An Acad Bras Cienc (2022) 94(4): e20200596 DOI 10.1590/0001-3765202220200596
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

CELLULAR AND MOLECULAR BIOLOGY

Preventive effects of fixed and progressive forced exercises on memory and brain electrical activity in morphine-addicted rats

NILOOFAR ASHTARI, MARYAM RADAHMADI & HOJJATALLAH ALAEI

Abstract: Exercise and addiction influence brain functions. The preventive effects of fixed and progressive forced exercises on both brain functions and body weight were investigated in morphine-addicted rats. Thirty-five rats were allocated to control, morphine, fixed exercise-morphine, and progressive exercise-morphine groups. Forced exercise was applied 1h/day for 21 days with morphine sulfate administered at doses of 10, 20, 30, 40, and 50 mg/kg for 5 consecutive days. The 50 mg/kg dose was repeated over the five subsequent days. Brain performance was evaluated using the passive avoidance test and EEG recordings. The passive avoidance test revealed no significant changes in brain functions (namely, latency, total dark stay time, and number of times entering the dark compartment). Compared to the control, the morphine group exhibited significantly lower alpha and beta waves but significantly higher delta and theta ones. Compared to the morphine group, the progressive and fixed exercise-morphine groups exhibited significant changes in their passive avoidance performance and only in the alpha wave of their EEG recordings. Progressive exercise improved learning, memory, and memory consolidation but reduced locomotor activity whereas fixed exercise affected EEG recordings in the addicted subjects. Clearly, different (fixed or progressive) exercise models produced different changes in brain functions.

Key words: addiction, brain waves, exercise, memory, morphine, rat.

INTRODUCTION

Drug addiction is nowadays known as the cause of behavioral abnormalities. Chemical drugs like morphine influence different brain functions as revealed by behavioral tests, new electrophysiological events (e.g., new waveforms of various frequencies) detected by electroencephalography (EEG) recordings (Yousif et al. 2008), and biochemical alterations (Bekheet et al. 2010, Chiang et al. 2015). EEG recording as an electrophysiological monitoring method disclosing the electrical activities of the brain cortex (Andrzejak et al. 2001, Kafa et al. 2010, Vorobyov et al. 2003) may be used to diagnose many brain dysfunctions (Hanslmayr et al. 2011, Uhlhaas & Singer 2006) such as sleeping disorders (Tang et al. 2007), memory impairment (Basar & Guntekin 2012, Knyazev et al. 2006), anxiety and depression (Blackhart et al. 2006), schizophrenia, and epilepsy (Arıkanoğlu 2011, Peng et al. 2013). Reports, however, indicate that unbalanced potency of EEG may be caused by administration of different doses of morphine (Li et al. 2016, Ferger & Kuschinsky 1995, Zanettini et al. 2018, Fischer et al. 2017).

On the other hand, useful methods have been proposed to counteract disturbances in EEG potency due to drug-induced synaptic changes (Aiyer et al. 2016) although no effective treatment is yet available for preventing relapse disorders due to the lack of a sound knowledge

of the mechanisms underlying such diseases (Ferger & Kuschinsky 1995).

A good approach to resolve this problem is exploiting the positive effects of exercise on brain functions (Bailey et al. 2008, Radak et al. 2007, Neeper et al. 1995). Exercise has been established to activate a variety of brain mechanisms (Radahmadi et al. 2015, 2016a) and it may, therefore, serve as an important strategy for improving the functions of the nervous system (Uchida et al. 2012). As a preventive measure in the treatment of addiction, exercise is additionally advantageous because it is inexpensive and readily available while it lacks the side-effects commonly associated with chemical drugs. Exercise protocols seem capable of preventing the destructive effects of addiction on the brain. Despite the extensive literature on exercise, no published report is yet available, to the best of the present authors' knowledge, on the preventive effects of fixed and progressive aerobic exercises on brain functions in addicted subjects. The present study was, therefore, designed and implemented to investigate the preventive effects of two exercise models (namely, fixed and progressive forced aerobic exercises) on memory, locomotor activity, memory consolidation, and brain electrical activity (as revealed by EEG recordings) in morphine-addicted rats.

MATERIALS AND METHODS

Experimental animals

Twenty-eight adults male Wistar rats (200–300 g in weight) were procured from Pasteur Institute (Tehran, Iran). The rats were maintained under 12-h light/dark cycles (lights on from 7:00 a.m. to 7:00 p.m.) under controlled temperature (22±2° C) and humidity (50±5%). Food and water were made available *ad libitum*, except during the exercise sessions. The procedures and protocols employed were approved by the Ethical Committee of Animal Use of Isfahan University of Medical Sciences (IR.MUI.MED.REC.1397.228) while all the experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, 2011 Revision). A period of one week was allowed for adaptation before the animals were randomly assigned to the following four groups (n = 7 rats/group) (Figure 1):

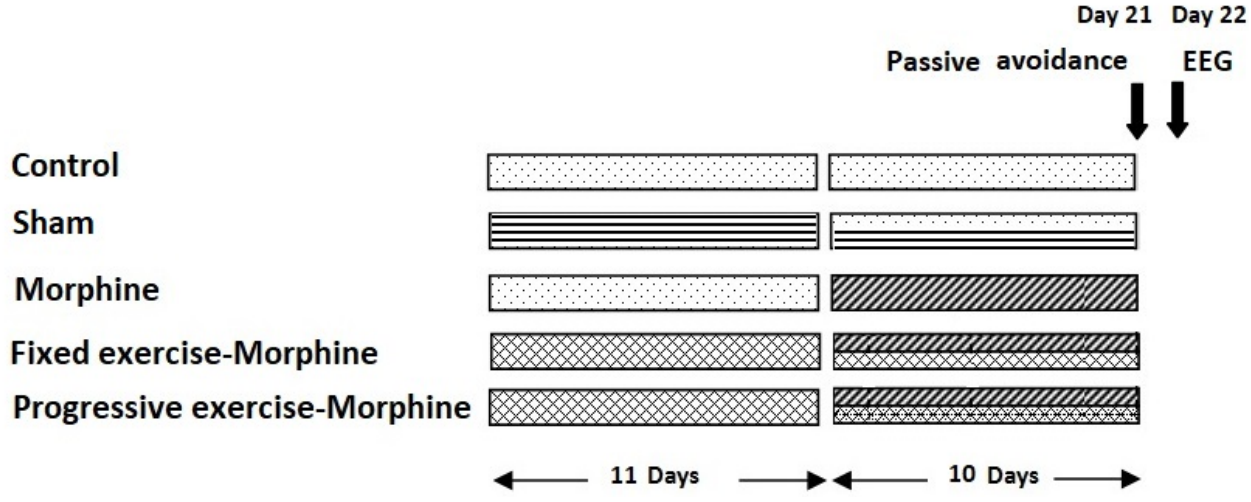

Figure 1. Schematic diagram of different groups. EEG: Electroencephalography.

– Control group: Rats were maintained in the laboratory for the first 11 days of the trial receiving no special treatment before they received normal saline (morphine vehicle) injections over the following 10 days.

– Sham group: Rats were maintained in the laboratory for the first 11 days of the trial receiving no special treatment before they received normal saline (morphine vehicle) injections over the following 10 days. Throughout the whole 21 days of the trail, the animals were placed on a motorized rodent running wheel for 1h/day without running.

– Morphine group: Rats were maintained in the laboratory for the first 11 days of the trial receiving no special treatment before they received morphine injections over the following 10 days.

– Fixed exercise-morphine group (Fix.Exe-Morphine): Rats were subjected to exercise over the first 11 days of the trial and subsequently received morphine injections over the following 10 days while the exercise was continued at the same speed.

– Progressive exercise-morphine group (Prog.Exe-Morphine): Rats were subjected to exercise over the first 11 days of the trial and subsequently received morphine injections over the following 10 days while the exercise was enforced at a higher speed.

Finally, all the rats were prepared for the passive avoidance test on day 21 and EEG recordings on day 22.

Experimental procedures

Exercise paradigms

All the rats in the exercised groups were run on a motorized rodent running wheel (Tajhiz Gostar Iranian Co., Tehran, Iran) as a forced aerobic exercise. The three-day adaption period prior to the experiments included a first day of running on the wheel in the off mode for 60 min once a day and two subsequent days of running on the wheel in the on mode with speed being increasing to 2.5 m/min and the exercise duration being raised from 15 to 30 min.

Two different intensity patterns (namely, fixed and progressive) of exercise were used. The fixed exercise protocol consisted of 1 h/day of running at a speed of 10 m/min over 21 consecutive days. The progressive exercise protocol consisted of 1h/day of running at a speed of 5 m/min for 11 consecutive days and 1h/day of running at a speed of 10 m/min over the remaining 10 days. Thus, the exercise continued both before and throughout the morphine administration period. The rats in the sham group, designed for evaluating the stress incurred due to the exercise apparatus, were placed on the motorized rodent running wheel for 1h/day throughout the trial period without having to run.

Drugs

Addiction was induced by single intraperitoneal (i.p) daily injections for 10 consecutive days of morphine sulfate (Temad Co. Tehran, Iran) dissolved in saline 0.9%. The drug was administered at doses of 10, 20, 30, 40, and 50 mg/kg for five consecutive days followed by administration of 50 mg/kg repeated over the last five days (in total, the rats received 400 mg/kg for 10 days). Morphine dependence of rats was assessed by Acon® urine morphine rapid test (strip) (Health Research Systems Inc., USA) for detecting positive urine morphine (Radahmadi et al. 2016b). Previous studies had reported induction of addiction by injecting lower doses of morphine over the same period as in the present study and testing to detect addition symptoms on the tenth day (Saedi Marghmaleki & Alaei 2016, Castilho et al. 2008, Marghmaleki et al. 2013). Finally, the control group received

equal volumes of saline (drug vehicle) for 10 days.

Behavioral paradigms

The passive avoidance test was used to assess learning, memory, memory consolidation, and locomotor activity in a shuttle box (64×25×35 cm) divided by sliding guillotine doors and grid floors into two compartments of identical sizes (32×25×35 cm). Each rat would be placed in the apparatus for 300s for habituation before a single learning trial would be performed after 1 day. In the learning trial, the rats would be placed individually in the light compartment for 60s before the guillotine door would be raised to allow the rat to enter the dark compartment. Once the door had been closed, a single electrical shock (0.5 mA, 2 s) would be delivered to the animal's foot through the grid floor using an isolated stimulator. The initial latency of entrance into the dark room would be recorded before inducing the electrical shock. In the memory trial, the latency of entrance into the dark compartment would be measured on day 21 at the end of the trail period (i.e., 1 day after the learning trial).

Foot shocks would be delivered for both habituation and memory tests (Dastgerdi et al. 2018). A delay time of up to 300s was recorded for entrance into the dark compartment in both the learning and memory trials. The ability of the animal to remember the foot shock received was determined in the passive avoidance task. Avoiding entry into the dark compartment or a longer duration of stay in the light compartment was interpreted as a positive response .Finally, the difference between initial latency and that after 1 day was interpreted as occurrence of learning (Radahmadi et al. 2017). Also, the total dark stay time was recorded as memory consolidation and/or storage of novel information (Dastgerdi et al. 2018) while the number of times the animal entered the dark compartment in 5 minutes was registered as locomotor activity (Vohora et al. 2000, Divsalar 2012).

Stereotaxic surgery and EEG electrophysiological study

Twenty-four hours after exposure to the last experimental session (i.e., on day 22), the rats were initially anesthetized with intraperitoneal injections of urethane (1.5 g/kg; Sigma, USA) and placed in a stereotaxic frame (Stoelting Co., USA). The skull was then exposed and two small holes were drilled over dura using a bregma reference point and a standard miniature drill 0.5 mm in diameter. The holes were 2 mm anterior to bregma and 1.5 mm lateral to midline. Then, electrodes were placed sub-cranially (at the level of the cortex), the relevant brain-device interfaces were connected to the rat and the device, and brain EEG waves were recorded. In the EEG system, the rats would be placed on a suitable pad and covered during the experiment in order to record better signals. The EEG activity of each anesthetized rat was recorded for approximately 20 min. Signals were low-pass filtered at 0.5–3 kHz and sampled at 1 kHz. They were additionally passed to a computer through an analog to digital interface and the data thus obtained were analyzed using eTrace Analysis before being transferred to the data acquisition system (Data Acquisition, Science Beam-D3111; eProbe- eTrace Experiment software; Parto Danesh Co.).

Electroencephalograms of brain waves are effective tools for investigating natural brain phenomena and various states of consciousness. The system processed the data to show the power of each of the alpha, beta, delta, and theta waves. The waves collected were filtered in the range of 0 – 30 Hz and the delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), and beta (13–30 Hz) waves were accepted (Jurkowlaniec et al. 2003).

The total power of the four frequency bands were taken to be 100% as the baseline, and the quantity of each frequency band (alpha, beta, theta, or delta) was calculated for all the groups examined and expressed as a percentage of the total power (Stein et al. 2017). In other words, all the waves accumulated after 1 wave were taken as 100% and every single wave was calculated as a percentage of the total power.

Body weight measurement

Body weights were measured on days 1 and 21 of the experiment and differences ($BWD=BW_{21Days}-BW_{1Day}$) were determined.

Statistical analysis

All the data were reported as means ±SEM. Moreover, the data were compared (between-group comparisons) using ANOVA followed by the LSD post-hoc test for multiple comparisons. Comparisons of initial latency and that after 1 day (within-group comparisons) were analyzed using the paired student's t-test. A P-value of less than 0.05 was considered as statistically significant. Ultimately, the calculations were performed using SPSS 21 (SPSS Inc., Chicago, IL, USA).

RESULTS

Since the Control (Co) and Sham (Sh) groups exhibited no significant differences in their test results or EEG recordings, the control group was selected as the reference for all the following comparisons.

Behavioral results

In the passive avoidance test, an ANOVA test followed by the LSD post hoc test indicated no significant differences in initial latency [$F(4, 30) =0.967$, $P>0.05$] (Figure 2), latency after 1 day [$F(4, 30) =2.411$, $P>0.05$] (Figure 3), dark stay time [$F(4, 30) =5.220$, $P<0.05$] (Figure 5), and number of times entering the dark compartment [$F(4, 30) =1.804$, $P>0.05$] (Figure 6).

Figures 2 and 3, respectively, show the data on initial latency and that after 1 day for all the groups subjected to the passive avoidance test. Based on the one-way ANOVA, no significant differences were observed in initial latency values among the experimental groups (Figure 2).

The progressive exercise-morphine group recorded significantly ($P<0.01$) higher values for latency after 1 day than the morphine group did (Figure 3), indicating their improved memory due

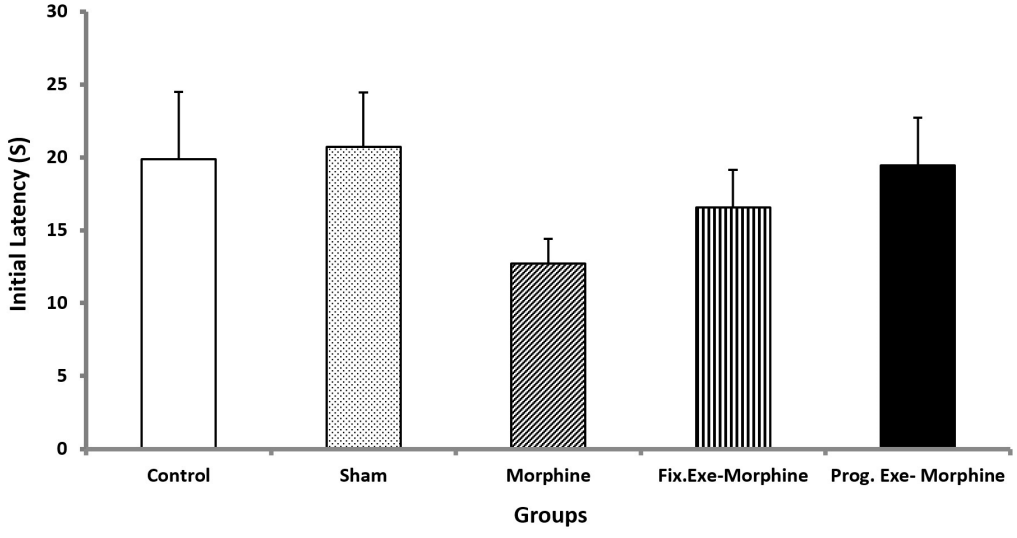

Figure 2. Initial latency to entrance into the dark room of the passive avoidance apparatus for all the groups before receiving a foot shock (n = 7). Results are expressed as means ± standard error of mean. No significant differences were observed among the groups.

to the protective effect of progressive exercise in addicted rats.

As shown in Figure 4, a paired sample t-test was used for evaluating changes in the within-group latency values obtained from the passive avoidance test. The results indicate differences between initial latency and latency after 1 day in the control [t(6)= -5.728, P<0.01], Sham [t(6)= -6.106, P<0.01], Morphine [t(6)= -11.596, P<0.01], Fix-Exe-Morphine [t(6)= -10.960, P<0.01], and Prog-Exe-Morphine [t(6)= -6.400, P<0.01] groups. The differences are clearly significant (P<0.01), indicating that learning happened in all the experimental groups.

As shown in Figure 5, significant decreases in total dark compartment (DS) stay time were recorded for both the fixed and progressive exercise-morphine groups (P<0.05 and P<0.01; respectively) when compared with the values measured in the control. Also, significantly (P<0.05) lower DS values were recorded for the progressive exercise-morphine group than those measured in the morphine group.

Finally, the progressive exercise-morphine group, compared with the morphine one, showed a significant (P<0.05) decrease in its number of entrances into the dark compartment as a locomotor activity (Figure 6).

Electroencephalography results

The ANOVA test followed by the LSD post hoc test of the EEG recordings revealed no significant changes in the theta [F(4, 30) = 2.275, P>0.05] (Figure 7a), delta [F(4, 30) = 2.350, P>0.05] (Figure 7b), beta [F(4, 30) = 2.069, P>0.05] (Figure 7c), or alpha waves [F(4, 30) = 2.175, P>0.05] (Figure 7d).

Figure 7 shows different EEG brain waves of the cortex. Based on the ANOVA test results, the percentages of delta, theta, beta, and alpha waves showed significant (P<0.05) differences between the morphine and the control groups (Figure 7a-d). A significant difference (P<0.05) was also detected in the percentages of alpha waves between the fixed exercise-morphine and the morphine groups (Figure 7a).

Body weight differences

The ANOVA test followed by the LSD post hoc test of body weight differences revealed no significant differences in alpha waves [F(4, 30)= 6.552, P>0.05] (Figure 8). Results indicated that body weights in the morphine (P<0.01), fixed exercise-morphine (P<0.05), and progressive exercise-morphine (P<0.01) groups showed significant differences ($BWD=BW_{21Days}-BW_{1Day}$) from those of the control (Figure 8).

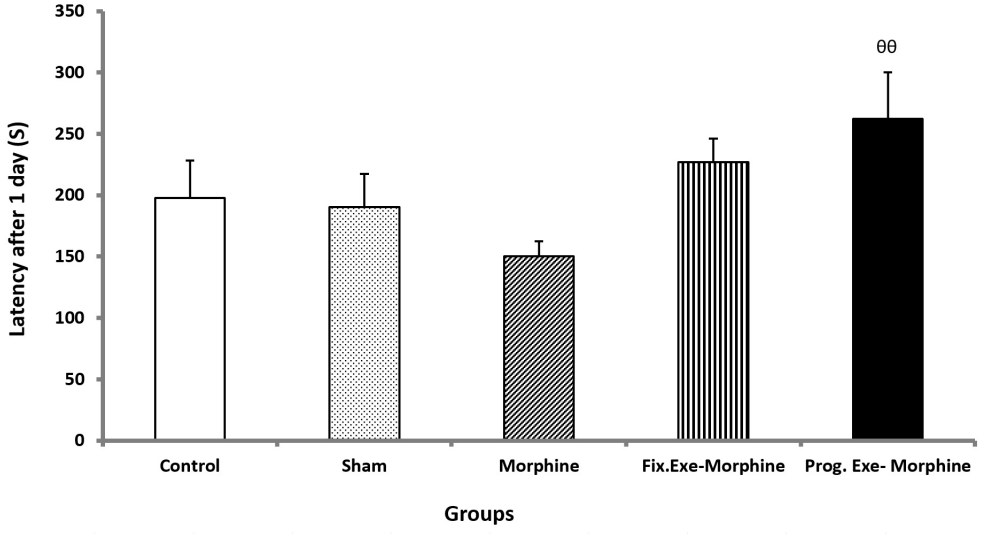

Figure 3. Latency after 1 day to entrance into the dark room of the passive avoidance apparatus for all the groups 1 day after receiving the foot shock (n = 7). Results are expressed as means ± standard error of mean. $^{\theta\theta}$P<0.01 compared to morphine group.

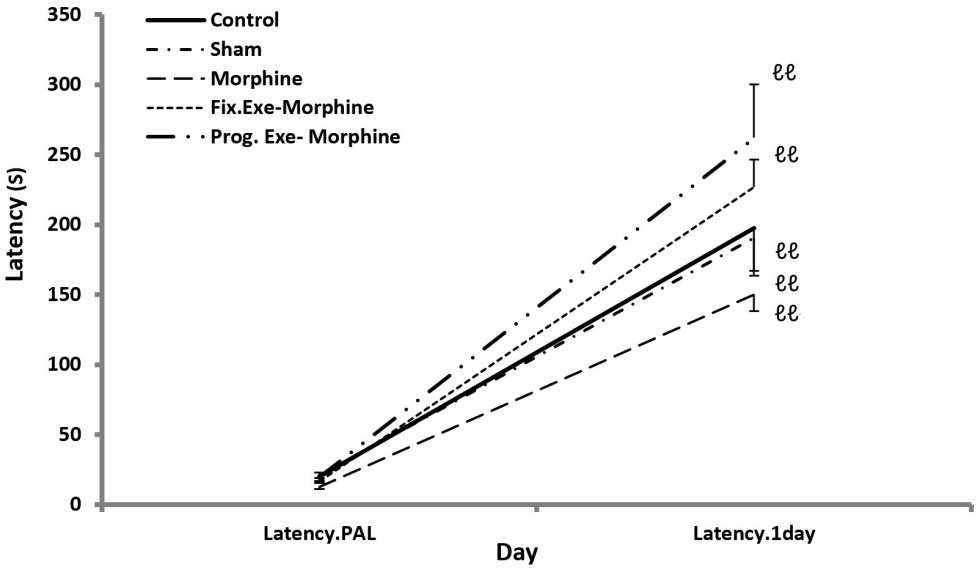

Figure 4. Initial latency and latency after 1 day to entrance into the dark room of the passive avoidance apparatus before and after the foot shock (within groups) (n = 7). Results are expressed as means ± standard error of mean. $^{\ell\ell}P < 0.01$ Initial latency relative to the latency after 1 day.

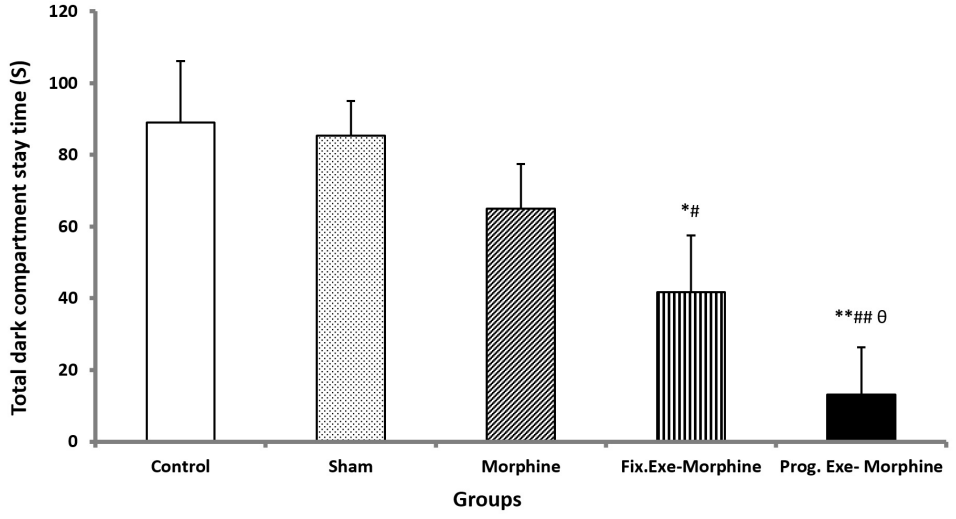

Figure 5. Total stay time in dark room of the passive avoidance apparatus for all the groups 1 day after receiving the foot shock (n = 7). Results are expressed as means ± standard error of mean. *P<0.05 and **P<0.01 compared to the sham group, $^{\#}P<0.05$ and $^{\#\#}P<0.01$ compared to the control group, $^{\theta}P<0.05$ compared to morphine group.

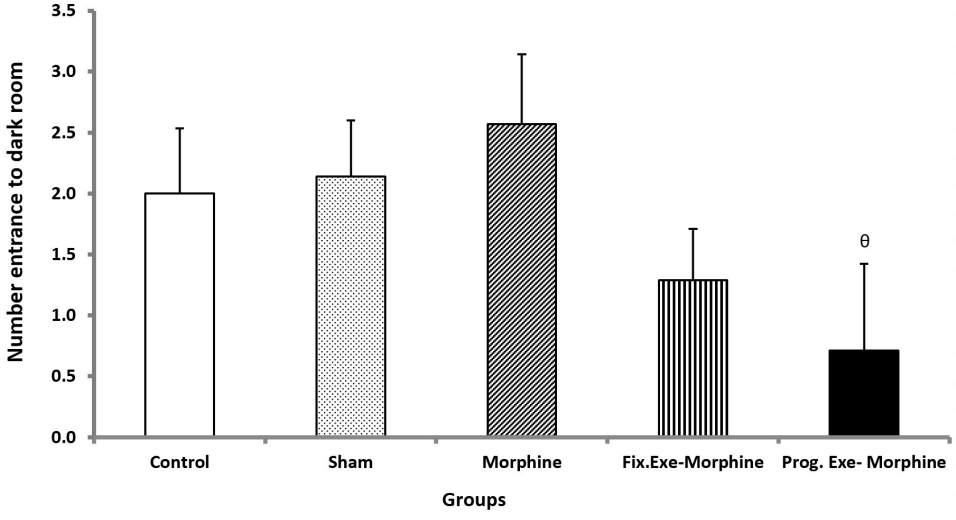

Figure 6. The number entrance to dark room of the passive avoidance apparatus for all the groups 1 day after receiving the foot shock (n = 7). Results are expressed as means ± standard error of mean. $^{\theta}P<0.05$ compared to morphine group.

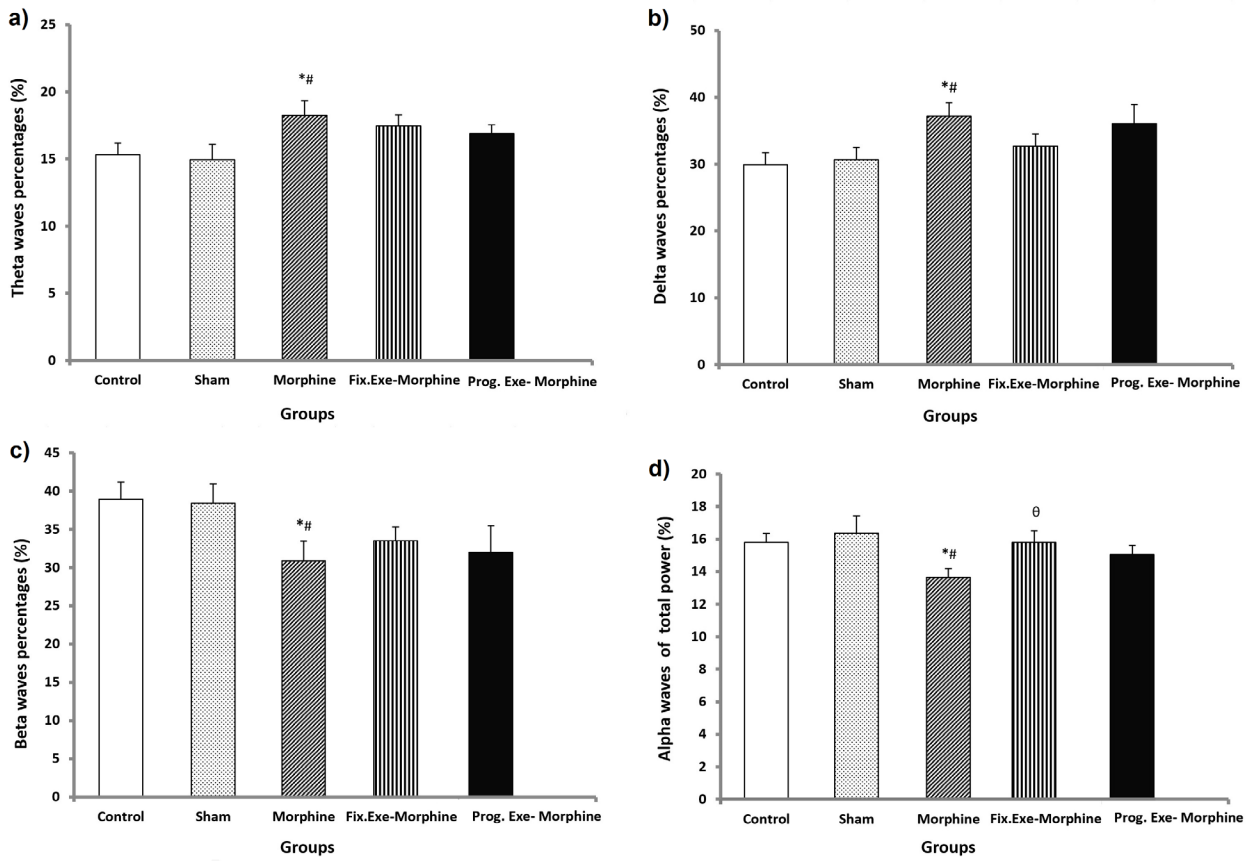

Figure 7. Comparison of percentages of different waves of total power (%) in all the groups. a) Theta waves percentages, b) Delta waves percentages, c) Beta waves percentages, d) Alpha waves percentages. Data represent means± SEM (One-way ANOVA followed by LSD post-hoc test). *P<0.05 compared to the control group, #P<0.05 compared to the sham group, and ΘP<0.05 compared to morphine group.

DISCUSSION

The preventive effects of the two forced (fixed and progressive) exercise models on abnormalities resulting from morphine addiction were investigated based on such brain functions as learning, memory, locomotor activity, memory consolidation, brain electrical activity (EEG recordings including alpha, beta, delta, and theta waves), and body weight differences in addicted rats. The findings showed that learning happened in all the groups tested, with the highest learning level recorded for the progressive exercise-morphine group and the lowest for the morphine one. This is in agreement with the findings reported by Saadipour et al. (2009) who reported learning occurring in their morphine-addicted rats. The present findings additionally revealed the greater effect of progressive exercise than the fixed one on learning improvement in addicted subjects. Some studies had reported that while treadmill running exercise improved learning rates in morphine rats (Azizi et al. 2005, Saadipour et al. 2009), it did not play up with different types of exercise.

Another finding of the present study indicated that the ten-day morphine administration did not produce any significant changes in the brain functions of memory, locomotor activity, and memory consolidation. Consistent with this finding, Babor et al. (1976) demonstrated that ten days of morphine administration was not

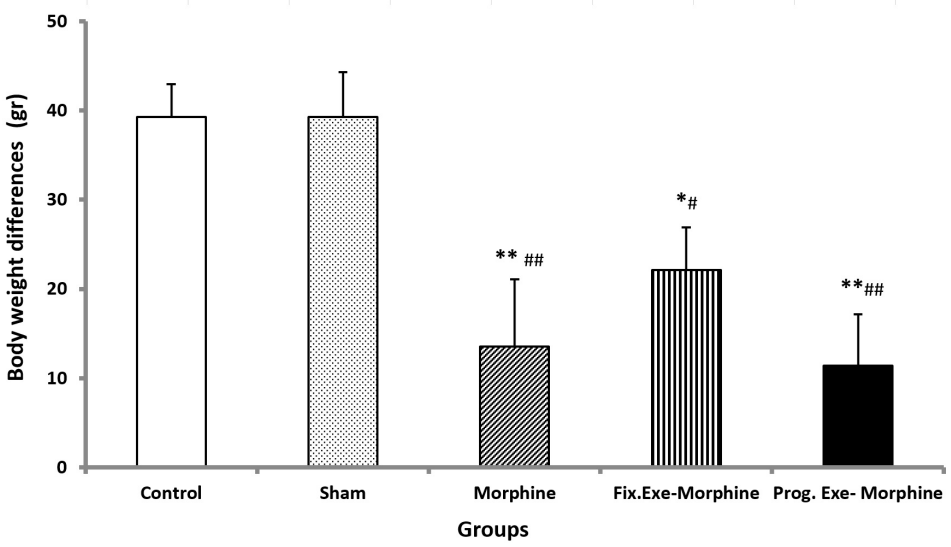

Figure 8. Body weight differences for all the groups (n = 7). Results are expressed as means ± standard error of mean. *P<0.05 and **P<0.01 compared to the control group, #P<0.05 and ##P<0.01 compared to the sham group.

adequate for impairing brain functions (Babor et al. 1976). This is while some studies reported that a ten-day morphine administration had impaired certain cognitive processes in their subjects (Gu et al. 2008, Rashidy-Pour et al. 2015). It is, therefore, possible that different brain regions might be involved in behavioral patterns and that the ten-day morphine administration is not adequate for changing these patterns.

Morphine administration in the current study was found to lead to a variety of activity patterns as revealed by different EEG (alpha, beta, theta, and delta) waves. For example, the alpha and beta waves were found to increase while the theta and delta waves to decline due to morphine administration. Enhanced alpha and beta waves could, therefore, indicate anti-stress effects and a relaxed mood (Murao et al. 2013), whereas declining theta and delta waves could be taken as reduced consciousness and loss of bodily awareness in addicted rats (Songsamoe et al. 2019). This is further supported by the results of previous studies that demonstrated different effects of morphine administration on EEG waves. For instance, Matejcek et al. (1988) reported reduced frequencies of slow EEG waves but increased frequencies of the beta wave as a result of morphine addiction. In contrast, Li et al. (2016) showed that acute morphine administration increased EEG potency in all the delta, theta, alpha, and beta bands. A moderate dose of morphine was reported to produce an unbalanced decrease in power across all the EEG frequency bands except for the beta band (Zanettini et al. 2018). In contrast, morphine administration was observed to give rise to an overall increase in all the band frequencies, but most notably in the alpha one (Ferger & Kuschinsky 1995). It, therefore, seems that changes in brain's electrical activity (EEG) should depend particularly on its dosage and duration (Radahmadi et al. 2017, Iwatsubo & Clouet 1977). The results of the present study, however, indicate that morphine administration seems to have stronger effects on changes in the EEG waves of the brain as a cellular mechanism rather than on changes in behavioral cognitive processes in which different brain regions are involved. Furthermore, while the mechanisms underlying cognitive processing and EEG generation are not fully understood, interactions among various brain regions and cortical networks are assumed to play the key roles in various rhythmical EEG activities under different conditions.

The progressive exercise in this study was found to be the only one to reverse the significantly adverse effects of morphine on memory, memory consolidation, and locomotor activity in addicted subjects. This is in contrast with the results obtained under the fixed exercise protocol that showed no beneficial effects on the parameters investigated in the passive avoidance test. In line with the current findings, one study reported that acute and chronic exercise would have positive effects on improving cognitive functions albeit the mechanisms involved are unknown (Gutmann et al. 2015, Alaei et al. 2006). Zarrinkalam et al. (2016) also showed that morphine-induced cognitive deficits were blocked by exercise. Also, Miladi-Gorji et al. (2011) demonstrated that a 10-day voluntary exercise was potentially effective in ameliorating some of the cognitive deficits revealed by the water maze task in rodents.

None of the brain waves in addicted subjects were found affected by the progressive exercise. This is while the fixed exercise enhanced only the alpha waves in the EEG recordings of addicted subjects but their beta, theta, and delta waves showed no changes relative to those recorded for the morphine group. Murao et al. (2013) also reported that alpha waves represented semantic information processing and good cognitive performance. They claimed that fixed exercise improved semantic information processing in addicted subjects.

It seems that the different preventive exercise protocols used in the present study had different effects on brain functions, as revealed by either behavioral tests or brain EEG recordings. This is confirmed by previous studies reporting different effects of exercise on the waves of the electroencephalogram. For example, one study indicated that exercise produced changes in the alpha wave (Gutmann et al. 2015) while another reported enhanced theta waves but declining delta waves in rats (Li et al. 2008). Bailey et al. (2008) demonstrated enhancements in the theta, alpha, and beta frequencies of EEG recordings during exercise although the changes were not specific to any special region of the brain (Bailey et al. 2008). However, the differences observed between EEG recordings and behavioral test results might be attributed to differences in the methods employed in EEG analysis (Radahmadi et al. 2017), exercise timings and durations (Bigliassi et al. 2017), exercise types, types of behavioral task, exercise protocols (Ranjbar et al. 2017), and the effects of morphine administration on brain responsiveness.

Sine both morphine administration and exercise were observed in the present study to affect body weight, differences in body weight were determined in quest of more accurate results. All the three experimental groups (namely, the fixed exercise group and the morphine and progressive exercise-morphine ones, in particular) in the present study exhibited loss of body weight. Some studies reported loss of body weight following exercise sessions in addicted subjects (Levin & Dunn-Meynell 2006, Droste et al. 2007, Mucha & Kalant 1979). Exercise started at a low speed seemed to lead to a greater body weight loss in addicted subjects than running started at a high speed, a finding that is confirmed by those reported in Donnelly et al. (2000).

CONCLUSIONS

In conclusion, the effects of morphine administration were found to be more strongly reflected in changes in brain electrical activity (EEG waves) as a cellular mechanism than in the test results of behavioral cognitive processes that involve different brain regions. Furthermore, compared to the fixed exercise

protocol, the progressive forced exercise was found to be far better at reversing the adverse effects of morphine addiction on memory, memory consolidation, and locomotor activity. The fixed exercise protocol used in this study was found capable of increasing only the alpha wave power involved in enhancing semantic information processing in addicted subjects. Finally, compared to running started at a high speed, exercise started at a low speed was found to lead to more losses of body weight in addicted subjects. Further studies are required to gain a better understanding of the possible mechanisms and etiologies explaining such changes.

Acknowledgments

The conduction of the present research was made possible through the supports received from Isfahan University of Medical Sciences, Isfahan, Iran.

REFERENCES

AIYER R, NOVAKOVIC V & BARKIN RL. 2016. A systematic review on the impact of psychotropic drugs on electroencephalogram waveforms in psychiatry. Postgrad Med 128: 656-664.

ALAEI H, BORJEIAN L, AZIZI M, ORIAN S, POURSHANAZARI A & HANNINEN O. 2006. Treadmill running reverses retention deficit induced by morphine. Eur J Pharmacol 536: 138-141.

ANDRZEJAK RG, LEHNERTZ K, MORMANN F, RIEKE C, DAVID P & ELGER CE. 2001. Indications of nonlinear deterministic and finite-dimensional structures in time series of brain electrical activity: dependence on recording region and brain state. Phys Rev E Stat Nonlin Soft Matter Phys 64: 061907.

ARIKANOĞLU A. 2011. Current approach to differential diagnosis of epileptic seizures and pseudo-seizures. J Clin Exp Invest 2: 330-334.

AZIZI MH, ALAEI H & ORYAN S. 2005. The effects of exercise (treadmill running) on passive-avoidance learning and memory in morphine dependent male rats. Iran J Basic Med Sci 4(28): 252-262.

BABOR TF, MEYER RE, MIRIN SM, MCNAMEE HB & DAVIES M. 1976. Behavioral and social effects of heroin self-administration and withdrawal. Arch Gen Psychiatry 33: 363-367.

BAILEY SP, HALL EE, FOLGER SE & MILLER PC. 2008. Changes in EEG during graded exercise on a recumbent cycle ergometer. J Sports Sci Med 7: 505.

BASAR E & GUNTEKIN B. 2012. A short review of alpha activity in cognitive processes and in cognitive impairment. Int J Psychophysiol 86: 25-38.

BEKHEET S, SAKER S, ABDEL-KADER A & YOUNIS A. 2010. Histopathological and biochemical changes of morphine sulphate administration on the cerebellum of albino rats. Tissue Cell 42: 165-175.

BIGLIASSI M, KARAGEORGHIS CI, WRIGHT MJ, ORGS G & NOWICKY AV. 2017. Effects of auditory stimuli on electrical activity in the brain during cycle ergometry. Physiol Behav 177: 135-147.

BLACKHART GC, MINNIX JA & KLINE JP. 2006. Can EEG asymmetry patterns predict future development of anxiety and depression? A preliminary study. Biol Psychol 72: 46-50.

CASTILHO V, BORELLI K, BRANDAO M & NOBRE M. 2008. Anxiety-like symptoms induced by morphine withdrawal may be due to the sensitization of the dorsal periaqueductal grey. Physiol Behav 94: 552-562.

CHIANG Y-C, YE L-C, HSU K-Y, LIAO C-W, HUNG T-W, LO W-J, HO K & TAO P-L. 2015. Beneficial effects of co-treatment with dextromethorphan on prenatally methadone-exposed offspring. J Biomed Sci 22: 19.

DASTGERDI HH, RADAHMADI M, REISI P & DASTGERDI AH. 2018. Effect of Crocin, Exercise, and Crocin-accompanied Exercise on Learning and Memory in Rats under Chronic Unpredictable Stress. Adv Biomed Res 7: 137-147.

DIVSALAR K. 2012. Destructive Effects of Prenatal WIN 55212-2 Exposure on Central Nervous System of Neonatal Rats. Addict Health 4: 9-19.

DONNELLY J, JACOBSEN D, HEELAN KS, SEIP R & SMITH S. 2000. The effects of 18 months of intermittent vs continuous exercise on aerobic capacity, body weight and composition, and metabolic fitness in previously sedentary, moderately obese females. Int J Obes 24: 566-572.

DROSTE SK, CHANDRAMOHAN Y, HILL LE, LINTHORST AC & REUL JM. 2007. Voluntary exercise impacts on the rat hypothalamic-pituitary-adrenocortical axis mainly at the adrenal level. Neuroendocrinology 86: 26-37.

FERGER B & KUSCHINSKY K. 1995. Effects of morphine on EEG in rats and their possible relations to hypo-and hyperkinesia. Psychopharmacology (Berl) 117: 200-207.

FISCHER IW, HANSEN TM, LELIC D, BROKJAER A, FRØKJÆR J, CHRISTRUP LL & OLESEN AE. 2017. Objective methods for the assessment of the spinal and supraspinal effects of opioids. Scand J Pain 14: 15-24.

GU C ET AL. 2008. Chronic morphine selectively impairs cued fear extinction in rats: implications for anxiety disorders associated with opiate use. Neuropsychopharmacology 33: 666-673.

GUTMANN B, MIERAU A, HÜLSDÜNKER T, HILDEBRAND C, PRZYKLENK A, HOLLMANN W & STRÜDER HK. 2015. Effects of physical exercise on individual resting state EEG alpha peak frequency. Neural Plast 2015: 717312.

HANSLMAYR S, VOLBERG G, WIMBER M, RAABE M, GREENLEE MW & BAUML KH. 2011. The relationship between brain oscillations and BOLD signal during memory formation: a combined EEG-fMRI study. J Neurosci 31: 15674-15680.

IWATSUBO K & CLOUET DH. 1977. Effects of morphine and haloperidol on the electrical activity of rat nigrostriatal neurons. J Pharmacol Exp Ther 202: 429-436.

JURKOWLANIEC E, TOKARSKI J & TROJNIAR W. 2003. Effect of unilateral ibotenate lesions of the ventral tegmental area on cortical and hippocampal EEG in freely behaving rats. Acta Neurobiol Exp (Wars) 63: 369-376.

KAFA IM, BAKIRCI S, UYSAL M & KURT MA. 2010. Alterations in the brain electrical activity in a rat model of sepsis-associated encephalopathy. Brain Res 1354: 217-226.

KNYAZEV GG, SAVOSTYANOV AN & LEVIN EA. 2006. Alpha synchronization and anxiety: implications for inhibition vs. alertness hypotheses. Int J Psychophysiol 59: 151-158.

LEVIN B & DUNN-MEYNELL A. 2006. Differential effects of exercise on body weight gain and adiposity in obesity-prone and-resistant rats. Int J Obes (Lond) 30: 722-727.

LI J, PAN Q, ZHU Z, LI M & YE Z. 2016. EEG characteristics of medial prefrontal cortex in rats with morphine dependent place preference under shuttling condition. Zhongguo Ying Yong Sheng Li Xue Za Zhi 32: 92-96.

LI J-Y, KUO TB, HSIEH SS & YANG CC. 2008. Changes in electroencephalogram and heart rate during treadmill exercise in the rat. Neurosci Lett 434: 175-178.

MARGHMALEKI VS, ALAEI HA, MALEKABADI HA & PILEHVARIAN A. 2013. Effect of Physical Activity on Symptoms of Morphine Addiction in Rats, after and before of Lesion of the mPFC Area. Iran J Basic Med Sci 16: 1091.

MATEJCEK M, POKORNY R, FERBER G & KLEE H. 1988. Effect of morphine on the electroencephalogram and other physiological and behavioral parameters. Neuropsychobiology 19: 202-211.

MILADI-GORJI H, RASHIDY-POUR A, FATHOLLAHI Y, AKHAVAN MM, SEMNANIAN S & SAFARI M. 2011. Voluntary exercise ameliorates cognitive deficits in morphine dependent rats: the role of hippocampal brain-derived neurotrophic factor. Neurobiol Learn Mem 96: 479-491.

MUCHA R & KALANT H. 1979. Increased weight gain as a morphine withdrawal response in rats. Pharmacol Biochem Behav 11: 197-201.

MURAO S, YOTO A & YOKOGOSHI H. 2013. Effect of smelling green tea on mental status and EEG activity. International Journal of Affective Engineering 12: 37-43.

NEEPER SA, GÓAUCTEMEZ-PINILLA F, CHOI J & COTMAN C. 1995. Exercise and brain neurotrophins. Nature 373: 109-109.

PENG H, HU B, ZHENG F, FAN D, ZHAO W, CHEN X, YANG Y & CAI Q. 2013. A method of identifying chronic stress by EEG. Pers Ubiquitous Comput 17: 1341-1347.

RADAHMADI M, ALAEI H, SHARIFI MR & HOSSEINI N. 2015. Preventive and therapeutic effect of treadmill running on chronic stress-induced memory deficit in rats. J Bodyw Mov Ther 19: 238-245.

RADAHMADI M, HOSSEINI DASTGERDI A, FALLAH N & ALAEI H. 2017. The effects of acute, sub-chronic and chronic psychical stress on the brain electrical activity in male rats. Phypha 21: 185-192.

RADAHMADI M, HOSSEINI N & ALAEI H. 2016a. Effect of exercise, exercise withdrawal, and continued regular exercise on excitability and long-term potentiation in the dentate gyrus of hippocampus. Brain Res 1653: 8-13.

RADAHMADI M, SHARIFI MR, AMINI M & FESHARAKI M. 2016b. Effect of the co-administration of glucose with morphine on glucoregulatory hormones and causing of diabetes mellitus in rats. Adv Biomed Res 5: 21-28.

RADAK Z, KUMAGAI S, TAYLOR AW, NAITO H & GOTO S. 2007. Effects of exercise on brain function: role of free radicals. Appl Physiol Nutr Metab 32: 942-946.

RANJBAR H, RADAHMADI M, REISI P & ALAEI H. 2017. Effects of electrical lesion of basolateral amygdala nucleus on rat anxiety-like behaviour under acute, sub-chronic, and chronic stresses. Clin Exp Pharmacol Physiol 44: 470-479.

RASHIDY-POUR A, FATHOLLAHI Y, MILADI-GORJI H & SAFARI M. 2015. Enhancing hippocampal neuronal numbers in morphine-dependent rats by voluntary exercise through a brain-derived neurotrophic factor-mediated

mechanism. Middle East J Rehabil Health Stud 2(1): e25589.

SAADIPOUR K, SARKAKI A, ALAEI H, BADAVI M & RAHIM F. 2009. Forced exercise improves passive avoidance memory in morphine-exposed rats. Pak J Biol Sci 12: 1206-1211.

SAEDI MARGHMALEKI V & ALAEI H. 2016. Effect of Treadmill Running on Morphine Dependence Before and After Medial Prefrontal Cortex Lesion in Rats. Asian J Sports Med 7: e35181.

SONGSAMOE S, SAENGWONG-NGAM R, KOOMHIN P & MATAN N. 2019. Understanding consumer physiological and emotional responses to food products using Electroencephalography (EEG). Trends Food Sci Technol 93: 167-173.

STEIN AM, MUNIVE V, FERNANDEZ AM, NUNEZ A & ALEMAN IT. 2017. Acute exercise does not modify brain activity and memory performance in APP/PS1 mice. PLoS ONE 12: e0178247.

TANG X, YANG L & SANFORD LD. 2007. Interactions between brief restraint, novelty and footshock stress on subsequent sleep and EEG power in rats. Brain Res 1142: 110-118.

UCHIDA S, SHIODA K, MORITA Y, KUBOTA C, GANEKO M & TAKEDA N. 2012. Exercise effects on sleep physiology. Front Neurol 3: 48.

UHLHAAS PJ & SINGER W. 2006. Neural synchrony in brain disorders: relevance for cognitive dysfunctions and pathophysiology. Neuron 52: 155-168.

VOHORA D, PAL S & PILLAI K. 2000. Effect of locomotor activity on the passive avoidance test for the evaluation of cognitive function. Indian J Pharmacol 32: 242-245.

VOROBYOV VV, SCHIBAEV NV, MORELLI M & CARTA AR. 2003. EEG modifications in the cortex and striatum after dopaminergic priming in the 6-hydroxydopamine rat model of Parkinson's disease. Brain Res 972: 177-185.

YOUSIF S, SAUBAMÉA B, CISTERNINO S, MARIE-CLAIRE C, DAUCHY S, SCHERRMANN JM & DECLÈVES X. 2008. Effect of chronic exposure to morphine on the rat blood–brain barrier: focus on the P-glycoprotein. J Neurochem 107: 647-657.

ZANETTINI C, SCAGLIONE A, KEIGHRON JD, GIANCOLA JB, LIN S-C, NEWMAN AH & TANDA G. 2018. Pharmacological classification of centrally acting drugs using EEG in freely moving rats: an old tool to identify new atypical dopamine uptake inhibitors. Neuropharmacology: 107446.

ZARRINKALAM E, HEIDARIANPOUR A, SALEHI I, RANJBAR K & KOMAKI A. 2016. Effects of endurance, resistance, and concurrent exercise on learning and memory after morphine withdrawal in rats. Life Sci 157: 19-24.

How to cite

ASHTARI N, RADAHMADI M & ALAEI H. 2022. Preventive effects of fixed and progressive forced exercises on memory and brain electrical activity in morphine-addicted rats. An Acad Bras Cienc 94: e20200596. DOI 10.1590/0001-3765202220200596.

Manuscript received on April 21, 2020;
accepted for publication on October 28, 2020

NILOOFAR ASHTARI
https://orcid.org/0000-0001-5700-7834

MARYAM RADAHMADI
https://orcid.org/0000-0002-1383-9572

HOJJATALLAH ALAEI
https://orcid.org/0000-0002-6346-5951

Department of Physiology, School of Medicine,
Isfahan University of Medical Sciences,
8174673461, Hezar Jerib Street, Isfahan, Iran

Correspondence to: **Maryam Radahmadi**
E-mail: m_radahmadi@med.mui.ac.ir

Author contributions

M. Radahmadi designed the experiments. N. Ashtari performed experiments. M. Radahmadi analyzed all data of experiments. N. Ashtari, M. Radahmadi and H. Alaei wrote the manuscript. All authors approved the final version of the manuscript.