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Nandrolone administration abolishes hippocampal fEPSP-PS potentiation and passive avoidance learning of adolescent male rats

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Abstract

Despite the chronic effects of Nandrolone decanoate (ND), the acute effects of ND on passive avoidance learning and memory (PAL) and its mechanism have not been investigated. This research examines the acute effect of ND on PAL, CA1 synaptic plasticity, testosterone and Corticosterone serum levels and the role of androgenic receptors (ARs). Adolescent male rats were treated with ND, 30 min before training and retention and after training test. Androgenic receptors (ARs) antagonist applied 15 min before ND. Hippocampal slices were perfused by ND. ND administration had an inverted U-shape effect on acquisition of PAL, testosterone and Corticosterone serum levels. The consolidation was only affected by high dose of ND. ND significantly decreased the retention of PAL across all doses. The magnitude of fEPSP-LTP was lower than that of control slices. In addition, an attenuation of fEPSP-PS coupling was also observed. Nilutamide could nullify the ND impairment effect. We concluded although a single dose of ND could affect all stages of PAL, its effects were more potent on retrieval, possibly arising from the acute effect of ND on the alterations of CA1 synaptic plasticity. In addition ND may induce its effects directly through ARs and indirectly through plasma testosterone and Corticosterone.

Key words: Adolescence, Hippocampus, Synaptic Plasticity, E-S-Coupling, Nandrolone, Nilutamide

1. Introduction

The abuse of anabolic androgenic steroids (AASs) for enhancing physical appearance and athletic performance has increased substantially in recent years among non-athletes, particularly adolescent males. It is estimated that 4%–12% of high school boys have misused AASs especially nandrolone (Anderson et al. 1997). In addition to the general adverse effects of AASs and particularly nandrolone decanoate (ND), as the most abused AAS, on sexual functions, a large number of experiments have reported that chronic administration of ND has an adverse effect on cognitive function including learning, memory, and anxiety (Kaufman et al. 2015; Kouvelas et al. 2008; Magnusson et al. 2009; Tanehkar et al. 2013).

However, the acute effects of ND have remained understudied especially during adolescence.

It has also been indicated that amygdala coupling with hippocampus declines by abusing AASs including nandrolone (Sarkey et al. 2008; Tabori et al. 2005; Westlye et al. 2017), suggesting that hippocampal function and fear behavior may be influenced by nandrolone. Further, previous studies have shown that intrahippocampal injection of testosterone has a dose-dependent effect on the acquisition, consolidation, and retrieval of PAL of adult male rats. It might also be the case regarding the effect of nandrolone on PAL of adolescents (Harooni et al. 2008). Another study has revealed that the detrimental effect of testosterone on spatial memory is induced through conversion to estradiol by aromatase enzyme (Moradpour F et al. 2006), but ND is metabolized less to 17- β -estradiol as compared with testosterone (Clark and Henderson 2003). In contrast, some studies have suggested that testosterone has positive effects on spatial learning and memory (Spritzer et al. 2011). It is also confirmed that the stages of PAL involve specific molecular and cellular mechanisms, though there are some common mechanisms as well (Abel and Lattal 2001). Therefore, one aim of this study is to study the effect of single dose intracerebroventricular (i.c.v.) injection of nandrolone on the PAL stages of adolescent male rats.

Long-term potentiation (LTP) is the most studied cellular mechanism regarding learning and memory (Abel and Lattal 2001). Some evidences suggest that sex steroids affect LTP through structural and functional plasticity and synaptic regeneration (Cunningham et al. 2007; Severi et al. 2005). For example, testosterone depletion following pre-pubertal gonadectomy increased LTP in the CA1 region of adult male rats, suggesting that the effect of androgens on LTP appears to be inhibitory (Harley et al. 2000; Skucas et al. 2013). On the other hand, administration of testosterone in hippocampal slices of adult male rats strengthened Schaffer collateral transmission in CA1 area (Smith et al. 2002). Another study reported that testosterone administration in gonadectomized rats elevated the number of spine synapses in the stratum radiatum of hippocampal CA1 area (Leranth et al. 2003). Nevertheless, there is not enough data on the effect of ND on CA1 synaptic plasticity. Overall, the collected data suggest that the effects of androgens on the synaptic plasticity of CA1 area are indeed mixed. Consequently, another aim of this study is to evaluate the effect of acute nandrolone perfusion on synaptic plasticity in the hippocampus CA1 area of male adolescent rats.

In addition, the characteristic feature of pathological anxiety is overstated fear, where the avoidance might contribute to initiation of irrational fears such as passive avoidance behavior which is related to maladaptive fear expression (Krimsky and Valek 2013). In this regard, understanding the biological effects of nandrolone on cognitive processes such as passive avoidance learning and its cellular mechanism may be a new/supplementary way to treating fear-related disorders such as post-traumatic stress disorder (PTSD).

2. Experimental procedures

2.1. Animals and surgery procedure

Male Wistar rats (22 days old, 22d) were purchased from the animal house of Kermanshah University of Medical Sciences (KUMS). The animals were housed in Plexiglas cage under controlled temperature (23 ± 2 °C), with daily exposure to a 12-hour light–dark cycle (lights on at 7:00 am) and free access to water and standard chow. The rats (27 ± 2 d) were placed in a stereotaxic apparatus (Stoelting, American), according to Paxinos and Watson atlas, and underwent Ketamine (100 mg/kg, i.p.) and Xylazine (25 mg/kg, i.p.) anesthesia. Guide cannulas were placed on their right lateral ventricles, and were fixed using a cold-cure acrylic material. All surgical processes were performed under a clean procedure, where the site of surgery was disinfected using Povidin Iodin 10% and treated with tropical analgesic agents (2.5% lidocaine, 2.5 % prilocaine). The treatments were initiated seven days (recovery time) after surgery. Drugs were dissolved in peanut oil as a vehicle and intracerebroventricular (i.c.v) injections of ND and Nilutamide were administered using a 5 μ l Hamilton microsyringe. ND gently microinjected thirty minutes before behavioral assessments. Nilutamide was microinjected 15 min before ND in a separate group. The injection needle was inserted 0.5 mm beyond the tip of the guide cannula. The injections (5 μ l volume/rat for ND and 2.5 μ l volume/rat for Nilutamide) were slowly performed during 3 min and the injection needles were left in place for one an additional minute. All procedures were conducted according to the Guide for the Care and Use of Laboratory Animals (Eighth Edition, 2011) and approved by the local Animal Ethics Committee of KUMS.

2.2. Behavioral study

The inhibitory avoidance apparatus (shuttle box) consisted of two light (Plexiglass) and dark compartments of the same size ($20 \times 20 \times 30$ cm³) separated by a movable guillotine door. The floor of the dark compartment was made of stainless-steel bars (0.5 cm diameter) placed 1

cm away from each other. Intermittent electric shocks (50 Hz, 3 s), with 0.7 mA intensity, were applied to the floor of the dark compartment by an isolated stimulator (Pourmotabbed and et al 2011).

On the training day, the rats were allowed to become familiar with the laboratory environment 30 min before each training session. To induce avoidance response, each animal was placed in the light compartment for 10 s, after which the door was raised, when the entrance latency into the dark compartment was recorded. Once the animal completely crossed to the next compartment, the door was closed and a 0.7 mA foot shock was delivered for 3 s. The rat was then removed from the apparatus and 2 min later, the procedure was repeated. The training was terminated when the rat remained in the light compartment for 120 s. All animals were trained with a maximum of three trials per day.

Twenty-four hours after the last training, a retention test was performed. Each animal was gently placed in the light compartment and after 10 s the door was opened. The latency time of animal to enter to the dark compartment (Step through latency, STL) was recorded and the session was continued up to 600 s. During this session, no electric shock was applied.

The aim of the behavioral studies was to evaluate the effect of intracerebroventricular (i.c.v) injection of ND on different stages of passive avoidance learning and memory. For this purpose, 96 rats were divided into three groups receiving the vehicle or a dose of ND 30 min before or immediately after acquisition trials or 30 min before retention test to evaluate the effect of nandrolone on acquisition, consolidation, or retrieval of PAL respectively. Each group was, in turn, divided into four subgroups which received ND 0, 73, 146 or 219 mM. For examine the role of androgenic receptor in retrieval of PAL, 16 rats were divided into two groups receiving the Nilutamide (6 mM) 15 min before ND administration. (n = 8 in all groups)

2.3.) Hormone assay

Twenty-four hours or 30 min after injections, 4-5 ml of blood were collected through heart puncture under ketamine-xylazine anesthesia then all rats were sacrificed by deep anesthesia. The blood samples were centrifuged at 2500 rpm for 15 min and the serum was stored at -80°C. The serum was used to measure the total serum testosterone and Corticosterone using the ELISA kit (STEADBIOPHARM, USA) according to manufacture guideline. The sensitivity, intra-assay, and inter-assay coefficient of variance for the ELISA kit of testosterone were 0.083 ng/ml, 4.16% and 8.94%, respectively. The sensitivity, intra-assay, and inter-assay coefficient of variance for the Corticosterone ELISA kit were 0.5 ng/ml, 4.6% and 6.9%, respectively. All blood samples were tested in duplicate (n = 5 in all groups).

2.4. Electrophysiological study

2.4.1. Slice preparation

We used the same protocol previously used in our laboratory with minor modifications (Moradpour et al. 2013). Briefly, male Wistar rats (40±2 days old) were anesthetized by chloroform and sacrificed through decapitation. Then, their brains were gently removed and stored in ice-cold artificial cerebrospinal fluid (aCSF) comprised of (in mM): NaCl 125, KCl 2.5, NaHPO₄ 1.25, NaHCO₃ 25, CaCl₂ 2, MgCl₂ 1.3, and glucose 10 (pH = 7.4 ± 0.5, 290–300 mOsm) and bubbled with a gas mixture of O₂ 95%: CO₂ 5% (carbogen) for 1 min. To prepare hippocampus slices, right hippocampi were gently removed, and 4 to 5 400µm thick slices of dorsal hippocampus were prepared through vibratome slicing. The slices were immediately placed in a recovery chamber containing aCSF at room temperature bubbled with carbogen with the pH controlled within 7.35- 4.45. All slices were incubated in the recovery chamber for at least 1.5 h before being transferred to a submerged recording chamber.

2.4.2. Electrophysiological recording

The slices were transferred on the recording chamber and perfused with carbogenated aCSF (warmed to $32^{\circ}\text{C} \pm 2$) at a rate of 3 to 4 ml/min. After 10 min, two aCSF-filled glass micropipettes (2–5 M Ω) were placed in the stratum radiatum. Also, the stratum pyramidale of the CA1 region of the hippocampal slices and a bipolar stimulating electrode (twisted stainless steel wire, Teflon coated) were placed in the stratum radiatum to stimulate the Schaffer collaterals. The stimuli consisted of constant current rectangular pulses (200 ms, 0.033 Hz, 10–120 μA), which were delivered by a stimulator-isolator, and the recording signals were amplified (D3111, Science beam, Iran) and displayed on a computer screen. They were then saved at a sampling rate of 10 kHz on a PC hard disk for offline analysis.

Evoked field potential recording was initiated 15 minutes after placement of electrodes. The recording continued until stable baseline recording (less than 10% variation) was obtained for 15 min. Then, eight gradual increments of stimulus intensities were applied to determine the input/output (I/O) curve. The lowest intensity that evoked a measurable response was considered as the threshold intensity (T), while the other stimulus intensities were determined as 2T, 3T....8T until the maximum stimulus intensity (120 μA) was reached. The slope of the downward deflection of field excitatory postsynaptic potential (fEPSP) was considered as an index of field synaptic response (fEPSP-I). The saturation of evoked response was obtained as over 120 μA ; therefore, it was possible to determine the stimulation intensity as the percentage of the maximum response. After development of I/O curve, the stimulus intensity which evoked 40–50% of the maximum response was determined as test pulse 1 (T1) and applied for 10 min. Then, nandrolone was perfused. Ten minutes after stable baseline recording, an additional I/O curve was created for defining the effect of nandrolone usage on the baseline responses. Then, a test pulse 2 (T2) was selected as what was described for T1 determination and applied until the end of experiment. After 10 min of stable baseline recording, high frequency stimulation (a train

of 100 stimuli at 100 Hz) was induced for inducing long-term potentiation (LTP), with the recording continuing for 60 min. Then, another I/O curve was created and the experiment was terminated. Nandrolone dissolved in pure DMSO as stock and was diluted in aCSF to achieve a final concentration of about 0.01 DMSO and final concentrations of 0 (14 slices/14 rats), 0.3 (6 slices/6 rats), 0.6 (8 slices/8 rats), or 3.6 (9 slices/9 rats) $\mu\text{M/L}$ of Nandrolone.

2.5. Statistics

Step-through latency (STL) was recorded and analyzed for behavioral assessment. The normality of the data distribution was analyzed using Shapiro–Wilk’s W-test. According to this test, the data were not normally distributed, and thus the data were transformed to Log10. They were then analyzed by one-way analysis of variance (ANOVA), followed by an LSD post hoc test to determine differences between groups. All data have been presented as mean $\pm$ standard error of the mean (SEM), with a p-value of less than 0.05 being considered as significant.

In the electrophysiological study, two responses were averaged at each stimulus intensity level. The averaged waveform was used for further analysis. The fEPSP slope (mV/ms) and the postsynaptic population spike amplitude (PSA) were measured based on the averaged waveform recorded from the stratum radiatum or the stratum pyramidale. The maximum fEPSP (fEPSP_{max}), minimum fEPSP (fEPSP_{min}), maximum PSA (PSA_{max}), minimum PSA (PSA_{min}), test pulse evoked fEPSP (fEPSP_{test pulse}), and test pulse evoked PSA (PSA_{test pulse}) were recorded and analyzed. A potentialize data analysis package (Science Beam, Iran) was used to determine the fEPSP slope or the PS amplitude, as previously described (Moradpour et al. 2016). The changes in the percentage of the PS amplitude and the fEPSP slope due to HFS were normalized in relation to the 10-min baseline recording. The averaged response obtained during the last 10-min baseline recording served as the reference, and was compared with the averaged response

acquired during the 60-min post-HFS recording period. fEPSP-PS coupling (E-S coupling) changes were quantified by the normalized PS amplitude versus Log_{10} fEPSP slope acquired during I/O experiments and analyzed using log. vs. normalized response (variable response). They were compared via extra sum of squares F-test by Prism 5 software. EC50s, the amount of fEPSP which evoked 50% of the maximum P, were compared using student t-test.

3. Results

3.1. Behavioral study

3.1.1. The effect of pre-training administration of nandrolone on the acquisition of PAL.

Fig. 1A demonstrates the result of pre-training i.c.v. microinjections of ND on the latency of entrance into the dark chamber at the retention test. One-way ANOVA indicated a significant difference in retention time among the groups ($F_{3, 31} = 4.78$, $p < 0.01$). Also, post hoc LSD test revealed that the groups receiving ND 73 mM ($p < 0.01$) and 219 mM ($p < 0.01$) spent less time to reenter the dark chamber as compared to the control group. However, ND 146 mM had no significant effect on the step-through latency ($p > 0.05$). The data suggested that there is an invert U-shape effect of nandrolone on the performance of adolescent male rats for a passive avoidance version of shuttle box.

3.1.2. The effect of post-training administration of nandrolone on the consolidation of PAL.

The results of post-training i.c.v. microinjection of ND are displayed in Fig 1B. One-way ANOVA test showed a statistically significant difference in STL score between the different doses of ND treatments ($F_{3, 30} = 4.27$, $p < 0.05$). Further analysis with post hoc LSD test revealed that ND 219 mM treated group took a significantly shorter time to re-enter to the dark chamber

($p < 0.05$) than vehicle treated rats. It suggests that the effect of ND on consolidation emerged only at the high doses.

3.1.3. The effect of pre-retrieval administration of nandrolone on the retrieval of PAL.

One of the aims of this study has been to investigate the effect of pre-retention test injection (i.c.v.) of nandrolone on the retrieval of passive avoidance learning. One-way ANOVA test indicated significant differences in STL among groups ($F_{3,31} = 3.48$, $p < 0.05$; Fig. 1C). LSD test showed that ND treatment across all doses significantly reduces STL compared to the vehicle treated group with $p = 0.006$ for ND 73 mM, $p = 0.018$ for ND 146 mM, and $p = 0.039$ for ND 219 mM. Although there is apparently a descending trend in STL with the increases of ND dosage, there were not any significant differences between ND treated groups ($p > 0.05$).

3.1.4. The effect of pre-retrieval administration of Nilutamide (Nilu.) on the retrieval of PAL.

Pre-retention test i.c.v. microinjection of Nilutamide was applied to examine the effect of androgenic receptor blocking on retrieval of PAL. Analysis of data by Unpaired t-test indicated that administration of Nilutamide at the dose of 6 mM had no significant effect on retention time compared to the control group ($t_{17} = -0.62$, $P = 0.53$; Fig. 2A).

3.1.5. Androgenic receptors mediate the impairment effect of Nandrolone on the retrieval of PAL

Nilutamide was microinjected 15 min before ND application to investigate the role of androgenic receptors in the impairment effect of ND on the retrieval of passive avoidance

learning. The results of one-way ANOVA analysis are presented in Fig 2B, indicating a statistically significant difference in STL score between the groups ($F_{2, 24} = 5.03$, $P = 0.01$). Post-hoc LSD analysis showed that ND 73 mM significantly decreases the STL ($P = 0.01$), while the group treated with Nilutamide (6 mM) plus Nandrolone showed no significant difference as compared to the control group ($P = 0.51$). Thus, pre-administration of Nilutamide could nullify the ND-induced reduction in the STL.

3.2. Hormone assay

The results of testosterone and Corticosterone measurements are presented in Fig. 3. One-way ANOVA analysis of the data collected 30 min after ND injection revealed no significant differences between the vehicle and ND treated groups in terms of serum level of testosterone ($F_{3,15} = 2.82$; $P = 0.08$; Fig. 3A) and Corticosterone ($F_{3,15} = 1.68$; $P = 0.22$; Fig. 3B). The results of one-way ANOVA test showed significant differences of serum testosterone ($F_{3,15} = 15.18$; $P = 0.00$; Fig. 3C) and Corticosterone level ($F_{3,15} = 20.59$; $P = 0.00$; Fig. 3D) between the data which taken from the samples collected 24 h after ND injection.

Post hoc LSD analysis revealed that the testosterone levels in groups treated with ND 73 ($P = 0.00$) and 219 ($P = 0.02$) mM rose significantly, but the in group treated with ND 146 mM ($P = 0.17$), there was no significant change compared to the control group. Also, the Corticosterone levels of ND 73 mM ($P = 0.03$) and 219 ($P = 0.00$) mM treated groups were higher than those of the control group. However, there were no significant differences between the vehicle and ND 146 mM treated groups ($P = 0.26$).

3.3. Electrophysiological study

3.3.1. The effect of ND on baseline responses

We examined the effect of nandrolone on the baseline fEPSP and PSA evoked by gradually increasing the stimulus intensity (for creating input/output curve) and test pulse applied to Schaffer collateral of the adolescent male rats' hippocampal CA1 area. Repeated measure ANOVA revealed no significant differences between input/output curves recorded from slices of the control and treatment groups: (fEPSP, $F_{3,33} = 1.88$; $P > 0.05$; Fig. 4A) and (PS, $F_{3,28} = 2.33$; $P > 0.05$; Fig. 4C). One-Way ANOVA demonstrated that other baseline parameters including fEPSP_{max}, fEPSP_{min}, PSA_{max}, PSA_{min}, fEPSP_{test pulse}, PSA_{test pulse} were not significantly affected by ND perfusion (Table 1).

3.3.2. The effect of ND on fEPSP-LTP and PS-LTP magnitudes

There was a significant difference in the magnitude of fEPSP-LTP between the groups ($F_{3,36} = 6.65$; $P < 0.01$; Fig. 5A,B). Tukey post hoc revealed that the magnitude of fEPSP-LTP in slices perfused with ND 0.6 ($P < 0.01$) and 3.6 μM ($P < 0.05$) was significantly lower than that of fEPSP-LTP in the control group. However, the magnitude of fEPSP-LTP in the group perfused with ND 0.3 μM did not significantly differ from that of the control slices ($P > 0.05$).

The analysis of PS showed that the PS-LTP magnitude of nandrolone-treated groups was not significantly different from that observed in the control group ($F_{3,30} = 0.84$; $P > 0.05$; Fig. 5C,D).

Repeated measure ANOVA indicated no significant difference in input/output curves after LTP induction for fEPSP ($F_{3,33} = 2.70$; $P > 0.05$; Fig. 4B). However, pairwise comparison by Tukey revealed a significant reduction of fEPSP in 0.6 and 3.6 μM treated group as compared with the vehicle-treated group across many stimulus intensities ($P < 0.05$). For PS, repeated measure ANOVA showed significant differences among input/output curves after LTP induction ($F_{3,26} = 3.73$, $p < 0.05$; figure 4D). Also, post hoc Tukey analysis showed that the effect of ND on I/O curve developed at the ND 0.6 ($P < 0.05$).

3.3.3. Effect of ND on E-S coupling curves

The discrepancy between the effect of ND on fEPSP slope and PS amplitude suggests that ND may cause some dissociation between fEPSP and PS, which has been tested by evaluating E-S coupling curves.

fEPSP slope was transformed to Log_{10} , and the PS amplitude was normalized. Then, the normalized PS amplitude was plotted vs. Log_{10} fEPSP slope to show the rate of E-S coupling. The rate of fEPSP slope at which PS amplitude reaches half-maximum is called as EC50. The best curve fit F-test analysis indicated a significant effect of HFS induction on the best curve fits (pre- vs. post-LTP induction) of the control groups ($F_{2,140} = 8.09$; $P < 0.001$; Fig. 6A). Further, unpaired t-test showed a significant drop in EC50 after LTP induction ($t_{16} = 2.67$; $P < 0.05$; Fig. 6A insertion), while ND nullified the effect of HFS on E-S coupling, where no significant differences were observed for the best curve fits at 0.3 ($F_{2,92} = 0.46$; $P > 0.05$; Fig. 6B), 0.6 ($F_{2,108} = 1.03$; $P > 0.05$; Fig. 6C), and 3.6 μM ($F_{2,108} = 0.30$; $P > 0.05$; Fig. 6D). The EC50s of ND treated groups were analyzed by unpaired t-test. It showed that EC50s of pre- and post-LTP induction had no significant differences for ND 0.3 μM ($t_{12} = 0.44$; $P > 0.05$), 0.6 μM ($t_{12} = 0.40$, $P > 0.05$), and 3.6 μM ($t_{12} = 0.27$; $P > 0.05$) (Fig. 6A-D insertion).

4. Discussion

This study has observed that a single dose administration of nandrolone causes an invert U-shape effect on acquisition of inhibitory (passive) fear learning in preadolescent rats. However, the effect of acute nandrolone exposure had negative dose-dependent effects on consolidation of inhibitory fear learning and it compromised the retrieval stage of memory across all doses. This result is consistent with another study (Harooni et al. 2008) reporting that intra-hippocampal injection of testosterone has a U-shape negative effect on the acquisition of passive avoidance

and detrimental effect of testosterone on the retrieval stage. However, here we show that ND dose dependently impairs PAL, while Harooni et al. reported an invert U-shape effect of testosterone on the consolidation of PAL. These discrepancy might be due to the fact that ND converts less to estrogenic substances than testosterone, and it is well established that some effects of testosterone are exerted through estrogenic receptors (Kokras et al. 2018; Moradpour F et al. 2006; Svensson 2010).

Subchronic ND administration has been reported to have the potential of nullifying the positive effect of exercise on CA1 area of hippocampus and dentate gyrus as well as Alzheimer's disease (Brännvall et al. 2005). Therefore, it should be investigated whether a single dose of ND could nullify the positive effect of exercise on neurogenesis and Alzheimer's.

A previous study reported that ND impairs spatial learning and memory through affecting androgen receptors (Kouvelas et al. 2008), which could explain negative effect of ND on retrieval of PAL, reported in this study. Importantly, this effect was eliminated when Nilutamide, an AR antagonist, was pre-administered with nandrolone. This finding suggests that exposure to nandrolone could impair the memory by stimulating AR within CNS.

However, the invert U-shape effect of ND on acquisition could not be justified by the effect on androgenic receptors. The short-term usage of ND has been reported to elevate the plasma levels of testosterone and cortisol through affecting hypothalamus-hypophysis-glands (Schlussman et al. ; Takahashi et al. 2004). In addition, it induces aromatase enzyme activity and increased conversion of intrinsic androgens to estrogenic neurosteroids (Roselli 1998). Therefore, the inverted U-shape effect of ND on PAL may be exerted through production of testosterone or/and Corticosterone. They improve learning and memory in an optimal level, while impairing learning and memory at lower or higher doses (Muller et al. 2005). This hypothesis was evaluated in the current study. There were no significant differences among the

groups' samples collected 30 min after ND treatment. However, ND treatment had a U-shape effect on the testosterone and Corticosterone level among samples collected 24 h later. Therefore, it could be suggested that ND had direct acute and indirect effects through testosterone and/or Corticosterone on the retrieval of PAL.

The detrimental effect of ND on PAL is in line with the electrophysiological data presented in this study, which showed acute perfusion of ND could nullify fEPSP-LTP and E-S potentiation.

LTP has been the most studied cellular mechanism of learning and memory (Abbas et al. 2015), which was explored by in-vitro field potential recording approach in this study. We indicated that ND could cancel the effect of HFS on fEPSP-LTP induction in a dose-dependent manner, but has no significant effect on the magnitude of PS-LTP. This suggests site-dependent effect of ND, which is consistent with previous study reporting site-dependent effect of castration on CA1 LTP (Moradpour et al. 2013). It also suggested that the mechanisms of fEPSP-PS coupling are affected by ND perfusion. This was evaluated by analyzing E-S coupling curve and shifts in EC50. It was observed that fEPSP-PS curve shifted to left and EC50 significantly decreased by LTP induction, in accordance with a previous report (Staff and Spruston 2003). Also, ND perfusion prevented the effect of HFS on both EC50 and leftward shift of fEPSP-PS curve. The GABAA activation effect of ND could explain these effects, which were discussed earlier in the current study.

Several neurotransmitter and cellular mechanisms such as GABAergic, glutamatergic, sigma1 receptors, neuropeptide Y, and calcineurin should be studied as a possible mechanism underlying ND effects on the PAL.

However, in the current study, we used intracerebroventricular microinjection of ND to avoid peripheral action of ND. Hence, it should be noted that this protocol is different from

human abuse of ND, and the results could not be directly related to the usual ND abuse effects. Furthermore, the effect of ND on behavioral performances may be exerted through the effect of testosterone or Corticosterone on the brain in general and also the effect of i.c.v. injection of ND is not site specific so while it affect hippocampus other structures may be involved.

5. Conclusion

The present study indicated ND administration could affect all stages of passive avoidance learning and memory, but its effect is more potent on the retrieval of the inhibitory memory, and its effect on retrieval develop at lower doses. Therefore, the ND might be a potential/complementary treatment for the disease related to fear memory such as PTSD. Nevertheless, the exact mechanisms require further investigation. It is also suggested that the effect of ND on CA1-LTP is site dependent and E-S coupling may be the better indicator of ND effect on CA1 synaptic plasticity.

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Table 1 –The field responses recorded in the CA1 region of hippocampus

Group fEPSP test pulse (mV/s)	Number of slices/rats (mV)	fEPSP max PSA test pulse (mV/s)	fEPSP min (mV/s)	PSA max (mV)	PSA min (mV)
Control 235.8±18.28	14 per 14 735.76±228.25	514.58±46.00	51.49±13.06	562.82±45.72	2.76±1.14
ND 0.3µM 446.12±134.19	6 per 6 882.95±302.41	837.7±234.7	29.1±11.01	2346.75±369.27	1.49± 1.03
ND 0.6µM 277.22±53.66	8 per 8 805.11±306.21	517.41±88.23	17.76±6.64	1809.85±363.14	9.91±5.04
ND 3.6µM 302.39±49.39	9 per 9 723.47±158.41	724.75±122.51	58.61±20.11	1806.25±120.31	17.61±13.1
The values are mean ±S.E.M. There was no significant difference in baseline parameters between control and dependent slices.					

Figure Captions

Fig.1. (A) Pre-training administration of ND 73 and 219 mM lead to decrease of STL significantly as compared to control group in adolescent male rats. But ND 146 mM had no significant effect on STL. (B) Post-training i.c.v. injection of ND 219 mM significantly decreased the STL, while ND 73 and 146 mM had no significant effect on STL. (C) Pre-retrieval administration of ND 73, 146 and 219 mM lead to significantly decrease of STL. Data represent the mean values $\pm$ SEM from control and ND groups (n=8). *P < 0.05 and **P < 0.01 VS control.

Fig.2. (A) Administration of Nilutamide 6 mM had no significant effect on STL in compared to control group. (B) Administration of Nandrolone 73 mM significantly decreases the STL, while Nilutamide (6 mM) nullified the Nandrolone effect on STL. Data represent the mean values $\pm$ SEM from control and ND groups (n=8). *P < 0.05 VS control.

Fig.3. Effects of ND on the serum testosterone and Corticosterone levels (A) In samples which blood collection was 30 min after ND injection, administration of ND 73, 146 and 219 mM had no significant effect on serum level of Testosterone and Corticosterone as compared to control group. (B) In samples which blood collection was 24 h after ND injection, administration of ND 73 and 219 mM lead to increase of serum Testosterone and Corticosterone in compared to control group in adolescent male rats, while ND 73 mM had no significant effect on serum Testosterone and Corticosterone. Data represent the mean values $\pm$ SEM from control and ND groups (n=5). *P < 0.05 and ***P < 0.001 VS control.

Fig.4. (A) The I/O curves of fEPSP before HFS induction were similar in DMSO and nandrolone perfused slices. (B) The I/O curve of the fEPSP after HFS induction in ND perfused slices (0.6 μ M) decreased significantly compared to the I/O curve of DMSO perfused slices. (C) The I/O curves of PS before HFS induction were similar in DMSO and ND perfused slices. (D) The I/O curve of the PS after HFS induction in ND perfused slices (0.6 μ M) significantly decreased compared to DMSO perfused slices. IMT, integer multiples of the threshold intensity. The data are presented as the mean $\pm$ SEM. *P < 0.05 and **P < 0.01 significant difference ND 0.6 VS control group.

Fig.5. The magnitude of the fEPSP-LTP (A) and PS-LTP (C) changes (%) versus time for hippocampal slices perfused with DMSO or nandrolone. (B) and (D) show the mean values (%) of the fEPSP-LTP and PS-LTP, respectively. Perfusion with nandrolone 0.6 and 3.6 μM significantly decreased the fEPSP-LTP magnitude compared to DMSO perfused slices; nandrolone 0.3 μM had no effect on fEPSP-LTP. PS-LTP had no significant changes in the presence of nandrolone compared to the control slices (Inserts). Representative superimposed fEPSPs and PSs trace at baseline (solid line) and after tetanus (dashed line). The data are presented as the mean $\pm$ SEM. * $P < 0.05$ and ** $P < 0.01$ significant difference ND 0.6 VS control group and # $p < 0.05$ significant difference between ND 3.6 VS control.

Fig.6. Nandrolone affects the E-S coupling in the hippocampal CA1. Before and 60 min after tetanic stimulation Normalized PS amplitude vs. Log_{10} fEPSP slope was plotted to show the rate of E-S coupling. The rate of fEPSP slope at which PS amplitude reaches half-maximum is named as EC50 (Insertions). Best curve fit F test analysis showed that in the control group curves before and 60 min after tetanic stimulation was differ significantly and EC50 decreased significantly (A). There were no significant effect of HFS induction on E-S coupling or EC50s of slices perfused with ND 0.3 μM , ND 0.6 μM and ND 3.6 μM (B-D).

Figure 1

Fig.1. (A) Pre-training administration of ND 73 and 219 mM lead to decrease of STL significantly as compared to control group in adolescent male rats. But ND 146 mM had no significant effect on STL. (B) Post-training i.c.v. injection of ND 219 mM significantly decreased the STL, while ND 73 and 146 mM had no significant effect on STL. (C) Pre-retrieval administration of ND 73, 146 and 219 mM lead to significantly decrease of STL. Data represent the mean values $\pm$ SEM from control and ND groups (n=8). *P < 0.05 and **P < 0.01 VS control.

190x153mm (300 x 300 DPI)

Figure 2

Fig.2. (A) Administration of Nilutamide at dose of 5 μ g had no significant effect on STL in compared to control group. (B) Administration of Nandrolone significantly decreases the STL, while Nilutamide (5 μ g) nullified the nandrolone effect on STL. Data represent the mean values $\pm$ SEM from control and ND groups (n=8). *P < 0.05 VS control.

181x76mm (300 x 300 DPI)

Figure 3

Fig.3. Effects of ND on the serum testosterone and Corticosterone levels (A) In samples which blood collection was 30 min after ND injection, administration of ND 73, 146 and 219 mM had no significant effect on serum level of Testosterone and Corticosterone as compared to control group. (B) In samples which blood collection was 24 h after ND injection, administration of ND 73 and 219 mM lead to increase of serum Testosterone and Corticosterone in compared to control group in adolescent male rats, while ND 73 mM had no significant effect on serum Testosterone and Corticosterone. Data represent the mean values $\pm$ SEM from control and ND groups (n=5). *P < 0.05 and ***P < 0.001 VS control.

182x138mm (300 x 300 DPI)

Fig.4. (A) The I/O curves of fEPSP before HFS induction were similar in DMSO and nandrolone perfused slices. (B) The I/O curve of the fEPSP after HFS induction in ND perfused slices (0.6 μM) decreased significantly compared to The I/O curve of DMSO perfused slices. (C) The I/O curves of PS before HFS induction were similar in DMSO and ND perfused slices. (D) The I/O curve of the PS after HFS induction in ND perfused slices (0.6 μM) significantly decreased compared to DMSO perfused slices. IMT, integer multiples of the threshold intensity. The data are presented as the mean $\pm$ SEM. * $P < 0.05$ and ** $P < 0.01$ significant difference ND 0.6 VS control group.

179x120mm (300 x 300 DPI)

Figure 5

Fig.5. The magnitude of the fEPSP-LTP (A) and PS-LTP (C) changes (%) versus time for hippocampal slices perfused with DMSO or nandrolone. (B) and (D) show the mean values (%) of the fEPSP-LTP and PS-LTP, respectively. Perfusion with nandrolone 0.6 and 3.6 μ M significantly decreased the fEPSP-LTP magnitude compared to DMSO perfused slices; nandrolone 0.3 μ M had no effect on fEPSP-LTP. PS-LTP had no significant changes in the presence of nandrolone compared to the control slices (Inserts). Representative superimposed fEPSPs and PSs trace at baseline (solid line) and after tetanus (dashed line).The data are presented as the mean $\pm$ SEM. *P < 0.05 and **P < 0.01 significant difference ND 0.6 VS control group and # p < 0.05 significant difference between ND 3.6 VS control.

221x227mm (300 x 300 DPI)

Figure 6

Fig.6. Nandrolone affects the E-S coupling in the hippocampal CA1. Before and 60 min after tetanic stimulation Normalized PS amplitude vs. Log 10 fEPSP slope was plotted to show the rate of E-S coupling. The rate of fEPSP slope at which PS amplitude reaches half-maximum is named as EC50 (Insertions). Best curve fit F test analysis showed that in the control group curves before and 60 min after tetanic stimulation was differ significantly and EC50 decreased significantly (A). There were no significant effect of HFS induction on E-S coupling or EC50s of slices perfused with ND 0.3 μM, ND 0.6 μM and ND 3.6 μM (B-D).

185x163mm (300 x 300 DPI)