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EFFECTS OF VOLUNTARY EXERCISE ON HIPPOCAMPAL LONG-TERM POTENTIATION IN MORPHINE-DEPENDENT RATS

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Abstract—This study was designed to examine the effect of voluntary exercise on hippocampal long-term potentiation (LTP) in morphine-dependent rats. The rats were randomly distributed into the saline–sedentary (Sal/Sed), the dependent–sedentary, the saline–exercise (Sal/Exc), and the dependent–exercise (D/Exc) groups. The Sal/Exc and the D/Exc groups were allowed to freely exercise in a running wheel for 10 days. The Sal/Sed and the morphine–sedentary groups were kept sedentary for the same extent of time. Morphine (10 mg/kg) was injected bi-daily (12 h interval) during 10 days of voluntary exercise. On day 11, 2 h after the morphine injection, the *in vivo* LTP in the dentate gyrus of the hippocampus was examined. The theta frequency primed bursts were delivered to the perforant path for induction of LTP. Population spike (PS) amplitude and the field excitatory post-synaptic potentials (fEPSP) slope were measured as indices of increase in synaptic efficacy. Chronic morphine increased the mean basal EPSP, and augmented PS–LTP. Exercise significantly increased the mean baseline EPSP and PS responses, and augmented PS–LTP in both saline and morphine-treated groups. Moreover, the increase of PS–LTP in the morphine–exercise group was greater (22.5%), but not statistically significant, than that of the Sal/Exc group. These results may imply an additive effect between exercise and morphine on mechanisms of synaptic plasticity. Such an interaction between exercise and chronic morphine may influence cognitive functions in opiate addicts.

Key words: voluntary exercise, morphine exposure, long-term potentiation, hippocampus.

INTRODUCTION

Hippocampal long-term potentiation (LTP) is a form of synaptic plasticity that proposed as a cellular substrate of learning and memory (Bliss and Collingridge, 1993). Previous studies, using *in vivo* and *in vitro* methods, have shown that chronic morphine can reduce LTP in rat hippocampal synapses (Pu et al., 2002; Salmanzadeh et al., 2003a; Bao et al., 2007). In contrast, other studies have shown that chronic morphine exposure augments *in vivo* LTP in Schaffer collateral–CA1 synapses (Mansouri et al., 1997, 1999), and *in vivo* LTP in the lateral perforant bath (PP)-granule cell synapses of the rat dentate gyrus (DG) (Ito et al., 2001; Lu et al., 2010b). The conflicts between these studies could be due to the fact that, different experimental protocols such as the pattern of stimulation, the time points of synaptic responses recording after the morphine injection, and the site of stimulation might be involved in the modulation of synaptic responses. For example, Pu et al. (2002) showed that chronic exposure of rats to morphine markedly reduced the capacity of hippocampal CA1 LTP during the period of drug withdrawal (9–12 h after the termination of chronic treatment), while Mansouri et al. (1997, 1999) demonstrated the augmented LTP in the Schalleral-CA1 synapses of the hippocampal slices taken from dependent, but not withdrawn rats.

Many studies have assumed that abused drugs can hijack synaptic machinery that are dedicated to plastic changes in the excitability of principal hippocampus circuits (Robbins and Everitt, 1999; Wolf, 2002; Bao et al., 2007; Kauer and Malenka, 2007), and may induce maladaptive plasticity in this structure (Eisch et al., 2000). Such maladaptive plasticity in the hippocampus and other brain structures may underline learning and memory impairment induced by chronic morphine (Miladi-Gorji et al., 2008, 2011; Lu et al., 2010a). Reversing or preventing these drug-induced synaptic modifications may prove beneficial in the treatment of relapse and other related disorders (Wolf, 2002; Lu et al., 2010a).

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Abbreviations: ANOVA, analysis of variance; AP, Anterior-Posterior; BDNF, brain-derived neurotrophic factor; D/Exc, dependent–exercise; DG, dentate gyrus; D/Sed, dependent–sedentary; DV, Dorssal-ventral; fEPSP, field excitatory post-synaptic potentials; I/O, input/output; LTP, long-term potentiation; ML, Medial-lateral; NMDA, *N*-methyl-D-aspartate; PBs, primed bursts; PP, perforant bath; PS, population spike; Sal/Exc, saline–exercise; Sal/Sed, saline–sedentary; TrkB, tyrosine kinase B.

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Recent studies have shown that physical activity can have a wide variety of beneficial effects in humans and experimental animals (Cotman and Engesser-Cesar, 2002; Kramer et al., 2006). It is increasingly clear that physical exercise maintains brain health and the synaptic plasticity (Alaei et al., 2006; Pietropaolo et al., 2008). Voluntary or forced exercises delay the onset of Alzheimer's disease in humans (Cotman and Berchtold, 2007) and promote neuro-genesis in the mice DG (van Praag et al., 1999b). Voluntary exercise can increase performance on spatial learning and memory tasks that need normal hippocampal functioning (van Praag et al., 1999a; Vaynman et al., 2004; Van der Borght et al., 2007) and it can also alter LTP in the hippocampus (Farmer et al., 2004).

We have recently demonstrated that voluntary exercise could ameliorate the spatial memory deficits in morphine-dependent rats through brain-derived neurotrophic factor (BDNF) receptors (tyrosine kinase B (TrkB)) (Miladi-Gorji et al., 2011). BDNF infusion has been shown to enhance LTP in the rat DG (Korte et al., 1998; Ying et al., 2002), while transgenic mice have been used to demonstrate that BDNF plays an important functional role in the expression of LTP in the hippocampus (Korte et al., 1995). BDNF levels are elevated during voluntary exercise which plays an important role in mediating exercise-induced enhancement of learning and memory as well as LTP (Vaynman et al., 2004).

One of the aims of the present study was to investigate whether chronic exposure to morphine could influence synaptic transmission and LTP in the DG of the hippocampus. Another aim was to examine whether voluntary exercise could alter hippocampal synaptic plasticity and LTP in morphine-dependent rats. Such studies can be helpful for understanding the neuro-physiological substrate of cognitive deficits seen in opiate addicts.

EXPERIMENTAL PROCEDURES

Animals

Adult male Wistar rats (220 ± 10 g) were individually housed in cages (50 × 26 × 25 cm) in a 12-h light/dark cycle at 22–24 °C, with food and water *ad libitum*. The experimental protocol was approved by the Ethical Review Board of Tarbiat Modares University (Iran). All the experimental procedures were conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. Additionally, care was taken to reduce number of rats in each experiment as far as possible.

Induction of morphine dependence

Morphine sulfate (Temad Company, Tehran, Iran) was dissolved in a physiological saline. On the basis of previous studies (Pu et al., 2002; Miladi-Gorji et al., 2011), morphine dependence was induced by chronic intermittent subcutaneous injections of morphine (10 mg/kg, 1 ml/kg) twice daily (07:00 and 19:00) for 10 days in the presence or absence of voluntary exercise (see below).The control rats were treated similarly, with injections of saline (1 ml/kg) replacing morphine.

Voluntary exercise paradigm

Each of the exercising rats was given all day/night access to a cage equipped with a running wheel (diameter = 34.5 cm, width = 9.5 cm) (Novidan.Tab, Iran) that was freely rotated against a resistance of 100 g. Each wheel was equipped with a magnetic switch that was connected to a separate counter, which was located outside the animal house and the number of revolutions per hour was monitored. The number of revolutions for each wheel was recorded every day at 6 a.m. The sedentary rats were confined to similar cages with no access to a wheel. The exercising groups were exposed to exercise during the development of dependence on morphine, which took 10 days before the start of the electrophysiology experiments (Akhavan et al., 2008; Miladi-Gorji et al., 2011). Exercising and sedentary rats were remained single housed throughout the entire experiment.

Experimental groups

Rats (n = 7–8 rats per group) were divided into the saline–exercise (Sal/Exc), the saline–sedentary (Sal/Sed), the dependent–exercise (D/Exc), and the dependent–sedentary (D/Sed) groups.

Electrophysiology

Surgery. The animals were anesthetized with urethane (1.5 g/kg, i.p.) 2 h after the last injection of morphine on day 11. For electrophysiological recording, a bipolar Teflon-coated stainless steel (125 μm diameter) in the medial PP (coordinates: Anterior-Posterior (AP), −8.1 mm; Medial-lateral (ML), −4.1 mm; Dorssal-ventral (DV), −3.3 mm, from skull surface) and a recording electrode (a bipolar Teflon-coated stainless steel) in the DG granule cell layer (coordinates: AP, −3.8; ML, 2; DV, 2.7–3.2 from skull surface) were implanted (Pu et al., 2002; Abrari et al., 2009).

Stimulation and recording. Fifteen minutes after electrode placement, constant current rectangular stimulus pulses (200 μs, 0.1 Hz) were delivered during a 20 min period after electrode placement (stabilization period). To achieve this, it was at times necessary to reposition the stimulating and/or recording electrodes until the highest potential could be obtained. When the variation in the population spike (PS) amplitude was less than ±10% for 20 min, the baseline recording was considered stable. An input/output (I/O) profile was established by increasing the stimulus intensity and measuring the PS amplitude.

The stimulus intensity that evoked a PS or field excitatory post-synaptic potentials (fEPSP) of 50–60% of the baseline maximum response was chosen for subsequent train stimuli. The evoked response was