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Authors
Dong, TN
Kramár, EA
Beardwood, JH
et al.

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Temporal endurance of exercise-induced benefits on hippocampus-dependent memory and synaptic plasticity in female mice

T.N. Dong[a], **E.A. Kramár**[b,c,d], **J.H. Beardwood**[b,c,d], **A. Al-Shammari**[b,c,d], **M.A. Wood**[b,c,d], **A. A. Keiser**[b,c,d,*]

[a]Nash Family Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY 10029, United States

[b]Department of Neurobiology and Behavior, School of Biological Sciences University of California, Irvine 92697-2695, United States

[c]Center for the Neurobiology of Learning and Memory (CNLM), University of California, Irvine 92697-2695, United States

[d]Institute for Memory Impairments and Neurological Disorders (UCI MIND), University of California, Irvine 92697-2695, United States

Abstract

Exercise facilitates hippocampal neurogenesis and neuroplasticity that in turn, promotes cognitive function. Our previous studies have demonstrated that in male mice, voluntary exercise enables hippocampus-dependent learning in conditions that are normally subthreshold for long-term memory formation in sedentary animals. Such cognitive enhancement can be maintained long after exercise has ceased and can be re-engaged by a subsequent subthreshold exercise session, suggesting exercise-induced benefits are temporally dynamic. In females, the extent to which the benefits of exercise can be maintained and the mechanisms underlying this maintenance have yet to be defined. Here, we examined the exercise parameters required to initiate and maintain the benefits of exercise in female C57BL/6J mice. Using a subthreshold version of the hippocampus-dependent task called object-location memory (OLM) task, we show that 14d of voluntary exercise enables learning under subthreshold acquisition conditions in female mice. Following the initial exercise, a 7d sedentary delay results in diminished performance, which can be re-facilitated when animals receive 2d of reactivating exercise following the sedentary delay. Assessment of

*Corresponding author at: 200 Qureshey Research Lab Building 506, Irvine, CA 92697, United States. akeiser@uci.edu (A.A. Keiser).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

T.N. Dong: Conceptualization, Methodology, Formal analysis, Investigation, Validation, Writing – original draft, Writing – review & editing. **E.A. Kramar:** ´ Formal analysis, Investigation, Writing – review & editing. **J.H. Beardwood:** Validation, Investigation, Formal analysis, Writing – review & editing. **A. Al-Shammari:** Validation, Investigation. **M.A. Wood:** Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition. **A.A. Keiser:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nlm.2022.107658.

estrous cycle reveals enhanced wheel running activity during the estrus phase relative to the diestrus phase, whereas estrous phase on training or test had no effect on OLM performance. Utilizing the same exercise parameters, we demonstrate that 14d of exercise enhances long-term potentiation (LTP) in the CA1 region of the hippocampus, an effect that persists throughout the sedentary delay and following the reactivating exercise session. Previous studies have proposed exercise-induced BDNF upregulation as the mechanism underlying exercise-mediated benefits on synaptic plasticity and cognition. However, our assessment of hippocampal *Bdnf mRNA* expression following memory retrieval reveals no difference between exercise conditions and control, suggesting that persistent *Bdnf* upregulation may not be required for maintenance of exercise-induced benefits. Together, our data indicate that 14d of voluntary exercise can initiate long-lasting benefits on neuroplasticity and cognitive function in female mice, establishing the first evidence on the temporal endurance of exercise-induced benefits in females.

Keywords

Exercise; Learning; Hippocampus; Synaptic plasticity; BDNF; Estrous cycle

1. Introduction

The effects of physical exercise on supporting and maintaining brain health are well documented. In the brain, the hippocampus is critical for learning and memory formation as disruption of this brain structure leads to memory impairments (Packard & McGaugh, 1996; Riedel et al., 1999; Scoville & Milner, 1957). Hence, aberrant morphological and functional alterations in the hippocampus are attributed to cognitive impairments associated with aging and disease states (Bettio et al., 2017). Studies in both humans (ten Brinke et al., 2015; Erickson et al., 2011; Pajonk et al., 2010; Teixeira et al., 2018) and animal models (Cooper et al., 2018; Neeper et al., 1996; O'Callaghan et al., 2007; Van Praag, 2008) have demonstrated the effects of exercise on supporting structural and functional integrity of the hippocampus, suggesting that physical exercise is an effective non-pharmacological intervention for cognitive impairment in both physiological and pathological conditions (Intlekofer & Cotman, 2013; Lauretta et al., 2021; Liu et al., 2011; Muscari et al., 2010). Despite ample research on beneficial effects of exercise and the relationship to hippocampus and cognitive function, little is known about the temporal dynamics of exercise-induced benefits. That is, what is the exercise duration required to augment hippocampal function, and how long do these benefits persist after exercise ceases? Even fewer studies have attempted to investigate the molecular mechanisms underlying the maintenance of exercise-induced benefits. Given that exercise regimens in humans are less rigid and consistent than those used in animal studies (Lee & Skerrett, 2001), it is important to develop exercise protocols that are flexible and do not require a daily exercise routine but can still engage and maintain exercise-induced cognitive benefits. With regard to animal studies, it is difficult to identify the minimal exercise duration required to initiate and maintain the benefits of exercise due to considerable variability in exercise protocols used across studies (Loprinzi et al., 2019). Hence, it remains important to examine the exercise threshold that induces and maintains cognitive benefits and the underlying mechanisms.

Exercise facilitates neuroplasticity, neurogenesis, and subsequently, learning and memory through mechanisms that induce the neurotrophic action of brain-derived neurotrophic factor (BDNF) in the hippocampus (Alomari et al., 2013; Cotman et al., 2007; Cotman & Berchtold, 2002; Ding et al., 2011; Triviño-Paredes et al., 2016; Van Praag, Christie, et al., 1999; Van Praag, Kempermann, et al., 1999). In both female and male rodents, exercise improves behavioral performance in a wide variety of memory tasks, including the heavily spatial-oriented, hippocampus-based tasks: Morris water maze (MWM), object-location memory (OLM) and radial arm maze in addition to other hippocampus-based memory tasks such as contextual fear conditioning, passive avoidance, and novel object recognition (Intlekofer et al., 2013; Lambert et al., 2005; O'Callaghan et al., 2007; Van Praag, Christie, et al., 1999; Van Praag, Kempermann, et al., 1999; Vivar, Potter, & Van Praag, 2012). In male rodents, our studies and others have identified that a minimal exercise duration of 2 weeks is required for benefits on learning and memory which can be maintained through a period of inactivity and re-engaged by re-introduction to a brief, subthreshold 2d exercise session (Berchtold et al., 2010; Butler et al., 2019; Intlekofer et al., 2013). On the molecular level, 2 weeks of exercise induces upregulation of hippocampal brain-derived neurotrophic factor protein in male rats (Berchtold et al., 2005). Following the decay of elevated BDNF levels to baseline, a subsequent re-introduction to a brief, subthreshold 2d exercise session can re-facilitate elevated levels of hippocampal BDNF (Berchtold et al., 2005). This suggests exercise-mediated BDNF upregulation as a potential mechanism that primes long-lasting neuronal changes to enable a lower exercise frequency stimulus to capitalize on these adaptations and facilitate cognitive benefits.

BDNF signaling promotes protein synthesis-dependent mechanisms to induce hippocampal long-term potentiation (LTP), a cellular correlate of learning and memory (Panja & Bramham, 2014; Silva, 2003). Therefore, enhancement of hippocampal LTP following exercise is suggested to underlie exercise-facilitated learning (L. Bettio et al., 2019; Liu et al., 2011). To our knowledge, no study thus far has investigated whether an enhancement of LTP persists following exercise cessation in either sex. Several studies have demonstrated exercise to efficiently enhance LTP in the dentate gyrus (Farmer et al., 2004; Van Praag, Christie, et al., 1999; Vasuta et al., 2007), however, there is a surprising dearth of research investigating how exercise modulates synaptic plasticity in the CA1 subfield (Cotman et al., 2007), which serves as the most studied area in the hippocampus in the context of spatial memory (Patten et al., 2015). Hence, the relationship between exercise and CA1 plasticity remains to be explored.

Several studies have supported the hypothesis that exercise-induced benefits are temporally dynamic (Berchtold et al., 2005, 2010; Butler et al., 2019; Y. P. Kim et al., 2003). Regarding female animals, our understanding of this process is less prominent as most of the work has been primarily conducted in males. Studies of learning and memory have provided evidence that similar behavioral outcomes between sexes might involve different neural mechanisms (Becker & Koob, 2016; Keiser & Wood, 2019; Sase et al., 2019). Despite data indicating that exercise improves neuroplasticity and cognition in both sexes, there is clear evidence for the sex-specific effects of exercise and the underlying mechanisms. In humans, exercise improves object-location memory in males and not females (Coleman et al., 2018). In rodents, exercise selectively improves the performance of one sex in

certain memory tasks (Barha et al., 2017). On the molecular level, distribution of BDNF across multiple brain structures and subregions of the hippocampus is different between sexes (Bakos et al., 2009; Franklin & Perrot-Sinal, 2006). Accumulating evidence has also highlighted sex differences in the functions and mechanisms of BDNF (Chan & Ye, 2017). For instance, the reproductive hormone, estrogen, can regulate BDNF expression through various mechanisms, one of which involves epigenetic modifications on the BDNF promoter (Fortress et al., 2014; Moreno-Piovano et al., 2014; Chan & Ye, 2017). In fact, hippocampal BDNF protein levels vary across the estrous cycle with the highest expression observed during estrus and proestrus, suggesting fluctuations in estrogen levels throughout the estrous cycle in females differentially affect BDNF expression (Scharfman et al., 2003). In addition, the estrous cycle can also modulate targets downstream of BDNF signaling pathways in the hippocampus (Spencer et al., 2008; Spencer-Segal et al., 2011). The expression of several genes involved in hippocampal function also undergoes dynamic changes throughout the estrous cycle (Iqbal et al., 2020). Collectively, these data emphasize the need to examine exercise-induced benefits separately in females and males.

As our previous study has established the exercise threshold that engages and maintains exercise-enhanced cognitive function in male mice (Butler et al., 2019), we utilized the same exercise paradigm to examine the temporal dynamics of exercise-induced benefits in female mice. In this study, we demonstrate that in female mice, 14d of voluntary exercise enables long-term memory formation under subthreshold acquisition conditions of the object-location memory (OLM) task. Similar to males (Butler et al., 2019), initial exercise-induced benefits are maintained through a 7d sedentary delay period and can be re-engaged by a brief 2d period of reactivating exercise to enable long-term memory formation under subthreshold acquisition conditions. OLM performance was not affected by phases of the estrous cycle at the time of training or test. However, voluntary wheel running activity was enhanced during the estrus phase. In addition, we demonstrate that exercise enhances LTP in the CA1 region of the hippocampus in females, and this effect persists even after a sedentary delay period, establishing the first evidence on the temporal endurance of exercise-induced neuroplasticity in females. To our surprise, assessment of hippocampal *Bdnf* expression following memory retrieval yielded no difference between experimental groups, suggesting persistent upregulation of BDNF may not be required to maintain exercise-induced cognitive benefits.

2. Materials and methods

2.1. Animals

Female, 8-week-old C57BL/6J mice (Jackson Laboratory) were individually housed under standard conditions (20 °C ± 1 °C; 70% ± 10% humidity; 12 h:12 h light and dark cycle) and provided *ad libitum* access to food and water. All experiments were conducted during the light phase. All experiments were conducted in accordance with the 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.

2.2. Exercise treatment

Mice were divided into groups (0–0–0: n = 8, 14–0–0: n = 7, 14–7–0: n = 8, 14–7–2; n = 8) and individually housed in either exercise cages (equipped with running wheel) or sedentary cages (standard cage). Exercise cages are made of 9.3 in × 13.9 in × 7.7 in (length × width × height) polycarbonate and equipped with a running wheel 40 cm in circumference, 12.7 cm diameter (Lafayette Instruments). Voluntary wheel running was monitored via an 86,110 Sensor/Counter plugged into a central interface connected to a dedicated PC with Scurry Activity Monitoring Software (Lafayette Instruments). Exercise parameters consist of an initial exercise period (0 or 14d) followed by a sedentary delay (0 or 7d), during which the running wheel was removed. Following this period of inactivity, some mice were assigned to receive a reactivating exercise session, consisting of 2d access to the running wheels (see Fig. 1).

2.3. Mouse estrous cycle stage identification

Phases of the estrous cycle were tracked and monitored around the same time (~10 am) daily, beginning on the first day of wheel running. Both visual assessment and vaginal smear were performed once a day as described by McLean et al. (2012) and Ajayi and Akhigbe (2020). Using a pipette, wet vaginal smears were taken in 5 uL of saline. To prevent pseudopregnancy effects (Adler & Zoloth, 1970), the pipette tip was carefully positioned to not penetrate the vaginal orifice. Collection fluid was placed on a glass slide and air-dried at RT. Once the smear was completely dry, slides were stained with 0.1% crystal violet stain (McLean et al., 2012). Cytology assessment was performed accordingly to identify phases of the estrous cycle (see Fig. 2E).

2.4. Subthreshold Object-Location memory (OLM) task

2.4.1. Apparatus—The subthreshold, 3-minute object-location memory (OLM) task was conducted using a set of 4 identical chambers. Each chamber was made of white-colored plastic with dimensions of 333 mm × 320 mm × 310 mm (length × width × height) and contained ~ 1 cm deep Sani-Chips (P. J. Murphy Forest Products). A vertical matte black marking strip was adhered to one side of each chamber to serve as the spatial navigation cue. Each context was illuminated by dim yellow light (~15 lx). Exploratory behavior was recorded and scored offline using ANY-maze tracking software (Stoelting Co.).

2.4.2. Experimental design—OLM training, testing, and analysis were performed as described by Vogel-Ciernia et al. (2015) with the acquisition duration adjusted to 3 min, which we have previously demonstrated to be subthreshold for encoding, resulting in poor performance in both short and long-term memory (Butler et al., 2019; Intlekofer et al., 2013; Malvaez et al., 2013; McQuown et al., 2011). Prior to OLM training, mice were handled and then habituated to the experimental context. Mice were handled for 2 min per day for 4 consecutive days with the last 2 days of handling overlapped with habituation. Habituation occurred over the course of 6 days, during which mice were exposed to the experimental context for 5 min per day. Habituation sessions were analyzed (to determine the distance traveled and speed) using ANY-maze behavioral analysis software. Reduced activity across days was used as an indicator for successful habituation (Supplementary

Fig. S1, S2). Regardless of the exercise regime, running wheels were removed the night prior to OLM training to eliminate the immediate effects of wheel running on behavior. Following habituation, mice received a 3 min acquisition session where two identical objects (100-mL glass beakers filled with cement) were placed in the distinct locations (location A1, upper left, and location A2, upper right). Objects were placed 9 cm away from each other, 6 cm from the sidewalls, and 1 cm from the front wall. To assess long-term memory, mice underwent a 5 min test, 24 h post-training during which one familiar object (counterbalanced) was moved to a novel location (location A3, bottom middle) positioned 2.5 cm from the bottom wall. Exploration of the object in the novel (location A3) vs. the familiar/fixed location (A1) was examined. Exploration was only scored when the mouse head pointed toward the object and came within 1 cm or when the nose touched the object. Total exploration time was recorded (t) and preference for the novel object was expressed as discrimination index (DI = ($t_{\text{novel}} - t_{\text{familiar}}$) / ($t$novel + t_{familiar}) × 100%). For training sessions, the object designated to be moved during the test session was used as the novel object to allow training and testing DI to be directly compared. Mice that explored <2 s during testing or training were excluded from the study. Mice that showed a preference for either object during training (DI > ± 20) were also excluded. All habituation, training, test, and scoring were performed by experimenters blinded to the experimental groups. Mice were sacrificed 60 min after the test and the dorsal hippocampi were dissected and stored at −80 °C until processing for RT-qPCR.

2.5. In vitro hippocampal slice preparation

Shortly following OLM acquisition, mice were anesthetized with isoflurane, decapitated, and the brains were quickly removed and submerged in ice-cold, oxygenated dissection medium containing (in mM): 124 NaCl, 3 KCl, 1.25 KH 2 PO 4, 5 MgSO 4, 0 CaCl 2, 26 NaHCO 3, and 10 glucose. Coronal hippocampal slices (340 μm) (n = 11 (0–0–0), 6 (14–0–0), 10 (14–7–0), 8 (14–7–2) slices from 6, 3, 5 and 5 mice) were prepared using a Leica vibrating tissue slicer (Model:VT1000S) before being transferred to an interface recording containing preheated artificial cerebrospinal fluid (aCSF) of the following composition (in mM): 124 NaCl, 3 KCl, 1.25, KH 2 PO 4, 1.5 MgSO 4, 2.5 CaCl 2, 26 NaHCO 3, and 10 glucose and maintained at 31 ± 10C. Slices were continuously perfused with this solution at a rate of 1.75–2 ml/min while the surface of the slices were exposed to warm, humidified 95% O2 / 5% CO2. Recordings began after at least 2 h of incubation.

Field excitatory postsynaptic potentials (fEPSPs) were recorded from CA1b stratum radiatum apical dendrites using a single glass pipette filled with 2 M NaCl (2–3 MΩ) in response to orthodromic stimulation (twisted nichrome wire, 65 μm diameter) of Schaffer collateral-commissural projections in CA1c stratum radiatum. Pulses were administered at 0.05 Hz using a current that elicited a 50% maximal spike-free response. After establishing a 10–20-minute stable baseline, long-term potentiation (LTP) was induced by delivering a single episode of 5 ‘theta’ bursts, each burst consisting of four pulses at 100 Hz and the bursts themselves separated by 200 ms (i.e., theta burst stimulation or TBS). The stimulation intensity was not increased during TBS.

The figures are presented as mean ± SEM. The fEPSP slope was measured at 10–90% fall of the slope and data in figures on LTP were normalized to the last 20 min of baseline. Electrophysiological measures were analyzed using a one-way ANOVA unless otherwise specified in the text and the level of significance was set at $p \leq 0.05$.

2.6. RT-qPCR

Dorsal hippocampus tissue was kept frozen at −80 °C until processing. RNA was isolated using RNeasy Mini Kits (Qiagen) according to the manufacturer's instructions and total RNA (50 ng) was reverse transcribed. cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Roche Applied Science). Primers were designed using the Roche Universal Probe Library; all primers were obtained from IDT and probes from the Roche Universal Probe Library and were used for multiplexing in the Roche Light-Cycle 480 II Machine (Roche). For *bdnf* exon VI transcript and *Hprt*, we designed a PrimeTime qPCR assay (IDT) as no Universal Probe Library assay was available. The following primers were used: *bdnf* exon I: forward primer (5′–3′): GCATCTGTTGGGGAGACAAG, reverse primer (5′–3′): TCACCTGGTG-GAACATTGTG, probe 56, *bdnf* exon IV: forward primer (5′–3′): GCTGCCTTGATGTTTACTTTGA, reverse primer (5′–3′): AAGGATGGT-CATCACTCTTCTCA, probe 31, *bdnf* exon VI: forward primer (5′–3′): CTGGGAGGCTTTGATGAGAC, reverse primer (5′–3′): GCCTTCATG-CAACCGAAGTA, probe: /56-FAM/AGGGACCAG/Zen/AAGCGTGA-CAACAAT/3IABkFQ/; *bdnf* exon IX: forward primer (5′–3′): GCCTTTGGAGCCTCCTCTAC, reverse primer (5′–3′): AAGGATGGT-CATCACTCTTCTCA, probe 31. All target probes were conjugated to the dye FAM. All values were normalized to *Hprt* expression, which used the following primers: forward primer (5′–3′): TGCTCGAGATGTCATGAAGG, reverse primer (5′–3′): CTTTTATGTCCCCCGTT-GAC, probe: /5HEX/AT CAC ATT G/Zen/T GGC CCT CTG T/3IABkFQ/. The normalization of values is adjusted to *Hprt* expression. Data and statistical analysis are done using Roche proprietary algorithms and REST 2009 software based on the Plaffl method (Pfaffl, 2001; Pfaffl et al., 2002).

2.7. Statistical analysis

Sample sizes in this study were similar to those generally used in the field, including those reported in previous publications (e.g. Butler et al., 2019; Keiser et al., 2021; Kwapis et al., 2018, 2020; López et al., 2019; Vogel-Ciernia et al., 2013) although no statistical methods were used to predetermine sample sizes. Statistical analyses were performed using either one-way ANOVA (Figs.1, 2D, 3B, 4, S1C–F, S2 C–F) or two-way ANOVA (Figs. 2A–C, S1A–B, S2 A–B) followed by Tukey- corrected t tests to compare individual groups. Simple planned comparisons to assess discrimination index (DI) scores were conducted within group to compare training and test DI using Student's *t* test (Fig. 1D). Two-way ANOVA had factors of Estrous Phase and Exercise Condition (Fig. 2A–B), Estrous Phase and Day (Fig. 2C) or Exercise Condition and Day (Fig. S1 A–B, S2 A–B). All statistics were performed with GraphPad Prism 8 software. Main effects and interactions for all ANOVA are described in the text. All analyses were two-tailed and required an α value of 0.05 for significance. Error bars in all figures represent SEM.

3. Results

3.1. Exercise enables long-term memory formation under subthreshold acquisition conditions in female mice

Previously we have demonstrated that 14d of exercise in male mice facilitates learning under subthreshold acquisition conditions of the OLM task which abolished following 7d of sedentary delay (Butler et al., 2019). Moreover, a subthreshold 2d of reactivating exercise after this sedentary delay period can re-engage initial benefits of 14d exercise to again induce the benefits on cognition. On a molecular level, exercise-induced hippocampal BDNF also diminishes significantly from 7 to 14d post-exercise and can be re-facilitated with a subsequent 2d bout of wheel running (Berchtold et al., 2005, Berchtold et al., 2010). Collectively, the exercise paradigm consisting of 14d of initial exercise, a 7d of sedentary delay, and 2d of reactivating exercise appears to capture the temporal dynamic of exercise-induced benefits in males. That is, the initial exercise engages cognitive benefits, which even though becomes dormant when exercise ceases, can be reactivated by a subsequent low-level exercise. Therefore, we examine whether similar exercise parameters also engage and maintain the cognitive benefits of exercise in females. We ran female mice through different exercise regimens that consist of an initial exercise (14d or 0d) followed by a sedentary delay (7d or 0d) and a reactivating exercise (2d or 0d) prior to OLM acquisition and test (Fig. 1A). To probe the beneficial effects of exercise on memory performance, mice received a 3-min subthreshold acquisition session during which the distinct locations (A1 and A2) of two identical objects were learned. In male mice, this training duration has been demonstrated as subthreshold for encoding in sedentary animals (Butler et al., 2019; Intlekofer et al., 2013; Malvaez et al., 2013; McQuown et al., 2011). On test day, one of the objects in the original locations was moved to a novel location (A3) and time spent exploring each object location was examined. Given that mice exhibit an innate preference for novelty, long-term OLM is evidenced by greater exploration of the novel object location compared to the object in the fixed location.

Exercise parameters affected memory performance on test day (Fig. 1D; one-way ANOVA, Group $F(3,27) = 7.66$, $p = 0.0007$), where 14 days of initial exercise (14–0–0) led to robust long-term memory performance relative to the sedentary control group (0–0–0) (Fig. 1D Tukey's post hoc test, $p = 0.003$). A 7-day sedentary delay resulted in diminished performance relative to 14–0–0 initial exercise group (Fig. 1D Tukey's post hoc test, $p = 0.007$) and a non-significant difference from control (0–0–0) (Fig. 1D Tukey's post hoc test, $p = 0.985$). To investigate whether the benefits of the initial exercise are maintained throughout the sedentary delay, a group of animals were subjected to 2d of reactivating exercise prior to OLM acquisition following a sedentary delay (14–7–2). Mice in the reactivating exercise cohort (14–7–2) displayed significantly higher DI scores compared to both sedentary control (0–0–0) (Fig. 1D Tukey's post hoc test, $p = 0.019$) and sedentary delay (14–7–0) cohorts (Fig. 1D Tukey's post hoc test, $p = 0.043$). Within-group comparisons (Fig. 1D, significance denoted with # symbol within bars) of the DI scores from acquisition day compared to test day reveal greater DI scores during test compared with training for only the 14–0–0 ($t(12) = 8.449$, $p = 0.0001$) and 14–7–2 groups ($t(14) = 4.991$, $p = 0.0002$), but not the 0–0–0 ($t(14) = 1.871$, $p = 0.082$) or 14–7–0 groups (t

(14) = 1.844, p = 0.086), further confirming successful long-term OLM formation only in the initial exercise (14–0–0) and reactivating exercise (14–7–2) cohorts. Test performance was not influenced by differences in object exploration time on test day (Fig. 1E; one-way ANOVA: F (3,27) = 2.264, p = 0.1038). Importantly, each exercise group habituated to the experimental context, measured by a significant reduction in distance traveled and mean speed across habituation sessions (Supplementary Fig. S1 A–B; Tukey's post hoc test habituation day 1 vs 6, $p < 0.05$ for 0–0–0, 14–7–0 and 14–7–2 groups; p = 0.07 for 14–0–0 group). During the acquisition session, mice in all groups exhibit relatively low and similar discrimination index (DI) scores, indicating a lack of preference for either object on training day in locations A1 and A2 (Fig. 1B; one-way ANOVA, DI: Group F (3,27) = 2.604, p = 0.0724). Overall object exploration was also similar between groups (Fig. 1C; one-way ANOVA, DI: Group F (3,27) = 1.243, p = 0.3137), indicating that object preference or differences in exploration do not contribute to observed differences in test performance. Together, these data demonstrate that 14d of an initial exercise enhances object-location memory formation under subthreshold acquisition conditions in female mice. This effect is maintained throughout the sedentary delay period and benefits can be re-engaged with a 2d reactivating exercise session.

3.2. Estrous phase affects voluntary wheel-running activity but not OLM performance

The estrous cycle of female rodents typically spans 4–5 days, during which reproductive hormones naturally fluctuate (Becker et al., 2017; Becker & Koob, 2016). Indeed, wheel running activity varies throughout the estrous cycle, suggesting the role of circulating sex hormones in modulating the physical activity of female rodents (Novak et al., 2012; Sherwin, 1998). Thus, we compare running distance across different phases of the estrous cycle to assess the role of estrous cycle on wheel running behavior. Regarding learning and memory, while some studies report no effect of estrous phase on behavioral performance of spatial memory tasks (Berry et al., 1997; Ter Horst et al., 2013; Keiser et al., 2017), others have suggested otherwise (Cordeira et al., 2018; Hokenson et al., 2021; Milad et al., 2009; Pompili et al., 2010; Tuscher et al., 2015; Warren & Juraska, 1997; Trask et al., 2020), including enhanced performance of the OLM task during proestrus and estrus phase (Frick & Berger-Sweeney, 2001; Frye et al., 2007; Paris & Frye, 2008; Pompili et al., 2010). Hence, we also compare OLM performance between estrous stages during acquisition and test days to identify any effects of estrous cycle on OLM performance within the group. Due to the low sample size, mice in proestrus and estrus phases were grouped together and mice in metestrus and diestrus were placed in another grouping in analysis of estrous on acquisition and retrieval. In analysis of running distance, daily running was graphed with estrous phases collapsed due to low sample size and analyzed with all phases separated when assessing mean running distance over the initial 14-day period.

An effect of estrous on running distance was not observed in assessments of daily running (Fig. 2A; two-way ANOVA, Phase F (1,63) = 0.10, p = 0.748; Phase × Day F (13,424) = 0.66, p = 0.798), but an effect of estrous phase was observed when estrous phases were separated and mean running distance was assessed (Fig. 2B; one-way ANOVA, Phase F (3,511) = 2.88, p = 0.035), where females in estrus ran significantly more than females in the diestrus (Fig. Tukey's post hoc test, p = 0.034), but not proestrus (p = 0.133) or

metestrus (p = 0.949) phases. Estrous phase on training (Fig. 2C; two-way ANOVA, Phase F (1,23) = 0.11, p = 0.738; Phase × Exercise Group F (3,23) = 0.70, p = 0.559) or test day (Fig. 2D; two-way ANOVA, Phase F (1,23) = 1.79, p = 0.193; Phase × Exercise Group F (3,23) = 0.20, p = 0.894) did not affect performance. Together, our data indicate that in line with other reports on estrous and running distance, mice in estrus results in greater overall running distance.

3.3. Exercise-enhanced hippocampal LTP is maintained throughout a period of inactivity

As shown above, our exercise paradigms facilitate long-term OLM following 14d of exercise and 2d of reactivating exercise. Hence, we asked whether hippocampal synaptic plasticity was also enhanced following exercise-facilitated learning in these same animals (Fig. 1A). Acute hippocampal slices were collected and processed to measure field excitatory postsynaptic potentials (fEPSP) recordings from the stratum radiatum of the CA1b in response to stimulation of Schaffer collateral-commissural projections in CA1c stratum radiatum. To examine changes in synaptic plasticity post-learning, we applied a single train of 5 theta-burst stimulation (TBS) to induce long-term potentiation (LTP) in slices collected during the memory consolidation window, 1 h after OLM acquisition. This BDNF-dependent form of stimulation has been previously reported to induce stable potentiation in mice (Acharya et al., 2019; Keiser et al., 2021; Kramár et al., 2004; Kwapis et al., 2018; Vogel-Ciernia et al., 2013; White et al., 2016). Twenty minutes post-TBS, fEPSP slope begins to stabilize as LTP enters the consolidation phase, during which dynamic synaptic events occur to maintain long-term synaptic strength (G. Lynch, 1998). In slices prepared from all mice, TBS produced immediate robust potentiation which decayed and then stabilized over the following 20 min (Fig. 3A). Stable LTP, measured 50–60 min post-TBS, was observed in exercise conditions (Fig. 3B; one-way ANOVA F (3,31) = 9.32, p = 0.0002) where all groups that underwent exercise displayed an increase in mean potentiation relative to sedentary control (14–0–0: p = 0.003, 14–7–0: p = 0.0002, 14–7–2: p = 0.033), indicating enhanced synaptic plasticity. To examine whether this synaptic strengthening was due to changes in baseline neuronal function within the hippocampus, we generated input/output curves and measured changes in paired-pulse facilitation. There was no significant difference between groups in the slope of the curves for fEPSP slope responses relative to fiber volley magnitude (Fig. 3C; one-way ANOVA F (3,31) = 0.35, p = 0.79) or paired-pulse facilitation (Fig. 3D; two-way ANOVA, group: F (3,31) = 1.49, p = 0.24, interaction: F (6,62) 1.70, p = 0.13). Together, these data suggest that an initial exercise period enhances hippocampal synaptic plasticity that persists even after exercise cessation and following a reactivating exercise session without altering baseline neuronal functions. It is worth noting that this long-lasting enhancement of hippocampal LTP is inconsistent with diminished OLM performance observed following the sedentary delay, which we address in the discussion.

3.4. Hippocampal BDNF is not elevated following OLM retrieval

BDNF signaling is a crucial mechanism underlying improved hippocampal synaptic plasticity and learning by exercise (Cotman et al., 2007; Intlekofer et al., 2013; S. Vaynman et al., 2004; S. S. Vaynman et al., 2006). Hence, we examine the hippocampal expression of *Bdnf* mRNA during retrieval, 1 h following behavioral testing. We decided to assess BDNF

levels at this time point for two main reasons. First, this time period following retrieval involves up-regulation of a number of immediate early genes (IEGs) in dorsal hippocampus (Keiser et al., 2017). Second, given the novel object location placement during the test session, new learning might also occur. Hence, 1 h post learning represents part of the consolidation window involving cellular consolidation and stabilization of a memory trace (McGaugh, 2000), during which IEGs crucial for consolidation are highly upregulated (e.g., Lonergan et al., 2010; Radulovic et al., 1998). Third, it is possible that the novel object location might be incorporated into the previous memory trace through reconsolidation rather than new learning (Kwapis et al., 2020). BDNF has been shown to be necessary for reconsolidation (Radiske et al., 2015), and BDNF protein levels are significantly elevated 3–4 h following retrieval (Radiske et al., 2015, Radiske et al., 2017). Hence, it is possible that upregulation of *Bdnf* mRNA might occur at an earlier timepoint. Collectively, we reasoned that 1 h following retrieval will best capture changes in BDNF expression.

In mice, the *Bdnf* gene consists of a 3′ common coding exon (exon IX) and eight 5′ noncoding exons (exon I-VIII) (Aid et al., 2007). Each 5′ untranslated noncoding exon is spliced to the 3′ common exon to generate multiple *Bdnf* transcripts (Aid et al., 2007), making exon IX a marker for total *Bdnf* transcripts. Exercise also selectively upregulates hippocampal Bdnf exon I, IV, and VI (Baj et al., 2012; Intlekofer et al., 2013; Sleiman et al., 2016); therefore, we assessed the hippocampal expression of these Bdnf exons. Different exercise regimes do not differentially affect the hippocampal expression of Bdnf exon XI (Fig. 4D; one-way ANOVA, $F(3,25) = 0.18$, $p = 0.90$), exon I (Fig. 4A; one-way ANOVA, $F(3,26) = 0.90$, $p = 0.45$), exon IV (Fig. 4B; one-way ANOVA, $F(3,26) = 0.03$, $p = 0.99$), or exon VI (Fig. 4C; one-way ANOVA, $F(3,26) = 0.57$, $p = 0.63$).

4. Discussion

The present study examined the effects of exercise on hippocampus-dependent learning and synaptic plasticity in female mice. We identified that a voluntary exercise period of 14d enables learning and long-term memory formation under subthreshold acquisition conditions of the hippocampus-dependent OLM task. Following the initial exercise, a 7d sedentary delay resulted in diminished performance, which was re-facilitated when animals received 2d of reactivating exercise following the sedentary delay. Female mice exhibited elevated running activity during the estrus phase relative to the diestrus phase, and no effect of estrous phase was observed on OLM performance. On a synaptic level, exercise facilitated LTP in the CA1 region of the hippocampus, which persisted throughout the sedentary delay and following the reactivating exercise session. Assessment of hippocampal expression of *Bdnf* following behavioral testing reveals no differences between groups. Together, our study establishes evidence that voluntary exercise can engage and maintain exercise-induced learning and neuroplasticity benefits in females.

Our data provide evidence for exercise-enhanced learning in female mice, findings that are consistent with previous reports demonstrating the ability of exercise to improve the performance of female animals in hippocampus-dependent short-term (Kim et al., 2010; Marosi et al., 2012; Siette et al., 2013) and long-term memory tasks (Aguiar et al., 2011; Harburger et al., 2007; Kim et al., 2010; Marlatt et al., 2012; Marosi et al., 2012; Van

Praag, Christie, et al., 1999). The majority of studies examining the effects of exercise on hippocampus-dependent learning in female rodents report a longer exercise duration, which can range from 6 wks to 8 months (see Barha et al., 2017; Uysal et al., 2014). Therefore, our data suggest a lower exercise duration, such as 2 wks (14d), is sufficient to induce enduring benefits on learning and memory in females. This is congruent with our previous study in male mice (Butler et al., 2019) that also reports 2 wks of voluntary exercise is the threshold for exercise-facilitated learning, whereas 1 wk or 2d of exercise alone fail to induce cognitive enhancement. Our study did not examine the effect of either 1 wk or 2d of exercise on cognition in females, thus, it remains a possibility that an even lower exercise duration can initiate cognitive benefits in females. In addition to the benefits induced by an initial 14d voluntary exercise session, an improvement in memory performance was not observed in animals that underwent a 7d sedentary delay following exercise. However, a 2d reactivating exercise following the sedentary delay re-facilitated learning, suggesting that exercise-induced cognitive benefits can be maintained and persist throughout the sedentary delay period and can be reactivated by re-exposing the animals to a lower exercise duration. This is similar to findings from Butler et al., 2019 using male mice, which demonstrate a 2d reactivating exercise session can re-facilitate learning following a 7d sedentary delay. Future studies will need to investigate whether 2d of exercise alone, without an initial bout of exercise can engage benefits on cognition. Furthermore, Butler et al., 2019 also shows that re-engagement of exercise-induced benefits is not observed following 14d of sedentary delay, proposing the hypothesis that exercise initiates a “temporal memory window” during which a subsequent subthreshold exercise session can capitalize on the neuronal adaptation established by the initial exercise to facilitate learning. In our study, we did not further investigate whether 2d of reactivating exercise can still re-facilitate the benefits of exercise after 14d of sedentary delay following exercise in females. Therefore, future studies can use a longer sedentary period to examine the extent of this “temporal memory window” in females. Together, our findings indicate that 14d of voluntary exercise can facilitate long-term memory formation under subthreshold acquisition conditions of the hippocampus-dependent OLM task, and these benefits are maintained throughout the 7d sedentary delay and can be re-engaged by 2d of reactivating exercise.

Our data also indicates similar running activity across phases of the estrous cycle with a significant increase in the total running distance during estrus relative to diestrus. In female rodents, the estrous cycle consists of a follicular phase (termed metestrus and diestrus), during which estradiol level gradually increases. Peak estradiol levels occur when animals are in the preovulatory phase (termed proestrus) and is followed by a surge in progesterone as animals enter the estrus phase, during which the animals are behaviorally receptive (Becker et al., 2017; Becker & Koob, 2016; Nilsson et al., 2015). This hormonal oscillation generates a day-to-day variability in exercise activity. Previous reports have shown variability in exercise engagement across the estrous cycle, with the highest running activity occurring during the proestrus phase when estrogen levels peak (Aguiar et al., 2018; Anantharaman-Barr & Decombaz, 1989; Manzanares et al., 2018). In ovariectomized rats, daily wheel running is significantly reduced and is restored with estrogen replacement, suggesting the role of estrogen in modulating exercise performance in females (Berchtold et al., 2001). Hence, our findings showing elevated wheel running

during estrus are inconsistent with these reports, given estradiol level is low during the estrus phase (Nilsson et al., 2015). Our data, however, are in line with other studies that observe increased wheel running during the estrus phase (Dixon et al., 2003; Eckel et al., 2000). An earlier study by Steiner et al., (1981) indicates peak wheel running occurred during the night between proestrus and estrus, suggesting a specific time point at which animals exhibit elevated activity rather than an effect of a singular estrous phase. Therefore, it is possible that inconsistent results might be due to variability in the time points at which estrous is examined between studies. In this study, estrous assessment was performed when animals are in the light phase (10 a.m ± 1 h). Given that mice are more active at night, and the transition between estrous stages can occur rapidly, our cytology data might not capture this transition during the dark phase, and thus do not fully represent the running activity throughout the 24 h period. Hence, future studies can assess the estrous stage of the animals closer to or during the dark phase when animal activity culminates to best represent daily exercise performance.

Fluctuation in the level of estradiol modulates hippocampal physiology in females. Throughout the estrous cycle, dendritic morphology, and spine density of CA1 pyramidal neurons vary, with spine density being highest during proestrus (González-Burgos et al., 2005; Gould et al., 1990; Kato et al., 2013; Prange-Kiel et al., 2008; Woolley et al., 1990; Woolley & McEwen, 1992). Alterations in hippocampal synaptic density are dependent on hippocampal estradiol synthesis (Prange-Kiel et al., 2009), which positively correlates with the plasma estradiol levels throughout the estrous cycle (Kato et al., 2013). Proestrus is also associated with enhanced LTP in CA1 neurons in response to Schaffer collateral inputs (Bi et al., 2001; Good et al., 1999; Warren et al., 1995), which may be mediated by changes in sensitivity to glutamate and GABA (Hamson et al., 2016). Jointly, these data provide the role of endogenous estradiol in modulating hippocampal network activity (Hamson et al., 2016), which might explain different behavioral performances throughout the estrous cycle when estrogen levels fluctuate (Triviño-Paredes et al., 2016; Walf et al., 2006; Warren & Juraska, 1997). In our study, when correlating estrous stages on either acquisition or test day with OLM performance within treatment groups, we did not see a significant effect of estrous on OLM performance. Given our sample size is small and the number of mice in a certain estrous phase were at random, future studies can improve the statistical power by increasing the sample size to allow for multiple animals in each estrous phase at the time of acquisition and test.

Learning engages dynamic neuronal events to facilitate neuronal communication at the synapse through a process termed long-term potentiation (LTP). Therefore, the induction and maintenance of LTP in the hippocampus is crucial for learning and memory consolidation (Elgersma & Silva, 1999; M. A. Lynch, 2004; Pastalkova et al., 2006). Previous studies demonstrate that enhanced hippocampal LTP is associated with improved cognitive function (e.g Keiser et al., 2021; Kwapis et al., 2018; Tang et al., 1999). In congruence with these studies, our findings show that 14d of voluntary exercise enhanced LTP in the CA1 subfield of the hippocampus relative to sedentary control, which corresponds to our behavioral findings of enhanced long-term memory formation in subthreshold acquisition conditions with exercise. The number of studies investigating exercise-mediated synaptic plasticity in the CA1 region is sparse, especially in females and

often provide conflicting results. In male rodents, exercise reverses impaired LTP in CA1 caused by stress (Dahlin et al., 2019), sleep deprivation (Zagaar et al., 2012, Zagaar et al., 2013), and in mouse models of Alzheimer's Disease (Dao et al., 2013, Dao et al., 2015). In females, exercise also attenuates LTP impairments in sleep-deprived rats (Saadati et al., 2014, 2015). However, in these studies, exercise in healthy controls does not further enhance CA1 LTP relative to sedentary controls, suggesting that exercise only exerts facilitated LTP in the CA1 region under aberrant conditions. Contrary to these reports and in line with our findings, recent studies in male rodents report enhanced CA1 LTP in healthy animals following exercise compared to sedentary control (D'Arcangelo et al., 2017; Ivy et al., 2020; Tsai et al., 2018). Considering the discrepancy between these studies, it is possible that variability in the type, length, and intensity of the exercise paradigms might contribute to different results. For instance, many of these studies use forced exercise (Dao et al., 2013, 2015; D'Arcangelo et al., 2017; Saadati et al., 2015; Tsai et al., 2018; Zagaar et al., 2012, Zagaar et al., 2013) with varied protocols, while only a couple studies employ voluntary wheel running (Ivy et al., 2020; Saadati et al., 2014) with different exercise duration. Provided that voluntary and forced exercise are not equivalent in their methodologies, and thus might differentially modulate hippocampal neurogenesis, neurotrophic factors, synaptic plasticity markers, and behavior (Barha et al., 2017; Leasure & Jones, 2008), it becomes necessary for future exercise research to be more homogeneous in exercise protocols used to allow for comparable results. Apart from this point, differences in age and strain of the experimental animals might also suggest different amounts of exercise required to engage certain benefits of exercise, further contributing to the dynamic regulation of exercise-mediated benefits. Altogether, our study is the first to show 2wks of voluntary exercise can indeed facilitate LTP in the CA1 region of the hippocampus in female mice.

In addition to the ability of exercise to enhance synaptic plasticity in the CA1 subfield of the hippocampus, we also demonstrate the persistence of an enhancement of LTP following exercise cessation, establishing the first evidence for the temporal endurance of exercise-induced benefits on neuroplasticity in females. The finding of long-lasting enhancement of LTP is surprising given diminished OLM performance following the sedentary delay. Given that our stimulation protocol consists of 5 theta-bursts and has been shown to induce stable potentiation in sedentary mice (Acharya et al., 2019; Keiser et al., 2021; Kwapis et al., 2018; Vogel-Ciernia et al., 2013; White et al., 2016), it is possible that a subthreshold stimulation consists of 3 theta-bursts (López et al., 2016), which may mimic the hippocampal activity during the subthreshold acquisition conditions more closely, might be able to capture the absence of exercise-induced benefits on the synaptic levels during the sedentary delay. It is worth noting that LTP was recorded from the hippocampal tissues collected 1 h following subthreshold OLM acquisition, during which synaptic plasticity-related products are upregulated (Carulli et al., 2011; Irvine et al., 2006; Wang & Peng, 2016), which might compensate for the reduction and/or loss of factors required for LTP enhancement that occur during the sedentary delay. Therefore, LTP recordings from mice that did not go through subthreshold OLM acquisition might yield results that are more in line with our behavioral data. Nevertheless, the persistence of enhanced LTP in female mice during the sedentary delay relative to control suggests the maintenance of benefits on synaptic functions from the initial exercise.

Exercise induces the hippocampal expression of brain-derived neurotrophic factor (BDNF) to facilitate neuroplasticity and learning (Cotman et al., 2007; Cotman & Engesser-Cesar, 2002; Loprinzi & Frith, 2018; S. Vaynman et al., 2004). BDNF signaling promotes structural synaptic alteration that in turn, modulates synaptic transmission and LTP (Kramár et al., 2004; Lu & Chow, 1999; Soulé et al., 2006). Expression of BDNF is crucial for long-term memory formation (Bekinschtein et al., 2008; Lubin, 2011), and BDNF induction is required for cognitive enhancement by exercise (Gomez-Pinilla et al., 2008; Intlekofer et al., 2013). Together, BDNF is a potential mechanism underlying the maintenance of exercise-induced benefits in females. To our surprise, *Bdnf mRNA* expression from hippocampal tissue collected 1 h post-OLM test was not significantly different between exercise conditions and sedentary control, rendering our findings inconsistent with other reports that show significant induction of hippocampal *Bdnf mRNA* following voluntary exercise in females (Berchtold et al., 2001; Gallego et al., 2015; Uysal et al., 2014). Our findings of exercise enhanced LTP despite the absence of hippocampal *Bdnf* upregulation are in line with that of Titterness et al., (2011), which provides evidence that exercise can enhance DG LTP in male rats and reduce the induction threshold for DG LTP in females in the absence of BDNF upregulation. In fact, time-course assessment of *Bdnf mRNA* following voluntary exercise in male rats reveals selective upregulation of *Bdnf* exons only following 1d and 28d of exercise but not 14d of exercise (Adlard et al., 2004), which is congruent with our observation of the lack of change in the hippocampal *Bdnf mRNA* expression following 14d of exercise in female mice. On the protein level, hippocampal BDNF in male rats is reported to be elevated with 7d of exercise and returns to baseline following 14d of exercise (Adlard et al., 2005). With long-term exercise, enhanced levels of mature BDNF are observed with 8 months of voluntary exercise (Marlatt et al., 2012) but not 5 months of exercise (Venezia et al., 2016). Though we did not examine hippocampal BDNF protein levels in our study, our data, along with aforementioned studies, suggest that *persistent* upregulation of BDNF might not be required to maintain the benefits of exercise, further supporting the dynamic mechanisms underlying exercise-induced benefits. It is also worth noting that hippocampal tissue was collected for *Bdnf* quantification during memory retrieval, 1 h after OLM test. It is possible that absence of BDNF upregulation between groups is due to learning induced *Bdnf* (Hall et al., 2000). This time point also represents 2d following the completion of the exercise schedule, thus, elevated *Bdnf* levels might have returned to baseline after animals stop exercising. Future studies can assess the time-course of BDNF expression in female hippocampus following exercise to further our understanding of how exercise-induced benefits are maintained across time.

To our knowledge, this is the first study to examine the temporal dynamics of exercise on neuroplasticity and cognition in female mice. These data contribute to the hypothesis of a “molecular memory window” of exercise-induced benefits established by an exercise threshold, during which benefits on neuroplasticity and cognition are maintained and ready for reactivation by future exercise stimuli. Our data also propose that exercise-induced benefits and the underlying mechanisms might be different between distinct subregions of the hippocampus. Given the growing body of evidence highlighting the involvement of epigenetic regulation in exercise-mediated synaptic plasticity and cognition (Fernandes et al., 2017; Intlekofer et al., 2013; Keiser & Wood, 2019), it is possible that exercise-induced

benefits are primed and maintained by epigenetic mechanisms. Hence, additional work is required to understand how exercise modulates and maintains cognitive benefits in both sexes. This includes examining the extent of the "molecular memory window" of exercise in females and the underlying mechanisms in both sexes to develop exercise regimens that optimally benefit brain health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

OLM	object-location memory
LTP	long-term potentiation
BDNF	brain-derived neurotrophic factor
DI	discrimination index
DG	dentate gyrus

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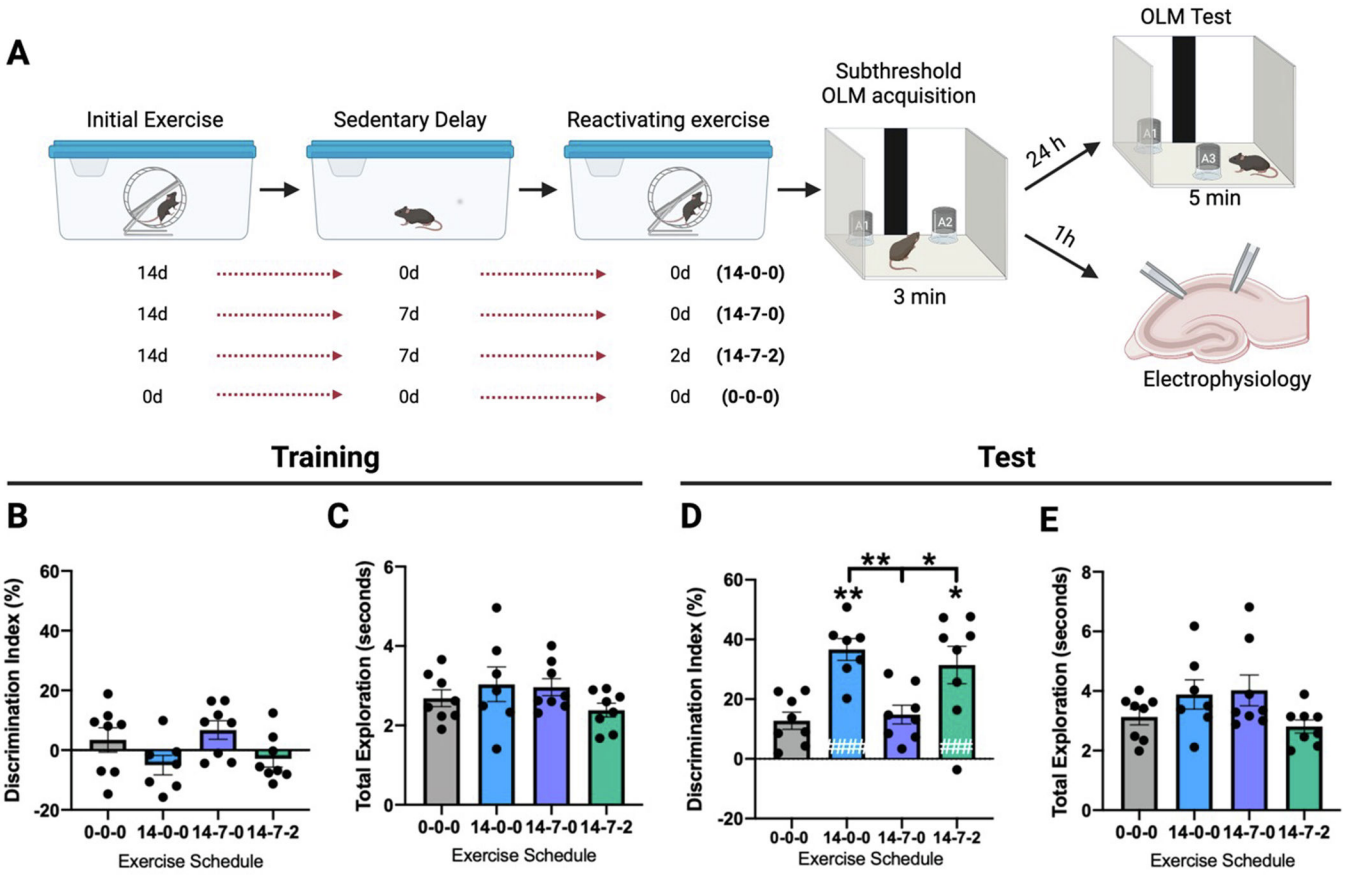

Fig. 1. Exercise enables long-term memory formation under subthreshold acquisition conditions in female mice.

(A) Schematic of different exercise regimes prior to OLM and electrophysiology. **(B)** Discrimination index (DI) score during the 3 min subthreshold OLM acquisition. **(C)** Total amount of time in seconds exploring objects during acquisition. Mice in all groups display low DI, indicating no preference for either object-location at training. **(D)** DI scores during test. Mice in 14–0–0 and 14–7–2 exhibit enhanced performance compared to 0–0–0 and 14–7–0. (**E)** Total amount of time in seconds exploring objects during test. Data are presented as mean ± SEM; (0–0–0: n = 8, 14–0–0: n = 7, 14–7–0: n = 8, 14–7–2; n = 8), ### $p < 0.001$, #### $p < 0.0001$ compared with acquisition session (within group). * $p < 0.05$, ** $p < 0.01$ compared to original object location.

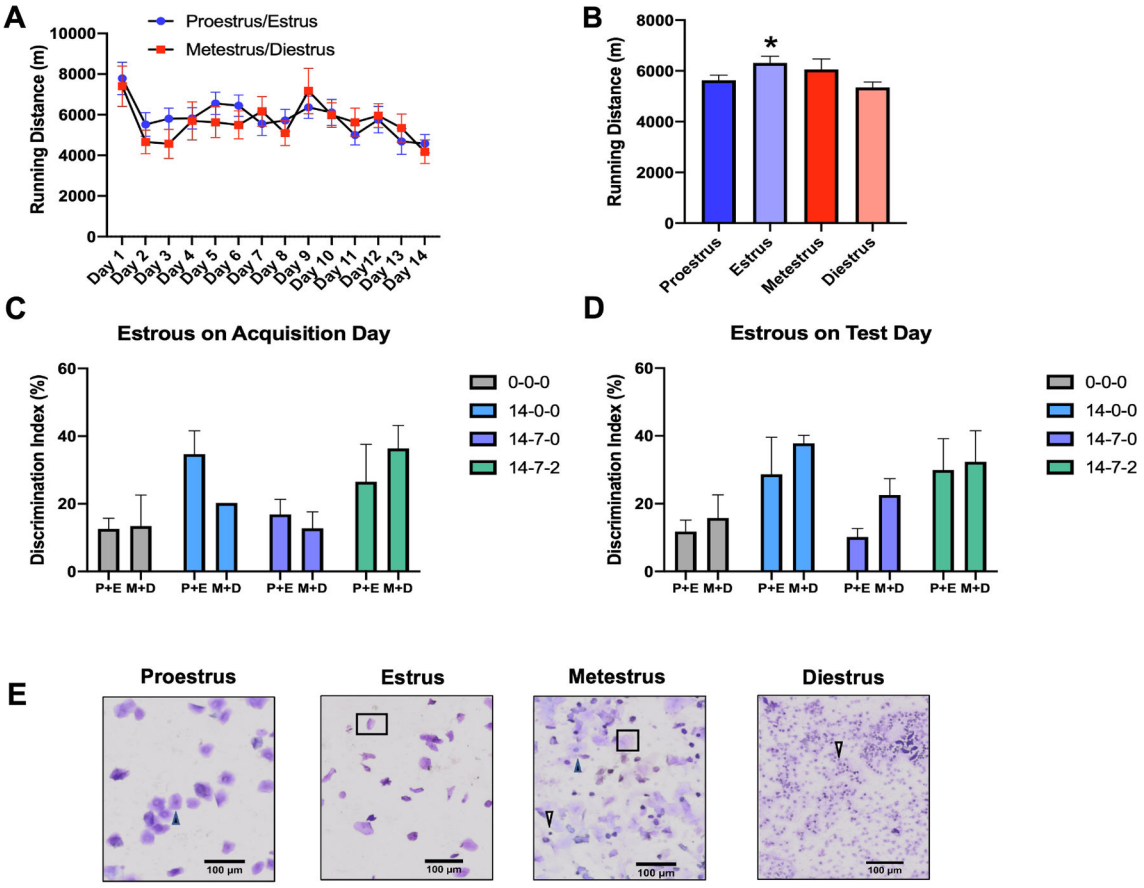

Fig. 2. Estrous cyclicity affects voluntary wheel-running activity but not OLM performance.

(A) Daily running distance in meters throughout the first 14d of the experiment with Proestrus/Estrus combined and Metestrus/Diestrus combined. **(B)** Average total daily running distance in meters separated by estrous phase. Mice in estrus phase show enhanced running distance relative to those in diestrus. **(C-D)** Discrimination index (DI) scores on test day stratified by estrous phase on acquisition **(C)** and test **(D)** day. Proestrus (P) and Estrus (E) are combined and Metestrus (M) and Diestrus (D) are combined similar to **A**. (**E**) Representative images of vaginal smears during (from left to right) proestrus, estrus, metestrus, and diestrus. The cell types are identified as follows: nucleated epithelial cells (black arrow), cornified epithelial cells (black box), and leukocytes (white arrow). * $p < 0.05$ compared to diestrus.

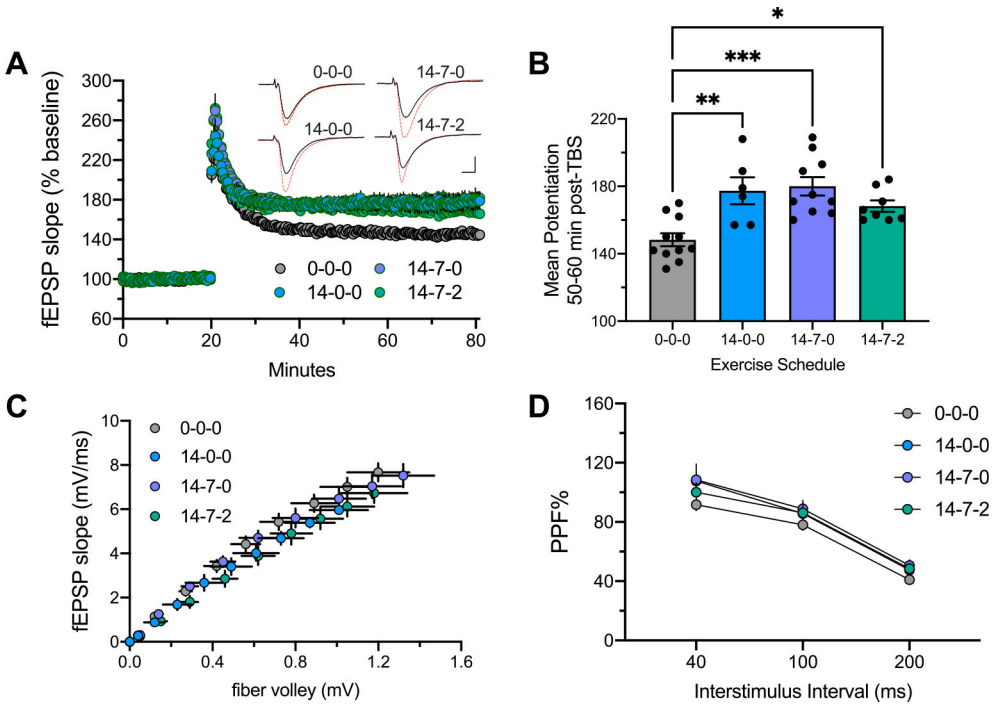

Fig. 3. Exercise-enhanced hippocampal LTP is maintained throughout a period of inactivity.

(A) Extracellular field recordings following stimulation of the Schaffer-commissural projections to the proximal apical dendrites of the CA1b field of the dorsal hippocampus after exercise and 1 h following subthreshold OLM acquisition. Following a stable 20-minute baseline recording, a single train of TBS was applied, and baseline recordings were resumed for an additional 60 min. The time course shows theta burst stimulation (TBS)-induced LTP was significantly enhanced in slices from 14–0–0, 14–7–0, and 14–7–2 compared with slices from sedentary control (0–0–0). Inset, representative traces collected during baseline (black line) and 60-minute post-TBS (red line). **(B)** Summary graph showing mean fEPSP slope 50–60 min after stimulation. Potentiation was significantly higher in slices from 14–0–0, 14–7–0, and 14–7–2 compared with 0–0–0 cohort. **(C)** Plot of fEPSP slope against corresponding fiber volley amplitude reveals no difference between groups. **(D)** Paired-pulse facilitation (PPF) was comparable between all groups. Data are presented as mean ± SEM; (n = 11 (0–0–0), 6 (14–0–0), 10 (14–7–0), 8 (14–7–2) slices from 6, 3, 5 and 5 mice). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared to control.

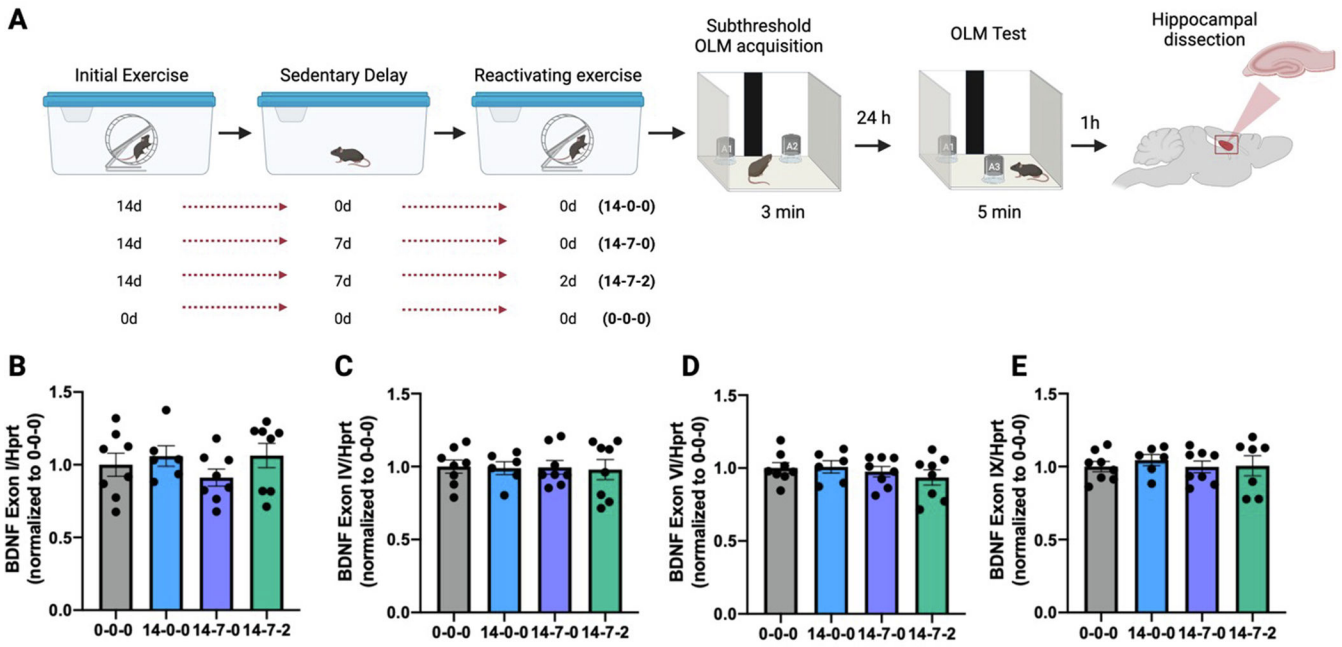

Fig. 4. Hippocampal *bdnf* is not enhanced following OLM retrieval.

(A) Schematic of the time point at which the hippocampus was dissected for Bdnf quantification. **(B-E)** Quantification of hippocampal expression of *bdnf* mRNA associated with **(B)** exon I, **(C)** exon IV, **(D)** exon VI, and **(E)** exon IX. Data are presented as mean ± SEM; (0–0–0: n = 8, 14–0–0: n = 6, 14–7–0: n = 8, 14–7–2; n = 8).