Basic Medical Sciences Doctoral School

Effects of short-term hypoxia and hyperoxia on the morphology and function of rat hippocampal neurons

PhD Thesis

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Pécs, 2024

I. Introduction

Unusual changes in oxygen concentration that differ from the physiological oxygen level and often go beyond the body's compensatory mechanisms. A decrease in the oxygen (O_2) level results in a hypoxic state in the tissues, which is also involved in the pathogenesis of several neurological disorders, such as Alzheimer's disease and Parkinson's disease [1, 2]. Neonatal hypoxia-ischemia is responsible for 23% of infant mortality and is a severe risk factor, especially in the development of cognitive and learning problems [3-5]. At the same time, mild hypoxic treatments are given an increasing role in the development of rehabilitation strategies. On the other hand, excess oxygen is often used to reduce the damage caused by severe hypoxia. Supplemental O_2 is widespread and frequently recommended, especially for critical care patients. Oxygen therapy can be life-saving, for example, in hypoxic-ischemic injuries, in lung patients (e.g. chronic obstructive pulmonary disease) and in premature neonates [6-8]. However, the advantages and disadvantages of different oxygen therapies are still controversial, as hypoxia and hyperoxia, which means higher oxygen levels in the tissues, can both lead to severe oxidative stress.

Alterations in metabolic processes are particularly dangerous for the brain. The human brain constitutes only a fraction of the total body mass (2%). Nevertheless, the brain is one of our largest energy-consuming organs, utilising 20% of oxygen metabolism [9, 10]. It is estimated that neurons use 75-80% of the energy produced in the brain [10]. Therefore, our nerve cells react very sensitively to changes in oxygen concentration. The hippocampus, which is involved in memory, learning and cognitive processes, is susceptible to disruption of O_2 homeostasis [11]. Oxygen levels different from the physiological concentration can significantly affect the neural activity of hippocampal neurons, thereby being responsible for long-term changes, primarily learning and spatial memory disorders. Consequently, it is crucial to understand the effect of short-term hypoxic and hyperoxic conditions on hippocampal neurons as thoroughly as possible to more accurately determine the optimal lower and upper limits of oxygen supply in clinical practice and during rehabilitation and to tailor oxygen therapy better.

II. Aims

The doctoral research was aimed at revealing the vulnerability of the hippocampus to short-term hypoxia and hyperoxia, as well as clarifying the underlying mechanisms, so we set the following goals in our work:

- Investigation of the damaging effects of acute, mild hypoxia (16% O_2) and acute, mild (30% O_2) and severe (100% O_2) hyperoxia in different layers of the hippocampus.
- Cell-specific identification of neurons damaged during hypoxia and hyperoxia using interneuron-specific markers.
- Determination of the effect of applied oxygen concentrations on network oscillations in the hippocampus.
- To identify the nerve cell populations most sensitive to changed O_2 concentrations based on their firing frequency.

III. Materials and methods

1. Animals

The experiments were performed on male Wistar rats (n = 60) weighing 250-300 g at the time of surgery. We always followed the guidelines and protocols approved by the National Scientific Ethical Committee on Animal Experimentation (license number: BA/73/0052-5/2022) and the directive of the European Communities Council (2010/63/EU) when using animals in experiments.

2. Oxygen treatments

At the beginning of the experiment, the rats were randomly divided into three groups: control group, hypoxia group and hyperoxia group. In the case of the hypoxia group, the applied O_2 concentration was 16%, while in the hyperoxia group, it was 30% and 100% O_2. For the histopathological and immunohistochemical studies, the animals (n = 10/group) were placed in an induction chamber, and the oxygen exposure was 1 hour. For the electrophysiological tests, the rats (hypoxia n = 10, hyperoxia n = 10) were anesthetized with urethane (1.1-1.3 g/kg). After the surgery, the animals breathed 21% O_2, and we took the baseline. Then, we changed

the O_2 concentration of the inhaled gas mixture through the anesthetic mask. The exposure time was 1 hour, and we made electrophysiological recordings during the last 15 minutes. At the end of the experiment, the animals were anesthetized with urethane (2 g/kg).

3. Histochemistry

Gallyas staining

This staining procedure stains the soma and neurites of degenerated neurons with high selectivity. Brain slices with a thickness of 50 μm were subjected to a series of dehydration steps (1-propanol, 1-1 min) and then incubated overnight in 1-propanol containing 1% sulfuric acid (esterification) at 56 °C. After rehydration, the samples were treated with 1% acetic acid for 5 minutes and then incubated in the physical developer solution. The reaction was stopped with 1% acetic acid (5 min).

Apoptosis detection

Two types of TUNEL tests were used to detect the DNA fragmentation of cell nuclei. In one case, the sections were incubated with a TdT reaction buffer for 10 minutes. It was incubated with a TdT reaction cocktail for 60 minutes at 37 °C. After washing with PBS, the samples were incubated with Click-iT reaction buffer for 30 minutes. For the other kit, the fixed brain slices were incubated with a permeabilization solution (0.2% Triton X-100; 0.1% sodium citrate) for 2 minutes on ice. The samples were then incubated with the TUNEL reaction mixture for 60 minutes at 37 °C in the dark.

4. Immunohistochemistry

We marked the brain slices with different interneuron markers (parvalbumin, somatostatin, neuropeptide Y, calbindin, calretinin, cholecystokinin) to identify the interneuron subclasses. We later performed the Gallyas silver staining described above on these samples. Furthermore, an anti-caspase-3 antibody was used to detect apoptosis. Sections were blocked in phosphate-buffered saline (0.1 M PBS, pH = 7.4) containing 1% Triton X-100 and 2% goat serum for 2 h at room temperature. After that, the solution containing the primary antibodies (primary antibody, 2% goat serum, 1% Triton X-100) was pipetted onto the slices and incubated overnight at 4 °C. After washing with PBS (3x5 minutes), the secondary antibodies were dissolved in PBS and placed on the sample. The incubation time was 2 hours.

5. Anesthesia and surgery

For the surgical intervention, the rats were anesthetized with an intraperitoneal injection of urethane (1.1-1.3 g/kg). The depth of anesthesia was checked, and then the heads of the animals were fixed in a stereotaxic device. For our electrophysiological tests, we selected the location of the electrodes based on our histochemical results. According to this, holes were made with a bone drill with a diameter of 2 mm on the skull bone according to the previously determined coordinates above the hippocampus. The electrode array used for conduction (A4x8-5mm-200-400-703, NeuroNexus Technologies, Inc., USA) was dipped in a 2% DiI solution, and then the electrode was inserted into the planned brain area using a micromanipulator. The electrode was connected to a 128-channel TDT amplifier system (Tucker-Davis Technologies Inc, Florida, USA). The electrophysiological data were digitalized using the LabChart virtual device (AD Instrument) and then recorded on a computer for later data processing. Oxygen concentration was monitored in the brain with a modified Clark-type oxygen microelectrode (OX-10, Unisense A/S, Aarhus, Denmark).

6. Processing of electrophysiological data

The raw data were processed and analyzed using MATLAB (The MathWorks, Inc., Natick, Massachusetts, USA). To analyze the multiunit activity (MUA), we used the automatic clustering algorithm of the recording software with 500-5000 Hz band filtering to detect the firing rate and inter-spike interval (ISI) values.

7. Statistical analysis

The statistical analysis of the measurement results was performed using the SPSS 28 program (SPSS Inc., Chicago, IL, US). If the assumptions of normal distribution and homogeneity of variance are met, we used a paired t-test to compare the average of two samples and, for more than two groups, analysis of variance (one-way ANOVA). For those populations where the condition for normality was not met, the Friedman test was performed for non-independent groups, and the Kruskal-Wallis test was used for independent samples. Differences were considered to be significant at the level of $p < 0,05$. Data are presented as mean ± SEM.

IV. Results and discussion

1. Effect of hypoxia and hyperoxia on neuronal damage in the hippocampus

Compacted neurons in the hippocampus have been previously observed, such as in cases of epilepsy and hyperglycemia [12, 13]. Furthermore, dark neurons can be formed in the hilus and CA1 regions during cerebral ischemia [14-16]. After mild (16% O_2) hypoxic treatment, we found that compacted neurons were formed within the dentate gyrus in the hilus and subgranular zone. Moreover, we observed compacted neurons in the CA1 and CA3 regions. In most healthy control animals, dark neurons did not occur at all. In the CA1 area, the damaged neurons were predominantly in the str. pyramidale (4.67 ± 2.43). The short-term hypoxic treatment resulted in the average number of compacted neurons in the CA3 region. Within this, str. oriens (17.57 ± 4.44), and str. pyramidale (17.57 ± 5.26) had the highest number of silvered neurons. Similar results were previously reported by Mahakizadeh et al. (2020), who observed dark neuron formation in the CA1 and CA3 regions during chronic hypoxia [17]. Based on literature data, the staining procedure we use (Gallyas silvering) practically does not stain the structures of healthy neurons. Thus, the rapid appearance of hyperargyrophilia shortly after the external initialization indicates damage to the affected cells [12-14].

To our knowledge, the formation of dark neurons in the brain has not yet been reported in the case of hyperoxia as opposed to hypoxia. Our studies established that mild (30% O_2) and severe (100% O_2) hyperoxia can cause the development of compacted neurons in the hippocampus. In the latter case, a significant increase in the dark neuron numbers (9.88 ± 0.66) could be observed compared to the control group and the group treated with 30% oxygen (2.32 ± 0.17). A higher number of damaged neurons were generated in the hilus (29.63 ± 1.33). In the CA1 area, we observed dark neurons in the str. oriens (13.80 ± 0.97), str. pyramidale (15.00 ± 1.17) and str. radiatum (1.03 ± 0.21). In the CA3 region, the most compacted neurons are str. radiatum (10.43 ± 0.96). The 16% and 100% O_2 applied in our experiment induced the formation of almost similar amounts of dark neurons, while it was significantly less at 30% O_2 exposure. The increased level of reactive oxygen and nitrogen species (ROS and RNS) may have played an essential role in developing the number of compacted neurons.

We performed a TUNEL test and caspase-3 labelling to detect apoptosis during our work. However, there was no specific labelling after the applied 1-hour normobaric hypoxic (16% O_2) and hyperoxic (30% and 100% O_2) treatments. Based on studies, 30 minutes of normobaric hypoxia (5% O_2) causes significant morphological changes in the cells of the CA3 region from

3. hours after the treatment, while the granule cells in the dentate gyrus are affected to a lesser extent, while the neurons of the CA1 region are mainly resistant to half an hour of hypoxic damage [18]. Furthermore, 1-hour 100% hyperoxia treatment increases the expression of pro-apoptotic Bax and Bad proteins in the cerebral cortex [19]. In light of this knowledge, we assume that the short-term O_2 treatments we use can potentially induce apoptosis. However, further studies are needed to clarify this.

2. Immunohistochemical characterization of damaged neurons

The samples were previously labelled with different interneuron markers to identify the compacted neurons. During the immunohistochemical staining, calbindin (CB), calretinin (CR), cholecystokinin (CCK), neuropeptide Y (NPY) and parvalbumin (PV) positive inhibitory cells were identified. Nevertheless, the fluorescent labelling did not overlap with the silvered neurons in either hypoxic or hyperoxic samples. On the other hand, somatostatin (SST) immunoreactive compacted neurons were observed during mild hypoxic treatment. After 1 hour of 16% hypoxia, 23.57% of dark neurons were somatostatin positive neurons. SST-positive neurons were located within the hilus. However, the CA1 and CA3 regions of the hippocampus did not contain SST immunoreactive compacted neurons. No SST-positive dark neurons were present in any of the hippocampal areas after hyperoxic exposure.

According to our hypothesis, the observed differences between hippocampal regions do not arise from differences between regions but from cell type-specific differences. Hippocampal interneurons differ in morphology, immunoreactivity, synaptic properties, laminar arrangement, and connectivity. It has been previously observed that a particular subpopulation of interneurons in the hilus is sensitive to Ca^{2+}-induced hyperexcitation [20, 21]. Thus, it is likely that a similar mechanism causes SST interneuron damage in the hilus during mild acute hypoxia. All SST immunopositive neurons in the hippocampus are GABAergic, and 14% of all inhibitory interneurons are SST-positive [22, 23]. In the dentate gyrus, SST immunopositive neurons are predominantly located within the hilus, primarily in the subgranular zone [24]. In the subgranular zone, SST neurons have a fusiform soma and their dendrites run parallel to the str. granulosum. They are predominantly described as hilar interneurons with perforant pathway-associated axon terminals (HIPP) [24]. HIPP cells target the PV-containing perisomatic basket cells and regulate the activity of the basket cells [25]. Consequently, a decrease in the number of SST immunoreactive neurons may lead to a decline in the activity of excitatory granule cells in the dentate gyrus.

3. In vivo measurement of oxygen concentration in the hippocampus

During our functional tests, the oxygen concentration was measured with an O_2 sensor embedded in the hippocampus. In hypoxic (16% O_2) treatment, tissue partial pressure of oxygen (PtO_2) decreased from 20.1 mm Hg to 8.71 mm Hg, which falls within the hypoxic threshold range determined by other studies. Regarding the hypoxic threshold, although there is no clearly defined "critical" PtO_2 for the rat brain, this value was estimated to be between 6-10 mmHg in the previous studies [26, 27]. It increased to 42.4 mm Hg at 30% O_2 and to 79.04 mm Hg when the oxygen concentration was further increased to 100%, indicating a higher than physiological oxygen level in the hippocampus.

4. The effect of hypoxia and hyperoxia on network oscillation

To investigate the effects of hypoxia (1 hour) and hyperoxia (1 hour) on network oscillation, local field potentials (LFP) were conducted from the layers of the hippocampus. In the case of the higher frequency alpha (8-12 Hz), beta (12-30 Hz) and gamma (30-100 Hz) waves, no significant differences were found during hypoxic exposure. However, in the lower frequency range (1-4 Hz), an activity around 2.18 Hz appeared, the peak frequency of which shifted with the change in oxygen concentration. The average peak frequency during baseline recording was 2.18 Hz, which increased to 2.28 Hz at 16% O_2 concentration. The difference was significant ($p < 0.05$) based on the analysis of variance and the subsequent post hoc test. We also examined the spectral power of the slow wave and found that the power spectrum increased, but the difference was not significant compared to the control. In agreement with our results, others have observed increased delta wave activity during hypoxia and ischemic [28-31]. An increase in delta activity may represent a sustained hyperpolarization and inhibition of cortical neurons, which affects the activity of the hippocampus via the entorhinal cortex [28, 29, 32-34]. Based on this, we assume that the activation level of neuron populations decreased due to mild hypoxia and that the effect of hypoxia on neuronal activity probably reduced or inhibited the activity of only specific neurons or neuron populations. In the hyperoxic group, a distinguishable slow activity around 2 Hz appeared in the delta frequency band, similar to the hypoxic experiment. At the 30% O_2 concentration, compared to the baseline (2.18 Hz), the peak frequency was significantly reduced to 1.92 Hz ($p < 0.05$). We increased the O_2 level further, and the slow oscillation was reduced considerably to 1.72 Hz at 100% O_2 exposure ($p < 0.001$). Delta wave performance decreased during oxygen treatment, but no significant difference was detected between normobaric and hyperoxic conditions. Based on the literature, the decrease in

delta activity is related to the increased firing rate of neurons [35]. Our results allow us to conclude that hyperoxia presumably increased the firing rate of many neurons, which manifested itself differently in individual neurons and neuron populations. During hypoxia and hyperoxia, reduced blood flow may develop [36]. Hence, the change in blood flow velocity may play a role in the shift towards lower frequencies. Neocortical discharges influence the activity of the hippocampal network via the entorhinal input [33], thereby presumably producing diverse patterns in hippocampal delta waves under altered O_2 concentrations. The change in the slow wave frequency during hypoxia and hyperoxia is assumed to be closely related to the secondary effects of oxygen, but further studies would be needed to understand this.

5. Changes in the firing activity of hippocampal neurons under hypoxia and hyperoxia

Because of their electrical activity, neurons require a lot of energy. Consequently, the lack of energy caused by mitochondrial damage plays a particularly significant role in the damage of nerve cells [37]. Our analyses found that hypoxia increased the firing frequency of pyramidal cells in the CA1 and CA3 regions ($p < 0.05$ and $p < 0.001$), which is related to the depolarization of hippocampal pyramidal cells. Hypoxic conditions cause a decrease in the amount of ATP, an increase in free calcium levels in the cytoplasm, and an accumulation of extracellular adenosine (generated during the breakdown of ATP). This causes a disturbance in the ionic balance, which leads to the early cessation of electrical activity and the disappearance of excitatory synaptic potentials [38]. Most neurons are known to be sensitive to hypoxia, but the reactions of different types of neurons can be different even within the same brain region [39-41]. As a result of hypoxia, the excitability of neurons in the CA1 region is reduced, which is probably explained by the strong expression of K_{ATP} channels in CA1 neurons, and the excitability is at least partly regulated by the availability and voltage dependence of voltage-gated potassium channels [42-45]. In our hypoxic model, there was a decrease in the electrical activation of hilus interneurons during the hypoxic treatment ($p < 0.005$). SST immunoreactive interneurons in the hilus are particularly sensitive to ischemia. Hypoxia induces presynaptic inhibition in dentate gyrus interneurons, partially mediated by activation of metabotropic glutamate receptors [46-48]. During our work, we divided the presumed interneurons into two groups based on ISI values and firing frequency (type I and type II). We observed that the electrical excitability of type I interneurons in the CA3 region was reduced in hypoxia ($p > 0.005$). Based on previous studies, inhibitory synapses are particularly sensitive to hypoxia, and

hypoxic hyperpolarization is often significant in the population of inhibitory interneurons [48, 49]. In our study, however, we observed an increase in the firing activity of another group of CA3 interneurons (type II) during hypoxic exposure ($p < 0.001$).

Similar results were obtained in the hyperoxia group. The firing activity of type I interneurons in CA3 decreased (30% O_2 $p < 0.05$ and 100% O_2 $p < 0.005$), while the electrical excitability of type II interneurons increased at the 30% O_2 concentration (30% O_2 $p < 0.001$). For pyramidal cells, the firing frequency in the CA1 region increased at 30% and 100% hyperoxia exposure, respectively (30% O_2 $p < 0.05$ and 100% O_2 $p < 0.05$). As a result of hyperoxia, a similar change in firing activity was observed in CA3 (30% O_2 $p < 0.001$ and 100% O_2 $p < 0.001$). In the case of hyperoxia, we have no results on the firing activity of hilus neurons because we had to discard most of the data due to the significant deviation of the ISI values. For reasons similar to hypoxia, no results were presented on the putative interneurons of CA1 either. Previous studies have shown that hyperbaric hyperoxia worsens neuronal excitability, but this is primarily due to the sensitivity of cells to atmospheric pressure [50-53]. In the CA1 region, a single transient hyperbaric hyperoxic stimulus raises neuronal activity, and normobaric hyperoxia has also been shown to increase the excitability of CA1 neurons [51]. Changes in ion channel characteristics or expression can affect neuronal excitability [54-58]. High oxygen levels increase the amount of ROS in mitochondria, especially in the mitochondrial respiratory chain complex I, which is particularly sensitive to reactive O_2 derivatives [59-61]. Furthermore, urethane anesthesia can alter neurotransmission [62, 63], thereby sensitizing neurons to responses to hypoxia and hyperoxia. We assume that normobaric 1-hour hypoxia (16% O_2) and hyperoxia (30% and 100% O_2) in the hippocampus results in a shift of the excitatory and inhibitory balance in the direction of excitation, i.e. lower activation of inhibitory interneurons may lead to a decrease in the regulation of pyramidal cells.

V. Summary

1. Using Gallyas' silver staining method, we were the first to demonstrate neuron damage (compacted neurons) in the hippocampus with ultrastructural changes after mild or severe hyperoxia exposure.
2. We showed that short-term, 1-hour exposure to hypoxia and hyperoxia can trigger the compaction of neurons, which damage is present in both the dentate gyrus, CA1 and CA3 regions. We analyzed the distribution of damaged neurons according to cell layers. Due to mild hypoxia (16% O_2), in the hilus and CA3 str. oriens and str. the pyramidale the neuron compaction was most significant. In contrast, the highest number of damaged neurons during mild hyperoxia (30% O_2) is in the CA3 str. radiatum, while in the case of severe hyperoxia (100% O_2), they were formed in the hilus.
3. To identify the compacted neurons, we combined silver impregnation with immunohistochemistry and showed that during mild hypoxia, some of the compacted neurons in the hilus are somatostatin-positive interneurons.
4. In the delta frequency range, we detected a peak frequency of around 2.2 Hz, which frequency increased during acute, mild hypoxia. In contrast, during acute hyperoxia, a decrease in frequency was observed at lower (30% O_2) and higher (100% O_2) oxygen concentrations.
5. We demonstrated that short-term hypoxic (16% O_2) and hyperoxic (30% and 100% O_2) exposure increases the firing frequency of pyramidal cells in the CA1 and CA3 regions. However, the firing response of interneurons in different layers of the hippocampus shows a more heterogeneous picture. Based on the observed trend in the firing properties of the interneurons, we divided the interneurons into two groups (I and II). Based on this, we determined that the firing activity of type I interneurons in the CA3 region decreased during both hypoxia and hyperoxia. In contrast, except for 100% O_2 exposure, the firing activity of type II interneurons increased. In addition, mild hypoxia induced a significant decrease in the firing activity of hilus interneurons.

VI. List of publications

Cumulative impact factor: **16.2**

1. Publications related to the thesis

Hencz AJ, Magony A, Thomas C, Kovacs K, Szilagyi G, Pal J, Sik A. (2024). Short-term hyperoxia-induced functional and morphological changes in rat hippocampus. Front Cell Neuroscience 18: 1376577. **[IF: 5,3]**

Hencz A, Magony A, Thomas C, Kovacs K, Szilagyi G, Pal J, Sik A. (2023). Mild hypoxia-induced structural and functional changes of the hippocampal network. Front Cell Neuroscience 29:17: 1277375. **[IF: 5,3]**

2. Other publications with impact factors

Hencz A, Szabó-Meleg E,1, Dayo MY, Bilibani A, Barkó Sz, Nyitrai M, Szatmári D.(2022). The p53 and Calcium Regulated Actin Rearrangement in Model Cells. Int J Mol Sci (2022) 13;23 (16):9078. **[IF: 5,6]**

3. Other publications

Hencz AJ; Somogyi P, Halász H; Szabó-Meleg E (2022).Visualization of the effect of TR100 anti-cancer compound on membrane nanotubes with SR-SIM microscopy. Resolution and Discovery 6: 12-19.

Hencz AJ, Szilagyi G, Gyorfi N, Tenzlinger K, Szechenyi A, Odry A, Odry P, Karadi Z, Vizvari Z, Toth A, Pal J. (2022). Grafén és indium-ón-oxid elektródák összehasonlítása alacsony frekvenciás elektromos impedancia spektroszkópia mérésekkel. In: Tóth A; Vizvári Z (szerk.) Orvosbiológiai kérdések - multidiszciplináris, bioimpedancia alapú válaszok: A PTE Anyagcsereszabályozás és Bioimpedancia Kutatócsoport publikáció gyűjteménye, konferenciakötet pp. 115-123. Pécsi Tudományegyetem, Pécs.

Filotas N, Helt R, **Hencz AJ**, Tenzlinger K, Odry A, Odry P, Karadi Z, Vizvar Z, Toth A, Gyorfi N, Szechenyi A, Pal J. (2022). Bioimpedancia mérő plate fejlesztése hipoxiás és hiperoxiás állapotok vizsgálatára sejtkultúrákban. In: Tóth A; Vizvári Z (szerk.) Orvosbiológiai kérdések - multidiszciplináris, bioimpedancia alapú válaszok: A PTE Anyagcsereszabályozás

és Bioimpedancia Kutatócsoport publikáció gyűjteménye, konferenciakötet pp. 104-114. Pécsi Tudományegyetem, Pécs.

4. Conference presentations

Hencz AJ, Magony A, Szilagyi G, Pal J, Sik A (2024). The impact of hypoxia and hyperoxia on the number of compacted neurons and brain activity. International Neuroscience Conference (INC), Pécs.

Hencz AJ, Szilagyi G, Gyorfi N, Tenzlinger K, Szechenyi A, Odry A, Odry P, Karadi Z, Vizvari Z, Toth A, Pal J. (2021). Comparative electrical impedance spectroscopy study of graphene and indium tin oxide electrodes during low-frequency measurements. IEEE 15th International Symposium on Applied Computational Intelligence and Informatics (SACI), pp. 000147-000152.

Filotas N, Helt R, **Hencz AJ**, Tenzlinger K, Odry A, Odry P, Karadi Z, Vizvar Z, Toth A, Gyorfi N, Szechenyi A, Pal J. (2021). Development of a Bioimpedance Measuring Plate for the Study of Hypoxic and Hyperoxic Conditions in Cell Cultures. Conference: 2021 IEEE 15th International Symposium on Applied Computational Intelligence and Informatics (SACI), pp. 153-158.

Gál AR, Tóth A,Vízvári Z, Kovács A, **Hencz A**, Klincsik M, Sári Z, Odry P, Vereczkei A, Kiss T, Fincsur A, Miseta A, Lénárd L, Karádi Z. (2019). Nem alkoholos eredetű zsírmáj előidézése és állatkísérletes vizsgálata új típusú diagnosztikai eljárás bevezetéséhez.
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Hencz A, Turmer K, Nyitrai M, Szabo-Meleg E. (2018). In vivo examination of membrane nanotubes in developing zebrafish embryos**.** Medical Conference for PhD Students and Experts of Clinical Sciences (MEDPECS), Pécs.

Hencz A, Turmer K, Nyitrai M, Szabo-Meleg E. (2018). In vivo examination of membrane nanotubes in developing zebrafish embryos. Visegrád Group Society for Developmental Biology: Inaugural Meeting (V4SDB), Brno.

Hencz AJ, Somogyi P, Madarász T, Nyitrai M, Matkó J, Bugyi B, Gunning P, Szabó-Meleg E. (2018). A T100 rákellenes szer hatása a membrán nanocsövek kialakulására és morfológiájára Membrán-Transzport Konferencia, Sümeg.

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Hencz A, Pernecker B, Boda R, Mauchart P, Móra A, Csabai Z.(2016). Seasonal and spatial differences in the trophic spectrum of Balkan Goldenring (Cordulagaster heros Theischinger, 1979) in the Mecsek Mountains, SW, Hungary. 2nd Central European Symposium for Aquatic Macroinvertebrate Research (CESAMIR), Pécs.

VII. References

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