# Evaluation of the results obtained in the course of studies

## *Overall evaluation of the research*

In the OTKA K116511 research support, both human and animal experiments were held. Animal studies were conducted in elderly rats mainly at the age of 28-32 months, corresponding to the age which is translational to the senescent age in human (Teglas et al., 2018a). The motor and cognitive performance during this later period of aging in rat shows a marked decline in functional effectiveness compared to younger ages (Teglas et al., 2019a). Our research concentrating on the characteristics of senescent age in rats revealed that this age is critical to define the appropriate preventive treatment options. It is assumed that this experimental approach fits to the aging of the human population and may also serve to create a guideline dealing with how and when we should start a comprehensive prevention process to assure a healthy and active aging. Prevention in this age period might be coupled with the so called prehabilitation (this is a novel but already used procedure in a number of European countries, and also with the usual rehabilitation procedures. This question is not only of health related but also of financial and social importance, i.e. "what is the most purposeful or targeted age period" to start the extended, age-specific and complex prevention of deteriorated aging?

The final aim is how to achieve healthy, mentally and physically active aging for the benefit of older generations and the entire society. It may be added that this objective includes versatile innovation attempts, because in addition to health care guidelines, targeted instrumental development might also be important. The title of present OTKA application (K116511) aimed at to develop: “Up-to date method to improve psychosomatic performance in elderly persons” points also into that direction.

In the present research, the notion that movement and cognitive brain functions are closely intertwined is further confirmed. That is, the brain organization of movement regulation and cognitive functions is closely related in both anatomically and functionally and support each other (see also here our previously published book chapter in the latest international encyclopedia of neuroscience: Luiten et al., 2013). In the course of our present research, we tried to confirm further this already accepted integrative concept by adding novel findings in both animal experiments and human studies.

***Human studies*** *(general approach):*

Ensuring healthy aging, acceptable quality of life expectancy and disease prevention requires the preservation of mobility and the healthy brain aging. Movement deteriorations in the course of aging support sarcopenia and osteoporosis, risk of fall and fracture, lead to decreased cognitive performance and day-to-day self-maintaining activities. To supporting healthy aging regular exercise is necessary which requires proper expertise based on gerontokinesiological aspects and practice. In the course of advancement of aging next to the different types of active exercise formats the so called passive exercise is going to gain more and more ground for maintaining the elderly’s physical and mental condition, since ageing is often hampered by motor, cognitive and psychosocial constraints which interfere with the options of practicing active physical training. One of the main features of passive exercise regarding aging is that it is complementary and worth to use in combination with active exercise, proper nutrition and psychosocial facilities.

At the time of the application of this research plan, we proposed to study the effects of electromagnetic field (EMF) stimulation for studying the so called passive exercise in human, by using the Sanza device, having a number of own previous positive experiences with this instrument. During the three years of this grant, there appeared another “passive” type of exercise, namely the whole body vibration (WBV, see Nyakas, 2018) for treatment of movement and age related diseases as well. This presently extending method also offers us many opportunities for the treatment of elderly people and more recently some childhood musculoskeletal disorders (Medveczky et al, 2018). The advantage was taken into this direction as well after starting the EMF stimulation type of aided exercise. Thus, the present research support on EMF stimulation experiments by the OTKA grant provided additional initiative stimulation into this direction too.

In the first year of the application of the instrumental approaches, methodological developments were aimed to follow in the field of human research (usage of body balance test, that of stabilometer with a self-developed instrument, development of waking analysis technology, etc.), which resulted in an internationally published article (Bretz et al., 2016). This article specialized in measuring body weight balance control and used target shooters (sports orientation as well) to improve to understand more the details of measurement technique. Furthermore, we developed earlier already to this grant application a differential choice reaction time measurement method to examine the interaction between the central nervous system function (discrimination, attention) and the muscular reaction time while pressing a pushbutton thoughtfully in the course of discrimination process. Some principle studies have already been carried out earlier with this method (Bretz et al., 2014), which helped to continue our further studies during the OTKA research period.

Furthermore, mainly during the coming other two years (2017 and 2018) the experimental principles were the followings: (1) measuring the kinesiological effects of electromagnetic field (EMF) stimulation on the knee extensor muscles (Nyakas and Bretz, 2018; Nyakas and Bretz, 2019) in order to develop a passive training technique suitable also for preventing falls during aging, (2) and applying the EMF treatment on the forearms in elderly peoples while testing the muscular choice reaction time and the brain-related differentiation abilities mentioned also above.

It may be added that the WBV research project is still in a preparatory phase for human studies, but that direction is not included directly to the tasks undertaken. As the first step we have carried out some measurements on the technical characteristics of a device (Marodyne LivMD), with the help of the company of Brüel & Kjaer. It may be noted here that this device has been used in our animal experiments related to aging, and we want to extend its usage to the ongoing human studies. We have adopted the basic principle that all mechanical vibration devices we use now and will use in the future should be validated regarding the technical indicators in order to track and know the exact mechanical parameters. This is important to disclose the potential adverse side effects especially those related to health, since a number of instruments are available on the market. This research process, which is certainly can be coupled to the health safety technology, can also form a separate part of further research, both in the general and in the sports health care. In more general sense: the novel technical treatment devices must be safe from a health point of view.

*Description of the main results achieved:*

In the line of human studies we carried out a number of measurements in a population from elderly houses by studying the action of EMF exposure on several movements and related neuronal capabilities (Nyakas, 2018; Nyakas and Bretz, 2018, Nyakas and Bretz, 2019, Nyakas et al. 2019). The basic lines of research were:

(1) To support muscular function of legs to prevent falling. In this line of research the muscular strength in knee extensors after EMF stimulation was measured and evaluated (Nyakas and Bretz, 2018). It may be mentioned that regarding the EU networking project in this topic from the side of MGGT (Magyar Gerontológiai és Geriátriai Társaság), C Nyakas is the Hungarian representative within the European scientific network organization. Part of the human results will be published in the "Idősgyógyászat" home journal (Nyakas et al., 2019, manuscript is accepted) and later in some other, international journals.

(2) Another principal approach of ours was to uncover preventive impact or progressive intervention by means of EMF stimulation as passive exercise on cognitive decline in the aging population. For these purposes first we further developed the complex walking analysis measuring method of ours in the elderly population (Nyakas et al., 2018), since walking habits and characteristics are predictive to the later decline of cognitive capabilities according to a large amount of published articles.

(3) Furthermore, we collected data on the characteristics of psychocardiac stress responses in elderly persons (with Vicardio instrument) which are under evaluation;

(4) and carried out discriminative reaction time measurements using m. digitorum communis stimulation with EMF exposure (consult also Fig. 2 and the description of results).

To present some examples from our results, Fig. 1 shows that EMF stimulation on the mid-thigh region increased knee muscle extension force on both the stimulated but also on the control side. It is concluded that one side stimulation acts on the other side as well, i.e. the action of stimulation is generalized in the body indicating a neuronal interaction in the central nervous system with the regulation of peripheral muscle activity. Since muscle strength is increased after stimulation chronic treatment protocol is worth to be used as treatment option to prevent falling during aging.

Fig. 2 shows that EMF stimulation on the arms is beneficial for reducing the number of errors in a discrimination setup where colors should be distinguished according to the differential reaction time measurement protocol. This result (see the table left side) suggests that cognitive functions (making errors during differentiation) even more influenced compared to muscle reaction time, i.e. the latency to press the pushbutton according to the appearance of colored signals. Here again, a cooperation between muscle and brain functions is exemplified which can be of a muscular origin as well after the direct EMF stimulation of the forearm muscles.

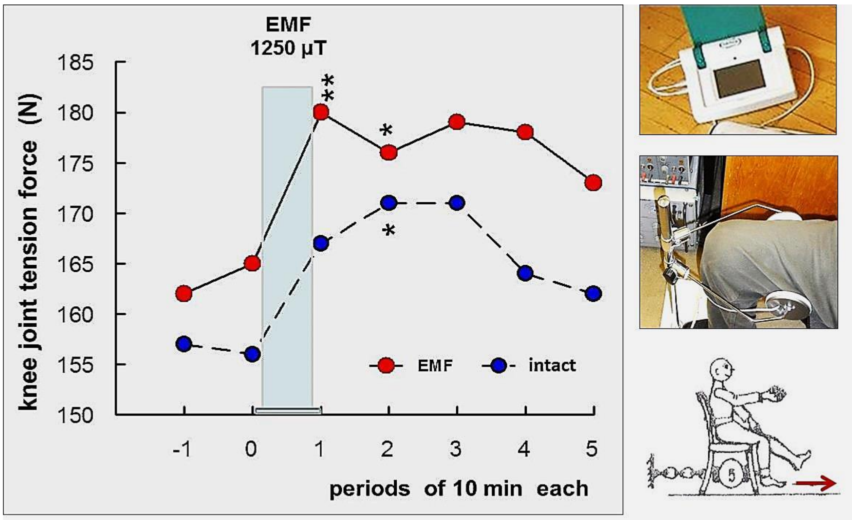


Fig. 1 Mid-thigh EMF stimulation of 10 min long increases knee extension force on both the stimulated and non-stimulated legs (age: 76y, n=22). *p<0.05 vs. prestimulation periods, against the averaged force of -1 and 0 values. (Nyakas et al., 2019). The stimulating instrument is Sanza and its Helmholtz adapter was applied for the stimulation (middle figure at the right side)

| choice reaction time | mean (ms) | SD (ms) | error A (%) | error B (%) | errors A+B (%) |
|---|---|---|---|---|---|
| EMF stimulation | 395.4 ± 10.70 | 89.8 ± 4.77 | 0.439 ± 0.167 | 0.375 ± 0.133 | 0.813 ± 0.208 |
| pseudo stimulation | 402.3 ± 10.37 | 91.8 ± 5.92 | 0.813 ± 0.208 | 0.625 ± 0.140 | 1.500 ± 0.284 |
| statistics | n.s. | n.s. | t=2.44 p<0.05 | t=1.85 p=0.07 | t=2.77 p<0.01 |

Fig. 2 Measurement of differential (choice) reaction time and errors during forearm EMF-stimulation vs. pseudo-stimulation in aged individuals (age: 76year, mean, n=32, see figure at right). Table at left: choice reaction time (mean ± SD) was measured during pressing a pushbutton, and a distinction was recorded between the green and red colors: error A – button was pressed after red (it was allowed only after green!), error B – button was not pressed after green (it was allowed only after red!).

***Animal experiments** (general evaluation and description of the main results):*

The present OTKA grant focused on the effects of "passive" exercise (electromagnetic field (EMF) stimulation) in terms of retaining functional capabilities in the senescent age in rats, and also aimed to compare the effects of active and so called passive exercises. These animal studies also served for translation type of experiments for the human experimentation to understand the mechanism of action of the passive exercise type of treatment.

(1) First we conducted aging related behavioral studies applying active type of exercise (treadmill running). It was applied for 6 months prior to 12, 24 and 32 months of age. Two prominent observations were found which influenced the further studies. First, it was recorded that the largest decline in the motility and behavioral functions including cognition could be obtained in the senescent age period (from 24 to 32 months); secondly, the chronic active exercise of a moderate intensity was effective almost exclusively in the senescent aging period, indicating that physical activity during the later period of aging is indeed the most effective (Teglas et al., 2019a). Thus, a principal of aged-dependent effect was confirmed pointing to the far more better sensitivity of exercise interventions in the senescent age (also close to the mean survival age).

(2) Accordingly, based on the "active" exercise experimental findings mentioned above, 32 months old aged rats were examined in our main experiment after a chronic EMF treatment of 6 weeks, i.e. after the "passive" exercise. The intensities of EMF treatment were 45, 92 microT, and a higher dose of 1200 microT was also introduced in order to detect occasional side effects and/or an additional high-dose effect. A number of functional tests have been used: open-field (OF) test for assessing psychomotility or somatosensory functions , novel object recognition (NOR) test to measure attention and discrimination capacity, and Morris water maze (MWM) test for evaluating spatial learning and memory. Positive effects were measurable in all behavioral tests, confirmed by increased psychomotility in OF, dose-dependent attention increment in NOR and enhanced spatial learning in the MWM test (Teglas et al., 2018a, see also Fig. 3 below).

(2) Processing animals' tissues after euthanasia for assays revealing adequate findings to the mechanism of action of treatment as also included in the experimental protocols. For that purpose (a) native tissues were frozen on dry ice: isolated hippocampus and separate hemispherical brain, (b) from the hind leg muscles: m. gastrocnemius and m. soleus, as striated muscle tissue samples were frozen (have not been subjected to biochemical analysis yet). (c) blood collection was performed and TNF-alpha was determined from the blood. The aim was to investigate the effects on the immune system, but no changes were found in TNF-alpha values as a result of training.

(3) Biochemical (Western blot) assays were performed in the hippocampus to measure the concentrations of BDNF, pAMPK / AMPK, pCREB / CREB, synaptophysin, ChAT, pS6 / S6 antigens. The results of the biochemical experiment were published party in 2018 showing that hippocampal BDNF content increased dose dependently after chronic EMF stimulation treatment (Teglas et al., 2018b) and will be published in another paper soon including the results obtained in the other biochemical markers as well (Teglas et al., 2019b).

*__Presentation of some selected results:__*

A) *__Behavioral results after chronic EMF stimulation__*

(1) Significant increase was seen in the effort of the hind legs in the 32-month-old treated animals after the chronic EMF stimulations, since the number of rearing, i.e. standing up on the hind legs increased in a dose-dependent manner (Fig. 3 – left side). Similarly, attention / discrimination ability

(cognitive performance) has increased significantly in the novel object recognition test (Fig. 3 – right side).

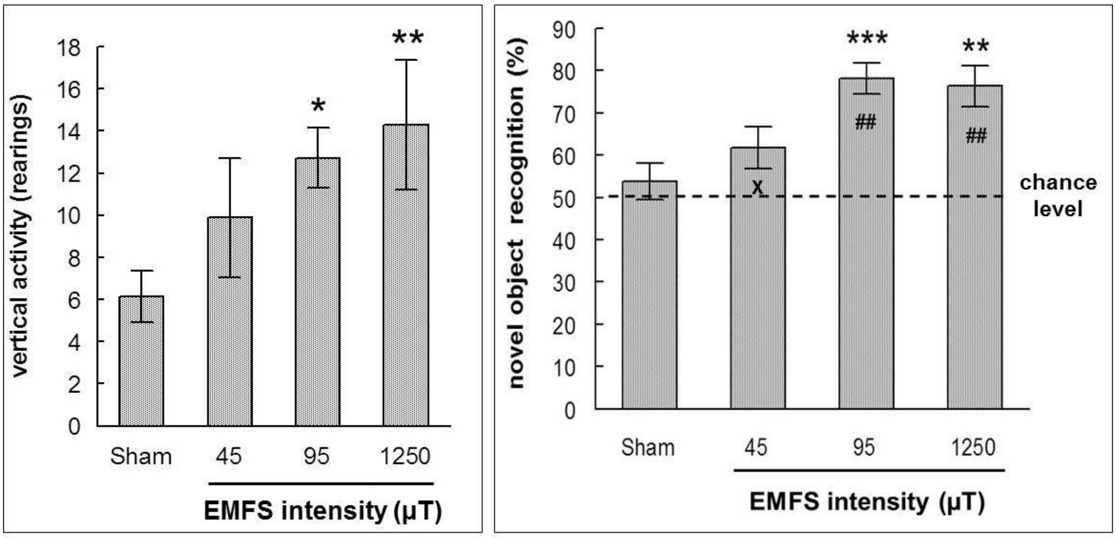


Fig. 3. EMF stimulation treatment increased novel environment induced vertical activity, i.e. rearing up on the hind legs (left panel) and cognitive, novel object recognition (right panel) ability in senescent rats in a dose-dependent manner (Teglas et al., 2018a). The increment in vertical activity was probably due to an increase in the hind leg muscle strength (left panel). On the right panel it is shown that the novel object recognition was not present in control rats (sham group) but after the two higher dose treatments the rats visited the novel object proportionally more (higher %) as the familiar object.

(2) Spatial learning performance has been increased in the MWM test, but the highest dose (1250 microT) was required for this effect (see Fig. 4). The figure also shows that the rats tolerated well the EMF stimulation (left panel), there was no obvious change in the behaviour of the animals during treatment.

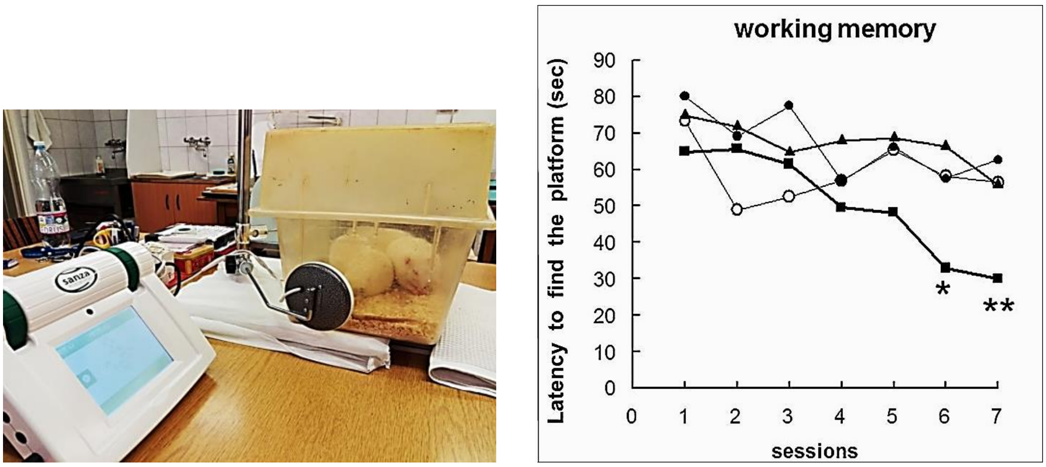


Fig. 4 EMF stimulation (1025 microT, left) enhances working memory performance (right panel) in senescent rats at the advanced phase of treatment (*p<0,05; **p<0.01; Teglas et al., 2018a). Left panel shows the experimental setup for chronic EMF stimulation.

Evaluating and discussing the behavioral findings, it can be concluded that the increase in the effort of the hind legs predicts the possibility that the results of chronic EMF treatment can also be achieved in human relation, i.e. by helping to prevent falls. Enhancement of cognitive performance is encouraging in the direction that cognitive decline in the elderly can also be addressed through preventive intervention. It is also important to note that the two measured functional improvements, the movement and brain activity changes are correlated in the treated elderly animals, which further strength that cognitive brain function and regulated motor behavior are closely interrelated ( Fig. 5).

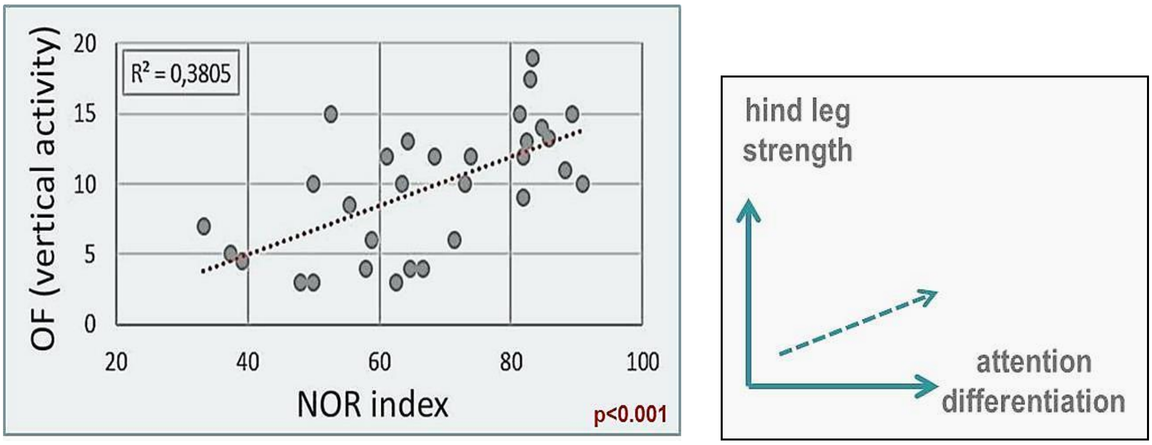


Fig. 5 There is a significant correlation between the NOR index (attention and discrimination abilities) and the vertical activity (standing up on hind legs which needs muscular strength). (Teglas et al., 2018b)

B*) Biochemical changes in the brain*

The molecular underlying mechanisms of EMF exposure-induced movement and cognitive supports were attempted to disclose in the hippocampus with Western blot techniques. The following marker proteins were assayed: BDNF, pAMPK/AMPK, pCREB/CREB, synaptophysine, ChAT, pS6/S6. The below figure (Fig. 6) shows that BDNF concentration increased in the hippocampus after chronic EMF stimulation in a highly dose-dependent manner, and there was an increment also in the ratio of pS6/S6 protein contents as the result of treatment. The BDNF (brain derived neurotrophic factor) is essential to maintain synaptic plasticity and neuronal regeneration, while the ribosomal S6 protein is a component of the 40S ribosomal subunit and is thought to be involved in regulating translation, important step in protein formation, furthermore also regulates cell size and cell proliferation. The results will be published in the near future, and the manuscript was sent to the journal of Neurochemistry International (Teglas et al., 2019b). Other investigated proteins were not changed significantly, a higher number of rats will be plausible to include into the experiments or other alternative intracellular signaling routs might be selected, especially those which are involved in the action of BDNF.

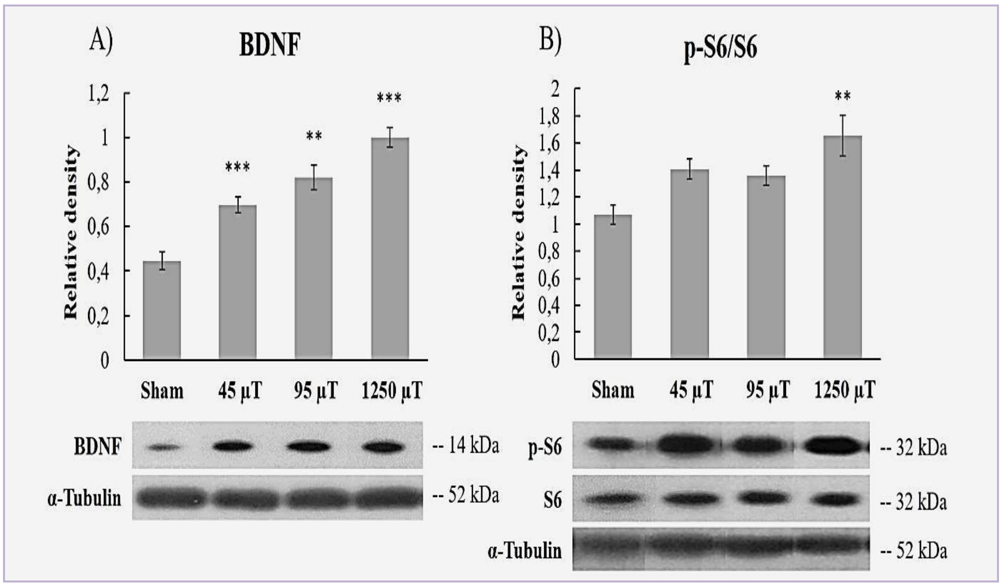


Fig. 6 Chronic EMF stimulation increases BDNF concentration (left panel) and protein synthesis throughout S6 phosphorylation (right panel) in the hippocampus of senescent rats (**p<0.01; ***p<0.001 vs Sham control, Teglas et al., 2019b)

The next figure (Fig. 7) shows that the BDNF concentration in the hippocampus correlated to the NOR index individually in the treated rats. Since BDNF is the main trophic factor in the brain and functionally involved in the maintenance of synaptic structure and function, and because during aging the deterioration of synapses are at the front line of neuronal morphological abnormalities (Luiten et al., 2013) to follow the function of BDNF is highly supported after different types of preventive therapies in the later elderly peoples. In human relation, a laboratory approach might also be important to work out circulating markers for diagnosis into this direction.

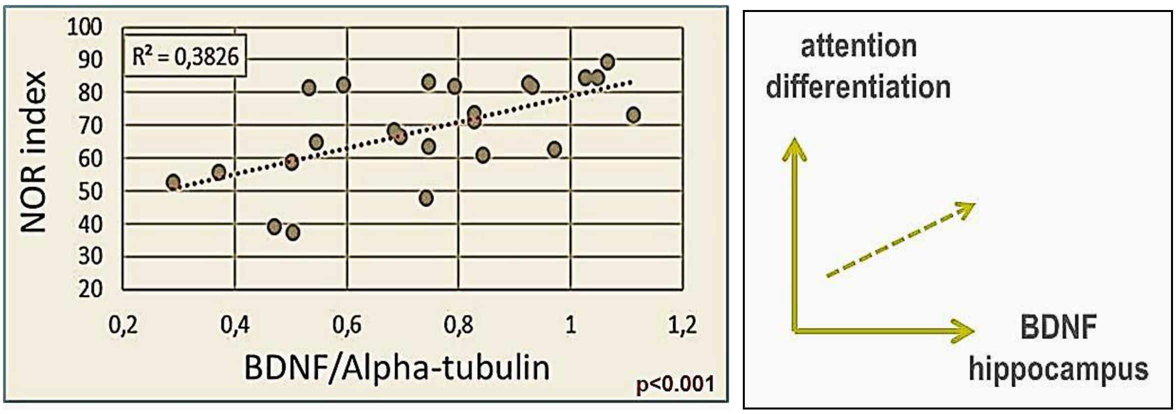


Fig. 7 There is a significant correlation between the NOR index and the hippocampal BDNF concentration after chronic EMF stimulation in senescent rats, indicating a close correlation between the cognitive function and the neurotrophic hormone BDNF level in the hippocampus (Teglas et al., 2018b)

**Literature**

our own publications are listed only which were related to the OTKA project


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