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Whole-body pulsed EMF stimulation improves cognitive and psychomotor activity in senescent rats

Tímea Téglás[a], Gabriella Dörnyei[b], Károly Bretz[c], Csaba Nyakas[a,b,*]

[a] Research Center of Sport and Life Sciences, University of Physical Education, Budapest, Hungary
[b] Department of Morphology and Physiology, Faculty of Health Sciences, Semmelweis University, Budapest, Hungary
[c] Department of Biomechanics, University of Physical Education, Budapest, Hungary

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ABSTRACT

During advanced aging passive exercise (PE) is becoming a valuable therapeutic intervention to improve physical and mental performances. In the present study chronic low frequency pulsed electromagnetic field (EMF) exposure was presented to senescent rats in order to clarify the behavioural effects related to cognitive and motility functions. Male Wistar rats of 30–32 months old were treated with EMF for six weeks, 3 times per week, 24 min per sessions prior to the age of 32 months. Stimulation intensities varied from 45 to 1250 μT. Psychomotility was estimated in an open field (OF), attention ability in novel object recognition (NOR), and spatial learning in the Morris water maze (MWM) tests. The results showed that EMF stimulation enhanced novelty-induced motility of vertical type, i.e. frequency of rearing activity was increased. In the cognitive tests EMF exposure increased attention-based discrimination in NOR and facilitated working memory type of spatial learning in the MWM tests. No undesirable type of side effects could be obtained even after the highest dose used. It is concluded that EMF stimulation in senescent age supports cognitive and psychomotor function in rats. The notion that PE may have complementary beneficial action on brain and motor functions in senescent age is strengthened by the present experimental results.

1. Introduction

Advanced aging is accompanied by functional deteriorations in different organs including the neuronal and sensory-motor systems. Aging in rats alters learning and memory functions leading to cognitive disturbances and movement instability. Along this period of life for both active and passive exercise may be a significant therapeutic intervention to maintain optimal physical and mental conditions. The active types of exercise are common in practice, but their applicability is often hampered by motor and psychosocial constraints of the elderly. Passive types of exercise tend to play an additional or complementary role in maintaining the elderly's physical and mental conditions [1–3]. On the other hand, translational experiments on animals may be a useful approach for detecting the underlying mechanisms of physiological action of passive exercise. In addition, animal experiments may more easily assist to uncover accidental unwanted side effects by outlining the physiological limits of interventions.

Among the passive forms of physical exercise the behavioural effects of low frequency electromagnetic field (EMF) exposure in animals already has gained interest. The variability of the EMF stimulation parameters like frequency in Hz and the experimental conditions like duration of EMF exposition moves along a wide spectrum in the previous studies dealing also with impact on the brain and behaviour functions [4–6]. Regarding novelty-induced behaviour several studies confirmed that EMF exposure increased activity of rats in the open field test [4,5]. Additional studies have indicated that EMF stimulation may interact with learning and memory processes. Some studies have described the inhibitory effects on different memory functions like object recognition [7] and spatial memory in rats [8,9]. Contrary, other studies have found stimulating effects in social [10] and spatial memory tasks [11] suggesting that the experimental variability of EMF stimulation studies need also closer considerations.

Regarding certain brain functions related to the underlining mechanisms in the hippocampus and forebrain it may be recalled here that using in vitro brain slice preparation EMF stimulation supported cortical synaptic function [12] and was able to modulate hippocampus excitatory and inhibitory processes [13]. Furthermore, a decrease in choline uptake was detected in the frontal cortex and hippocampus in rats following an acute EMF exposure for 45 min and 0.75–1.00 mT intensity at 60 Hz [14]. Experiments of Liu et al. [15] have shown that

* Corresponding author at: Research Center of Sport and Life Sciences, University of Physical Education, Alkotas Str. 44, H-1123 Budapest, Hungary.
E-mail address: nyakas@tf.hu (C. Nyakas).

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continuous EMF exposure for 60 days partially improved cognitive and clinicopathologic symptoms using an Alzheimer's disease rat model.

The aim of present study was undertaken to reveal novel findings of pulsed EMF exposure on psychomotor, attentional and spatial learning in senescent rats, which may highlight the usefulness of PE in this very advanced age. It is known from human studies that in the senescent age cognitive capacities and sensory-motor functions might decline with an accelerated speed as compared to earlier ages and this way becomes more responsive to therapeutic interventions [16–18]. In one of our previous experiments, we have found that active exercise of moderate intensity exerted significantly more improvements on motor and brain functions in the 32 months old rats as compared to younger rats of 12 and 24 months of ages (Unpublished results). Similarly, mice in the very old age are more responsive regarding enhancement in motor activity, learning and memory by heat shock protein treatment as compared to middle aged animals [19]. The aging induced cognitive decline is partly caused by increased movement variability, reduced coordination abilities, and slowing of movement speed in both rodents and human beings [20,21]. In relevant available literature on rodent studies, the age limit barely exceeded the two years of age to the behavioural effects of physical exercise. Consequently, senescent age also in rats can be considered as a unique age period, thus in this study we selected this very old age of 30–32 months exclusively. It is hypothesized based on our former experience as well that in the senescent age rats are highly reactive to passive type of physical exercise carried out by pulsed EMF treatment.

2. Materials and methods

2.1. Animals

All experiments were carried out by using 30–32 months old male Harlan-Wistar rats (430–500 g) from our own colony. Animals were housed under standard laboratory conditions (12:12 h light–dark cycle with lights on at 07.00 h, 23 °C temperature, and 40–50% relative humidity), food and water was provided ad libitum. Two rats were housed per cage. All experimental procedures which were carried out on the animals had been approved by the Animal Examination Ethics Council of the Animal Protection Advisory Board at the Semmelweis University, Budapest; and comply with the principles of EU Directive 2010/63/EU for animal experiment.

2.2. Pulsed EMF stimulation

The pulsed electromagnetic stimulation with its signal configuration influences positively the membrane polarization, submolecular effects, the activation of atoms, the increase of the oxygen saturation and the cell regeneration. Various organic system components can be stabilized against disturbances. In the scientific interpretation of the physical and physiological effects of the magnetic field therapy, the Maxwell's equations constitute the mathematical background leading to the understanding of the physical and biochemical mechanisms implicated in the biological processes [22,23]. The pulsed EMF stimulation was provided by the instrument Santerra MCR System (Piding, Germany) having a control circuit, which produces the carrier frequencies (narrow impulses) belonging to the EMG spectra and the modulation with different wave shapes, the frequencies of which belong to the EEG spectra. The characteristics of the EMF stimulation were the following: (1) low frequency EMF exposure was applied, i.e. frequencies were below 300 Hz, (2) the stimulating square wave pulse consisted of a carrier signal of 200 Hz and a bipolar modulating signal of 20 Hz. The stimulating system included one central unit with touch screen display connected to a pillow sized 40 × 30 cm. The rats were randomly divided into four groups: control group (sham exposure, n = 11), 45 μT group (with 45 μT EMF exposure, n = 8), 95 μT group (with 95 μT EMF, n = 11), and 1250 μT group (with 1250 μT EMF n = 10). It may be added for comparison that the EMF potential of the Earth is varying from 30 to 60 μT. A single plastic animal cage was placed on the pillow in the case of applying the two lower intensities. The Helmholtz adapter of the instrument was applied for providing the high intensity of 1250 μT. From the age of 30 months the rats were treated with pulsed EMF stimulation for six weeks as it is indicated in Table 1. Each stimulation session lasted for 24 min. For the control animals of the same treatment condition was set up without EMF exposure (sham stimulation).

Table 1
Experimental design is summarized showing the timing of different interventions throughout the 6 weeks period.

	Days						
Weeks	1	2	3	4	5	6	7
1	EMF		EMF		EMF		
2	EMF		EMF	MWM	EMF		
3	EMF	MWM	EMF	MWM	EMF		
4	EMF	MWM	EMF	MWM	EMF		
5	EMF	MWM	EMF	MWM	EMF		
6	EMF	OF	EMF	NOR			

Abbreviations: EMF - electromagnetic field exposure were provided three times per week; MWM - Morris water maze spatial learning test were carried out through seven daily sessions; open field behaviour (OF) and novel object recognition performance (NOR) were tested at the end phase of EMF treatment.

2.3. Behavioural tasks

All behavioural tests were performed blindly and the individual animals were selected randomly for the behavioural testing. The animals' behaviour was followed by an optical camera positioned 1.5 m above the test area. All behavioural tests were started in the morning and 24 h after the last EMF exposure as indicated in the experimental design (Table 1).

2.3.1. Open field test

In the open field test, we studied vertical and horizontal activity of rodents as described by us earlier [24]. The open field test box consisting of a circular arena with 80 cm in diameter, which was subdivided into 20 sectors by faint brown concentric and radial lines, and surrounded by a 45 cm high aluminum wall. Illumination was provided by a 30 W bulb, positioned 60 cm above the floor of the apparatus. Each animal was placed in the center of the open field and the novelty-induced psychomotor activity was measured by direct visual observation for 5 min. The arena was cleaned with a wet sponge and a dry paper towel between testing each animal. We counted the intensities of horizontal and vertical exploratory activities, i.e. number of line crossings by walking and duration of rearing activity in sec, respectively.

2.3.2. Novel object recognition

Novel object recognition test was utilized principally as described earlier [25,26] and was tested in the habituated open field arena 48 h after the OF behavioural test and 24 h after the last pulsed EMF exposure (consult Table 1). Briefly, during the first session (sample trial) two identical objects were placed into the arena keeping equal distances from the wall in an asymmetric position regarding the center of arena. These objects became familiar objects during the 5 min free exploration period. After a 120 min inter-session interval spent in the home-cage, the rats were replaced into the open-field arena for the 2nd session lasted another 5 min (test trial). During the second session, one of the familiar objects was replaced by a novel object. Frequency (total number of visits in scores) and duration (total time spent with visiting objects in seconds) were registered. Any animal is not exploring the objects at least five times during the second 5 min period was excluded from the statistical analysis because they did not reach the behavioural criterion for this test. For evaluating behavioural performance recognizing novel object (NOR, recognition index in percent) against the familiar one of the following calculation was applied:

$$\text{Recognition index}(\%) = \frac{\text{Duration of visits to novel object}}{\text{Duration of visits to novel + familiar objects}} \times 100$$

2.3.3. Morris water maze spatial learning

The Morris water maze learning test (MWM) was performed according to Morris [27] in a round black water tank (diameter 153 cm, height 63 cm) filled to a depth of 53 cm with water of 26 ± 1 °C. A black hidden platform (diameter 10.8 cm) was located at a fixed position and submerged 1.5 cm below the surface of the water. Four trials with different starting positions were equally spaced around the perimeter of the tank from the middle of each quadrant. The animals had to find out the place of the hidden platform guided by different cues on the wall of the experimental chamber and the surroundings. Altogether 7 sessions were applied (see Table 1). The rats received four trials per each session. The order of starting positions varied randomly by trials, but was constant during a session. Rats were placed into the water at a starting position facing towards the wall of the tank. Each trial lasted until rats found the platform. If the platform was not found within 90 s, the experimenter led the animal to it. Rats spent 30 s on the platform at the end of each trial. Duration to find the platform was recorded for each trial and registered as latency in seconds. During each session, the latency time of the first trial served on reference memory recording and the mean latency time of the daily 4 trials for working memory.

2.4. Statistics

The Statistica 13.2 program was applied for evaluation of the numerical results by using one- or two-way ANOVA followed by paired comparisons between two groups by the Tukey's *post hoc t*-test. NOR performance in each group separately against chance level was evaluated by a dependent samples *t*-test, counting means against reference constant of 50%. Means ± SEMs are shown in the figures where *post hoc t*-test results are all depicted. For correlation analysis between sensory-motor parameters in OF (walking and rearing) versus cognitive performances in NOR and MWM tests we used a parametric correlation test of Statistica 13.2. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Open field activity

This type of behavioural activity reflecting psychomotor stimulation to novelty is summarized in Fig. 1. Number of line crossings as horizontal activity did not change significantly [F (3.35) = 1.33, p = 0.28] only a tendency of the increment in this behaviour could be found. Numbers of rearing as vertical activity, however, was influenced by the chronic pulsed EMF exposure [F (3.35) = 3.18, p = 0.036]. The higher doses increased this type of activity, i.e. standing up on two feet was more frequent in the middle and high dose treated groups (p = 0.020 and p = 0.0090, respectively, see also Fig. 1).

3.2. Novel object recognition

Pulsed EMF exposure improved novel object recognition [F (3.37) = 7.82, p = 0.00040] and both the middle (p = 0.00087) and the high (p = 0.0043) doses were effective on the ability to discriminate between novel versus familiar objects compared to Sham controls. Since basic recognition capability for discrimination is only present in the individual groups if the ratio of visiting novel vs. familiar objects is significantly above 50% (chance level as a reference constant, shown by the broken line in Fig. 2). This criterion was met only by the middle and high intensity groups (p = 0.000009 and p = 0.00063, respectively, calculated by testing the mean performances against the reference constant of 50%). At the level of 45 μT intensity the difference only approached significance (p = 0.056). The Sham control group clearly did not show any sign of recognition which is usual at this very advanced old age.

Evaluating the interaction between sensory-motor abilities in OF test vs. NOR cognitive capability was estimated by correlation analysis including all groups. It was found that both the vertical and horizontal activities positively correlated to NOR performance (n = 38; rearing: r = 0.626, p = 0.000; walking: r = 0.407, p = 0.021) suggesting the presence of a positive impact of motor abilities on cognition.

3.3. Morris water maze learning

Reference and working memory along the 7 sessions of this test are depicted in Fig. 3. Regarding all groups the reference memory (left panel) improved along the sessions (repeated measures ANOVA: F (6.204) = 7.21, p = 0.000001). However, there was no significant differences among the groups [F (3.34) = 0.40, p = 0.75]. In case of working memory (right panel) there could also be found an improvement along the sessions (repeated measures ANOVA: F (6.204) = 5.11,

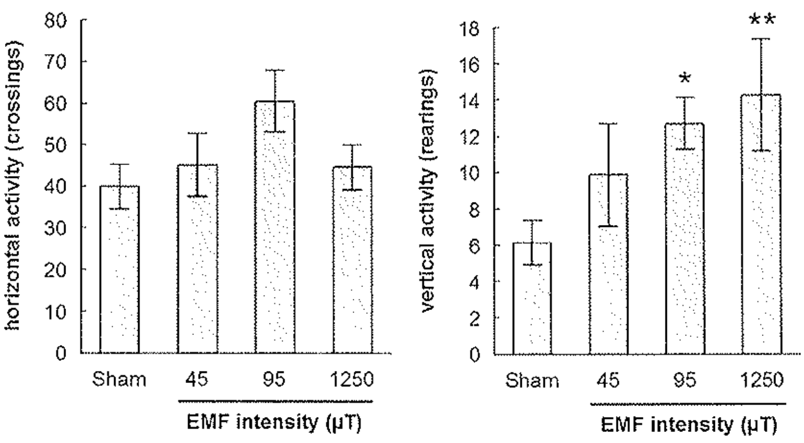

Fig. 1. Open field activity of 32 months old rats following chronic pulsed EMF stimulation. Horizontal activity is shown on the left and vertical one at the right side. The number of animals varied 8–11 per experimental groups. *Post hoc t*-test results: *: p < 0.05; **: p < 0.01 vs. Sham control group.

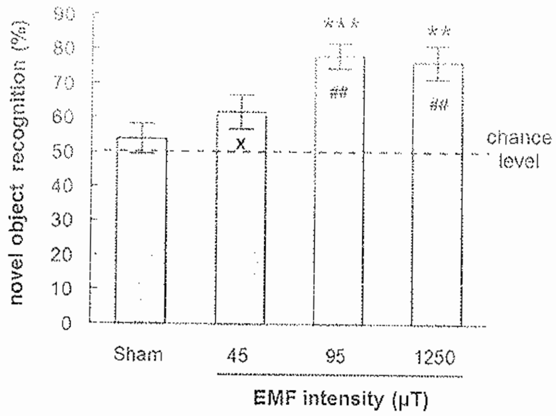

Fig. 2. Novel object recognition was improved by the chronic EMF stimulation and both the middle ($p < 0.001$) and the high ($p < 0.01$) doses were effective increasing NOR against Sham controls (**: $p < 0.01$ and ***: $p < 0.001$). Furthermore, testing NOR performance against the chance level in each group separately the middle and high intensity treated groups showed significant discrimination capability (##: $p < 0.01$, *t*-test for dependent samples). At the level of 45 μT intensity the difference in discrimination only approached significance (x: $p = 0.056$, dependent samples *t*-test).

$p = 0.000065$). However, in this memory parameter a difference among the groups could be revealed: F (3.34) = 3.30, $p = 0.032$. Furthermore, the interaction between the two factors, i.e. session and group, approached significance: F (18.204) = 1.47, $p = 0.10$. Lastly, *post hoc t*-test disclosed that at the last two sessions only the group treated with the highest intensity performed better as compared to the sham control group ($p = 0.012$ at session 6, and $p = 0.0090$ at session 7, see Fig. 3).

The spatial learning performance in MWM test was also compared by correlation to the spontaneous sensory-motor activity levels in OF test, i.e. walking and rearing scores. Regarding RM no correlations were found. Correlation analysis regarding WM, however, revealed that rearing correlated with the cumulative latency time counted throughout all the seven sessions ($n = 38$; rearing: $r = -0.498$, $p = 0.002$). This means that the higher rearing activity is correlated to a decreased latency time founding the hidden platform.

4. Discussion

Our aim in this study was to reveal the biological action of a chronic type of low frequency pulsed EMF stimulation on cognitive and psychomotor behaviour of senescent rats. The main findings indicated that EMF exposure facilitated a number of behavioural responses in senescent rats, i.e. vertical ambulation was increased in open-field, discrimination behaviour in novel object recognition test was enhanced, and working memory in Morris spatial learning task could also be partly facilitated at least by the highest EMF intensity of 1250 μT. These results strongly suggest that both spontaneous behaviour in novel environment and cognitive capabilities in different learning tasks can be favorably altered. In the present study we selected the age of 30–32 months in our experimental rats which is a unique age in the literature for studying age-related behaviour and exercise-induced behavioural changes. In addition, this age, i.e. the senescent age in rats possesses a number of unique physical and mental characteristics similarly to human and by this way can be viewed as a translational experimental model. Majority of studies in the past already emphasized that physical exercise support learning and memory including spatial learning behaviour in aged rats [28–30], although some published results showed no effect [31]. In these studies using active type of exercises, mainly treadmill training was selected. Applying the pulsed EMF exposure protocol in the present case can be considered as a passive type of physical exercise. In human relation there is a need to provide this kind of exercise for aged persons especially in case of advanced movement difficulties or immobility.

During the spontaneous mobility test in the open field (see Fig. 1) no significant increment was found in horizontal activity represented by ambulation on four legs, but intensity of rearing activity was increased after treatment with both the middle and high EMF intensities. It may be noticed that through ageing [32], and especially by the advanced age over 30 months in rats rearing activity declines more intensively as compared to horizontal ambulation, i.e. walking on four legs. It might be assumed that muscle strength required for standing up on hind legs declines most intensively during very advanced aging resulting a particularly degraded motor regulation of the hind limbs. It has been shown that disuse atrophy coupled with the increased protein degradation against synthesis may be a causal factor in response to hind limb unloading in aged rats [33]. An early study of Van der Zee et al. [20] already showed that the hind limb movements of aged rats significantly deteriorated, especially after the age of 2 years, which could have been alleviated pharmacologically in this study through enhancement of the sciatic and caudal nerve conduction velocities. Others revealed that the reduced maximum force generation despite minimal changes in muscle mass is the main course in the degradation of hind foot posture in aged rats [34]. In the present experiment, we may assume that the EMF treatment primarily increased muscle strength and/or movement coordination in the hind legs which resulted in an obvious

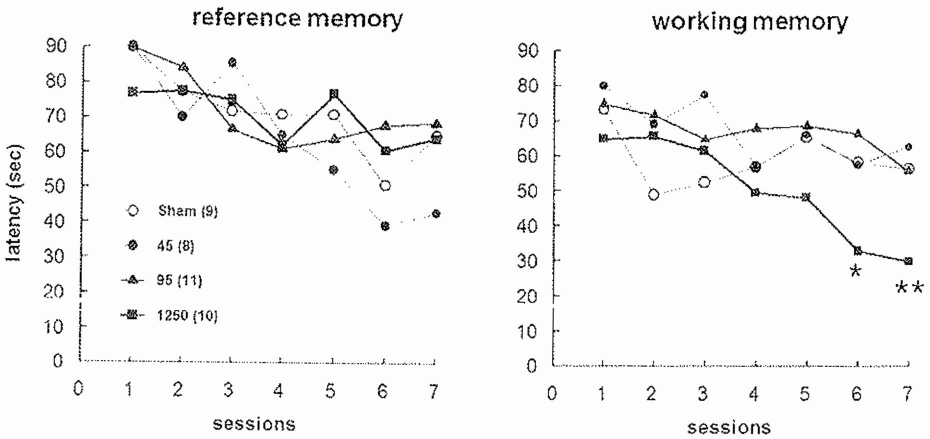

Fig. 3. Morris water maze learning and memory performances are shown trough seven daily sessions. Left panel presents the reference memory and the right one the working memory performance. Improvement could be detected in the working memory by the highest dose applied: *: $p < 0.05$; **: $p < 0.01$ vs. Sham control group.

increase in rearing activity. Further studies are awaited to investigate how muscle strength and mass and the neuromuscular functions are influenced by EMF stimulation.

In the NOR test by recognizing novel object compared to a familiar one at the two higher doses showed again equal effectiveness to enhance discrimination learning behaviour (see Fig. 2). This kind of learning behaviour is based on attention ability and memory retrieval coupled to working memory during the second behavioural session. The sham control animals showed no discrimination ability, i.e. did not discriminate between the novel and familiar objects, which are common in this very old age of 32 months. Others also revealed that long-term visual object recognition memory failed in aged 25–27 months old Wistar rats [35]. In the present study the dose-related enhancement of NOR performance was clearly detectable though increasing the EMF stimulation intensity from 45 to 1250 μT. According to the literature, working memory as reflected in NOR behaviour is organized in the prefrontal cortex which brain area is known for interconnecting motoric and cognitive brain functions through regulation of working memory [36].

The Morris water maze test is widely used to evaluate spatial learning and memory in rats guided by environmental cues in the surroundings [27,37]. One option for the evaluation of this behaviour is to distinguish between reference and working memory abilities. Reference memory is based on long-term memory and tested here counting each first trial of the sessions. Working memory was calculated by averaging the four trials at each session. Although in the course of consecutive sessions there was a significant improvement of both reference and working memories in the groups the treatment effect was restricted only to the highest dose of intensity and only in the advanced stage of training (see Fig. 3). Furthermore, reference memory was not influenced while working memory improved by the highest dose. For an explanation of the relatively moderate effect several aspects of experimental design (Table 1) may also be considered. Firstly, it might be possible that certain number of EMF stimulation sessions are necessary prior to detect the initial treatment effect. Secondly, only two MWM sessions were given weekly which might have slowed down the progression of spatial learning and made less effective building up mainly the reference memory because of the long intertrial intervals.

Decline in spatial learning performance in the old age of 24–28 months compared to young age in rats found earlier [38,39] confirms the concept that advanced aging in rats is accompanied by cognitive disturbances and aged rats can serve as a pharmaceutical target for translational model of cognitive decline. In Alzheimer's disease (AD) rodent models learning and memory impairments are also present, like in aged animals, and some laboratories reported a memory improving effect of EMF exposure on both aged AD mice [40] and adult AD rats in Morris maze [41]. In another study hippocampus injury was induced by trimethyltin chloride neurotoxin and it was presented that the EMF stimulation enhanced spatial learning and memory in the MWM test which was accompanied by a facilitated neurogenesis in the hippocampus [42]. A further experimental work revealed that EMF stimulation enhanced repair following peripheral nerve transection injury [43]. In our future aims, more experimental analyses are waiting to uncover the actual mechanisms involved at molecular and cellular levels in response to pulsed EMF exposure in both the brain and motor system. These notions are based on recognition that there is a close cooperation between the motor and cognitive functions demonstrated in both rodent [44] and human studies [45–47].

It is a novel aspect of the present findings that a correlation was found between the sensory-motor abilities tested in the open field and the cognitive performance in NOR and MWM tests. The vertical movement ability, i.e. the frequency of rearing correlated well in both cognitive tests with the cognitive performance. Here again may be put forward to the probable option that the hind limb functioned better in these old rats after the chronic EMF exposure. The relation between sensory-motor and cognitive functions is highly investigated during aging also in human [16]. Clinical studies already have confirmed that EMF exposure is beneficial in sclerosis multiplex [48], Alzheimer [49] and Parkinson [50] diseases. Further studies in translational animal models are waiting to highlight the cellular and molecular mechanisms of action regarding the neuronal - muscular interactions of a broadest sense.

In conclusion, in this study the chronic pulsed EMF stimulation was introduced to rats reaching the senescent age of 32 months in order to develop a translational model for supporting healthy aging, to enhance psychomotility and attenuating cognitive decline. The results showed that EMF exposure increased motility in novel surroundings, mainly the upraise position on the rear feet was highly facilitated, which support the idea that the muscle strength of hind legs has been increased. On the other hand cognitive functions like object recognition and even spatial maze learning enhanced by EMF stimulation in the senescent rats which support an improvement in discrimination and learning behaviours.

Declarations of interest

None.

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