# A Unique Mouse Model of Early-Life Exercise Enables Lasting Hippocampal Synaptic Plasticity and Memory

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**Abstract:** Exercise is a powerful modulator of learning and memory function in humans. The cognitive benefits of aerobic physical activity, as well as underlying molecular mechanisms, are well documented in studies on adult rodents. For example, voluntary running wheel activity in adult mice allows for the formation of long term, hippocampus-dependent memory of typically subthreshold learning events [1]. One of the primary mechanisms by which exercise enables long term memory is via the regulation of the brain-derived neurotrophic factor (BDNF), a molecule critical for hippocampal-dependent learning and memory formation in adult rodents. Less is known about the short and long-term consequences of *early life exercise (ELE)* on hippocampal-dependent memory and synaptic plasticity, specifically if the exercise is taking place during periods of postnatal hippocampal maturation. We hypothesize that ELE during one week (postnatal days P21-27) or three weeks (P21-41) can enable long term spatial memory formation in adolescent mice. To address this hypothesis, we tested spatial memory formation in the Object Location Memory task, which is hippocampus-dependent. Further, we test for changes in hippocampal synaptic plasticity after these two periods of ELE with electrophysiological studies. Our results suggest early-exercise has a lasting impact on hippocampal memory and synaptic plasticity when occurring during periods of postnatal hippocampal maturation.

## Introduction

Exercise is highly effective in improving memory functions during adulthood in both humans and rodents (reviewed in [2]). It may be argued that the potency of exercise in preserving and improving cognition may equal or exceed that of any currently existing pharmacological intervention [3]. Preclinical studies using rodent models have identified underlying neurobiological mechanisms by which exercise improves brain, and specifically hippocampal function. Exercise in adulthood augments neurogenesis [4], vascularization [5], synapse number [6], facilitates long-term potentiation (LTP; [7]), and induces a number of synaptic genes and proteins, including the key plasticity-modulating growth factor brain-derived neurotrophic factor (BDNF; [8]). The effects of exercise are transient in adults, fading in days. Given the inherent plasticity of the developing hippocampus, as well as its sensitivity to early-life environmental signals, it is critically important to explore the possibility that physical exercise during early-life may lead to enduring benefits in hippocampal function.

The mammalian brain undergoes protracted postnatal development [9,10] and is particularly sensitive to early-life environmental signals [11]. Clinical studies demonstrate a strong correlation between early-life experiences and cognitive outcomes later in life. Early-life adversity has been linked to increased risk for AD [12], PTSD [13] and other adult-onset learning and memory impairments [14]. Rodent models allow for the study of causation; indeed, early-life stress in rats and mice lead to structural atrophy of dendrites, attenuated long-term potentiation, and impaired hippocampus-dependent memory in adulthood [15-17]. In contrast, positive early-life experiences such as augmented maternal care have salubrious effects on synaptic plasticity and memory that persist into adulthood [18,19]. These experience-dependent outcomes suggest a bidirectional plasticity of the lasting effects of early-life experience on hippocampal function [20,21]. Although a large research focus has been on understanding the consequences of maladaptive early-life experiences, there has been less attention paid to understanding the molecular and functional sequelae of positive early-life signals; this area of study is vitally important for the ultimate development of early-life interventions to optimize cognitive development.

In this study, we develop a model of early-life exercise upon weaning to address the hypothesis that early-life exercise can lead to benefits in cognitive function and synaptic plasticity in the adolescent. We show that both male and female mice undergoing exercise between 3$^{rd}$-6$^{th}$ postnatal weeks of life exhibit an enabling of hippocampus-dependent spatial memory, increased long-term potentiation and changes in basal synaptic physiology.

Importantly, this model can be used to uncover developmentally-specific molecular mechanisms engaged by early life exercise to influence neuronal function and behavior in a lasting manner.

## Materials and Methods

### Animals

Mice were progeny of timed-pregnant C57Bl6J dams, obtained from Jackson Laboratories. Mice had free access to food and water and lights were maintained on a 12 h light/dark cycle. Upon weaning on postnatal day (P) 21, mice were housed in either standard bedding cages or cages equipped with a running wheel. Both male and female mice were used for these studies. All behavioral testing was performed during the light cycle. All experiments were conducted according to US National Institutes of Health guidelines for animal care and use and were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

### Running Wheel Paradigm

Upon weaning on P21, mice were pair-housed in standard cages equipped with a stainless steel running wheel and free access for a duration specified (either one week during P21-27, or three weeks during P21-41). Each wheel is outfitted with a polyurethane mesh so the small limbs of juvenile mice do not slip through the rungs of the wheels. Running wheel activity was tracked via digitally monitored sensors attached to each cage that counted. Wheel revolutions were counted daily and converted to distance ran per cage (km).

### Object Location Memory (OLM) Protocol

The object location memory protocol used in this study has been modified from previous protocols described elsewhere [22]. On postnatal day 36, mice were brought into a testing room with room brightness approximately ~45 LUX. Mice were handled for a total of 6 days prior to the OLM training session: twice a day for the first 3 days followed by once a day for the next 3 days. Habituation sessions were 5 min and occurred within chambers containing unique spatial cues on each wall. Habituation overlapped with the last 3 days of handling. During the training phase, mice were placed into the same chambers with two identical objects and exposed to either a subthreshold (3 minutes) or threshold (10 minutes) training period. On testing day, one of the two objects was moved to a novel location inside the chamber, and mice were then allowed to explore the objects for 5 minutes. The times spent with each object was determined by hand-scoring, and values were converted into a Discrimination Index (DI): [(time spent

exploring novel object – time spent exploring familiar object)/(total time exploring both objects) × 100%]. Mice with uneven exploration times between the two objects during the training phase (DI > 20) or showed no decrease in total distance traveled across habituation sessions were excluded from analysis.

Electrophysiology studies

Male mice were sacrificed between P42-51, and slices were collected from the rostral hippocampus. Slices were placed in an interface recording chamber with preheated (31 ± 1 °C) artificial cerebrospinal fluid (124 mM NaCl, 3 mM KCl, 1.25 mM $KH_2PO_4$, 1.5 mM $MgSO_4$, 2.5 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM d-glucose). Slices were continuously perfused at a rate of 1.75–2 ml per min while the surface of the slices was exposed to warm, humidified 95% $O_2$/5% $CO_2$. Recordings began after at least 2 h of incubation. Field excitatory postsynaptic potentials (fEPSPs) were recorded from CA1b stratum radiatum using a single glass pipette (2–3 MΩ) filled with 2 M NaCl. Stimulation pulses (0.05 Hz) were delivered to Schaffer collateral-commissural projections using a bipolar stimulating electrode (twisted nichrome wire, 65 μm) positioned in CA1c. Current intensity was adjusted to obtain 50% of maximal fEPSP response. After a stable baseline was established, LTP was induced with a single train of five theta bursts, in which each burst (four pulses at 100 Hz) was delivered 200 ms apart (i.e., at theta frequency). The stimulation intensity was not increased during TBS. Data were collected and digitized by NAC 2.0 Neurodata Acquisition System (Theta Burst). Data in LTP figure were normalized to the last 10 min of baseline and presented as mean ± SE. Baseline measures on I/O curves, paired-pulse facilitation and LTP were analyzed using a two-way ANOVA.

Statistical Analyses

Statistical analyses were performed using either a two-tailed Student's t test or two-way ANOVAs followed by Bonferroni's *post hoc* tests were used to make specific comparisons when significant interactions and/or main group/test effects were observed. Two-way ANOVAs had factors of postnatal day and sex. All statistics were performed with GraphPad Prism 7 software. Main effects and interactions for all ANOVAs are described in the text, along with the specific number of animals of each sex used in each individual experiment. All analyses were two-tailed and required an α value of 0.05 for significance. Error bars in all figures represent SEM. For all experiments, values ±2 SD from the group mean were considered outliers and were removed from analyses.

## Results

### Juvenile male and female mice gradually increase their running distance during three weeks of voluntary exercise.

In our juvenile exercise model, male and female mice were weaned directly into cages equipped with a running wheel (Fig. 1A) and allowed to run for either 3rd-4th postnatal week (P21-27), or 3rd-6th postnatal weeks (P21-41). Each digitally-monitored wheel tracked running distance for the cage (two mice housed per cage, and distance traveled per cage represents the total distance ran between two mice). Mice ran on average about 3-4 kilometers per night by the end of the first postnatal week (Fig. 1B). A two-way repeated-measures ANOVA revealed a significant effect of postnatal day ($F_{(20, 120)} = 13.67$; $p < 0.0001$) and significant interaction with sex ($F_{(20, 120)} = 2.138$; p = 0.0063). Exercising female mice also gained significantly less weight when compared to exercising males (Fig. 1C; n = 11-12 mice per group, $t_{(21)} = 10.61$; $p < 0.001$) and sedentary females ($t_{(16)} = 3.437$; $p < 0.0034$).

### Early-life exercise enables long-term memory formation in adolescent mice.

Our initial experiments address the hypothesis that aerobic exercise occurring during specific periods of postnatal hippocampal development can have enduring consequences on hippocampal memory. We tested if a weak training paradigm that is normally insufficient (sub-threshold) for long-term memory formation can become sufficient for memory formation after an early-life exercise experience. Hippocampus-dependent learning was assessed with the Object Location Memory (OLM) task (Fig. 2A), with either a 3-min or 10-min exposure to 2 identical objects on day of training (memory acquisition). This training exposure was performed during the adolescent period, on P41. The 3-min exposure is a duration previously established as sub-threshold, or insufficient, for supporting long-term memory formation in adult mice, whereas 10 minutes of training is usually sufficient [23,24]. There were no significant differences in total object exploration times when comparing males to females (Fig. 2B). 24 h later (on P42), memory was tested by re-exposing the mice to the same context with one object moved to a new location, as mice that remember OLM training will show increased exploration of the object in the novel location [22]. Using wild-type male and female mice, 24 hrs after the 3-min sub-threshold training session, adolescent mice that exercised during the 3rd-4th postnatal week (P21-27 ELE) as well as those that exercised for 3 weeks (P21-41 ELE) showed strong discrimination in this spatial memory task (Fig 2C). Sedentary mice housed with a locked wheel were unable to discriminate

between familiar and novel object locations. This suggests an enduring effect of early-life exercise on learning and memory when undertaken during critical postnatal developmental periods.

**Synaptic plasticity and basal synaptic physiology are modulated after early-life exercise.**

Long-term potentiation (LTP) studies were performed in acute hippocampal slices from both P21-41 ELE and P21-27 ELE groups. All electrophysiological studies were performed between P42-P51, and induced with a single train of 5 theta bursts to Schaffer collateral inputs and recorded field EPSPs from apical dendrites of CA1b. LTP was increased in adolescent mice that underwent early life exercise for three weeks, but not in the one-week early-exercise group (Fig 3A). There were no differences in burst area across groups (Fig. 3B). Interestingly, input/output curves from both P21-41/ 3 week ELE and P21-27/ 1 week ELE mice showed greater fEPSP responses in early-life exercised groups compared to sedentary controls, suggesting a modulation of basal post-synaptic physiology in both early-life exercised groups (Fig 3C). Paired-pulse facilitation (PPF) experiments demonstrated that both exercise groups had significantly reduced facilitation of the presynaptic circuit when compared to controls (Fig 3D). These results suggest that even a one week period of early-life exercise can lead to lasting changes in hippocampal circuit maturation and function.

**Discussion**

We have created a mouse model of early-life voluntary exercise for the evaluation of hippocampus-dependent memory and synaptic plasticity. We found that during three weeks of exercise, female mice gain a significantly less weight when compared to males. Concomitant with this finding was increased daily running distances in female mice over the three week running period. These studies also revealed that exercise during postnatal days 21-41, as well as a shorter period of early life exercise from P21-27, can promote hippocampal-dependent learning and enable long-term memory formation following a subthreshold learning event. Most notably the discover that enhancements in synaptic plasticity and enduring changes to the basal physiology of hippocampal circuitry endure after early-life exercise require further investigation of underlying molecular and structural mechanisms.

Capitalizing on the possibility that exercise during early-life has lasting beneficial effects on cognitive outcomes requires the rigorous study of 1) the *timing* of exercise during specific periods of postnatal neurodevelopment, and 2) *temporally-specific cellular and molecular mechanisms* uniquely engaged by exercise during early-life. In children, studies positively associate higher physical activity levels with improved working memory [25,26], structural brain health [27] and academic performance [28], however, there remains a need for more prospective, randomized-controlled trials implementing pediatric-specific exercise interventions. Despite the general public acknowledgement that exercise is good for physical and mental health, childhood sedentary behaviors are increasingly prevalent worldwide and linked to obesity and poorer cognition [29]. Optimizing cognitive development in childhood is an important goal not only for future academic performance and cognitive outcomes, but also for children to achieve their full potential as future contributors to our larger society [30]. Overall, the goal of this research is to use mouse models to identify the precise timing of early-life exercise that can lead to lasting cognitive benefits, and investigate the underlying mechanisms that enable early-life exercise to promote enhanced cognition.

In summary, exercise is a highly potent, positive modulator of cognitive function. Exercise parameters for optimal cognitive development in typically developing children, and cognitively impaired children, currently do not exist. Yet it is precisely during this development period that exercise may have the most long-term benefit. It is clear that more data are needed in the field of exercise neurobiology during development to inform future pharmacological, and non-pharmacological interventions. Mouse models can provide mechanistic approaches and opportunities for understanding developmental mechanisms influenced by exercise. Further understanding of the timing and biological mechanisms governing circuit development and neuronal function after early-life exercise will ultimately aid in the design of therapeutic strategies.

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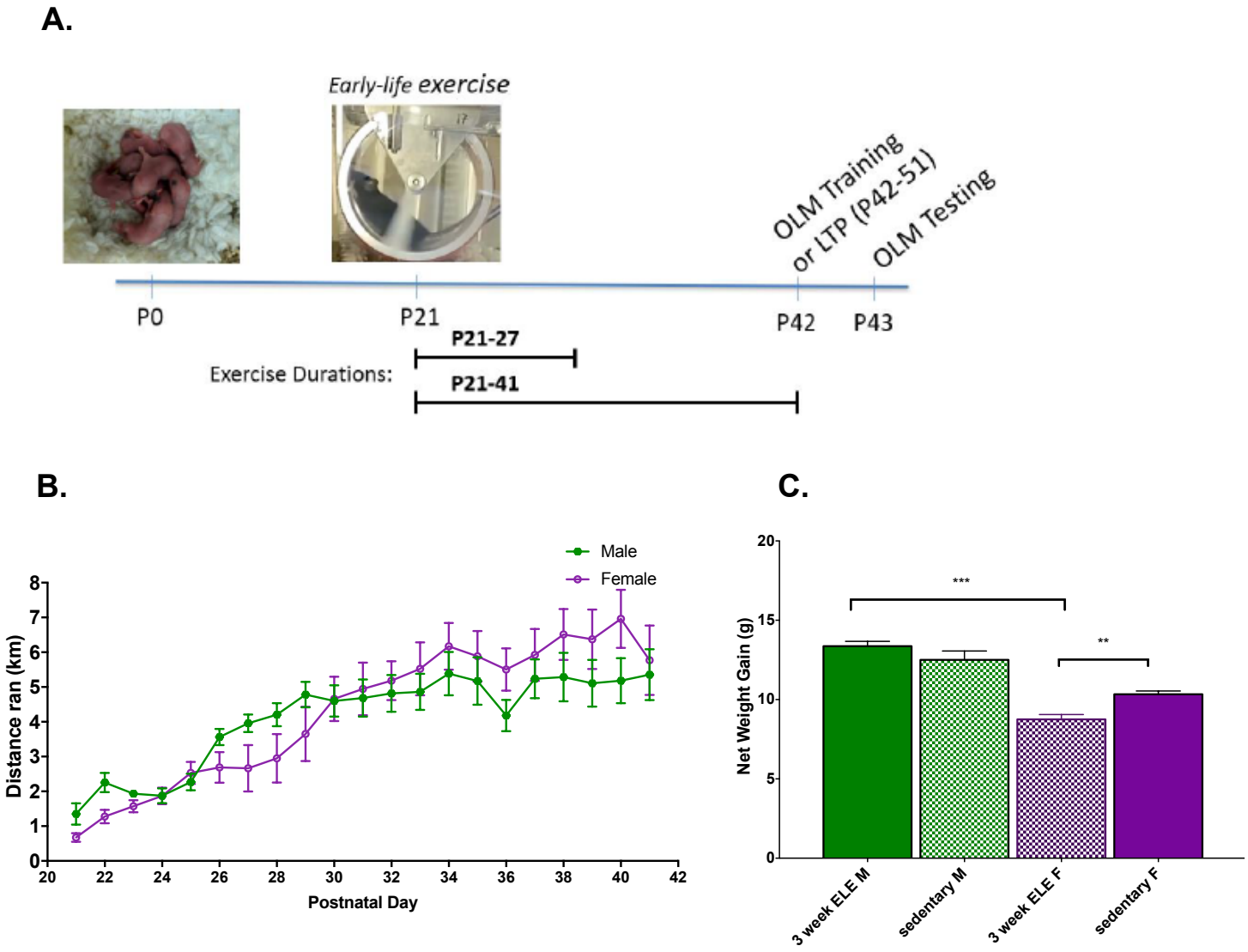


**Figure 1**. **Experimental Design, Running Behavior and Weight Trends in Early Exercise Model**. (A) Experimental design diagram. (B) Females ran significantly further distances across days than males during P21 to P41 (n = 3 cages per group, 2 mice per cage). Distances were recorded as number of wheel revolutions and converted into kilometers. Statistical analysis by 2-way ANOVA. **** $p \leq 0.001$. (C) Females gained significantly less weight over the three week voluntary exercise period (n= 6–12/group). A significant difference in weight gain was also observed between exercise and sedentary females, but no difference detected in males. Data were analyzed using Student's t tests: ** $p \leq 0.01$, and *** $p \leq 0.001$.

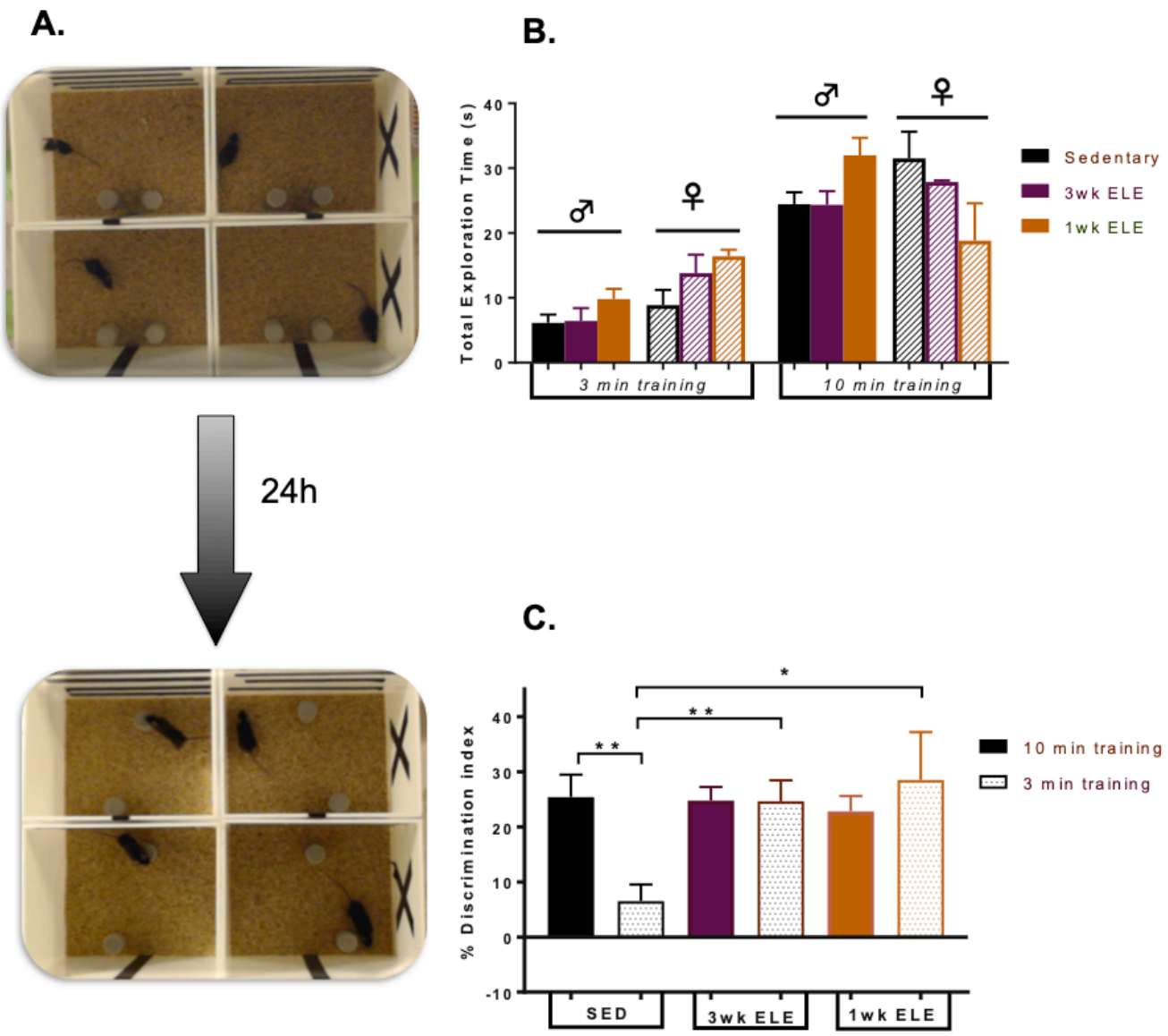


**Figure 2. ELE enables long-term memory formation in adolescent mice.** Object Location Memory Task training (A, B) Mice were placed in training chambers for either 3 or 10 minutes and allowed to explore two identical objects. No difference was observed in total exploration times between groups, or between males and females, during training. Data were analyzed using Student's t tests: * $p \leq 0.05$; ** $p \leq 0.01$. (C) Both mice from the 3-min training + 3 wk ELE group and the 3-min training + P21-27 ELE group showed a significantly higher DI than the 3-min training sedentary group. In addition, no significant difference was observed between the 3-

min training + 3 wk ELE group and the 10-min training + 3 wk ELE group. Data were analyzed using Student's t tests: * p ≤ 0.05; ** p ≤ 0.01.

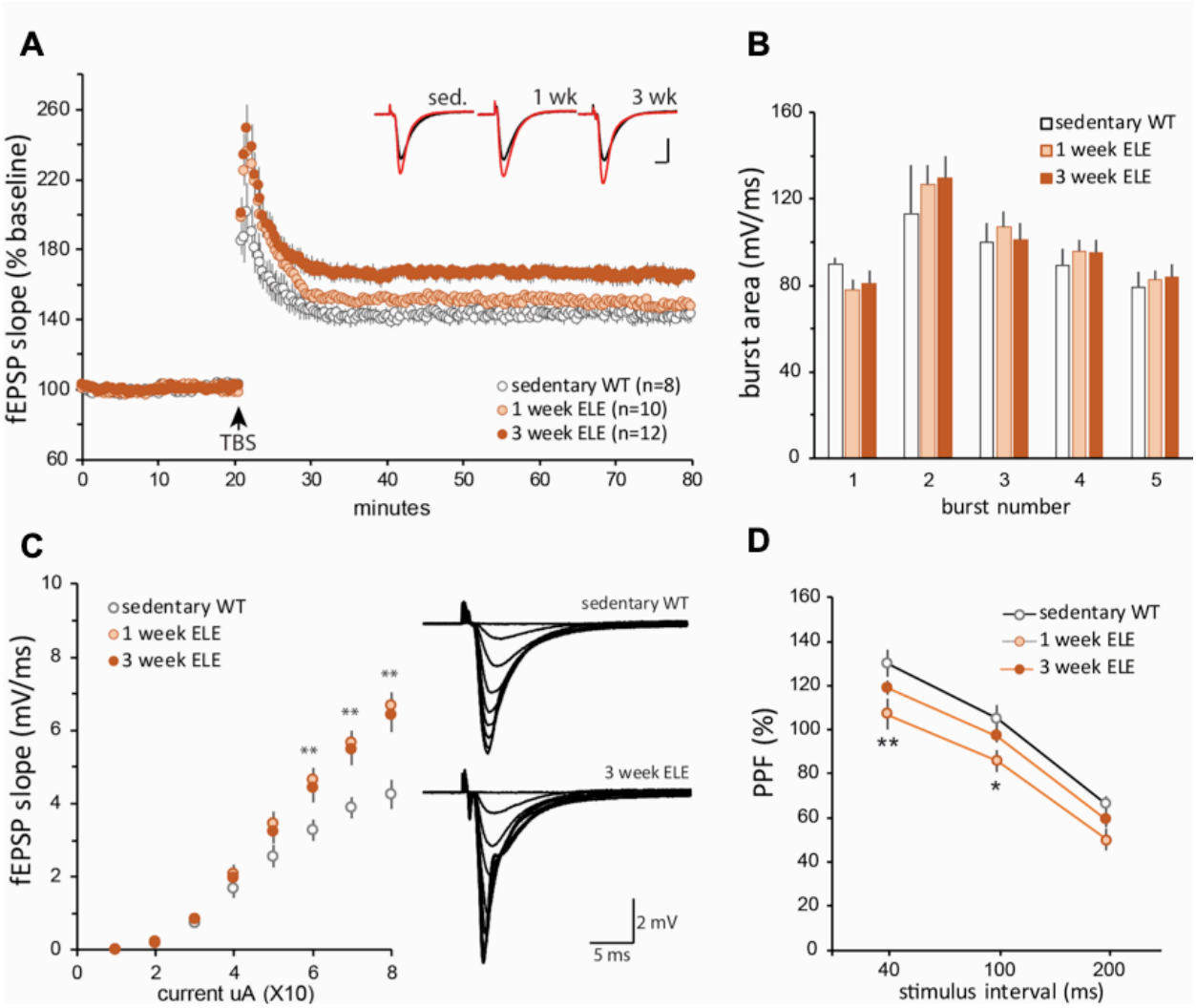


**Figure 3. Early-life exercise leads to enhanced hippocampal synaptic plasticity in CA1.** Mice undergoing 3 weeks of early-exercise had a significant increase in LTP, compared to sedentary controls and 1 week early-exercise mice (A). No differences detected in burst area during theta-burst stimulation (B). I/O curves generated in the Schaffer collaterals showed greater fEPSP responses in both 1- and 3-week early life exercised groups compared to sedentary controls (C). Paired-pulse facilitation was also significantly reduced in both early exercise groups (D) when compared to sedentary mice. * $p \leq 0.05$, ** $p \leq 0.01$.