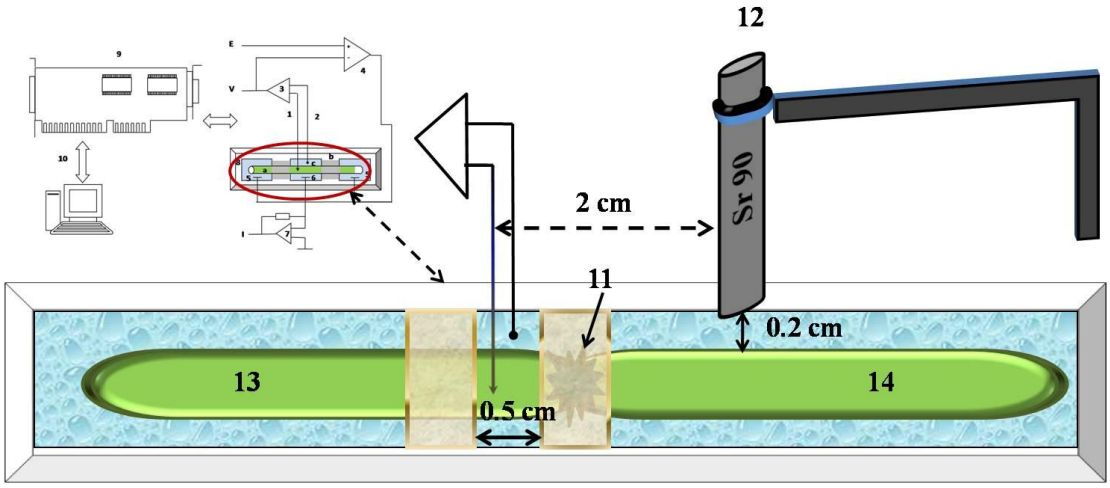


**Fig. 2.1. Scheme of experiments on RIBE investigation in two adjoining internodal cells of Nitellopsis obtusa: inset in upper left corner of the figure depicts schematic diagram of conventional experimental set-up used in all our electrophysiological investigations consisting of 1- glass microelectrode, 2 – reference electrode, 3 – amplifier of bioelectrical signals, 4- differential amplifier, 5 – external Ag/AgCl current electrodes, 6 – virtual - ground electrode, 7 – current/voltage converter, 8 – plexiglass chamber with three compartments (a – cell, b – vaseline gap (isolation), c – central compartment of the chamber, where electrophysiological measurements were done), 9– universal system of data acquisition, 10 – computer, E- command voltage, V – recorded membrane potential, I –recorded transient current; 11 –multicellural node connecting two internodal cells (13 and 14), placed under vaseline gap, 12 – sealed source of ionizing radiation (radioactive isotope of strontium Sr 90 74 kBq), fixed under target cell; 13 – non target cell, in which electrophysiological measurements were performed; 14 – target cell for direct irradiation.**

The medium of both intermodal cells was strongly separated by vaseline gaps isolating three compartments of plexiglass chamber. The multicellural node connecting two intermodal cells was placed between central and lateral compartments under the vaseline gap (**fig 2.1. 11**). Cell placed in right lateral compartment of plexiglass chamber was irradiated with sealed source of radioactive strontium isotope (Sr 90 74 kBq) fixed 0.2 cm above target cell (**fig 2.1. 12**), thus ensuring ~2.4 kBq activity of β radiation on the target cell surface. Electrophysiological measurements were conducted in

neighbouring adjoined cell placed in both (**fig 2.1. 13**) left lateral and central compartments of plexiglass chambers. Microelectrode was inserted into the part of non target cell located in the central compartment and the source of IR under the target cell was positioned so, that the distance between irradiation and measurement places was maintained for 2 cm. Electrophysiological parameters of not irradiated cells were recorded under previously described current and voltage clamp procedures in control condition, then after target cell irradiation for 30 min and subsequently after 24 h of exposure.

Two types of pilot control experiments were also performed. Firstly, in order to verify whether duration of keeping cells in control solution could influence bioelectrical properties of internodes we conducted experiments comprising electrophysiological examination of bioelectrical parameters at the beginning of experiments and after cells exposition to APW for 24 h. To provide the similar experimental conditions, two adjoining internodal cells were also used in these examinations. Experimental set-up was the same as described for RIBE investigations (**fig 2.1**) but in the absence of irradiation source (**fig 2.1 12**).

The second type of control experiments was dedicated to examine bioelectrical changes in the target cell directly irradiated for 30 min. In order to maintain uniform experimental conditions two adjoining internodal cells were investigated under the similar experimental set-up as for RIBE investigations (**fig 2.1**) but with some necessary changes. Two adjoining internodes were placed in plexiglass chamber so, that irradiation source (**fig 2.1 12**) was fixed above the cell in which electrophysiological measurements will be performed (**fig 2.1 13**) maintaining the same distances between measurements and irradiation places as well as between irradiation source and target cell surface as in RIBE experiments. During these experiments irradiation and electrophysiological examination took place at the same cell, and the electrophysiological parameters were recorded under control condition and then after irradiation for 30 min.

All experimental data are expressed as the mean values ± standard errors (SE). The statistical significance of the differences was tested using a paired Student's t-test, and a p level equal to or less than 0.05 was considered to be statistically significant. The data analysis was performed using Microcal ORIGIN 8.5 and Statistica 6.0 (StatSoft).

## 3. RESULTS

### 3.1. Effect of tritium on the membrane resting potential, specific membrane resistance, action potential dynamics, $Ca^{2+}$ and $Cl^-$ transport in *Nitellopsis obtusa* cells

Investigating the effect of tritium on the plasma membrane bioelectrical properties of charophyte cell, first we evaluated how different activity concentrations of tritium affect steady bioelectrical properties of PM, such as membrane resting potential and specific membrane resistance.

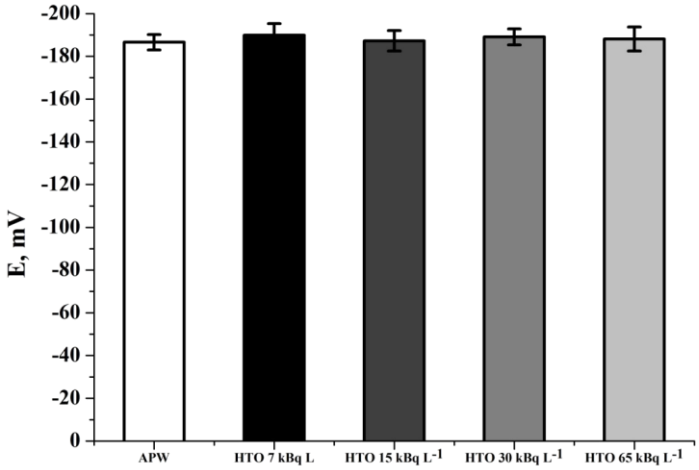


**Figure 3.1. Effect of HTO of different activity concentrations on the resting potential of *Nitellopsis obtusa* cells: for each HTO activity concentrations n = 10, APW n = 40**

Examination of membrane resting potential in *Nitellopsis obtusa* cells showed that at activity concentration from 7 kBq $L^{-1}$ to 65 kBq $L^{-1}$ tritium dose not significantly affect this parameter (**fig. 3.1**).

During the investigations of tritium effect on the specific membrane resistance (R) of charophyte cells, it was revealed that tritium with 7 kBq $L^{-1}$ activity concentration (n = 10) do not alter R (**fig. 3.2.**). However, tritium of 15 kBq $L^{-1}$ activity concentration enhanced specific membrane resistance by about 52 % ($\Delta$=17.72 ± 1.9 k$\Omega$ $cm^{-2}$) (n = 10, p = 0.005). The similar effect was observed during cell exposure to tritium with 30 kBq $L^{-1}$ and 65 kBq $L^{-1}$ activity concentrations, thus the magnitude of tritium effect on R did not depend on applied activity concentration at the interval from 15 kBq $L^{-1}$ to 65 kBq $L^{-1}$.

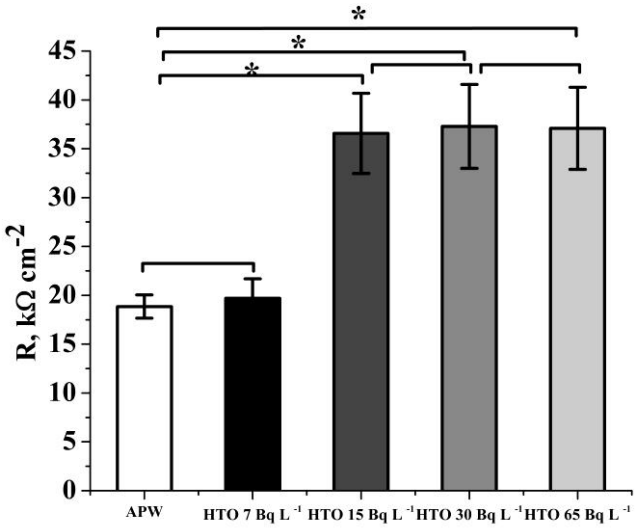


**Figure 3.2. Effect of HTO of different activity concentrations on the specific membrane resistance *Nitellopsis obtusa* cells: for each HTO activity concentrations n = 10, APW n = 40 40; * - p < 0.05 vs. control conditions**

After the evaluation of tritium effect on steady PM bioelectrical characteristics of *Nitellopsis obtusa*, we investigated the impact of tritium at different activity concentrations on the mechanisms, involved in generation of electrical excitation (action potential –AP) of charophyte cells.

First of all, we examined tritium effect on the membrane potential recovery dynamics after electrical excitation of PM. The mechanism for the electrical excitation of the plant cell PM is complex and involves various ion transport systems. The conventional model indicates that an AP comprises a three-phase process in plant cells. The AP consists of an ascending phase determined by a voltage-dependent $Ca^{2+}$ influx that leads to the activation of an outward $Cl^-$ current that triggers steep depolarization of the membrane potential; the biphasic recovery of the membrane potential that follows the excitation is called repolarization. This descending phase of the AP contains two components: fast and slow. The fast repolarization reflects a passive outward $K^+$ flux, and the slow one is related to the activation of $H^+$-ATPase and a gradual decrease in the potassium current (Sukhov et al., 2011; 2009). Thus, alteration of the electrical signalling pattern and dynamics can indicate the disturbance of some ion transport systems within the cell. Thus, we first of all evaluated the effect of HTO on the AP kinetics. Fig. 3.3 shows the changes in the pattern of the membrane potential descent

from the peak of the AP due to an exogenous HTO of different activity concentrations treatment.

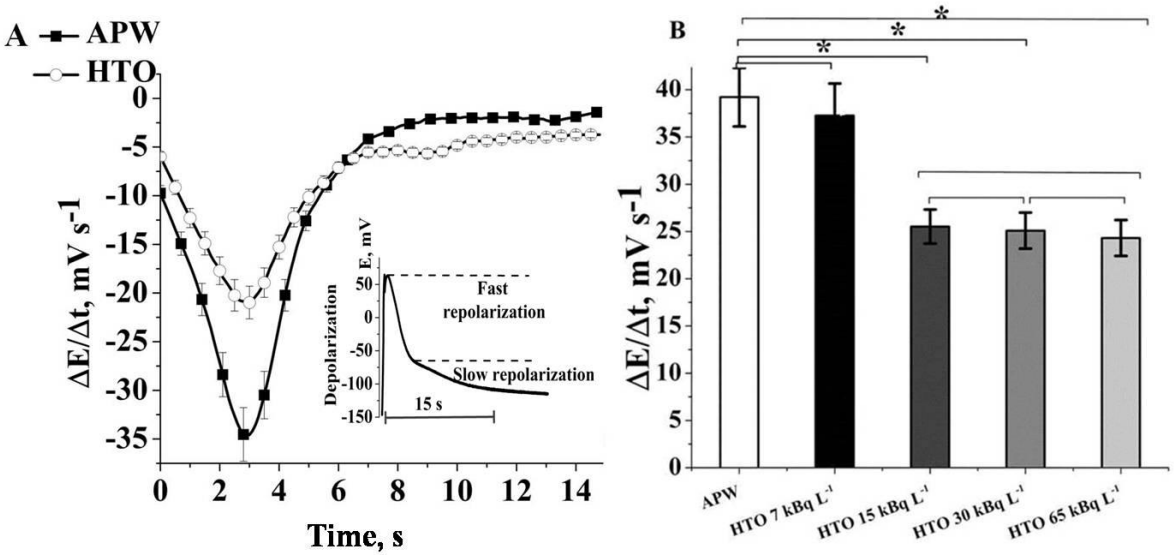


**Figure 3.3. Effect of different HTO activity concentrations on the pattern of an action potential in a giant alga cell (APW - artificial pond water, HTO – solution of tritiated water and APW with an activity concentration of 7 kBq $L^{-1}$, 15 kBq $L^{-1}$, 30 kBq $L^{-1}$, 65 kBq $L^{-1}$): A - kinetics of the repolarization phase (HTO - 15 kBq $L^{-1}$+ APW solution): *inset*: a record of a typical action potential (AP) in Nitellopsis obtusa showing three stages of AP generation; B - absolute maximum fast repolarization rate (n=10, *-p<0.05 vs. control conditions)**

The data presented in **fig. 3.3** show that the 15 kBq $L^{-1}$ HTO significantly slowed down the membrane potential recovery after the AP. This retardation occurred during both the fast and the slow repolarization stages, but the main changes occurred in the fast repolarization phase (2 - 4 s after AP peak). The averaged maximum rate of membrane potential recovery decreased from 39.2 ± 3.1 mV $s^{-1}$ in APW to 25.5 ± 1.8 mV $s^{-1}$ with the 15 kBq $L^{-1}$ HTO treatment (**fig. 3.3B)**. Thus, the 15 kBq $L^{-1}$ HTO exposure caused a deceleration of the repolarization by approximately 35% (13.7 ± 1.2 mV $s^{-1}$, p = 0.006) These investigations as well as examination of membrane resting potential and R changes due to tritium exposure, showed that 7 kBq $L^{-1}$ HTO do not significantly affect repolarisation rate. Cells treatment with HTO 30 kBq $L^{-1}$ and HTO 65 kBq $L^{-1}$ caused equal effect on fast repolarization rate of AP as was estimated due to exposure to 15 kBq $L^{-1}$ HTO. These results allow to conclude that tritium effects at 15 kBq $L^{-1}$ ÷ 65 kBq $L^{-1}$

activity concentration interval is not dose dependent, therefore HTO activity concentration of 15 kBq $L^{-1}$ has been selected for further tritium effect investigations as experimentally estimated lowest effective activity concentration.

After examining the effect of HTO on the membrane potential recovery after excitation, we investigated the inward voltage dependent $Ca^{2+}$current triggering the initiation of AP in charophyte cells.

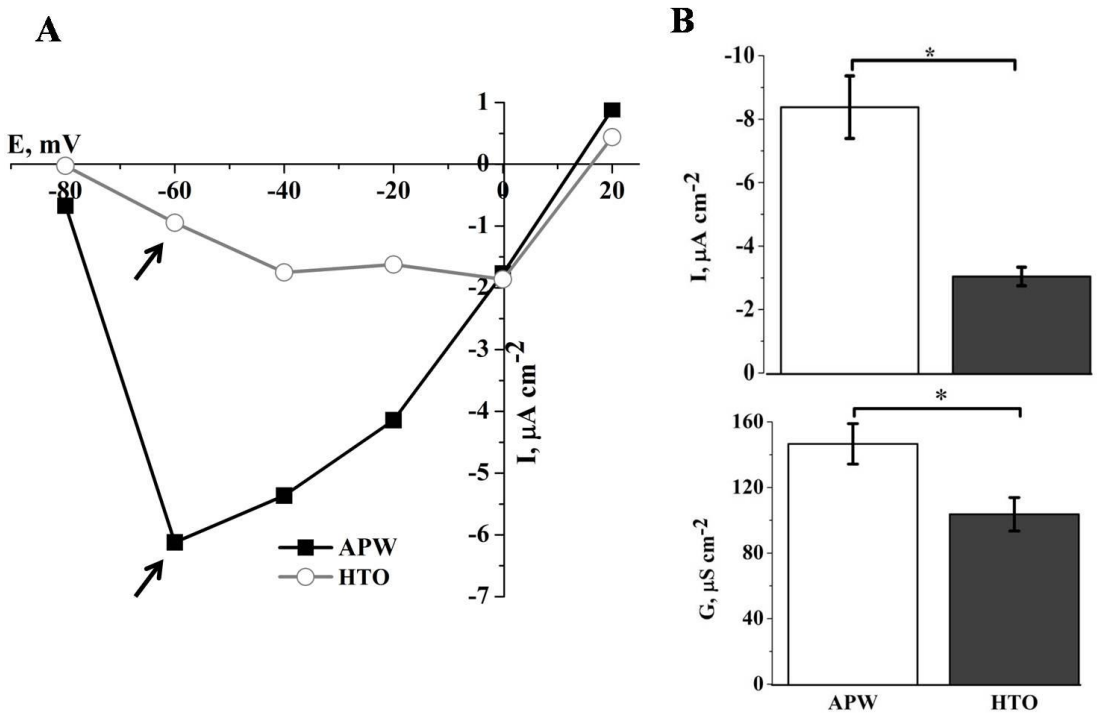


**Figure 3.4. Effect of HTO on the voltage-gated $Ca^{2+}$ channels (APW - artificial pond water, HTO – solution of tritiated water and APW with an activity concentration of 15 kBq $L^{-1}$): A – typical current-voltage profile for $Ca^{2+}$, arrows points voltages where the value of the inward $Ca^{2+}$ current were evaluated; B – upper part depicts value of the maximum inward $Ca^{2+}$ current (at E= -60mV); bottom part represents averaged maximum value of the membrane chord conductance for $Ca^{2+}$ (n=10, *-p<0.05 vs. control conditions).**

**Figure 3.4** depicts the effect of 15 kBq $L^{-1}$ HTO on the voltage-dependent $Ca^{2+}$ transport mechanisms involved in electrical signalling. The exogenous application of HTO resulted in a reduction in the calcium ion influx **(fig. 3B)** by 64 % ($\Delta$= -5.3 ± 0.4 µA $cm^{-2}$, p = 0.001) along with a decrease in the PM chord conductance for $Ca^{2+}$ by approximately 30 % (from 150 ± 10 µS $cm^{-2}$ in APW to 100 ± 9 µS $cm^{-2}$ in HTO, p = 0.02).

Although the changes in the calcium influx during electrical excitation were determined, it was necessary to examine the activity of the $Cl^-$ transport involved in the depolarization stage of the AP and considered to be $Ca^{2+}$-dependent.

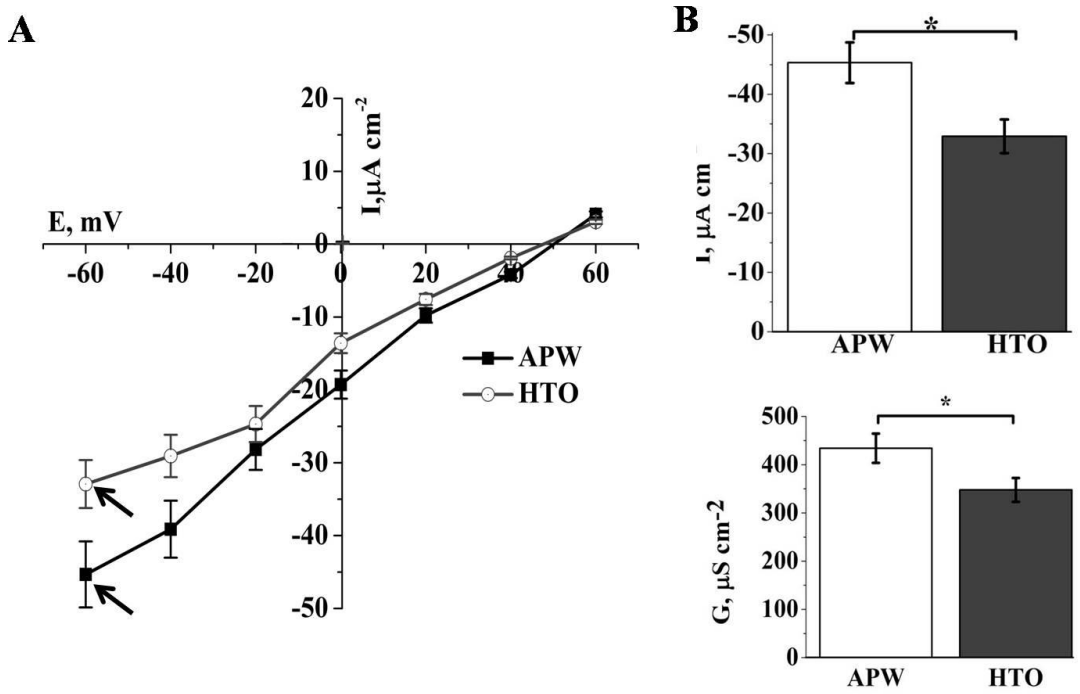


**Figure 3.5. Effect of HTO on the outward voltage- and $Ca^{2+}$ -dependent $Cl^-$ current (APW - artificial pond water, HTO – solution of tritiated water and APW with an activity concentration of 15 kBq $L^{-1}$): A –typical current-voltage profile for $Cl^-$, arrows points voltages where the value of the outward $Cl^-$ current were evaluated; B – upper part depicts value of the outward $Cl^-$ current (at E= -60mV); bottom plot represents maximum membrane chord conductance for $Cl^-$ (n=10, *-p<0.05 vs. control conditions).**

**Figure 3.5 A** illustrates the HTO effect on the outward $Cl^-$ current-voltage relationships. The application of exogenous 15 kBq $L^{-1}$ HTO reduced the averaged maximum amplitude of the outward $Cl^-$current from the -45.3 ± 3.6 μA $cm^{-2}$ recorded under control conditions to -32.9 ± 2.4 μA $cm^{-2}$. The tritium exposure decreased the magnitude of the chloride efflux by approximately 27 % (Δ = 12.4 ± 1.1 μA $cm^{-2}$, p = 0.004) (**fig. 3.5 B upper part**). The effect of HTO was also demonstrated in the changes of the PM chord conductance of $Cl^-$. The results presented in **fig. 3.5 B** (bottom) demonstrate the inhibitory properties of HTO on the chloride channel activity. The membrane permeability to $Cl^-$ decreased from 430 ± 13 μS $cm^{-2}$ in the APW

environment to 340 ± 12 μS $cm^{-2}$ in HTO. This change involved a decrease in the PM chord conductance of $Cl^-$ (at E= -60 mV) by approximately 23% (90 ± 7 μS $cm^{-2}$, p = 0.02).

### 3.2. Combined effect of tritium and aluminium on $Cl^-$, $Ca^{2+}$ and $K^+$, ions transport across the membrane of *Nitellopsis obtusa* cells

Taking into account the model of the electrical signalling in plant cell described in 3.1 section, we focused on the examination of combined effect of aluminium (Al) and tritium on the mechanisms involved in the AP initiation, such as voltage and $Ca^{2+}$ dependant $Cl^-$ current.

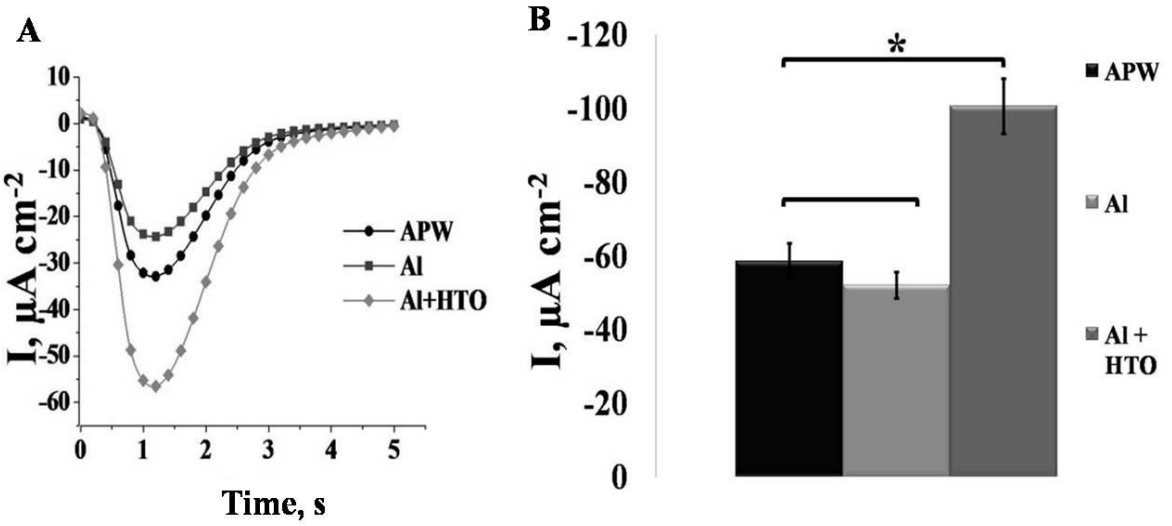


**Figure 3.6. Effect of Al and mixture of Al and HTO on the outward $Cl^-$ current: A – the typical record of $Cl^-$ current pattern at E= -60 mV; B –the value of outward $Cl^-$ current at E= -60 mV (APW - artificial pond water, „Al" – 1 $10^{-3}$ M Al solution, „HTO" – tritiated water solution with activity concentration of 15 kBq $L^{-1}$, „Al + HTO" – mixed solution consistent of 1 $10^{-3}$ M Al and 15 kBq $L^{-1}$ HTO; all results are presented in values normalized under control condition, n=9, *- p<0.05 vs. control condition)**

The **fig. 3.6 B** demonstrates the decline of the magnitude of $Cl^-$ current for about 11% ± 8 due to application of Al. This tendency is similar to single 15 kBq $L^{-1}$ HTO evoked effect as seen in depression of chloride efflux (**see fig. 3.5**). In contrast, administration of Al and HTO mixture caused amplification of outward $Cl^-$ current magnitude by about 71%±10. Whereas, $Cl^-$ current triggering depolarisation phase of AP is considered to be dependant from the voltage as well as intracellular $Ca^{2+}$ concentration, we assumed that alterations of this current could be reflected in the

voltage-dependent $Ca^{2+}$ channels activity. The results presented in **fig.3.7**. provide an evidence of Al and Al and HTO mixture to have impact on the $Ca^{2+}$ channels involved in AP generation.

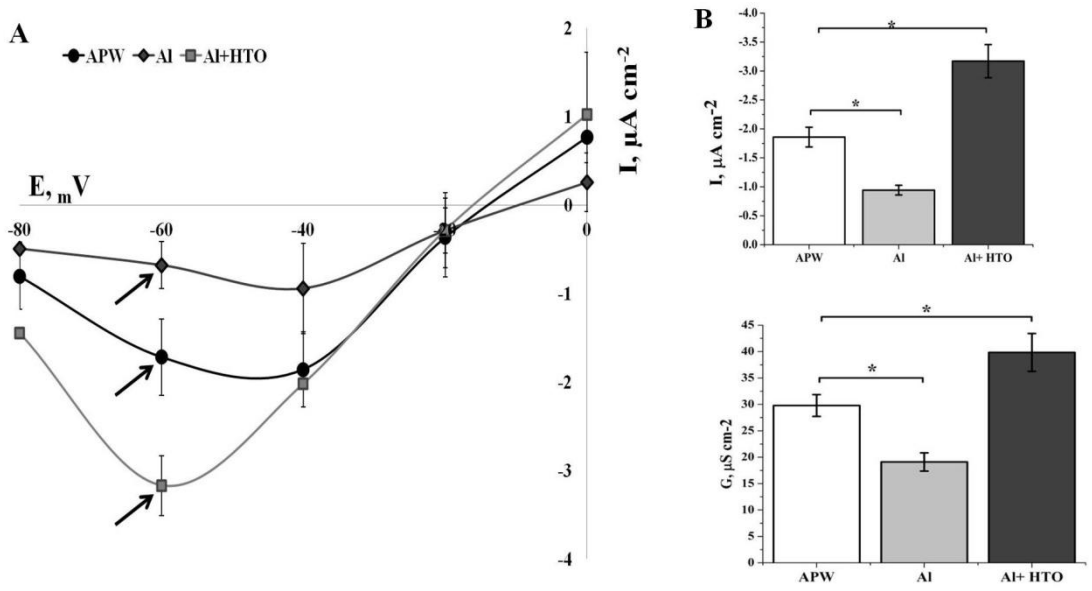


**Figure 3.7. Effect of Al and mixture of Al and HTO on the voltage gated $Ca^{2+}$ channels activity: A – the typical $Ca^{2+}$ current - voltage relationship, arrows points voltages where the value of the inward $Ca^{2+}$ current were evaluated; B – upper part depicts the value of inward $Ca^{2+}$ current at E= -60 mV; bottom part depicts maximal plasma membrane chord conductance for $Ca^{2+}$ (APW - artificial pond water, „Al" – 1 $10^{-3}$ M Al solution, „HTO" – tritiated water solution with activity concentration of 15 kBq $L^{-1}$, „Al + HTO" – mixed solution consistent of 1 $10^{-3}$ M Al and 15 kBq $L^{-1}$ HTO; all results are presented in values normalized under control condition, n=9, *-p<0.05 vs. control condition)**

The **fig.3.7** shows that in this case single Al also acts in the similar manner as a single HTO (**see fig 3.4**) causing the reduction of the inward $Ca^{2+}$ current for about 49%±9 ($p<0.05$ vs. control condition) and chord conductance for about 38%±9 ($p<0.05$ vs. control condition) (**fig. 3.7B**). Meanwhile, Al and HTO mixture magnifies $Ca^{2+}$current by about 69%±9 and the chord conductance for 33%±9 ($p<0.05$ vs. control condition).

The last component of AP generation we investigated was outward $K^+$ current, involved in repolarization, dynamics. The **fig. 3.8** illustrates the effect of Al and its combination with HTO on the outward $K^+$ I-V relations.

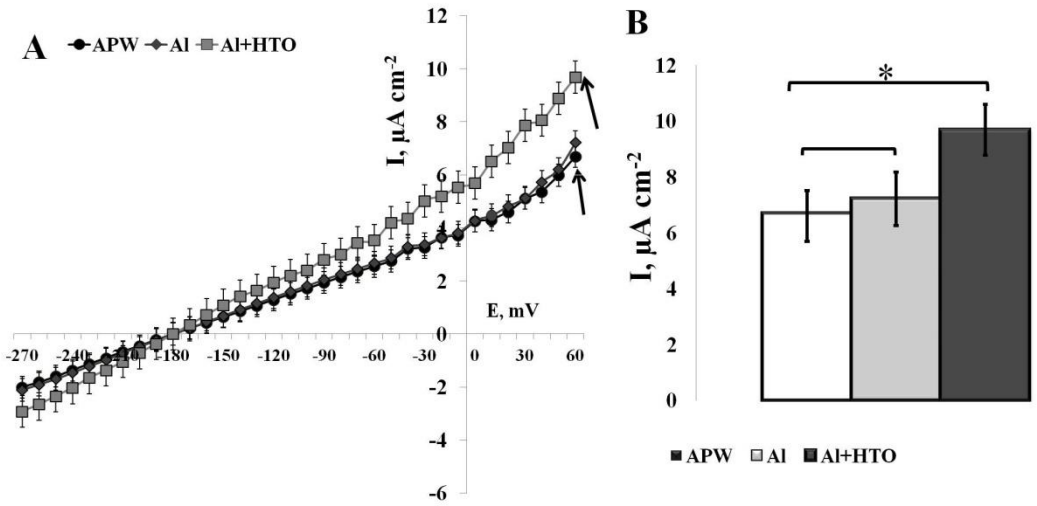


**Figure 3.8. Effect of Al and mixture of Al and HTO on the outward on the $K^+$ transport: A – the $K^+$ current - voltage relationship, arrows points voltages where the value of the inward $K^+$ current were evaluated; B –the averaged value of inward $K^+$ current at E= 60 mV(APW - artificial pond water, „Al" – 1 $10^{-3}$ M Al solution, „HTO" – tritiated water solution with activity concentration of 15 kBq $L^{-1}$, „Al + HTO" – mixed solution consistent of 1 $10^{-3}$ M Al and 15 kBq $L^{-1}$ HTO; all results are presented in values normalized under control condition, n=9, *-p<0.05 vs. control condition)**

The single Al does not significantly affect $K^+$ efflux, but Al in combinations with HTO behave differently by enhancing the magnitude of the maximum outward $K^+$ current for about 45%±9 **fig. 3.8B**. Based on the results of investigation of HTO effect on the repolarisation rate it can be assumed that single HTO could inhibit $K^+$ efflux, since fast repolarization rate determined mainly by $K^+$ transport was declined due to HTO (**see fig. 3.3**)

### 3.3. Radiation induced bystander effects (RIBE) in two adjoining internodal cells of *Nitellopsis obtusa*

During RIBE experiments we investigate the changes of bioelectrical activity in not directly exposed internodal cell after 30 min and 24 h lasting exposure to sealed source of radioactive strontium of adjoined cell. We observed no changes in resting potential and specific membrane resistance after neighbouring cell exposure for 30 min and 24 h (Table 3.1).

Table 3.1. Effect of exposition of one adjoining intermodal cell to sealed radioactive source (Sr 90 74 kBq) at different durations on the resting potential and specific membrane resistance of non target neighbouring cell (n = 6)

| Target cell environmental conditions | Non target cell bioelectrical parameters | |
|---|---|---|
| | Specific membrane resistance R, kΩ $cm^{-2}$ | Membrane resting potential RP, mV |
| APW | 13.4 ± 0.4 | -223 ± 3.1 |
| Sr 90 (30 min.) | 13.4 ± 0.4 | -226.1 ± 3.7 |
| Sr 90 (24 h.) | 13.6 ± 0.2 | -225.1 ± 6.5 |

Investigating the dynamics of action potential, we found that irradiation of one adjoining cell caused changes in the pattern of AP neighbouring not directly irradiated cell (**fig. 3.9**)

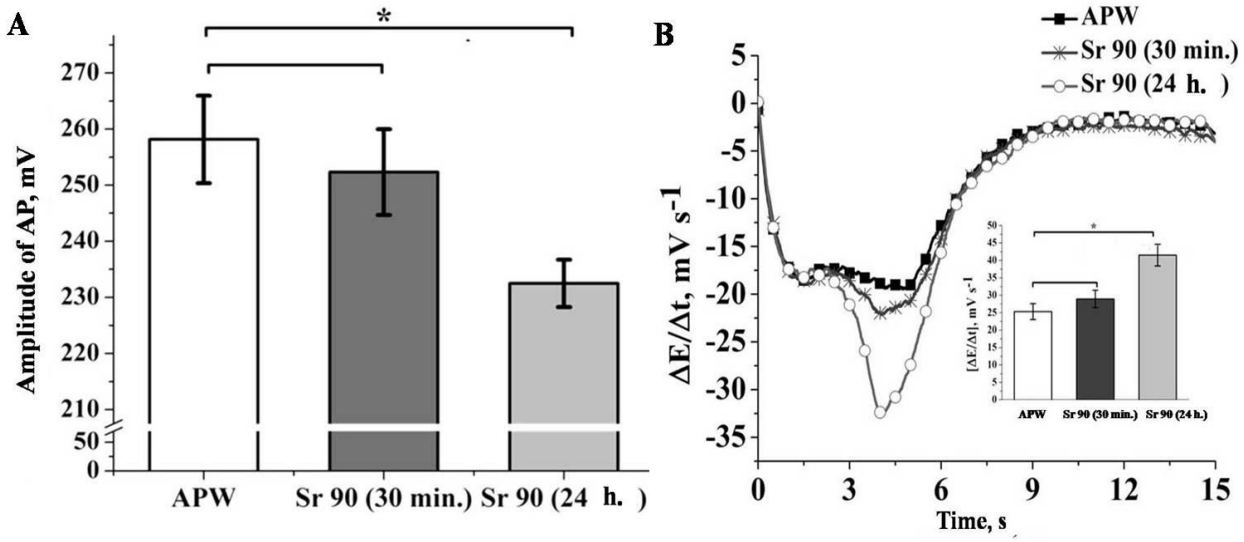


**Figure 3.9. Effect of exposition of one adjoining intermodal cell to sealed radioactive source (Sr 90 74 kBq) at different durations on the dynamics of action potential in the non target neighbouring cell (n = 6, *-p<0.05 vs. control condition): A – amplitude of AP; B- typical pattern of repolarization rate: *inset*: absolute maximum rate of fast repolarization**

We observed that irradiation of target cell for 30 minutes did not significantly affect the amplitude of the action potential in neighbouring not irradiated cell (**fig 3.9A**).

However, after the increasing exposure time to 24 hours, the amplitude of action potential in neighbouring cell was significantly reduced by about 23.3 ± 2.6 mV (p = 0.015). Continuing to analyze the action potential pattern, we found that repolarization dynamics was also altered in non irradiated cell (**Fig.3.9B**). It was estimated that irradiation of target cells for 30 min. also had no significant effect on the on the velocity of membrane potential changes during repolarization in the adjacent non target cell meanwhile, elongation of irradiation time up to 24 hours accelerated fast repolarization in not irradiated cell by 64% (Δ = 16.2 ± 1.8 mV s-1) compared with the control conditions (p = 0.027). Whereas the fast repolarization of AP in *Characeae* reflects the steep $K^+$ efflux, we studied the activity of potassium transport involved in $K^+$ transport out of the non target cell after the irradiation of its neighbouring cell (**fig. 3.10**)

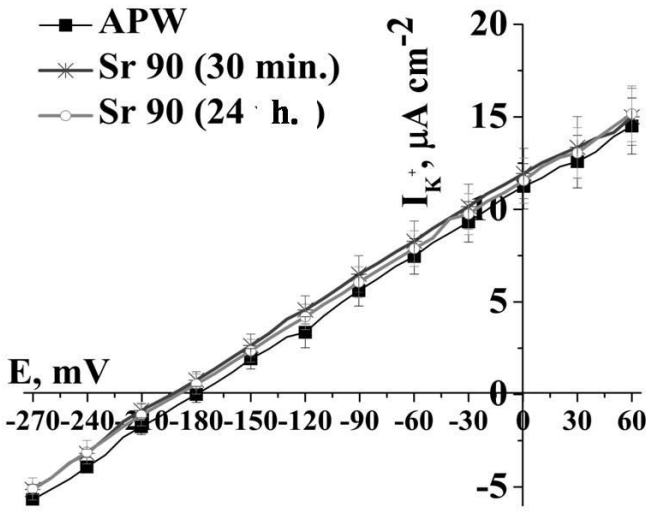


**Figure 3.10. Effect of exposition of one adjoining intermodal cell to sealed radioactive source (Sr 90 74 kBq) at different duration on the outward $K^+$ current – voltage relation in the non target neighbouring cell, n = 6**

Investigating transient outward potassium current - voltage characteristics, we found that irradiation of o charophyte cell for 30 minutes, as well as for 24 hours, did not result in significant changes of $K^+$ outward transport in the neighbouring not irradiated cell at steady state.

We have also investigated $Cl^-$ transport activity during generation of AP in not irradiated cell adjacent to cell exposed to radioactive strontium (**fig. 3.11**)

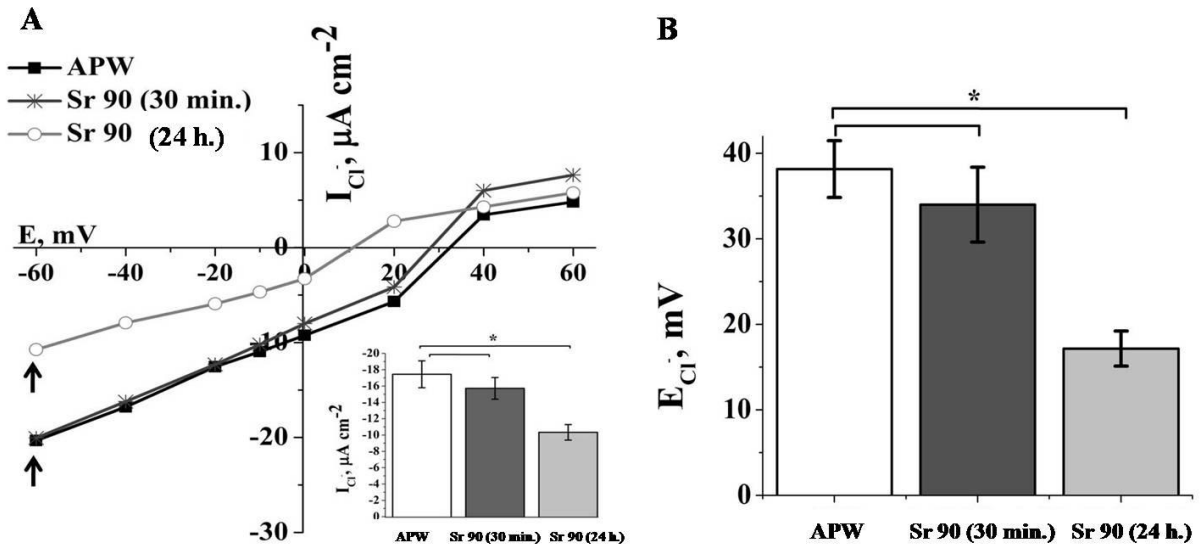


**Figure 3.11. Effect of exposition of one adjoining intermodal cell to sealed radioactive source (Sr 90 74 kBq) at different duration on the outward chloride current in the non target neighbouring cell: A – typical I-V curve of outward $Cl^-$ current during AP generation, arrows points voltages where the value of the outward $Cl^-$ current were evaluated: *inset*: value of the outward $Cl^-$ current at E= -60mV (n = 6, *-p<0.05 vs. control condition): B – reversal potential for $Cl^-$ current (n = 6, *-p<0.05 vs. control condition)**

Chloride transport studies revealed that irradiation of target cell for 24 h. significantly inhibited $Cl^-$ transport from neighbouring not exposed cell during electrical excitation (**fig. 3.11A**). It was estimated that $Cl^-$ efflux from non target cell decreased by 41% ($\Delta$ = -7.11 ± 0.75 μA cm$^{-2}$, p = 0.03). Moreover, analysis of I-V curve of outward $Cl^-$ current showed that reversal potential for $Cl^-$ was also altered. We found that target cell irradiation for 24 hours determined shift of $Cl^-$ reversal potential in decreasing direction from the value 38.17 ± 3.32 mV in control conditions to 17.16 ± 2.05 mV after irradiation (**fig. 3.11B**). The reversal potential for $Cl^-$ was changed by 45% (p = 0.009). After the irradiation of target cell for 30 minutes, the similar tendencies in changes of chloride efflux and reversal potential in neighbouring not irradiated cell was observed, but differences of studied parameters values were statistically significant.

In order to evaluate possible contribution of prolonged exposition in APW to estimated bioelectrical changes in non irradiated cell, we implemented control experiments to investigate parameters in control conditions at the beginning of experiments and after keeping cells in APW for 24 h (**fig 3.12**).

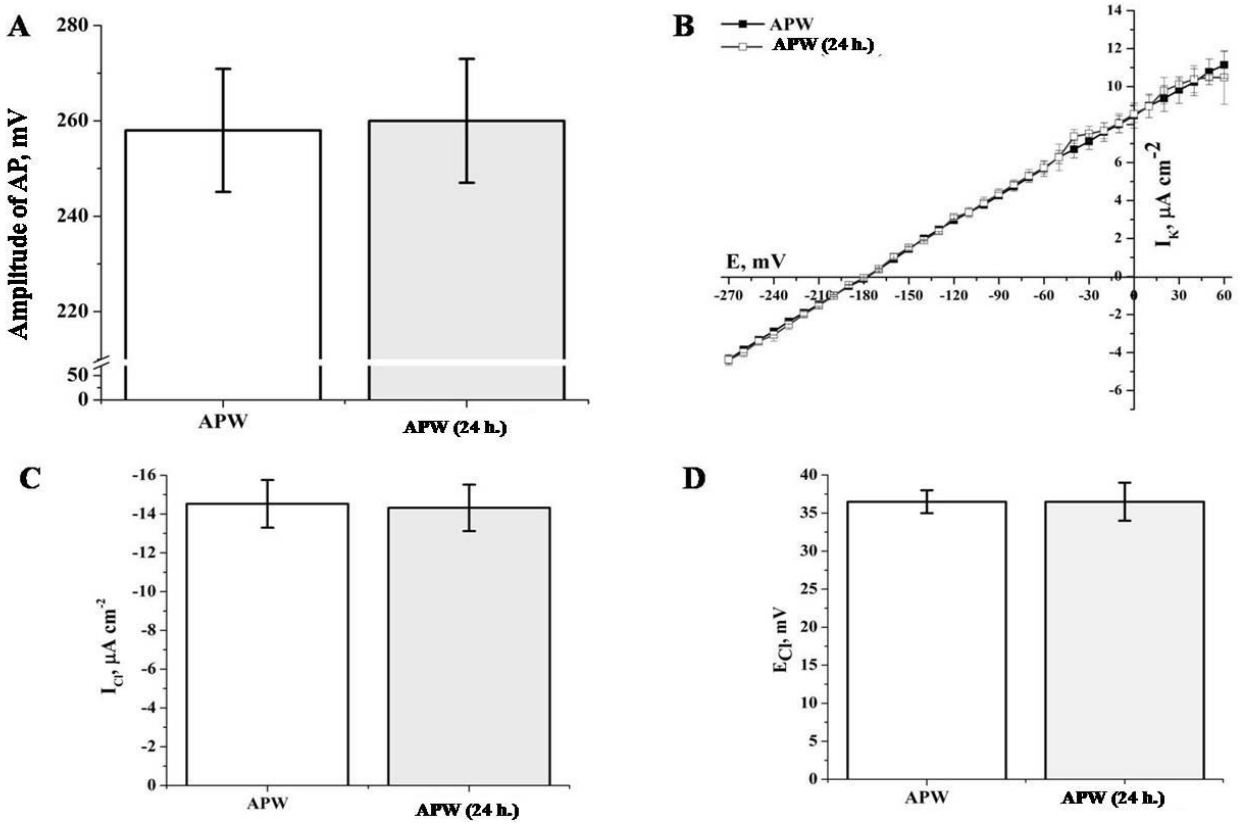


**Figure 3.12. Effect of duration of *Nitellopsis obtusa* cells exposition in APW on the bioelectrical parameters of one of the adjoining charophyte cells (APW – bioelectrical parameters evaluated at the beginning of experiments, APW (24 h.) - bioelectrical parameters evaluated after 24 hours keeping cells in APW, n=2): A – amplitude of AP; B – outward $K^+$ current - voltage relationship; C – magnitude of outward $Cl^-$ current at E= -60mV, D – reversal potential of outward chloride current.**

Control trials have shown that for all of the investigated parameters values in APW solution at the beginning of the experiment and after 24 hours keeping cells in APW were not significantly different. In this way, it can be assumed that the cells prolonged exposition to APW could not affect the estimated bioelctrical changes in non target cell due to irradiation of adjoined neighbouring cell.

We also evaluated changes of bioelectrical parameters of the charophyte cell directly irradiated by sealed source of radioactive strontium (Sr 90 74 kBq) for 30 minutes. During these experiments two adjoining charophyte cells were used, but in contrast to RIBE, the irradiation and electrophysiological measurements were performed in the same internodal *Nitellopsis obtusa* cell (**fig 3.13**).

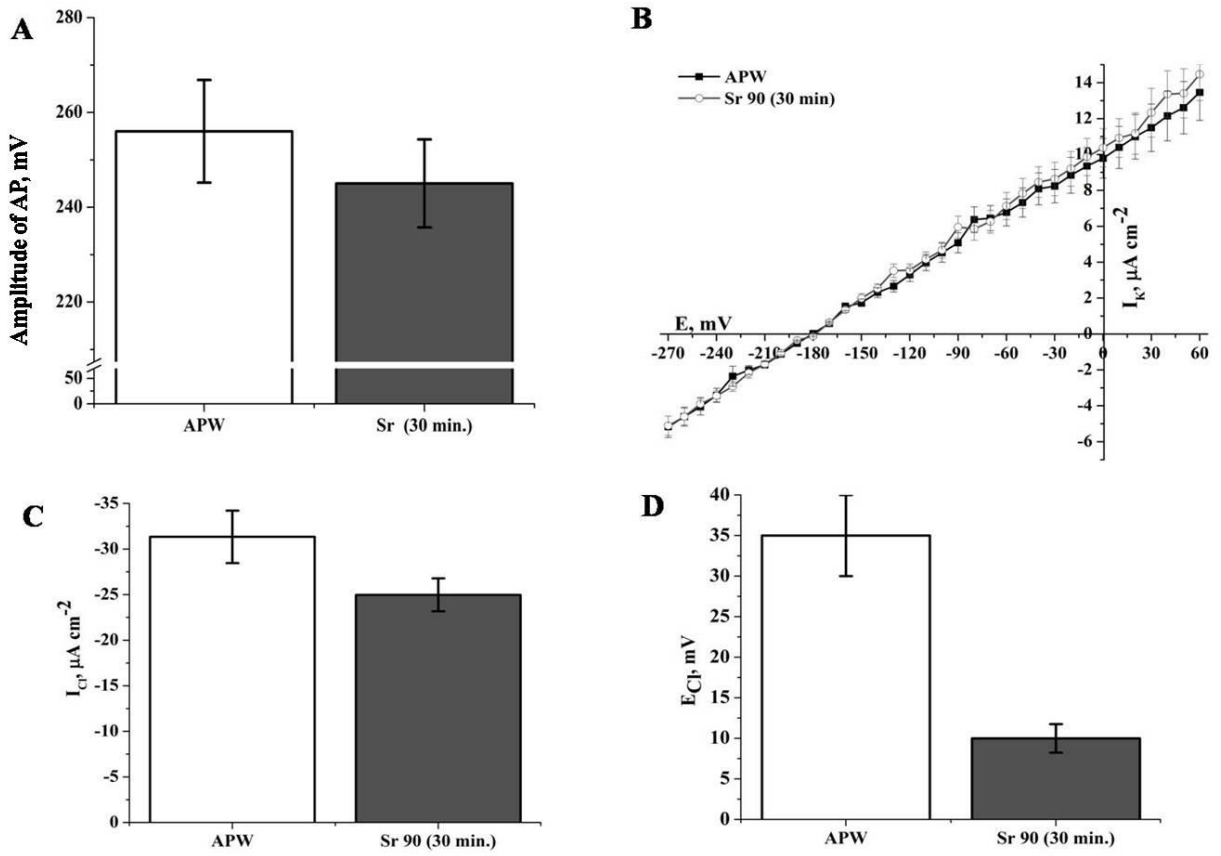


**Figure 3.13. Effect of exposure from sealed source of radioactive strontium (Sr 90 74 kBq) on the bioelectrical parameters of directly irradiated *Nitellopsis obtusa* cells in two adjoining charophyte cells (APW – bioelectrical parameters evaluated under control conditions, Sr 90 (30 min.) - bioelectrical parameters evaluated after 30 minutes of investigated cell irradiation, n = 3): A – amplitude of AP; B – outward $K^+$ current - voltage relationship; C – magnitude of outward $Cl^-$ current at E= -60mV, D – reversal potential of outward chloride current.**

These experiments showed that the similar tendencies in changes of bioelectrical parameters to those estimated in RIBE experiments after 24 hours of target cell irradiation, were observed in directly irradiated *Nitellopsis obtusa* cell even after 30 minutes of direct exposure to Sr 90 (**fig 3.13**). We have found that after 30 min. of direct irradiation amplitude of AP (**fig 3.13A**), magnitude of chloride outward current (**fig 3.13C**) together with reversal potential (**fig 3.13D**) in the irradiated cell tended to decline; meanwhile outward $K^+$ transport expressed a marginal trend to increase (**fig 3.13B**)

## 4. DISCUSSION

### 4.1. Effect of low-dose tritium on electrophysiological properties of *Nitellopsis obtusa* cells

In summary, we observed that HTO affected the *Characeae* electrogenesis. The HTO activity concentration of 15 kBq $L^{-1}$ used in our experiments was effective despite only providing an external dose rate that is approximately 0.05 μGy $h^{-1}$ above the background radiation (Ulanovsky et al., 2008), which is much lower than the recently proposed dose rate of 10 μGy $h^{-1}$ for the predicted no-effect value for freshwater ecosystem (Larsson, 2008). Thus, we found that low-dose HTO affects the kinetics of electrical signalling by decreasing the recovery rate of the membrane potential after excitation of the cell PM (**see fig. 3.3**). These phenomena could be interpreted to be associated with the reduced activity of the $K^+$ transport system that is involved in the fast repolarization; a similar low-energy X-ray exposure was shown to reduce the potassium permeability during an action potential in *Nitella flexilis* (Röttinger et al., 1972). The HTO incorporated by the cell can be partially transformed into organically bound tritium (OBT) and behaves in a very complex manner. Plants mostly produce OBT via photosynthesis. The plant cell bioelectrochemical system regulates photosynthesis, and its disturbance can interfere with photosynthetic activity, consequently affecting tritium assimilation in the plant. Thus, it is possible that HTO can interact with PM channel-forming proteins, including those for potassium transport systems. Tritium has been demonstrated to be more radiotoxic when combined with other molecules (Bradshaw et al., 2013); however, the binding of tritium to an ion channel molecule can affect the channel structure and function, thus modifying the PM permeability to specific ions. Similarly, the active $H^+$-ATPase transport system that is involved in repolarization can be disturbed. The inactivation of $H^+$-ATPase could explain the observed changes in the slow repolarization, shown in **fig. 3.3 A**. During fast repolarization in *Charophytes*, the $K^+$ flow rate is faster, and $H^+$-ATPase requires minutes to restore the normal $K^+$ state by lowering the $K^+$ concentration (Beilby, 2007).

Therefore, we suppose that the altered $K^+$ concentration could also contribute to the prolonged slow repolarization observed during the first 15 s. In addition to the possible direct effect of HTO on the molecular structures of the ion transport systems, HTO can act indirectly through water radiolysis, resulting in increased oxidative stress and

reactive oxygen species (ROS) production in plant cells. ROS in higher plants and algae are continuously produced in chloroplast, mitochondria, peroxisomes, by cell wall peroxidase and plasma membrane NADPH oxidase (Pottosin et al., 2014). Under normal conditions ROS generation and scavenging are balanced by defence mechanisms within the plants, however different biotic and abiotic stress factors could impair this balance resulting in oxidative burst as a common response of plants to environmental stress (Gill & Tuteja, 2010). The suggested molecular targets of ROS include membrane phospholipids, proteins, regulators of ion transport mechanisms and combinations of these targets in animals, as well as in plant cells. ROS affect the electrical properties in animal cells via a decrease in the AP depolarization rate, amplitude and duration; this decrease has been demonstrated to result from inhibition of $Na^+$, inward $K^+$, or ATP-sensitive $K^+$ and $Ca^{2+}$ channels or from activation of a delayed outward rectifying $K^+$ current, inward non-selective cation channels, and the $Ca^{2+}$ current associated with changes in the intracellular $Ca^{2+}$ concentration (Kourie, 1998). Through the production of ROS, such as hydrogen peroxide ($H_2O_2$) and the hydroxyl radical (OH•), HTO in plants can lead to inactivation of outward rectifying $K^+$ channels (via exogenous $H_2O_2$) or can cause a continuous voltage-independent $K^+$ leak current across the PM and the vacuole membrane (via ROS, particularly $OH^{\bullet}$). Through catalyses of the influx and efflux of monovalent cations and the influx of divalent cations, HTO can also affect the depolarization-dependent non-selective cation channels that determine this current (Demidchik et al., 2007; 2010). Therefore, ROS could be generated and act as second messengers that regulate $K^+$ channel activity during the plant cell response to ionizing radiation. Thus, changes in the activity of these channels could be reflected in the repolarization rate behaviour.

The estimated toxicity of HTO on the mechanisms of AP initiation may also include reduced anion efflux and thus be linked to the direct interaction of $Cl^-$ transport systems. Plant anion channels serve as switchers of stress response and make this effect possible (Roelfsema et al., 2012). In addition to the direct interaction between ionizing radiation and chloride channels, the toxicity could also be associated with HTO-induced ROS production that leads to inactivation of chloride channels (Jeulin et al, 2000; Barth et al., 1993, Thomas et al., 2007.) In contrast, the observed decrease in the chloride current can reflect not only the direct HTO action on anion channels but also the results

of radiation-induced impairment of calcium homeostasis. The latter is possible in plants because the calcium ion is a very important secondary messenger that is involved in the response to many environmental factors and biotic and abiotic stresses (Song et al., 2008). Moreover, the amplitude of the $Cl^-$ current is known to be proportional to the square of the cytoplasmic $Ca^{2+}$ concentration in plants (Berestovsky et al., 2005). Our results showing the changes in the inward $Ca^{2+}$ current (**fig. 3.4**) do not contradict the above mentioned proposition. We observed that the changes in the $Ca^{2+}$ ion transport interfered with the $Ca^{2+}$-dependent $Cl^-$ current in our experiments: the decrease in the inward $Ca^{2+}$ current decreased the $Ca^{2+}$ concentration in the cytoplasm, making it less conducive to opening voltage- and $Ca^{2+}$-dependent $Cl^-$ channels and thus reducing the $Cl^-$ efflux. In addition, calcium ions have been reported to play an important role in preventing the plant cell wall from structural damage due to γ irradiation at higher doses (Kovács et al., 2002). Therefore, the estimated HTO-induced decrease in the inward $Ca^{2+}$ current that regulates the cytosolic calcium concentration in plant cells can also portend the reduced resistance of the external cell barrier to environmental stressors.

It can be concluded that *Nitellopsis obtusa* internodal cells present a suitable model for monitoring HTO toxicity in an intact organism at the cellular level as the bioelectrical characteristics of its plasma membrane appear to react sensitively to low-dose ionizing radiation. The application of rapid electrophysiological techniques allowed the identification of weak-radiation-induced effects soon after irradiation; therefore, it was possible to investigate the impact of HTO on the algae cell PM bioelectricity at a very low external radiation dose rate for aquatic ecosystem. The estimated low-dose HTO-induced alterations in the electrical activity of plant cell membranes play a significant role, because it reflect not only functional or structural changes of PM, but also damage to other macromolecules that are important to intercommunication processes. Unfortunately, the mechanisms by which tritium, as well as other sources of low-dose ionizing radiation, affects the electrical properties of the Characeae PM are poorly studied and require more extensive examination.

## 4.2. Combined effect of tritium and aluminium on ions transport systems involved in of *Nitellopsis obtusa* electrogenesis

Phytotoxicity of Al was estimated in different plant species (Vardar et al., 2007) and is considered to be pH-dependant and appears due to predomination of its toxic form $A1^{3+}$ in acidic environment (pH < 5) (Vitorello et al., 2005). Aluminium toxicity in plants could be expressed in alteration of membrane potential, surface potential, $H^+$ - ATPase activity, $K^+$, $Ca^{2+}$, $Cl^-$ transport system functionality (Bose et al., 2010). Al induced inhibition of $H^+$ - ATPase coupled with decreased surface negativity of plasma membrane due to Al high affinity to PM surface resulted in subsequent impairment of ion transport across the membrane has been observed in squash roots (Ahn et al, 2001; Ahn, 2002). Depolarization of membrane potential due to Al treatment has been estimated in *Arabidopsis* (Illés et al., 2006), wheat (Papernik et al., 1997) Characeae *Chara carolina* (Takabatake et al, 1997) and *Nitellopsis obtusa* (Kisnieriene et al., 2005). Al treatment stimulated an increase in $K^+$ efflux and $Cl^-$ and $H^+$ influx in root apices of intact wheat seedlings (Wherrett et al., 2005). Investigation of protoplasts from wheat root cells revealed the existence of Al activated anion channel that allows Cl- efflux (Ryan et al., 1997). It has also been found that that Al can enter the plant cells through a $Ca^{2+}$ channel and inhibit $K^+$ uptake by blocking inward rectifying potassium channels from the cytoplasm site (Piñeros et al., 2001). Aluminium induced slowering of repolarization phase of AP has been showed in the internodal cells of *Nitellopsis obtusa* (Kisnieriene et al., 2007). Disruption of cytoplasmic $Ca^{2+}$ homeostasis has been suggested as a primary trigger of Al toxicity. Al can disrupt $Ca^{2+}$-dependent metabolism by maintaining elevated free $Ca^{2+}$ level in the cytoplasm. These could be due to both Al induced influx of $Ca^{2+}$ through depolarization-activated PM calcium channels and by enchantment of $Ca^{2+}$ release into the cytoplasm from tonoplast and endoplasmic reticulum (Rengel et al., 2003). But influence of other stress factors including IR on Al phytotoxicity remains poorly studied. Just few studies revealed enhancement of Al toxic effect due to common action with IR. In the present study we evaluated possible modifying effect of tritium on giant algal cell bioelectrical response pattern due to Al exposure. As might have been expected, we determined that at neutral pH Al alone did not significantly affect chloride and potassium transport systems involved in electrical

excitation of PM. On the other hand, we observed that inward $Ca^{2+}$ current together with membrane chord conductance for $Ca^{2+}$ decreased due to single Al exposure (**fig 3.7**). The shifted equilibrium potential for the calcium seen at **fig.3.7A** evidenced the interaction of Al with the gating mechanisms or selectivity filter of $Ca^{2+}$ channels resulted in decrease of opened channels quantity (Лапковская и др., 2010). This assumption does not contradict the data on the Al inhibitory properties dealing with $Ca^{2+}$ channels and the alteration of $Ca^{2+}$ homeostasis via competitive interaction between Al and $Ca^{2+}$ (Poschenrieder et al., 2008). Regarding the effect of HTO on PM electrical parameters in charophyte cells, it is important to note, that single 15 kBq $L^{-1}$ HTO caused significant depression of $Cl^-$ and $Ca^{2+}$currents (**fig 3.4 and 3.5**), and is considered to inhibitory affect $K^+$ transport, since it slowed the fast repolarization rate (**fig. 3.3**) determined by $K^+$ efflux. But what can be the reason of different or sometimes even inverse action of Al in combination with HTO we determined? It was reported that combined action of Al and IR cause higher increase of glial fibrillary acidic protein (GFAP) content in the rat brain then those evoked by single Al or single irradiation suggesting combined contribution of both factors to reorganization of glial cytoskeleton and influence on the level of free $Ca^{2+}$ (Nedzvetskii et al., 2001). Our results can also support this prediction, since estimated magnification of $Ca^{2+}$ current due to Al and HTO combined treatment could be associated with increase of concentration of free $Ca^{2+}$ in cytoplasm. Possible explanation of modifying action HTO on the Al toxicity on *Nitellopsis obtusa* electrogenesis might also occur through the oxidative stress promotion. It was shown that HTO and copper have a synergetic effect on reactive oxygen species (ROS) production (Rety et al., 2010). It has also been found that exposure of salmon skin and gill samples to of Al together with low-dose γ radiation determined stronger reduction in the clonogenic survival of HPV-G reporter cells than Al and IR alone suggesting their synergism in ROS production (Mothersill et al., 2014)

HTO toxicity can be expressed via local direct action on PM structures and also trough the water radiolysis resulting in induction of oxidative stress and ROS production in plant cell. Since IR induced water radiolysis leads to the production of hydroxyl radical (OH•) and particularly hydrogen peroxide ($H_2O_2$) this could appear as a probable basis of modifying action on the Al effect expression. Al induces oxidative stress indirectly through the Fenton reaction (Vitorello et al., 2005) and in contrast to HTO,

induces a slighter ROS increase in not acidic environment. Thus HTO-induced increase of $H_2O_2$ content enhances the possibility of Al to stimulate production of $OH^{\bullet}$. Apart from this, HTO could also influence the uptake of Al by the algae cell or affect antioxidant system enzymes through $H_2O_2$ oxidation of their thiol groups (Gill & Tuteja, 2010). As a consequence, the Al effect in combination with HTO could be modified comparing with single Al action.

So, results of Al effect on the voltage dependent outward rectifying $K^+$ current presented in **fig. 3** could be interpreted in accordance to above mentioned aspects. While $K^+$ efflux and voltage - independent $K^+$ leak current increased due to Al and HTO mixed treatment, but did not change after single Al exposure, we can predict that implication of HTO enhanced Al induced production of $OH^{\bullet}$ resulted in activation of additional $K^+$ channels (Demidchik et al., 2010).

Regarding to the amplification of outward $Cl^-$ current evoked by Al and HTO combined treatment, this reverse effect comparing with single Al (**fig. 3.6**) or single HTO (**fig. 3.5**) action could be caused by direct affect on chloride channels responsible for depolarisation phase of AP or activation of additional anion channels sensitive to ROS (Jeulin et al., 2000). On the other hand, modification of Al behaviour being in mixture with HTO may occur due to ROS induced intracellular acidification (Chao et al., 2002) leading to occurrence of $Al^{3+}$ resulting in increase of $Cl^-$current (Ryan et al., 1997). However, it is possible that contribution to the increase of $Cl^-$current belongs to the imbalance of $Ca^{2+}$ homeostasis due to Al and HTO exposure and can imply increment of internal $Ca^{2+}$ concentration (Berestovsky & Kataev, 2005). This proposition could be confirmed by our results of the investigations of $Ca^{2+}$ channels behaviour presented at **fig. 3.7** Al and HTO combined application in contrast to single Al (**fig. 3.7**) or single HTO (**fig. 3.4**) significantly increases inward $Ca^{2+}$ current. This effect could occur through $Ca^{2+}$ channels activated by $OH^{\bullet}$ similar to identified in *Arabidopsis* roots or non-selective cation channels involved in $Ca^{2+}$ influx.

Summarising the obtained results it could be concluded that the *Nitellopsis obtusa* PM bioelectrical properties provide informative indicators of the fast cell response to the Al and HTO stress. The estimated changes in the ion transport mechanisms especially in $Ca^{2+}$ homeostasis due to Al and HTO mixture could enable to predict further impairment of the electrogenesis, signal transduction and other essential biochemical conversations

resulted in cell dysfunction. It is very important while separate influence of Al is rather exception than routine in our mixed contaminated environment. The obtained results suggest, that except acidity, tritium can also be considered as an important factor for Al toxicity expression in plants. But the mechanisms of interaction of Al and HTO either other ionising radiation sources remain far from understanding, so further comprehensive investigations are necessary.

### 4.3. Bioelectrical expression of radiation induced bystander effects in *Nitellopsis obtusa*

RIBE in plants were evidenced for the first time very recently, in 2007 by Chinese research group, who showed that microbeam proton irradiation of shoot apical meristem in *Arabidopsis* embryo caused inhibition of root postembryonic development suggesting that RIBE in plants can occur at the long-distances within whole organism (Yang et al., 2007). Further experiments of this research group evidenced the existence of mutagenic RIBE in plants. They revealed the long-distance RIBE in growing seedlings of *Arabidopsis thaliana,* expressed in an elevated induction of homologous recombination, upregulated expression of the AtRAD54 gene, due to root irradiation with α-particles; suggesting that ROS are involved in mediation of RIBE in plants, since significant inhibition of mutagenic RIBE due to ROS scavengers treatment was observed (Li F et al., 2010). It has also been showed that some of RIBE signals might be transported only unidirectionally through the plasomdesmata via symplastic pathway in plants (Wang et al., 2012). Separate or interlinked ROS, $Ca^{2+}$ and MAPK signalling is considered as important candidate for RIBE mediation in animals and plants. ROS signalling in all plants occurs in response to different stress factors (Bailey-Serres et al., 2006). Plants employ these short - living reactive molecules as signal transducers and mediators of RIBE (Li F et al., 2010). $Ca^{2+}$ signalling is widely expressed in higher plants and Characeae (Tazawa et al., 2001). MAPK signalling in higher plants is widely showed, however less data could be found on MAPK involvement in algae stress response. Activation of some elements of MAPK signalling was found in *Volvox, Chlamydomonas, Osterococcus, Dunaliella viridis* ir *Dunaliella salina* (Chitlaru et al., 1997; Lei et al., 2008; Merchant et al., 2007; Palenik et al., 2007). Our previous study has shown that *Nitellopsis obtusa* osmotic and temperature stress induces generation of spontaneous action potentials together with activation of two small MAPKs (~ 48 kDa

and ~ 30 kDa) (our unpublished data). Therefore we can suppose that MAPK, as well as ROS and $Ca^{2+}$, could be involved in mediation of observed RIBE in *Nitellopsis obtusa* cells.

In order to investigate RIBE in *Nitellopsis obtusa* cells, tritium as a source of irradiation could not be used. Tritium due to its unique mobility in biological systems could be transported alone or together with other substances from directly irradiated to non target adjoining cell through symplast. I such case bioelectrical changes in non-target cell could be associated with direct action of tritium on this cell but not related to RIBE phenomenon. Therefore, in the study we used another radioactive β -emitter Sr 90. Sr 90 is a radioactive isotope of strontium with a half-life of 28.79 years. It undergoes $\beta^-$- decay with emission of electrons ($\beta^-$ energy of 546 keV) and yttrium isotope $^{90}$Y with half-life of 64 hours, which subsequently also undergoes β− decay with emission of electrons (β− energy of 2280 keV) and stable zirconium isotope ($^{90}$Zr). Sr 90 could be considered as a pure beta source since γ emission from the decay of 90Y is quite rare and usually could be ignored. In our experiments we used sealed source of Sr 90 with nominal activity of 74 kBq. Application of sealed radioactive source enabled us to avoid direct irradiation of neighbouring non target cell. Our study was designed to show the direct signal transmission between cells within plasmodesmata between *Nitellopsis obtusa* cells, therefore the outer mediums of both adjoining cells were isolated by vaseline gaps (**fig. 2.1**); such experimental conditions ensured that transmission of RIBE signals through external medium would be impossible.

In our RIBE experiments we found that irradiation of target intrenodal *Nitellopsis obtusa* cell for 30 min did not significantly affect bioelectrical activity of its neighbouring adjoined cell, while prolongation of exposure time up to 24 h significantly alerted some electrophysiological properties of non irradiated cell. Meanwhile, in directly irradiated cell the similar tendencies in bioelectrical parameters changes were observed already after 30 minutes of direct exposure to Sr 90. Time differences in appearance of effect in directly irradiated target cell and RIBE could occur since it takes longer time, starting from the effect occurrence in the target cell, subsequently signal transduction to the neighbouring cell and finally RIBE appearance in non target cell reflected in its bioelectrical response. During the analysis of action potential pattern in non irradiated cell, we found that AP amplitude decreased after the 24 h neighbouring

cell exposure (**fig. 3.9A**). It should be noted that decrease of AP amplitude was determined by decrease of AP peak value that is known to be dependent on electrochemical potential for chloride ions in plants ( Воденеев и др., 2009). Estimated shift of outward $Cl^-$ current reversal potential in not irradiated cell from ~38 mV to ~17 mV due to adjoined cell irradiation for 24 h (**fig. 3.11B**) could promote the presumption of possible changes in electrochemical potential for $Cl^-$. While chloride reversal potential strongly depends on chloride concentration differences inside and outside the cell, its shit to less positive voltage value could imply the decrease of $Cl^-$ intracellular concentration. Decrease of intracellular $Cl^-$ concentration could be determined by inhibition of $H^+/Cl^-$ - ATPase involved in maintenance of chloride intracellular concentration catalysing $2H^+/Cl^-$ simport to the cell after steep depolarisation of PM during AP in plants (Beilby et al., 1981). Inhibition of this proton pomp is possible due to elevation of ROS production related to RIBE. ROS inhibitory effect on PM proton pomp activity was found in *Arabidopsis* (Trouverie et al., 2008). On the other hand, altered homeostasis of intracellular $Cl^-$ could also be linked to possible activation of protective mechanisms in response to RIBE. Plant anion channels act as a stress response switchers and can be activated to exclude toxic substances from the cell. Some of these channels are preamble for organic acids as well as for $Cl^-$ (Roelfsema et al., 2012). There are known plant chloride channels activating by ROS (Trouverie et al., 2008). Thus, chloride ions partly could be transported out of the cell through these channels during 24 h in response to neighbouring cell irradiation resulting in decrease of intracellular chloride concentration. We suppose that depression of $Cl^-$ efflux during AP generation observed in not irradiated cell after irradiation of neighbouring cell for 24 h (**fig. 3.11A**) could also be linked to intracellular $Cl^-$ concentration decrease.

We found that irradiation of one of the adjoining *Nitelopsis obtusa* cells caused the enhancement of membrane potential recovery rate during fast repolarization after electrical excitation of neighbouring not exposed internodal cell (**fig. 3.9B**). AP fast repolarisation rate in *Characeae* in general is determined by steep $K^+$ efflux. Whereas we do not estimate significant changes in outward $K^+$ current in the steady state non target cell after its neighbouring cell irradiation, $K^+$ transport could not play a crucial role in observed changes of fast repolarization rate. However, AP repolarization in plants involves activation of outward $K^+$ transport together with inhibition of transport systems

providing $Cl^-$ efflux (Thiel et al., 1995). Thus, estimated depression of outward $Cl^-$ current together with decrease of AP amplitude could determine increase in repolarisation rate, while under this circumstance, smaller changes in membrane potential during depolarisation require less cellular resources to be recovered. Crucial contribution of chloride transport systems in RIBE could be assumed, since plant anion channels are known to play obvious role in long distance signalling due to their ability to serve in cell-to-cell communication, to synchronize signals within a plant; plant cells adjoined through plasmodesmata influence each other's membrane potential; can depolarize their neighbouring cells by simultaneous activation of anion channels in both (Roelfsema et al., 2012).

Observed bioelectrical changes in non target *Nitellopsis obtusa* cell could be determined by transduction of RIBE mediators of biochemical nature as well as electrical signal through plasmodesmata from irradiated adjoining intermodal cell. Transmitted electrical signal could directly alter bioelectrochemical system or induce physiological response in non-target cell (Pyatygin et al., 2011), for example, via triggering stress hormone biosynthesis (Davies, 2004). Commonly RIBE interpretation is based on appeared genetic effects within non irradiated organisms or cells. Our study is inconsistent with this, since estimated bioelectrical alterations of non target cell could result in or imply both subgenetic and genetic effects within the cell, whereas changes in gene expression could affect membrane regulatory and barrier functions (Cramp et al., 1994; Santini et al., 1992; Somosy et al., 2000; Stanković et al., 1996); meanwhile, electrical signals could influence gene expression (Volkov, 2012)

In summary, we can conclude that irradiation of one of the adjoining internodal could result in bioelectrical changes in its neighbouring not exposed cell. Results of control experiments eliminated possibility of contribution of prolonged cells exposition in APW to estimated changes of investigated bioelectrical parameters of non target cell. Revealed similarity in changes of AP amplitude, rate of repolarisation, chloride outward transport and reversal potential to $Cl^-$ in both directly irradiated target cell and in non target cell adjoined to irradiated cell let to presume that RIBE can be expressed in electrophysiological alterations within plant cells. Moreover, strong separation of investigated cells medium shows that that RIBE in two adjoining charophyte cells could occur due to cell-to-cell signal transduction through plasmodesmata, without medium

contribution. Our proposed applied experimental protocol suggests an approach to investigate propagation of RIBE due cell-to-cell intercommunication without medium contribution *in vivo* without any chemical intervention to cell functionality.

## 5. CONCLUSIONS

1. 15 kBq $L^{-1}$ tritium inhibited $Cl^-$ and $Ca^{2+}$ transport and decreased the rate of fast repolarization phase of the action potential in *Nitellopsis obtusa* cells.
2. *Nitellopsis obtusa* cells expressed different bioelectrical responses to tritium, aluminium and combined aluminium and tritium treatment: combined tritium and aluminium exposure caused activation of $K^+$, $Ca^{2+}$ ir $Cl^-$ transport systems involved in Charophyte electrogenesis.
3. Radiation induced bystander effects in two adjoining internodal cells of *Nitellopsis obtusa* was expressed in changes of bioelectrical activity of non target cell: ionizing radiation exposure of target internodal cell caused inhibition of $Cl^-$ transport, changes in reversal potential of chloride ions and increase in the rate of fast repolarization phase during action potential in neighbouring non target internodal cell
4. Radiation induced bystander effects in Charophyte cells could be associated with cell-to-cell signal transduction through plasmodesmata

## SANTRAUKA (SUMMURY IN LITHUANIAN)

Šiuolaikiniame pasaulyje mažų dozių jonizuojančioji spinduliuotė turi reikšmingą poveikį žmogui ir aplinkai, nes jos šaltiniai gali būti padidėjęs gamtinis radiacinis fonas, padidėjusi aplinkos antropogeninė tarša, medicininė, profesinė ir avarinė apšvita. Šiame darbe pirmą kartą parodyta, kad mažos išorinės apšvitos dozės, sąlygotos radioaktyvaus vandenilio izotopo tričio, veikia *Nitellopsis obtusa* ląstelių membranų elektrines savybes. Kadangi buvo nustatyta, kad menturdumblių elektriniai atsakai į egzogeninį aliuminio poveikį kinta dėl tričio buvimo menturdumblių ląstelių aplinkoje, tritis galėtų būti laikomas reikšmingu veiksniu aliuminio fitotoksiškumo pasireiškimui. Taip pat buvo parodyta, kad tiesioginės apšvitos nepaveiktoje *Nitellopsis obtusa* menturdumblių ląstelėje dėl tarpląstelinio signalų perdavimo per plazmodezmas vyksta bioelektrinio aktyvumo pokyčiai, sąlygoti per bamblį sujungtos ląstelės švitinimo mažomis jonizuojančiosios spinduliuotės dozėmis, skleidžiamomis uždaro radioaktyvaus stroncio izotopo šaltinio. Šio darbo metu gauti rezultatai leidžia daryti prielaidą, kad menturdumblių elektrofiziologiniai tyrimai galėtų prisidėti prie mažų jonizuojančiosios spinduliuotės sukeliamų fenomenų augaluose *in vivo* tyrimų ląsteliniame lygmenyje, o menturdumblių bioelektrinės reakcijos galėtų būti naudojamos kaip jautrūs jonizuojančiosios spinduliuotės poveikio indikatoriai aplinkos radioekologinėje stebėsenoje ir biotos apšvitos vertinime.

**PUBLICATIONS**

**Publications listed in the *Thomson Reuters Web of Science* database:**

1. **Olga Sevriukova**, Auste Kanapeckaite, Vilma Kisnieriene, Rima Ladygiene, Indre Lapeikaite, and Vidmantas Sakalauskas. Modifying action of tritium on the charophytes bioelectrical response to anthropogenic pollution. Trace Elements and Electrolytes. 2014, 31 (2): 60-66, ISSN 0946-2104.

2. **Olga Sevriukova**, Auste Kanapeckaite, Indre Lapeikaite, Vilma Kisnieriene, Rima Ladygiene, Vidmantas Sakalauskas. Charophyte Electrogenesis as a Biomarker for Assessing the Risk from Low-Dose Ionizing Radiation to a Single Plant Cell. Journal of Environmental Radioactivity, 2014. Status: accepted. DOI 10.1016/j.jenvrad.2014.04.016, ISSN 0265-931X

3. **Olga Sevriukova**, Auste Kanapeckaite, Indre Lapeikaite, Vilma Kisnieriene, Vidmantas Sakalauskas. Impact of low dose Tritium on the bioelectrical activity of Charophyte cell. Proceedings of 11th International Conference on Medical Physics Kaunas University of Technology, 2013, 61-66, ISSN 1822-5721

**Other peer-reviewed publications (not included in thesis):**

1. Olga Sevriukova, Ieva Lebednikienė, Jūratė Vaicekavičiūtė. Radiacinės saugos mokymo žinių poreikių analizė Lietuvoje. Visuomenės sveikata. 2009, 3/(46): 33-38. ISSN 1392-2696

2. O. Sevriukova, V. Sakalauskas, V. Kisnierienė. Application of intracellular microelectrode measurements and voltage clamp techniques for the investigation of the effect of acetylcholine on plant cells membrane parameters. Proceedings of 13$^{th}$ International conference Biomedical engineering, 2009, 124-127. ISSN 2029-3380

**Presentations in conferences:**

1. **О. Севрюкова**, В. Сакалаускас, В. Киснериене. Воздействие малых доз трития на механизмы генерации потенциала действия в растительной клетке. Сборник докладов 17 международной школы – конференции молодых ученых « Биология – наука 21 века», 2013, стр. 149-150

2. **Olga Sevriukova**, Vidmantas Sakalauskas, Indrė Lapeikaite, Jonas Burneika, Kristina Panaciova, Vilma Kisnieriene. The combined effect of aluminium and tritium on the plant cell membrane bioelectrical parameters. 10th Keele Meeting Abstracts Book, 2013, p. 48

3. Vilma Kisnieriene, Jonas Burneika, Indrė Lapeikaite, **Olga Sevriukova**, Vidmantas Sakalauskas. Influence of $Al^{3+}$ on light-induced membrane potential changes of Nitellopsis obtusa cells. 10th Keele Meeting Abstracts Book, 2013, p. 41

4. **О. Севрюкова**, В. Сакалаускас, В. Киснериене, Й. Бурнейка, И. Лапейкайте. Воздействие малых доз ионизирующего излучения на биоэлектрические параметры растительной клетки. Сборник докладов международной научно – практической конференции «Клеточная биология и биотехнология растений». 2013, 109

**Presentations in conferences (not included in thesis):**

1. Kisnieriene V., Sakalauskas V., **Sevriukova O**., The application of action potential analysis to define metals impact on plants cells. Proceedings of 3rd International Symposium on Metallomics Münster/Germany, June 15 - 18, 2011

2. V. Kisnierienė, V. Sakalauskas, **O. Sevriukova**. Paveldimas spazminis paralyžius. Ar Arabidopsis thaliana šakniaplaukių augimo viduląsteliniai procesai gali padėti suprasti neuronų degeneracines ligas. Antrosios mokslinės Lietuvos neuromokslų asociacijos konferencijos pranešimų medžiaga. 2010, p.16

3. **О. Севрюкова**, В. Сакалаускас, В. Киснериене. Воздействие ацетилхолина на биоэлектрические параметры растительной клетки. Сборник докладов 14 Международной школы – конференции молодых ученых « Биология – наука 21 века», 2010, Том 1, стр. 216

4. **O. Sevriukova**, V. Sakalauskas, V. Kisnierienė. Acetylcholino įtaka bioelektrinių signalų perdavimui augalinėse ląstelėse. Pirmosios mokslinės Lietuvos neuromokslų asociacijos konferencijos „Nervų sistemos tyrimai Lietuvoje“pranešimų medžiaga, 2009, p. 23

## REFERENCES


1. Adelstein, S, J. (2001). Biologic Responses to Low Doses of Ionizing Radiation : J Nucl Med, 42(9), 26–37.
2. Adliene, D., Rääf, C., Magnusson, A., Behring, J., Zakaria, M., Adlys, G., Mattsson, S. (2006). Assessment of the environmental contamination with long-lived radionuclides around an operating RBMK reactor station. Journal of Environmental Radioactivity, 90(1), 68–77.
3. Ahn, S. J. (2002). Aluminium-induced growth inhibition is associated with impaired efflux and influx of H+ across the plasma membrane in root apices of squash (Cucurbita pepo). Journal of Experimental Botany, 53(376), 1959–1966.
4. Ahn, S. J., Sivaguru, M., Osawa, H., Chung, G. C., & Matsumoto, H. (2001). Aluminum inhibits the H(+)-ATPase activity by permanently altering the plasma membrane surface potentials in squash roots. Plant Physiology, 126(4), 1381–90.
5. Alvim, M. N., Ramos, F. T., Oliveira, D. C., Isaias, R., & França, M. G. C. (2012). Aluminium localization and toxicity symptoms related to root growth inhibition in rice (Oryza sativa L.) seedlings. Journal of Biosciences, 37(S1), 1079–1088.
6. Bailey-Serres, Julia, R. M. (2006). The Roles of Reactive Oxygen Species in Plant Cells. Plant Physiology, 141, 311.
7. Baluska, F., & Mancuso, S. (2013). Ion channels in plants From bioelectricity, via signaling, to behavioral actions. Plant Signaling & Behavior, 8(1), 176–179.
8. Barth C., Stark G., W. M. (1993). Inactivation by ionizing radiationof ion channels formed by polyene antibiotics amphotericin B and nystatin in lipid membranes: An inverse dose-rate behavior. Biophysical Journal, 64, 92–97.
9. Beilby, M. J. (2007). Action potential in charophytes. International Review of Cytology, 257(07), 43–82.
10. Beilby, M. J., & Casanova, M. T. (2014). The Physiology of Characean Cells. Berlin, Heidelberg: Springer Berlin Heidelberg.
11. Beilby, M. J., & Walker, N. a. (1981). Chloride Transport in Chara. Journal of Experimental Botany, 32(1), 43–54.

12. Berestovsky, G. N., & Kataev, A. A. (2005). Voltage-gated calcium and Ca2+-activated chloride channels and Ca2+ transients: voltage-clamp studies of perfused and intact cells of Chara. European Biophysics Journal : EBJ, 34(8), 973–986.
13. Boyer, C., Vichot, L., Fromm, M., Losset, Y., Tatin-Froux, F., Guétat, P., & Badot, P. M. (2009). Tritium in plants: A review of current knowledge. Environmental and Experimental Botany, 67(1), 34–51.
14. Bose, J., Babourina, O., Shabala, S., & Rengel, Z. (2010). Aluminum-dependent dynamics of ion transport in Arabidopsis: specificity of low pH and aluminum responses. Physiologia Plantarum, 139(4), 401–12.
15. Bradshaw, C., & Jaeschke, B. C. (2013). Bioaccumulation of tritiated water in phytoplankton and trophic transfer of organically bound tritium to the blue mussel, Mytilus edulis. Journal of Environmental Radioactivity, 115, 28–33.
16. Burlakova EB. (1995). Low Intensity Radiation : Radiobiological Aspects. Radiation Protection Dosimetry, 62(1-2), 13–18.
17. Chao, C.-M., Jin, J.-S., Tsai, C.-S., Tsai, Y., Chen, W.-H., Chung, C.-C., & Loh, S.-H. (2002). Effect of hydrogen peroxide on intracellular pH in the human atrial myocardium. The Chinese Journal of Physiology, 45(3), 123–9.
18. Chitlaru, E., Seger, R., & Pick, U. (1997). Activation of a 74 kDa plasma membrane protein kinase by hyperosmoti ... Activation of a 74 kDa plasma membrane protein kinase by hyperosmotic shocks in the halotolerant alga D un a l i el l a sa lin a Activation of a 74 kDa plasma membrane protein kinase, 157, 429–436.
19. Cramp W. A., Yatvin M. B., H.-R. M. (1994). Recent developments in the radiobiology of cellular membranes. Acta Oncologica Vol., 33(8), 945–952.
20. Dallas, L. J., Keith-Roach, M., Lyons, B. P., & Jha, A. N. (2012). Assessing the Impact of Ionizing Radiation on Aquatic Invertebrates : A Critical Review. Radiation Research, 177, 693–716.
21. Daňová, D., Kafka, I., Kaleničová, Z., & Nováková, J. (2010). The Effect of Low Dose Ionizing Radiation and Cadmium Chloride on Glucose Metabolism in Broiler Chickens. Acta Veterinaria Brno, 79(3), 415–418.

22. Darbre, PD, Bakir, A, Iskakova, E. (2013). Effect of aluminium on migratory and invasive properties of MCF-7 human breast cancer cells in culture. Journal of Inorganic Biochemistri, 128, 245–249.
23. Davies, E. (2004). New functions for electrical signals in plants. New Phytologist, 161, 607–610.
24. Delistraty, D. (2008). Radioprotection of nonhuman biota. Journal of Environmental Radioactivity, 99(12), 1863–1869.
25. Demidchik, V., Cuin, T. A., Svistunenko, D., Smith, S. J., Miller, A. J., Shabala, S., … Yurin, V. (2010). Arabidopsis root K+-efflux conductance activated by hydroxyl radicals: single-channel properties, genetic basis and involvement in stress-induced cell death. Journal of Cell Science, 123(Pt 9), 1468–1479.
26. Demidchik, V., & Maathuis, F. J. M. (2007). Physiological roles of nonselective cation channels in plants: from salt stress to signalling and development. The New Phytologist, 175(3), 387–404.
27. Demidchik, V, Sokolik, A, Y. (1997). The Effect of Cu2 + on lon Transport Systems of the Plant. Plant Physiology, 114, 1313–1325.
28. Dmitriev, A., Shevchenko, O., Polischuk, V., & Guscha, N. (2009). Effects of low dose chronic radiation and heavy metals on plants and their fungal and virus. Data Science Journal, 8, 49–66.
29. Fairbrother, A, Wenstel, R, Sappington, K, Wood, W. (2007). Framework for metals risk assessment. Ecotoxicology and Environmental Safety, 68(2), 145–227.
30. Feinendegen, L. E. (2005). Evidence for beneficial low level radiation effects and radiation hormesis. British Journal of Radiology, 78(925), 3–7.
31. Fromm, J., & Lautner, S. (2007). Electrical signals and their physiological significance in plants. Plant, Cell & Environment, 30(3), 249–257.
32. Galeriu, D., Melintescu, A., Strack, S., Atarashi-Andoh, M., & Kim, S. B. (2013). An overview of organically bound tritium experiments in plants following a short atmospheric HTO exposure. Journal of Environmental Radioactivity, 118, 40–56.
33. Garnier-Laplace, J., Geras'kin, S., Della-Vedova, C., Beaugelin-Seiller, K., Hinton, T. G., Real, A., & Oudalova, A. (2013). Are radiosensitivity data derived from natural field conditions consistent with data from controlled exposures? A

case study of Chernobyl wildlife chronically exposed to low dose rates. Journal of Environmental Radioactivity, 121, 12–21.

34. Gen Yang a, Lijun Wu , Lianyun Chen, Bei Pei , Yugang Wang, Furu Zhan, Yuejin Wu, Z. Y. (2007). Targeted Irradiation of Shoot Apical Meristem of Arabidopsis Embryos Induces Long-Distance Bystander/Abscopal Effects. Radiation Research:, 167(3), 298 – 305.
35. Geras'kin, S. A., Oudalova, A. A., Kim, J. K., Dikarev, V. G., & Dikareva, N. S. (2007). Cytogenetic effect of low dose gamma-radiation in Hordeum vulgare seedlings: non-linear dose-effect relationship. Radiation and Environmental Biophysics, 46(1), 31–41.
36. Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry : PPB / Société Française de Physiologie Végétale, 48(12), 909–30.
37. Gupta, N., Gaurav, S. S., & Kumar, A. (2013). Molecular Basis of Aluminium Toxicity in Plants : A Review. American Journal of Plant Science, 4, 21–37.
38. Horváth, F., Erdei, L., Wodala, B., Homann, U., & Thiel, G. (2002). Deltamethrin rescues run down of K + outward rectifying channels in Vicia faba guard cells, 46(2 mM), 19–20.
39. ICRP. (2008). Environmental Protection - the Concept and Use of Reference Animals and Plants. ICRP Publication 108. Ann. ICRP, 38 (4-6).
40. Illés, P., Schlicht, M., Pavlovkin, J., Lichtscheidl, I., Baluska, F., & Ovecka, M. (2006). Aluminium toxicity in plants: internalization of aluminium into cells of the transition zone in Arabidopsis root apices related to changes in plasma membrane potential, endosomal behaviour, and nitric oxide production. Journal of Experimental Botany, 57(15), 4201–13.
41. Jean-Baptiste, P., & Fourré, E. (2013). The distribution of tritium between water and suspended matter in a laboratory experiment exposing sediment to tritiated water. Journal of Environmental Radioactivity, 116, 193–196.
42. Jeulin, C., Fournier, J., Marano, F., Dazy, A.-C. (2000). Effects of hydroxyl radicals on outwardly rectifying chloride channels in a cultured human bronchial cell line. Eur. J. Physiol., 439, 331–338.

43. Kawahara, M., & Kato-Negishi, M. (2011). Link between Aluminum and the Pathogenesis of Alzheimer's Disease: The Integration of the Aluminum and Amyloid Cascade Hypotheses. International Journal of Alzheimer's Disease, 2011, 276 - 393.
44. Khan, N., Gray, I. P., Obejero-Paz, C. a, & Jones, S. W. (2008). Permeation and gating in CaV3.1 (alpha1G) T-type calcium channels effects of Ca2+, Ba2+, Mg2+, and Na+. The Journal of General Physiology, 132(2), 223–38.
45. Kim, S. B., Baglan, N., & Davis, P. A. (2013). Current understanding of organically bound tritium (OBT) in the environment. Journal of Environmental Radioactivity, 126C, 83–91.
46. Kisnieriene, V., Ditchenko, T. I., Kudryashov, A. P., Sakalauskas, V., Yurin, V. M., & Ruksenas, O. (2012). The effect of acetylcholine on Characeae K+ channels at rest and during action potential generation. Central European Journal of Biology, 7(6), 1066–1075.
47. Kisnierienė, V., & Sakalauskas, V. (2005). Al 3 + induced membrane potential changes in Nitellopsis obtusa cells, (1), 31–34.
48. Kisnieriene, V., & Sakalauskas, V. (2007). The effect of aluminium on bioelectrical activity of the Nitellopsis obtusa cell membrane after H+-ATPase inhibition. Central European Journal of Biology, 2(2), 222–232.
49. Kisnierienė, V., Sakalauskas, V., Pleskačiauskas, A., Yurin, V., & Rukšėnas, O. (2009). The combined effect of Cd2+ and ACh on action potentials of Nitellopsis obtusa cells. Central European Journal of Biology, 4(3), 343–350.
50. Koteles, G. (1982). Radiation Effects on Cell Membranes *. Radiation and Environmental Biophysics, 21, 1–18.
51. Kourie, J. I. (1998). Interaction of reactive oxygen species with ion transport mechanisms. American Journal of Physiology - Cell Physiology, 275, C1–C24.
52. Kovács, E., & Keresztes, A. (2002). Effect of gamma and UV-B/C radiation on plant cells. Micron (Oxford, England : 1993), 33(2), 199–210.
53. Larsson, C.-M. (2008). An overview of the ERICA Integrated Approach to the assessment and management of environmental risks from ionising contaminants. Journal of Environmental Radioactivity, 99(9), 1364–1370.

54. Lei G, Qiao D, B. L. (2008). Isolation and characterization of a mitogen-activated protein kinase gene in the halotolerant alga Dunaliella salina. Journal of Applied Phycology, 20, 13–17.
55. Li F , Liu P, Wang T, Bian P, Wu Y, Wu L, Y. Z. (2010). The induction of bystander mutagenic effects in vivo by alpha-particle irradiation in whole Arabidopsis thaliana plants. Radiat Res., 174(2), 228–237.
56. Little, M. P., & Lambert, B. E. (2008). Systematic review of experimental studies on the relative biological effectiveness of tritium. Radiation and Environmental Biophysics, 47(1), 71–93.
57. Lukšienė, B., Marčiulionienė, D., Gudelienė, I., & Schönhofer, F. (2013). Accumulation and transfer of 137Cs and 90Sr in the plants of the forest ecosystem near the Ignalina Nuclear Power Plant. Journal of Environmental Radioactivity, 116, 1–9.
58. Manusadzianas, L., Maksimov, G., Darginaviciene, J., Jurkoniene, S., Sadauskas, K., & Vitkus, R. (2002). Response of the charophyte Nitellopsis obtusa to heavy metals at the cellular, cell membrane, and enzyme levels. Environmental Toxicology, 17(3), 275–283.
59. Marples, B, Lambin, P, Skov , K, A, Joiner, M, C. (1997). Low dose hyper-radiosensitivity and increased radioresistance in mammalian cells. International Journal of Radiobiology, 71(6), 725–735.
60. Martac L, Podgorac J, S. S. (2010). Evaluation of the neurotoxical effect of aluminum on the wistar rat. Arch. Biol. Sci., Belgrade, 62(3), 585–588.
61. Melintescu, A., & Galeriu, D. (2011). Dynamic model for tritium transfer in an aquatic food chain. Radiation and Environmental Biophysics, 50(3), 459–473.
62. Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ, Witman GB, Terry A, Salamov A, Fritz-Laylin LK, M.-D. L. et al. (2007). Display Settings : The Chlamydomonas genome reveals the evolution of key animal and plant functions . Journal of Applied Phycology, 318, 245–250.
63. Mossor-Pietraszewska, T. (2001). Effect of aluminium on plant growth and metabolism. Acta Biochimica Polonica, 48(3), 673–86.

64. Mothersill, C., & Seymour, C. (2005). Display Settings : Radiation-induced bystander effects : are they good , bad or both ? Med Confl Surviv, 21(2), 101–110.
65. Mothersill, C., & Seymour, C. (2006). Radiation-induced bystander effects: evidence for an adaptive response to low dose exposures? Dose-Response : A Publication of International Hormesis Society, 4(4), 283–90.
66. Mothersill, C., Smith, R. W., Heier, L. S., Teien, H.-C., Land, O. C., Seymour, C. B., Salbu, B. (2014). Radiation-induced bystander effects in the Atlantic salmon (salmo salar L.) following mixed exposure to copper and aluminum combined with low-dose gamma radiation. Radiation and Environmental Biophysics, 53(1), 103–14.
67. Nedveckaite, T., Filistovic, V., Marciulioniene, D., Kiponas, D., Remeikis, V., & Beresford, N. A. (2007). Exposure of biota in the cooling pond of Ignalina NPP: hydrophytes. Journal of Environmental Radioactivity, 97(2-3), 137–147.
68. Nedveckaite, T., Filistovic, V., Marciulioniene, D., Prokoptchuk, N., Plukiene, R., Gudelis, A., Beresford, N.-A. (2011). Background and anthropogenic radionuclide derived dose rates to freshwater ecosystem: nuclear power plant cooling pond: reference organisms. Journal of Environmental Radioactivity, 102(8), 788–795.
69. Nedveckaite, T., Gudelis, A., & Vives i Batlle, J. (2013). Impact assessment of ionizing radiation on human and non-human biota from the vicinity of a near-surface radioactive waste repository. Radiation and Environmental Biophysics, 52(2), 221–234.
70. Nedveckaite, T., & Marciulioniene, D. (2011). Radiological and Environmental Effects in Ignalina Nuclear Power Plant Cooling Pond – Lake Druksiai : From Plant put in Operation to Shut Down Period of Time. In P. Tsvetkov (Ed.), Nuclear Power - Operation, Safety and Environment (pp. 261–286). In Tech.
71. Nedzvetskii V. S., Nerush P. A., Tikhomirov A. A., R. L. A. (2001). Effects of Ionizing Radiation and Aluminum Chloride on Protein of Glial intermediate Filaments in the Rat Brain. Neurophysiology, 33, 33–38.
72. Opritov, V. A., Pyatygin, S. S., & Vodeneev, V. A. (2002). Direct Coupling of Action Potential Generation in Cells of a Higher Plant ( Cucurbita pepo ) with the

Operation of an Electrogenic Pump. Russian Journal of Plant Physiology, 49(1), 160–165.

73. Palenik, B., Grimwood, J., Aerts, A., Rouzé, P., Salamov, A., Putnam, N., … Grigoriev, I. V. (2007). The tiny eukaryote Ostreococcus provides genomic insights into the paradox of plankton speciation. Proceedings of the National Academy of Sciences of the United States of America, 104(18), 7705–10.
74. Panda, S. K., Baluska, F., & Matsumoto, H. (2009). Aluminum stress signaling in plants. Plant Signaling & Behavior, 4(7), 592–7.
75. Papernik, L. a., & Kochian, L. V. (1997). Possible Involvement of Al-Induced Electrical Signals in Al Tolerance in Wheat. Plant Physiology, 115(2), 657–667.
76. Piñeros, M. a, & Kochian, L. V. (2001). A patch-clamp study on the physiology of aluminum toxicity and aluminum tolerance in maize. Identification and characterization of Al(3+)-induced anion channels. Plant Physiology, 125(1), 292–305.
77. Pyatygin, S. S., Opritov, V. a., & Vodeneev, V. a. (2011). Signaling role of action potential in higher plants. Russian Journal of Plant Physiology, 55(2), 285–291.
78. Poschenrieder, C., Gunsé, B., Corrales, I., & Barceló, J. (2008). A glance into aluminum toxicity and resistance in plants. The Science of the Total Environment, 400(1-3), 356–68.
79. Pottosin, I., Velarde-Buendía, A. M., Bose, J., Zepeda-Jazo, I., Shabala, S., & Dobrovinskaya, O. (2014). Cross-talk between reactive oxygen species and polyamines in regulation of ion transport across the plasma membrane: implications for plant adaptive responses. Journal of Experimental Botany, 65(5), 1271–83.
80. Prise, K. M., Folkard, M., & Michael, B. D. (2003). A review of the bystander effect and its implications for low-dose exposure. Radiation Protection Dosimetry, 104(4), 347–55.
81. Rengel, Z., & Reid, R. J. (1997). Uptake of Al across the plasma membrane of plant cells, 31–35.
82. Rengel, Z., & Zhang, W.-H. (2003). Role of dynamics of intracellular calcium in aluminium-toxicity syndrome. New Phytologist, 159(2), 295–314. d

83. Rety, C., Gilbin, R., & Gomez, E. (2010). Induction of Reactive Oxygen Species and Algal Growth Inhibition by Tritiated Water with or Without Copper. Environmental Toxicology, 1, 155–165.
84. Ryan, P. R., Skerrett, M., Findlay, G. P., Delhaize, E., & Tyerman, S. D. (1997). Aluminum activates an anion channel in the apical cells of wheat roots. Proceedings of the National Academy of Sciences of the United States of America, 94(12), 6547–52.
85. Ryan, RP, Skerrett, M, Findlay , GP, Delhaize, E, Tyerman, D. (1997). Aluminium activates an anion channel in the apical cell of wheat roots. Plant Biology, 94, 6547–6552.
86. Roelfsema, M. R. G., Hedrich, R., & Geiger, D. (2012). Anion channels: master switches of stress responses. Trends in Plant Science, 17(4), 221–229.
87. Röttinger, E. M., & Hug, O. (1972). The effects of low energy x-rays on membrane potential, membrane resistance and action potential of Nitella flexilis. Radiation Research, 50(3), 491–503.
88. ROUT, GR, SAMANTARAY, S, DAS, P. (2001). Aluminium toxicity in plants : a review. Agronomie, 21, 3–21.
89. Santini, M. T., Cametti, C., Bonincontro, a, Napolitano, M., Indovina, P. L., Durante, M., Grossi, G. F. (1992). Differences in membrane electrical properties between C3H 10T1/2 mouse embryo fibroblasts and their ionizing radiation and chemically transformed counterparts. European Biophysics Journal : EBJ, 20(6), 305–9.
90. Shemetun, O. V., & Pilins'ka, M. a. (2007). Radiation-induced "bystander" effect. Cytology and Genetics, 41(4), 251–255.
91. Shimmen, T., Mimura, T., Kikuyama, M., & Tazawa, M. (1994). Characean Cells as a Tool for Studying Electrophysiological of Plant Cells Characteristics, 278, 263–278.
92. Silva, S., Pinto, G., Mc, D., & Cm, C. (2012). Aluminium long-term stress differently affects photosynthesis in rye genotypes. Plant Physiology and Biochemistry, 54, 105–112.
93. Somosy, Z. (2000). Radiation response of cell organelles. Micron (Oxford, England : 1993), 31(2), 165–181.

94. Song, W.-Y., Zhang, Z.-B., Shao, H.-B., Guo, X.-L., Cao, H.-X., Zhao, H.-B., Hu, X.-J. (2008). Relationship between calcium decoding elements and plant abiotic-stress resistance. International Journal of Biological Sciences, 4(2), 116–25.
95. Spiewla, E. (1995). Dependence of oscilatory changes in electrical resistance of Nitellopsis obtusa cell membrane on the vegetation season. Acta Societatis Botanicorum Polanie, 64(4), 375–377.
96. Stanković, B., & Davies, E. (1996). Both action potentials and variation potentials induce proteinase inhibitor gene expression in tomato. FEBS Letters, 390(3), 275–9.
97. Sukhov, V., Nerush, V., Orlova, L., & Vodeneev, V. (2011). Simulation of action potential propagation in plants. Journal of Theoretical Biology, 291, 47–55.
98. Sukhov, V., & Vodeneev, V. (2009). A mathematical model of action potential in cells of vascular plants. The Journal of Membrane Biology, 232(1-3), 59–67.
99. Takabatake, R., & Shimmen, T. (1997). Inhibition of Electrogenesis by Aluminum in Characean Cells. Plant and Cell Physiology, 38(11), 1264–1271.
100. Tazawa, M., Kikuyama, M., & Okazaki, Y. (2001). Different cytoplasmic calcium contents among three species of Characeae. Plant & Cell Physiology, 42(6), 620–6.
101. Tekpli, X., Holme, J. a, Sergent, O., & Lagadic-Gossmann, D. (2013). Role for membrane remodeling in cell death: implication for health and disease. Toxicology, 304, 141–57.
102. Thiel, G., Institut, P., Gtttingen, U., Karsptile, U., & Gtttingen, D.-. (1995). Dynamics of chloride and potassium currents during the action potential in Chara studied with action potential clamp, 85–92.
103. Thomas, M.P., Chartand K., Reynolds, A., Vitvitsky, V., Banerjee, R., Gendelman, H. E. (2007). ROS ir K ir chloras.pdf. Journal of Neurochemistry, 100, 503–519.
104. Trouverie, J., Vidal, G., Zhang, Z., Sirichandra, C., Madiona, K., Amiar, Z., … Brault, M. (2008). Anion channel activation and proton pumping inhibition involved in the plasma membrane depolarization induced by ABA in Arabidopsis thaliana suspension cells are both ROS dependent. Plant & Cell Physiology, 49(10), 1495–507.

105. Ulanovsky, a, Pröhl, G., & Gómez-Ros, J. M. (2008). Methods for calculating dose conversion coefficients for terrestrial and aquatic biota. Journal of Environmental Radioactivity, 99(9), 1440–8.

106. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). (2012). Biological mechanisms of radiation actions at low doses. (U. NATIONS, Ed.). New York.

107. Vardar, F., & Ünal, M. (2007). Aluminum toxicity and resistance in higher plants. Advances in Molecular Biology, 1, 1–12.

108. Vitorello, VA, Capaldi, FR, Stefanuto, V. (2005). Recent advances in aluminum toxicity and resistance in higher plants. Braz. J. Plant Physiol., 17(1), 129–143.

109. Volkov, A. G. (Ed.). (2012). Plant Elektrophysiology: Signaling and Responses (pp. 152–159). Springer-Verlag Berlin Heidelberg.

110. Volkov, A. G., & Ranatunga, D. R. A. (2006). Plants as environmental biosensors. Plant Signaling & Behavior,, 1(3), 105–15.

111. Wang, T., Li, F., Xu, W., Bian, P., Wu, Y., & Wu, L. (2012). Novel features of radiation-induced bystander signaling in Arabidopsis thaliana demonstrated using root micro-grafting. Plant Signaling & Behavior, 7(12), 1566–72.

112. Wherrett, T, Ryan, PR, Delhaize, E, Shabala, S. (2005). Effect of aluminium on membrane potential and ion fluxes at the apices of wheat roots. Functional Plant Biology, 32(3), 199–208.

113. Лапковская, ЕМ, Филипцова, ГГ, Соколик, АИ, Юрин, В. (2010). Влияние синтетических аналогов простаноидов на Ca2+ проницаемые каналы плазматической мембраны растительной клетки. Физиология Растений. Труды БГУ, 5(1), 95–99.

114. Воденеев В.А.,. Сухов В.С, Опритов В.А., Царева Ю.В.,. Яковлева Е.В,. Неруш В.Н, Акинчиц Е.К. (2009) Динамика внеклеточной концентрации ионов хлора при генерации холодоиндуцированного потенциал действия у высших растений. Вестник Нижегородского университета им. Н.И. Лобачевского, 2, 111–115

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