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Forced Exercise Improves Passive Avoidance Memory in Morphine-Exposed Rats

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Abstract: The aim of this study was to investigate the effect of short-term forced exercise protocol on passive avoidance retention in morphine-exposed rats. Effects of morphine on acquisition and retrieval of retention have been proven in the avoidance paradigms. Twenty four male Wistar rats weighing 250-300 g were used in this study. Animals were randomly divided into four groups including: (1) non-morphine-exposed without exercise (nA.nE) (2) non-morphine-exposed with exercise (nA.E) (3) morphine-exposed without exercise (A.nE) and (4) morphine-exposed with exercise (A.E). Rats ran as forced exercise on the motorized treadmill 1 h daily for ten days. Morphine-exposed animals received intraperitoneal morphine during first 5 days of the exercise period and their dependence to morphine was confirmed by naloxane admistration (10 mg kg^{-1}, i.p.) and withdrawal test. After 10 days of forced exercise, step down latency was tested and Inflexion Ratio (IR) was evaluated in each rat. Baseline step down latencies before any morphine exposing or exercise have shown no significant alteration in all groups. Inflexion Ratio (IR) of nA.E group has increased significantly ($p<0.001$) at 1, 3, 7 and 14 days after receiving shock (learning) compared to nA.nE and A.E groups. Our data showed that short-term forced exercise on treadmill improved retention in both morphine-exposed and non morphine-exposed rats at least up to 7 days and more than 14 days, respectively. Alteration in retention between exercised groups may attribute the release of adrenal stress hormones such as epinephrine and corticosterone because of the emotional arousal.

Key words: Morphine, forced exercise, passive avoidance retention, rat

INTRODUCTION

Both voluntary and forced exercise protocols have been used to explore the effect of exercise on brain function (Albeck *et al*., 2006). The positive effects of exercise on many physiological systems, including the central nervous system, are well-established. Exercise-included improvements in learning and retention have been directly associated with improved neurogenesis, an increase in activity-dependent synaptic plasticity and altered gene expression (Cotman and Berchtold, 2002; Farmer *et al*., 2004; Van Praag *et al*., 1999a; Shors *et al*., 2001). Many of these improvements have been observed in the hippocampus, a highly plastic structure located in the medial temporal region of the brain that is vital to activity-dependent learning and retention (Lynch, 2004). Opioids play an important role in learning and memory (Izquierdo *et al*., 1980). Accumulating evidence has demonstrated that morphine, an opioid receptor agonist, impaired retention memory in passive avoidance tasks (Zarrindast and Rezayof, 2004; Nishimura *et al*., 1990; Galeotti *et al*., 2001). Several lines of studies showed that pre-training administration of morphine caused memory deficit (Ragozzino and Gold, 1994; Aguilar *et al*., 1998; Nishimura *et al*., 1990; Tayebi *et al*., 2005). Previous studies reported that post-training morphine induced impairment on the retrieval process in a passive avoidance paradigm (Malekmohamadi *et al*., 2006). However, there were reports demonstrating that pre-testing injection of morphine reversed the retention impairment that was induced by pre-training scopolamine and naloxone in rodents (Tayebi *et al*., 2005; Nishimura *et al*., 1990; Shiigi *et al*., 1990). Some brain regions are now also considered as important sites for the molecular and cellular plasticity underlying the addiction and retention. Complex circuits involving the

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hippocampus, cerebral cortex, ventral and dorsal striatum and amygdala implicated in both addiction and retention (Nestler, 2002; Kauer, 2001). There is evidence to suggest that neurogenesis, an increase in which has been linked to exercise, may be necessary only in specific types of hippocampal-dependent learning (O'Callaghan *et al*., 2007). Over recent years, most of its effects have extensively studied; but its effects on the Central Nervous System (CNS) and especially learning and retention in morphine-exposed subjects, have remained largely unexplored. Animal studies on rats and mice reported better cognitive performance because of increased physical activities (Anderson *et al*., 2002). In support of this hypothesis, it appears that exercise could enhance neurogenesis (Van Praag *et al*., 1999b). Additionally, chronic opiate treatments including heroin and morphine can reduce rodents' hippocampal Long-Term Potentiation (LTP) which is proposed to underlie learning and memory (Spain and Newsom, 1991; Pu *et al*., 2002). Davis *et al*. (2002) suggested that as in the days of withdrawal, the neuropsychological impairment might extinguish. To our knowledge, very little works have done on the effect of short-term forced exercise on retention in morphine-exposed subjects. So, the aim of the present study was to determine the effect of 10 days forced exercise on the passive avoidance retention in morphine-exposed rats.

MATERIALS AND METHODS

Subjects: Twenty-four male Wistar rats (250-300 g; 4-5 months old obtained from Jondishapour University animal breeding center) were used in this study from November 20, 2007 to December 13, 2008, Neurobehavioral Lab., Ahwaz Physiology Research Center. Animals housed in standard cages with food and water *ad libitum* and experienced a 12; 12 h light-dark cycle in a temperature-controlled environment (20-22°C). Experiments approved by the Jondishapour Academy of Sciences Ethics Local Committee Ahwaz-Iran. All rats were gentle handled for 5 days (5 min daily), the animal care and experimental protocols done prior to the onset of behavioral tests. Rats were divided randomly into four groups: (1) non-morphine-exposed without exercise (nA.nE), which received intraperitoneal (i.p.) 1 mL normal saline (0.9% NaCl) twice daily during the first 5 days of 10 days exercise. 2) Non-morphine-exposed with exercise (nA.E), which received normal saline and forced exercise on treadmill. 3) Morphine-exposed without exercise (A.nE), which received intraperitoneal injection of morphine during first 5 days of 10 days exercise (5, 10, 20, 40, 50 mg kg^{-1}, two times daily, 8 am and 5 pm) and 4) Morphine-exposed with exercise (A.E) which received same doses of morphine and forced exercise on treadmill. Animals received 10 mg kg^{-1}, sc naloxone at the 5th day (45 min after last injection of morphine for withdrawal syndrome test.

Speed and duration of exercise in both nA.E and A.E groups were kept constant at 17-18 m/min (belt speed, 1 km h^{-1}), 60 min daily for 10 days, respectively. Inclination was varied during 60 min forced exercise. In brief, slope was 0°at first 10 min, 5° at second 10 min, during secondary and tertiary 20 min periods it was settled on 10 and 15°, respectively. The nA.nE and A.nE groups always placed in neighboring lanes without switching on the treadmill motor for the exact duration but the runners not forced to run. Electrical part of treadmill was delivered light electric shocks (about 0.3 mA D.C.) when the rats entered the rear of the test chamber. The runners could avoid of shock by running on the treadmill belt and non-runners could avoid by going away from electric shock wires (Alaei *et al*., 2006).

Passive avoidance learning and retention: Animals were familiar to the instrument 24 h before training. Next day rats placed on the elevated platform situated in the centre of the floor of the passive avoidance test box and the latency to stepping down recorded. Third day of the experiment immediately after stepping down, animals received mild electric shock (0.5 mA, 3 sec duration, D.C.) through the grid floor and then returned to their home cages. On the following day (as 24 h retention interval) rats placed on the platform again while no electric shock was gave to them. Latency to stepping down recorded. If the rat remained on the platform for the 5 min, it assigned a maximum score of 300 sec. Latency to stepping down assessed again up to 14 days later in order to evaluate the short, mid and long-term retention of passive avoidance memory. The results have been expressed as retention scores after 1, 3, 7 and 14 days of receiving shock for each rat, by calculating the Inflexion Ratio (IR) using following formula:

$$\text{Inflexion Ratio (IR)} = (L_1-L_0)/L_0$$

where, L_0 is initial stepping down latency (sec) of learning test and L_1 is stepping down latency (sec) of 1, 3, 7 and 14 days after learning as retention (Jaiswal and Bhattacharya, 1992). The experimental protocol from 1st to 27th days of the research has shown in Table 1.

Drugs: Morphine hydrochloride and naloxone (Temad's Pharmaceutical Company, Iran) were diluted in sterile normal saline 0.9%.

Table 1: Shows the experimental protocols

Days	Experimental protocols
1st	Made the animals familiar to experimental instruments for exercise on treadmill
2nd	Starting Exercise one hour daily and exposes To morphine
2nd-6th	Continually exposing to morphine and forced exercise on 6th day withdrawal test was taken (afternoon 45 min after admission of final injection of morphine)
6th-10th	Continually giving forced exercise without exposing to morphine and same afternoon make the animals familiar to step-down apparatus
11th	Evaluated latency of stepping down without shock in all animals
12th	Starting giving shock (conditioning)
13th	Test of retention (1st day after shock delivery)
16th	Test of retention (3rd day after shock delivery)
20th	Test of retention (7th day after shock delivery)
27th	Test of retention (14th day after shock delivery)

Statistical analysis: Data expressed as Mean±SEM and analyzed by using the SPSS (version 15). Differences between groups were considered significant at $p<0.05$. Baseline latencies (before given shock or learning) and inflexion ratio in 1st, 3rd, 7th and 14th days after learning were analyzed by two-ways ANOVA. Post hoc comparisons made using the Tukey's test.

RESULTS AND DISCUSSION

Early our data have shown that jumping number was decreased significantly in A.E vs A.nE. group. Teeth's chattering was decreased significantly in A.E when compared to A.nE group (Saadipour *et al*., 2008). Baseline stepping down latencies (Before any receiving shock, or morphine exposure and exercise in all groups) showed no significant alteration between all groups (Fig. 1).

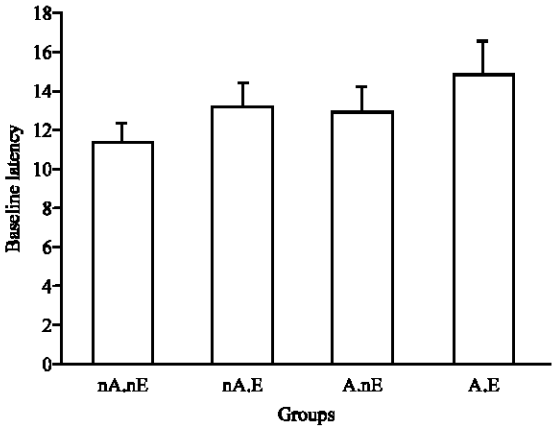

Fig. 1: Mean±SEM of baseline latency (before shock delivery, morphine exposure and exercise) in all groups (ANOVA, Tukey's test, n =six in each group)

Inflexion Ratio (IR) of both nA.E and A.E groups at 1 and 3 days after shock delivery (learning) was significantly increased ($p<0.05$, $p<0.001$) compared to nA.nA and A.nE groups respectively (Fig. 2a, b). But IR was raised with less rate of enhancement in A.E group compared to nA.E (Fig. 2a, b).

IR in 7 and 14 days after shock delivery of nA.E group was increased significantly ($p<0.001$) compared to nA.nE group (Fig. 2c, d). Surprisingly, IR in 7 day after shock delivery was increased no significantly ($p>0.05$) in A.E compared to A.nA group, while IR showed no change and increase at 14 days (long-term retention) between A.E and A.nE groups (Fig. 2c, d). In contrast with nA.E group these data showed that effect of short-long forced exercise on retention did not persisted for long time in morphine dependent rats.

Our results have shown a positive effect of 10 days forced exercise protocol on passive avoidance retention in both non morphine-exposed and morphine-exposed rats, as assessed by stepping-down task. Physical exercise influences all organs and systems. Over recent years most of morphine effects have been extensively studied but, its effects on the Central Nervous System (CNS) and especially learning and retention, have remained largely unexplored. Human and animal studies have suggested that exercise has benefits overall health and cognitive function (Van Praag *et al*., 1999b). In this study, we report that the forced running paradigm is effective in enhancing passive avoidance learning and retention in rats. At the end of 10 days of treadmill running, the runners (morphine-exposed and non morphine-exposed groups) would have covered an approximate total distance of 10.8 km, averaging about 1.08 km day^{-1}. Presented results indicate that forced treadmill running improved passive avoidance retention at 14th days in non morphine-exposed rats. Also, it was potentiated learning and retention for short time (3rd days) in morphine-exposed rats. This study has been suggested that expose to morphine impaired passive avoidance retention in rats. Others have indicated that injection of morphine into medial septum of animals impairs the retention both for avoidance training and during spontaneous alternation (Talley *et al*., 2002; Papageorgiou *et al*., 2001). Most studies involving exercise based on voluntary running paradigms (Fordyce *et al*., 1991; Fordyce and Wehner, 1993;

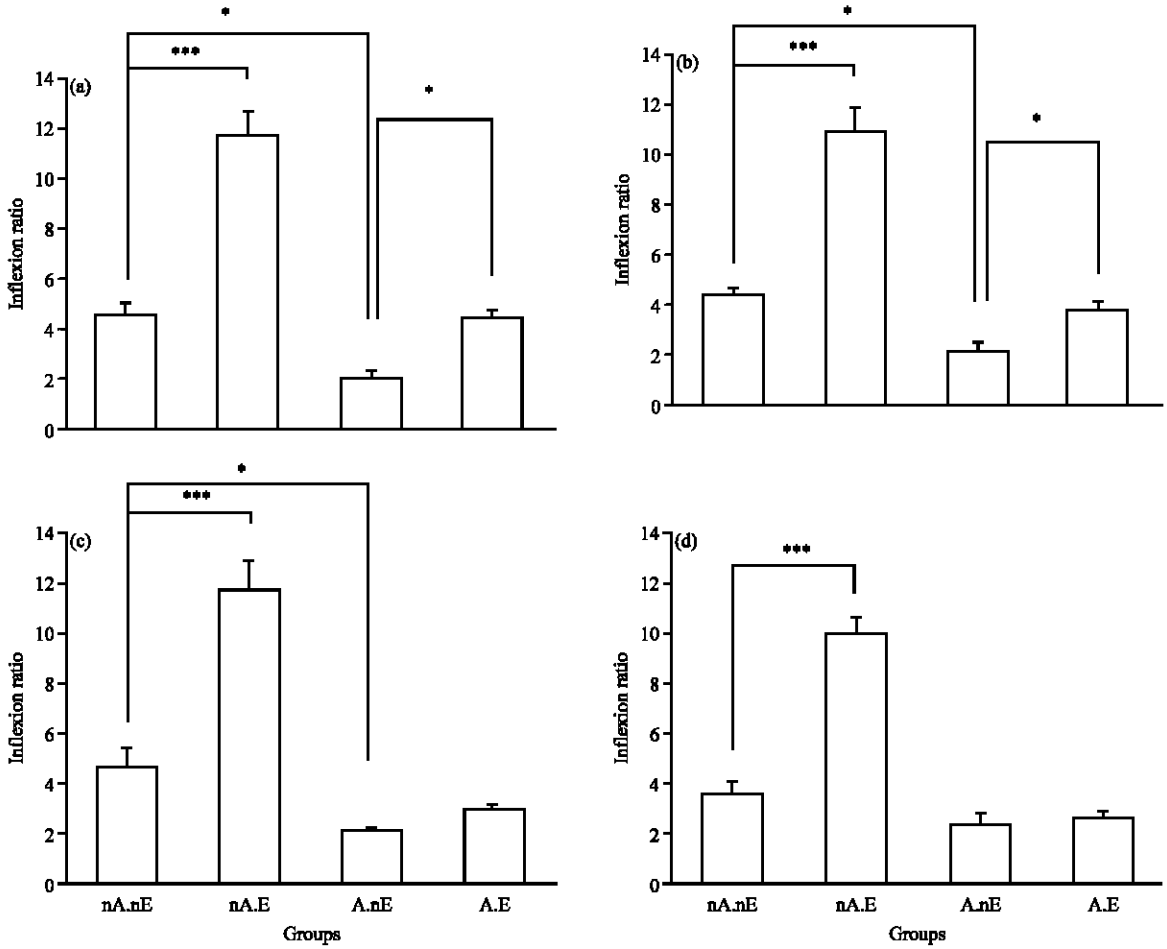

Fig. 2: (a), Mean±SEM inflexion ratio at 24 hour after shock delivery in all groups (n=6, ***p<0.001, *p<0.05). (b), Inflexion ratio (Mean±SEM) 3 days after learning in all groups (n=6). (***p<0.001 nA E vs. nA.nE), (*p<0.05 A.nE vs. nA.nE), (*p<0.05 A.E vs A nE). (c), Mean±SEM inflexion ratio at 7 days after learning in all groups (n=6). (***p<0.001 nA E vs. nA.nE), (*p<0.05 nE.A vs. nA.nE). (d), inflexion ratio at14 days after learning in all groups (ANOVA, Tukey's test, n=six, ***p<0.001, ***p<0.001)

Levine *et al*., 1995). Also it has reported that increased levels of exogenous opiates (such as morphine and heroin), adrenal steroids and stress during exercise are known to be implicated in cell proliferation and differentiation into neurons (Eisch *et al*., 2000) and the opiate peptides inhibit neuroblast replication and differentiation in the developing cerebellum *in vivo* (Hauser and Mangoura, 1998). Studies in rodents have shown that chronic opiate treatments including morphine and heroin reduce hippocampal LTP which may play a crucial role in learning and memory (Spain and Newsom, 1991; Pu *et al*., 2002). Moreover, repeated administration of morphine impaired spatial and working memory in Morris water maze and radial maze tests during early withdrawal (Li *et al*., 2001; Sala *et al*., 1994). It previously reported that the forced running paradigm is associated with a certain level of stress (Arida *et al*., 2004). On the other hand, such acute stress is likely to affect the learning process since it previously reported that emotionally arousing experiences such as stress often facilitate the formation of retention more readily (McEwen, 2000). Mechanistically, this could be attributed to the release of adrenal stress hormones, epinephrine and cortisol (corticosterone in the rat), because of the emotional arousal stimulus (McEwen, 2000; McGaugh, 2000). It speculated that treadmill forced exercise influences on the passive avoidance retention in morphine-exposed and non morphine-exposed rats. Also, many studies showed that one of the most important mechanisms of memory enhancement in the exercise groups is neurogenesis and neuroplastic changes in the brain especially in hippocampus that may affected by exercise (McGaugh, 2000).

CONCLUSION

The present study provides evidence of a forced exercise-related improvement of passive avoidance retention in rats. Retention deficit caused by morphine also improved by forced running. We suggest that this could attribute to the release of adrenal stress hormones as consequences of the emotional arousal stimulus. In addition, exercise could improve learning and retention in animals by neurogenesis and neuroplastic changes in brain especially in hippocampus. By the present study, it confirmed that morphine impaired the acquisition and retrieval of passive avoidance recognition depending on dose and state.

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