Effects of different exercise modes and intensities on cognitive performance, adult hippocampal neurogenesis, and synaptic plasticity in mice

Jiang, Hanlin ; Kimura, Yusuke ; Inoue, Shota ; Li, Changxin ; Hatakeyama, Junpei ; Wakayama, Masahiro ; Takamura, Daisuke ; Moriyama…

(Citation)
Experimental Brain Research,242(7):1709-1719

(Issue Date)
2024-07

(Resource Type)
journal article

(Version)
Accepted Manuscript

Effects of different exercise modes and intensities on cognitive performance, adult hippocampal neurogenesis, and synaptic plasticity in mice

Hanlin Jiang [a], Yusuke Kimura [a], Shota Inoue [a], Changxin Li [a,b], Junpei Hatakeyama [a], Masahiro Wakayama [a], Daisuke Takamura [a, c], Hideki Moriyama [d*]

[a] Department of Rehabilitation Science, Graduate School of Health Sciences, Kobe University, Kobe, Japan

[b] Department of Rehabilitation, Affiliated Hospital of Zunyi Medical University, Zun Yi, China

[c] Department of Rehabilitation, Kobe City Medical Center General Hospital, Kobe, Japan

[d] Life and Medical Sciences Area, Health Sciences Discipline, Kobe University, Kobe, Japan

[*] **Correspondence:**

Hideki MORIYAMA, Ph.D.

Professor

Life and Medical Sciences Area, Health Sciences Discipline, Kobe University

Tomogaoka 7-10-2, Suma-ku, Kobe, Hyogo, 654-0142, Japan

Tel & Fax +81 78 796 4574

E-mail morihide@harbor.kobe-u.ac.jp

Abstract

Exercise can induce beneficial improvements in cognition. However, the effects of different modes and intensities of exercise have yet to be explored in detail. This study aimed to identify the effects of different exercise modes (aerobic and resistance) and intensities (low and high) on cognitive performance, adult hippocampal neurogenesis and synaptic plasticity in mice. A total of 40 C57BL/6J mice were randomised into 5 groups (n = 8 mice per group): control, low-intensity aerobic exercise, high-intensity aerobic exercise, low-intensity resistance exercise, and high-intensity resistance exercise. The aerobic exercise groups underwent treadmill training, while the resistance exercise groups underwent ladder climbing training. At the end of the exercise period, cognitive performance was assessed by the Y-maze and Barnes maze. In addition, adult hippocampal neurogenesis was evaluated immunohistochemically by 5-bromo-2'-deoxyuridine (BrdU)/ neuronal nuclei (NeuN) co-labeling. The levels of synaptic plasticity-related proteins in the hippocampus, including synaptophysin (SYP) and postsynaptic density protein 95 (PSD-95), were analyzed by western blotting. Our results showed no significant differences in cognitive performance among the groups. However, high-intensity aerobic exercise significantly increased hippocampal adult neurogenesis relative to the control. A trend towards increased adult neurogenesis was observed in the low-intensity aerobic group compared to the control group. No significant changes in synaptic plasticity were observed among all groups. Our results indicate that high-intensity aerobic exercise may be the most potent stimulator of adult hippocampal neurogenesis.

Keywords: Cognitive function, Aerobic exercise, Resistance exercise, Hippocampus, Adult neurogenesis, Mice

Introduction

The cognitive benefits of exercise are well-documented, yet identifying the optimal conditions to maximize these effects remains unclear (Mandolesi et al. 2018; Biazus-Sehn et al. 2020). Human studies face the challenges in isolating exercise effects from variables such as diet, sleep, and lifestyle. Additionally, the difficulty of conducting hippocampal biochemical analyses—a key area for cognition—complicates understanding potential benefits. Utilizing animal models, especially rodents, offers a solution. These models replicate human hippocampal anatomy and physiological responses to exercise, providing a framework for examining the mechanisms behind exercise-induced cognitive improvements.

Aerobic and resistance exercises are distinguished as 2 primary modes of exercise in enhancing cognitive function (Mandolesi et al. 2018; Biazus-Sehn et al. 2020). Aerobic exercise refers to activities such as walking or cycling that involve continuous, rhythmical muscle contractions. In contrast, resistance exercise uses muscle power to lift weights against resistance. While both modes are associated with cognitive performance improvements, the extent and mechanisms of their effectiveness are subjects of ongoing debate (van Uffelen et al. 2008). A research in animal models indicates that aerobic exercise may significantly enhance cognition (Lan et al. 2018). However, contrasting findings indicate that the cognitive benefits of exercise cannot be solely attributed to exercise mode; the intensity of the activity also plays a crucial role (Shih et al. 2013; Wang and Wang 2016). These conflicting results underline the importance of a comprehensive approach that evaluates both exercise mode and intensity.

Exercise-induced cognitive improvement is mainly mediated by increased adult neurogenesis and hippocampal synaptic plasticity (Vivar et al. 2012; Voss et al. 2013), with the upregulation of neurotrophic factors such as brain-derived neurotrophic factors (BDNF) and Insulin-like Growth Factor 1 (IGF-1) playing a pivotal role. These factors not only support the proliferation of neurons but also enhance the efficacy of synaptic connections through the regulation of proteins such as Synaptophysin (SYP) and postsynaptic density protein 95 (PSD-95), which are crucial for synaptic plasticity (Savioz et al. 2014; Nie and Yang 2017). The precise aerobic exercise intensity optimal for adult neurogenesis remains to be clarified. While low-intensity exercise is generally considered beneficial (Lou et al. 2008), the evidence also supports that high-intensity exercise elevates the expression of some key proteins (Cefis et al. 2019). Conversely, the impact of resistance exercise on neurogenesis is yet to be conclusively determined, as studies have yielded inconsistent

findings (Nokia et al. 2016; Codina-Martínez et al. 2020). Moreover, while the influence of aerobic exercise on the levels of synaptic proteins such as SYP and PSD-95 has been explored, findings remain contradictory (Fang et al. 2013; Segabinazi et al. 2020). The impact of resistance exercise is not well-established (Fernandes et al. 2016; Segabinazi et al. 2020). Consequently, further research is imperative to ascertain the effects of exercise mode and intensity on adult neurogenesis and synaptic plasticity (Shih et al. 2013).

Our study aimed to elucidate the impacts of different exercise modes and intensities on cognitive performance, hippocampal neurogenesis, and synaptic plasticity in mice. We employed behavioral assessments through Y-maze and Barnes maze tests, quantified neurogenesis in the adult hippocampus via immunohistochemical techniques, and determined the levels of synaptic proteins SYP and PSD-95 through Western blotting.

Materials and Methods

Experimental design and animal care

A total of 40 male C57BL/6J mice (7 weeks old) were obtained from Japan SLC (SLC, Japan). The mice were housed in standard cages under a 12 h dark/light cycle at a constant temperature of 22 ± 1°C. All experimental procedures were examined by the Institutional Animal Care and Use Committee, approved by the president of Kobe University, and performed according to the Kobe University Animal Experimentation Regulations (approval number: P190814).

After a one-week adaptation to the laboratory environment, the mice were randomly divided into 5 groups (n = 8 per group): control, low-intensity aerobic exercise, high-intensity aerobic exercise, low-intensity resistance exercise and high-intensity resistance exercise. The aerobic exercise groups underwent treadmill training, while the resistance exercise groups underwent ladder climbing training. The control group did not participate in any exercise. These exercise protocols were implemented across a duration of 37 days. To assess the proliferation phase of neurogenesis, 4 mice in each group were randomly selected for histological analysis and received daily injections of 5-bromo-2'-deoxyuridine (BrdU, 50 mg/kg) during the first 10 days of the exercise regimen. The remaining four mice in each group were allocated for western blotting analysis. During the final week of the exercise period, cognitive performance assessments were administered to all mice. The whole experimental design is shown in Fig. 1A.

Aerobic exercise protocols for low and high intensities

An MK-680 treadmill device (Muromachi kikai Co, Ltd., Japan) with no incline was used for the aerobic exercise. During a five-day acclimatization period, the mice were first exposed to a 15-minute rest on the treadmill. The treadmill speed was maintained at 8 m/min for four days. The daily running time was increased in 15-minute increments to a maximum of 60 minutes (Fig. 1B). After a 3-day rest post-acclimatization, all aerobic exercise mice underwent treadmill treatment for 60 minutes each day for 37 days. The continuous daily training was designed to investigate the long-term effects of exercise on animals, in accordance with our established methodologies (Inoue et al. 2023). The 37-day training was chosen to ensure the functional incorporation of newborn neurons into the hippocampal circuitry, a process that takes approximately 4-5 weeks to complete (Bettio et al. 2019). This period is crucial for assessing the long-term effects of exercise on brain health, including the changes in neuronal survival, synaptic plasticity, and cognitive performance. Previous research identified the lactate threshold for untrained normal C57BL/6 mice as 17.8 m/min (Billat et al. 2005). In our study, the treadmill speed for the low-intensity aerobic exercise group was set at 12 m/min, typically below the average lactate threshold for mice. On the other hand, the speed for the high-intensity group was set at 20 m/min, which may exceed the average threshold. In response to variability in individual lactate thresholds, as highlighted by recent researches (Constans et al. 2021; Hugues et al. 2022), we recognize that our uniform speed protocol does not fully accommodate individual physiological differences. Thus, while our study employs terms such as low or high intensity, these should be considered relative within the spectrum of potential exercise intensities. Electrical stimulation of the treadmill was activated while the mice were running. To minimize stress, gentle back tapping was incorporated as recommended by (Khataei et al. 2021) before the mice were exposed to sustained electroshock during running.

Resistance exercise protocol

We modified the resistance exercise program presented by the previous study (Inoue et al. 2023). The resistance exercise used a 1.1-meter ladder with 1-centimeter grid steps inclined at 80 degrees. Mice were familiarized with the ladder by practicing climbing from the bottom to the top for two non-consecutive days. The acclimatization included ladder climbing with no loading, comprising four sets of three climbs (Fig. 1B). After a 72-hour rest period, the mice began a 37-day daily training session. For these sessions, weights

corresponding to a certain percentage of their body weight were attached to the base of their tails. For the low-intensity resistance exercise group, these weights were 30% of their body weight, while for the high-intensity resistance exercise group, they were 120% of their body weight. The climbing for both groups consisted of four sets of three climbs each day, with a rest period of 1-2 minutes at the top of the ladder inside a shelter.

Y-maze tests

The maze, constructed from white polyvinyl chloride, featured three equidistant arms, each measuring 40 centimeters in length, 6 centimeters in width, and 9.5 centimeters in height. The arms were oriented 120 degrees apart. On the 31st day of the exercise regimen, mice were individually placed in one arm of the Y-maze and allowed to explore the apparatus for 8 minutes. A "perfect alternation" was defined as the sequential exploration of two distinct arms before returning to the starting arm, irrespective of the initial direction chosen. The percentage of spontaneous alternations was calculated using the equation:

Spontaneous alternation (%) = [(Number of perfect alternations)/ (Total arm entries - 1)] x 100.

On the 37th day, the forced-alternation test was conducted. One arm of the Y-maze (the novel arm) was blocked. Mice were placed in the central region and allowed to explore the two open arms for 5 minutes. After a 5-hour interval, the novel arm was unblocked, and the mice could explore all three arms for another 5 minutes. The exploration time in each arm was recorded. Forced alternation was calculated with the formula:

Forced alternation (%) = [(number of novel alternations)/ (total arm entries - 1)] x 100.

Barnes maze

The Barnes maze experiment was conducted from days 32 to 36 of the exercise regimen. The maze consisted of a white circular polyvinyl chloride platform (90 centimeters in diameter) with 20 equally spaced holes (5 centimeters in diameter). Beneath one of these holes, an escape box was positioned. Each mouse aimed to find this escape box using spatial cues around the maze. For 4 successive days, each mouse underwent 4 trial sessions daily ensuring sufficient learning opportunity (Patil et al. 2009). The following parameters were recorded: escape latency (the time each mouse took to find the escape hole), distance traveled (the total distance a mouse covered in its attempt to locate the escape hole), and search errors (the number of times a mouse mistakenly inspected a hole that was not the

target). 24 hours after the last trial, each mouse was placed at the center of the maze and given 90 seconds to explore. During this exploration phase, the target hole was inaccessible to the mice. The time spent by each mouse in the target quadrant, where the escape box had previously been situated, was monitored. The data was tracked and analyzed using the Python-based tracking system ezTrack (Pennington et al. 2019).

Immunofluorescence

48 hours after the last exercise training, mice were deeply anesthetized with a cocktail consisting of 4 mg/kg midazolam, 0.75 mg/kg medetomidine hydrochloride, and 5 mg/kg butorphanol tartrate. The mice were then perfused transcardially with 4% paraformaldehyde in 0.1M phosphate-buffered saline (PBS). Brain tissue was removed, fixed in 4% paraformaldehyde at 4°C, and dehydrated with a gradient concentration sucrose solution. The brain tissue was rapidly frozen and stored at -80°C. The cryoprotected brains were sectioned serially at 20 μm in the coronal plane (−2.06 to −2.36 mm from Bregma) using a freezing microtome (CM1860, Leica Biosystems, Japan). Sections were denatured in 2 N HCl for 30 minutes at 37°C, then neutralized in 0.1 M borate buffer for 10 min. Next, all sections were incubated with 10% normal goat serum (VEC S-1000, VECTOR Laboratories, USA) and 0.3% Triton X-100 in 0.01M PBS (PBSTx). The sections were then incubated with 1:1000 rat anti-BrdU antibody (ab6326; Abcam Japan, Japan) and 1:2000 rabbit anti-NeuN antibody (ab177487; Abcam) overnight at 4°C. Employing NeuN staining alongside BrdU labeling is consistent with established protocols for investigating the proliferation and maturation phases of neurogenesis (Gusel'nikova and Korzhevskiy 2015; Kim et al. 2017). After washing in PBSTx, sections were incubated in 1:1000 fluorescence-labeled secondary antibodies: Alexa Fluor 568-conjugated anti-rat IgG (A11077; Life Technologies, Japan) or Alexa Fluor 488-conjugated anti-rabbit IgG (A11034; Life Technologies) at room temperature for 1 hour. Sections were Then incubated with DAPI (D21490; Thermo Fisher Scientific, Japan) to stain cell nuclei. ProLong Diamond Antifade Mountant (P36965; Life Technologies) was applied, followed by a cover slip, and analyzed by immunofluorescence microscope (Olympus BX53-33P-DPH2, Olympus, Japan). The number of BrdU-positive cells co-localized with NeuN in the dentate gyrus of each mouse section was calculated for statistical analysis. To estimate the total number of such cells in the dentate gyrus, the total number across all sections was multiplied by 10 (Zhao and van Praag 2020).

Western blotting

42 hours after the final exercise, mice were anaesthetized using the same cocktail as previously described. They were then decapitated, and their brains were quickly removed. The hippocampi were carefully dissected out and immediately stored at -80°C. Hippocampus tissue was homogenized in RIPA buffer and protein concentration was normalized using the using the TaKaRa BCA Protein Assay Kit (Takara Bio, Japan). For each sample, 10 micrograms of protein were loaded and separated on Any kD™ Mini-PROTEAN TGX™ precast gel (Bio-Rad, USA). After separation, proteins were transferred onto a Polyvinylidene fluoride membrane (Trans-Blot® Turbo™ Transfer Pack, Bio-Rad) and blocked with Tris-buffered saline (TBS) containing 5% skim milk for 1 hour at room temperature. The membranes were then probed with primary antibodies at 4°C overnight: anti-PSD-95 (1:2000, ab18258; Abcam), anti-SYP (1:100000, ab32127; Abcam) and anti-GAPDH (1:50000, CST 2118S; Cell Signaling Technology, USA) as a normalization control. The choice of GAPDH for normalization is based on its consistent expression in the hippocampal tissue and its reliability as a housekeeping protein (Chen and Russo-Neustadt 2009; Rahmati and Kazemi 2019). Following primary antibody incubation, the membranes were washed with TBS containing 0.1% Tween 20, and then incubated with a secondary goat anti-rabbit antibody (A16110; Thermo Fisher Scientific) for detection. The bands on the PVDF were treated with Immunostar LD (Wako Pure Chemical Industries, Japan) and imaged using the OptimaShot CL-420α system (FUJIFILM Wako Pure Chemical, Japan). To ensure reliable comparisons across different gels, we adhered to our established protocol for transfer, antibody incubation, and imaging (Inoue et al. 2023). Band intensities were quantified using ImageJ software (National Institutes of Health, USA).

Statistical analysis

All data are presented as mean ± standard error of the mean (SEM). Data were analyzed using GraphPad Prism 9. Normality was assessed using the Shapiro–Wilk test. All data were analyzed using a one-way ANOVA with Turkey's HSD post hoc test. Results were considered statistically significant when $P < 0.05$.

Results

Cognitive performance

198 The spontaneous and forced alternations in Y-maze showed no statistical differences among
199 the groups (Fig. 2A-B). The escape latency, search errors, and distance traveled in the Barnes
200 maze training trial were recorded, and no significant differences were found (Fig. 2C-E). In
201 the Barnes maze probe trial, time in the target quadrant (Fig. 2F) did not reveal any
202 differences among the groups.
203
204 Adult *hippocampal neurogenesis*
205 Typical BrdU/NeuN-positive co-labeled cells in the dentate gyrus of each group are
206 presented in Fig. 3A. More co-labeled cells were observed in the high-intensity aerobic
207 exercise group than in the control and low-intensity resistance exercise groups ($P < 0.01$).
208 Although not reaching statistical significance, the low-intensity aerobic exercise group
209 exhibited a rising tendency in the number of cells co-labeled with BrdU/NeuN compared to
210 the control group ($P = 0.076$). Similarly, the high-intensity aerobic exercise group showed a
211 rising trend in co-localization relative to the low-intensity resistance exercise; however, this
212 was not statistically significant ($P = 0.070$). No significant changes in BrdU/NeuN-positive
213 cells were observed in the resistance exercise groups compared to the control (Fig. 3B).
214
215 *Hippocampal Synaptic plasticity*
216 No statistical differences in synaptic plasticity-associated proteins SYP and PSD-95 levels
217 were detected among the groups, as illustrated in Figure 4.
218
219 **Discussion**
220 Our study investigated the effects of various modes and intensities of exercise on cognitive
221 performance, adult neurogenesis, and synaptic plasticity-related proteins in mice. We
222 observed no changes in cognitive performance or hippocampal synaptic plasticity in mice,
223 regardless of exercise mode or intensity. Aerobic exercise, particularly at high intensity,
224 significantly increased adult neurogenesis in the hippocampus; however, this did not
225 translate into detectable improvements in cognitive performance.
226 Contrary to numerous studies indicating that exercise enhances cognitive functions
227 (Costa et al. 2012; Li et al. 2013), our results did not reveal significant enhancements,
228 supporting similar recent research (Morgan et al. 2018; Constans et al. 2021). Consistent
229 with studies of animals at peak cognitive condition (Segabinazi et al. 2020; Kelty et al.

2022), our results suggest a ceiling effect where high baseline cognitive function limits the detectable impact of exercise. Some studies further support our results by showing cognitive improvements post-exercise, especially in models with cognitive deficits (de Meireles et al. 2019; Kelty et al. 2022). These findings suggest that the beneficial effects of exercise on cognition are more pronounced in mice with impaired cognition. On the other hand, maximizing cognitive benefits from exercise could be achieved by integrating exercise with specific cognitive training strategies (Heisz et al. 2017). Investigating the synergistic effects of combined exercise and cognitive training in future research could offer important insights.

Aerobic exercise has been demonstrated to enhance adult hippocampal neurogenesis, with intensity levels playing a critical role (Vivar et al. 2012; Nokia et al. 2016). Our study suggests that high-intensity aerobic exercise is more effective than low-intensity exercise in promoting the proliferation phase of neurogenesis. This benefit is potentially linked to higher lactate production during high-intensity exercise (Ferreira et al. 2007). Lactate may act as a signaling molecule that initiates neurogenesis by stimulating neurotrophic factors, including brain-derived neurotrophic factor (BDNF) (El Hayek et al. 2019). Nevertheless, it is crucial to acknowledge that individual differences in physiological responses to exercise intensities can significantly affect neurogenesis outcomes. Although intense aerobic exercises, such as running at 30 or 40 m/min, are known to elevate BDNF levels (Cefis et al. 2019), their effects on neurogenesis are not consistently superior to those of lower intensities (Inoue et al. 2015; Okamoto et al. 2015). This variation may arise from exceeding the optimal exercise intensity for BDNF production. Furthermore, lower-intensity exercises may be more beneficial for neurogenesis by reducing corticosterone levels, a known inhibitor of BDNF (Wu et al. 2020). Consequently, intense exercise could establish a metabolic environment that impedes adult hippocampal neurogenesis (Okamoto and Soya 2012). These findings highlight the importance of designing future studies to develop personalized exercise programs that consider individual lactate thresholds and physiological responses to maximize neurogenic benefits.

For resistance exercise, consistent with previous studies (Harri and Heiskanen 2014; Nokia et al. 2016), our research found no significant effects of resistance exercise on the proliferation phase of neurogenesis, irrespective of intensity. Resistance exercise, which involves anaerobic, short-duration muscle contractions, triggers unique molecular responses that differ from aerobic exercise. This includes releasing insulin-like growth factor 1 (IGF-

1), potentially influencing neurogenesis (Cassilhas et al. 2012). Our results suggest that aerobic exercise is more effective than resistance training in promoting the proliferation phase of adult hippocampal neurogenesis, likely due to its ability to increase neurotrophic factors such as BDNF through lactate signaling (Vilela et al. 2017).

This study corroborates the pivotal role of aerobic exercise in augmenting the proliferation phase of adult neurogenesis. However, the observed increase in neurogenesis did not translate to improved cognitive performance, aligning with an earlier study (Clark et al. 2008). This discrepancy suggests that the cognitive benefits of increased adult neurogenesis are complex, reflecting the broad aspects of cognition (Leuner et al. 2006).

Synaptic plasticity, the foundational mechanism underpinning learning and memory, is significantly influenced by key proteins, notably SYP and PSD-95 (El-Husseini et al. 2000; Kwon and Chapman 2011). Our findings reveal that neither aerobic nor resistance exercise modulates the expression levels of SYP and PSD-95 in healthy mice. This aligns with findings from similar studies in adult rodents (Fang et al. 2013; Segabinazi et al. 2020; Bartra et al. 2022). Notably, in models of Alzheimer's disease, exercise has been shown to restore levels of these proteins, indicating potential therapeutic implications (Shin et al. 2016; Liu et al. 2022). The differential responses between healthy and diseased states could imply that synaptic plasticity in healthy mice is optimized, limiting the scope for further enhancement through exercise. While our investigation primarily measured the synaptic plasticity-related proteins SYP and PSD-95, it is crucial to acknowledge the role of a broader molecular network in synaptic plasticity. Proteins such as BDNF and IGF-1 are known to respond to synaptic plasticity and are implicated in the physiological responses to exercise. Although our study did not directly quantify changes in these proteins, it is reasonable to hypothesize that their activities could be modulated by exercise (Cetinkaya et al. 2013; Afzalpour et al. 2015). Thus, while we observed no significant changes in SYP and PSD-95, this does not diminish the possibility that exercise may exert its beneficial effects on synaptic plasticity through other proteins.

This study has several limitations. First, because our research focused on healthy mice, the findings may not directly apply to models of aging or cognitive dysfunction (van Praag et al. 2005; Kelty et al. 2022). Nonetheless, studying healthy mice allowed us to examine intrinsic physiological changes induced by exercise. These results provide baseline data for recommending exercise regimens and serve as a foundation for future therapies to

counteract aging and cognitive decline (Cassilhas et al. 2012; Segabinazi et al. 2020). Second, our methodology standardized exercise intensity for all mice, which simplifies comparisons but overlooks individual variability of lactate threshold. The absence of post-exercise lactate confirmation also means we cannot ensure uniform intensity achievement. These factors may hinder the application of our findings to personalized human exercise programs. Future research should use individualized intensity protocols, as demonstrated by previous studies (Constans et al. 2021; Hugues et al. 2022). These protocols utilize incremental exercise tests to determine lactate threshold for each animal, allowing for exercise intensities that adapt to physiological changes. Third, we employed a gentle tapping to encourage running before electrical stimulation, aiming to reduce the stress typically associated with forced exercise. However, we were not able to completely exclude the effects of stress hormones. Our results may reflect a nuanced interplay between the stress response to exercise and the neurogenic benefits of exercise. Fourth, our study focused on exercise intensity due to its documented influence on neurogenesis (Ratey and Loehr 2011; Ludyga et al. 2020). However, we cannot ignore the potential effects of exercise distance on brain plasticity. Finally, the small sample size for the BrdU/NeuN measurements limits the generalizability of our neurogenesis findings. However, the preliminary results are promising and underscore the necessity for robust validation of the observed trends.

In conclusion, our study highlights the role of high-intensity aerobic exercise in enhancing adult hippocampal neurogenesis in mice, though its effects on cognitive performance and synaptic plasticity remain elusive. This underscores an imperative for additional research to identify the optimal exercise conditions to maximize neurogenesis and cognitive enhancement. Future research should focus on elucidating the underlying mechanisms and expand the study to include models of aging and cognitive decline.

Acknowledgements

We thank Bin Liu, Daisuke Eguchi, Chunxiao He, and Peng Liu for their skilled technical assistance.

Funding

This work was supported by the Japan Society for the Promotion of Science KAKENHI Grant No. 19H04050.

Conflict of interest

The authors have no conflict of interest to declare.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Reference

Afzalpour ME, Chadorneshin HT, Foadoddini M, Eivari HA (2015) Comparing interval and continuous exercise training regimens on neurotrophic factors in rat brain. Physiol Behav 147:78–83

Bartra C, Jager LA, Alcarraz A, et al (2022) Antioxidant molecular brain changes parallel adaptive cardiovascular response to forced running in mice. Antioxidants (Basel) 11:1891

Bettio L, Thacker JS, Hutton C, Christie BR (2019) Modulation of synaptic plasticity by exercise. In: Yau S-Y, So K-F (eds) International Review of Neurobiology. Elsevier, San Diego, CA, pp 295–322

Biazus-Sehn LF, Schuch FB, Firth J, Stigger F de S (2020) Effects of physical exercise on cognitive function of older adults with mild cognitive impairment: A systematic review and meta-analysis. Arch Gerontol Geriatr 89:104048

Billat VL, Mouisel E, Roblot N, Melki J (2005) Inter- and intrastrain variation in mouse critical running speed. J Appl Physiol 98:1258–1263

Cassilhas RC, Lee KS, Fernandes J, et al (2012) Spatial memory is improved by aerobic and resistance exercise through divergent molecular mechanisms. Neuroscience 202:309–317

Cefis M, Prigent-Tessier A, Quirié A, et al (2019) The effect of exercise on memory and BDNF signaling is dependent on intensity. Brain Struct Funct 224:1975–1985

352 Cetinkaya C, Sisman AR, Kiray M, et al (2013) Positive effects of aerobic exercise on
353 learning and memory functioning, which correlate with hippocampal IGF-1 increase in
354 adolescent rats. Neurosci Lett 549:177–181
355 Chen MJ, Russo-Neustadt AA (2009) Running exercise‐induced up‐regulation of
356 hippocampal brain‐derived neurotrophic factor is CREB‐dependent. Hippocampus
357 19:962‐972
358 Clark PJ, Brzezinska WJ, Thomas MW, et al (2008) Intact neurogenesis is required for
359 benefits of exercise on spatial memory but not motor performance or contextual fear
360 conditioning in C57BL/6J mice. Neuroscience 155:1048–1058
361 Codina-Martínez H, Fernández-García B, Díez-Planelles C, et al (2020) Autophagy is
362 required for performance adaptive response to resistance training and exercise‐induced
363 adult neurogenesis. Scand J Med Sci Sports 30:238–253
364 Constans A, Pin-Barre C, Molinari F, et al (2021) High-intensity interval training is superior
365 to moderate intensity training on aerobic capacity in rats: Impact on hippocampal plasticity
366 markers. Behav Brain Res 398:112977
367 Costa MS, Ardais AP, Fioreze GT, et al (2012) The impact of the frequency of moderate
368 exercise on memory and brain-derived neurotrophic factor signaling in young adult and
369 middle-aged rats. Neuroscience 222:100–109
370 de Meireles LCF, Galvão F Jr, Walker DM, et al (2019) Exercise modalities improve
371 aversive memory and survival rate in aged rats: Role of hippocampal epigenetic
372 modifications. Mol Neurobiol 56:8408–8419
373 El Hayek L, Khalifeh M, Zibara V, et al (2019) Lactate mediates the effects of exercise on
374 learning and memory through SIRT1-dependent activation of hippocampal brain-derived
375 neurotrophic factor (BDNF). J Neurosci 39:1661–1618

El-Husseini AE, Schnell E, Chetkovich DM, et al (2000) PSD-95 involvement in maturation of excitatory synapses. Science 290:1364–1368

Fang ZH, Lee CH, Seo MK, et al (2013) Effect of treadmill exercise on the BDNF-mediated pathway in the hippocampus of stressed rats. Neurosci Res 76:187–194

Fernandes J, Soares JCK, do Amaral Baliego LGZ, Arida RM (2016) A single bout of resistance exercise improves memory consolidation and increases the expression of synaptic proteins in the hippocampus. Hippocampus 26:1096–1103

Ferreira JCB, Rolim NPL, Bartholomeu JB, et al (2007) Maximal lactate steady state in running mice: Effect of exercise training. Clin Exp Pharmacol Physiol 34:760–765

Gusel'nikova VV, Korzhevskiy DE (2015) NeuN as a neuronal nuclear antigen and neuron differentiation marker. Acta Naturae 7:42–47

Harri M, Heiskanen K (2014) Aerobic exercise increases hippocampal neurogenesis more compared to resistance exercise in rats selectively bred for high/low response to training

Heisz JJ, Clark IB, Bonin K, et al (2017) The effects of physical exercise and cognitive training on memory and neurotrophic factors. J Cogn Neurosci 29:1895–1907

Hugues N, Pin-Barre C, Pellegrino C, et al (2022) Time-dependent cortical plasticity during moderate-intensity continuous training versus high-intensity interval training in rats. Cereb Cortex 32:3829–3847

Inoue K, Okamoto M, Shibato J, et al (2015) Long-term mild, rather than intense, exercise enhances adult hippocampal neurogenesis and greatly changes the transcriptomic profile of the hippocampus. PLoS One 10:e0128720

Inoue S, Matsuura K, Eguchi D, et al (2023) Effects of different modes and intensities of exercise on longevity proteins in middle-aged mouse skeletal muscle. Physiol Int 110:150–159

Kelty TJ, Mao X, Kerr NR, et al (2022) Resistance-exercise training attenuates LPS-induced astrocyte remodeling and neuroinflammatory cytokine expression in female Wistar rats. J Appl Physiol 132:317–326

Khataei T, Romig-Martin SA, Harding AMS, et al (2021) Comparison of murine behavioural and physiological responses after forced exercise by electrical shock versus manual prodding. Exp Physiol 106:812–819

Kim JW, Nam SM, Yoo DY, et al (2017) Comparison of adult hippocampal neurogenesis and susceptibility to treadmill exercise in nine mouse strains. Neural Plast 2017:1–13

Kwon SE, Chapman ER (2011) Synaptophysin regulates the kinetics of synaptic vesicle endocytosis in central neurons. Neuron 70:847–854

Lan Y, Huang Z, Jiang Y, et al (2018) Strength exercise weakens aerobic exercise-induced cognitive improvements in rats. PLoS One 13:e0205562

Leuner B, Gould E, Shors TJ (2006) Is there a link between adult neurogenesis and learning? Hippocampus 16:216–224

Li H, Liang A, Guan F, et al (2013) Regular treadmill running improves spatial learning and memory performance in young mice through increased hippocampal neurogenesis and decreased stress. Brain Res 1531:1–8

Liu Z-T, Ma Y-T, Pan S-T, et al (2022) Effects of involuntary treadmill running in combination with swimming on adult neurogenesis in an Alzheimer's mouse model. Neurochem Int 155:105309

Lou S-J, Liu J-Y, Chang H, Chen P-J (2008) Hippocampal neurogenesis and gene expression depend on exercise intensity in juvenile rats. Brain Res 1210:48–55

Ludyga S, Gerber M, Pühse U, et al (2020) Systematic review and meta-analysis investigating moderators of long-term effects of exercise on cognition in healthy individuals. Nat Hum Behav 4:603–612

425 Mandolesi L, Polverino A, Montuori S, et al (2018) Effects of physical exercise on cognitive
426 functioning and wellbeing: Biological and psychological benefits. Front Psychol 9:.
427 https://doi.org/10.3389/fpsyg.2018.00509
428 Morgan JA, Singhal G, Corrigan F, et al (2018) The effects of aerobic exercise on
429 depression-like, anxiety-like, and cognition-like behaviours over the healthy adult lifespan of
430 C57BL/6 mice. Behav Brain Res 337:193–203
431 Nie J, Yang X (2017) Modulation of synaptic plasticity by exercise training as a basis for
432 ischemic stroke rehabilitation. Cell Mol Neurobiol 37:5–16
433 Nokia MS, Lensu S, Ahtiainen JP, et al (2016) Physical exercise increases adult hippocampal
434 neurogenesis in male rats provided it is aerobic and sustained. J Physiol 594:1855–1873
435 Okamoto M, Soya H (2012) Mild exercise model for enhancement of hippocampal
436 neurogenesis: A possible candidate for promotion of neurogenesis. J Phys Fit Sports Med
437 1:585–594
438 Okamoto M, Yamamura Y, Liu Y-F, et al (2015) Hormetic effects by exercise on
439 hippocampal neurogenesis with glucocorticoid signaling. Brain Plast 1:149–158
440 Patil SS, Sunyer B, Höger H, Lubec G (2009) Evaluation of spatial memory of C57BL/6J
441 and CD1 mice in the Barnes maze, the Multiple T-maze and in the Morris water maze. Behav
442 Brain Res 198:58–68
443 Pennington ZT, Dong Z, Feng Y, et al (2019) ezTrack: An open-source video analysis
444 pipeline for the investigation of animal behavior. Sci Rep 9:19979
445 Rahmati M, Kazemi A (2019) Various exercise intensities differentially regulate GAP-43 and
446 CAP-1 expression in the rat hippocampus. Gene 692:185–194
447 Ratey JJ, Loehr JE (2011) The positive impact of physical activity on cognition during
448 adulthood: a review of underlying mechanisms, evidence and recommendations. Rev
449 Neurosci 22:171–185

450 Savioz A, Leuba G, Vallet PG (2014) A framework to understand the variations of PSD-95
451 expression in brain aging and in Alzheimer's disease. Ageing Res Rev 18:86–94
452 Segabinazi E, Gasperini NF, Faustino AM, et al (2020) Comparative overview of the effects
453 of aerobic and resistance exercise on anxiety-like behavior, cognitive flexibility, and
454 hippocampal synaptic plasticity parameters in healthy rats. Braz J Med Biol Res 53:e9816
455 Shih P-C, Yang Y-R, Wang R-Y (2013) Effects of exercise intensity on spatial memory
456 performance and hippocampal synaptic plasticity in transient brain ischemic rats. PLoS One
457 8:e78163
458 Shin M-S, Jeong H-Y, An D-I, et al (2016) Treadmill exercise facilitates synaptic plasticity
459 on dopaminergic neurons and fibers in the mouse model with Parkinson's disease. Neurosci
460 Lett 621:28–33
461 van Praag H, Shubert T, Zhao C, Gage FH (2005) Exercise enhances learning and
462 hippocampal neurogenesis in aged mice. J Neurosci 25:8680–8685
463 van Uffelen JGZ, Chin A Paw MJM, Hopman-Rock M, van Mechelen W (2008) The effects
464 of exercise on cognition in older adults with and without cognitive decline: a systematic
465 review. Clin J Sport Med 18:486–500
466 Vilela TC, Muller AP, Damiani AP, et al (2017) Strength and aerobic exercises improve
467 spatial memory in aging rats through stimulating distinct neuroplasticity mechanisms. Mol
468 Neurobiol 54:7928–7937
469 Vivar C, Potter MC, van Praag H (2012) All about running: Synaptic plasticity, growth
470 factors and adult hippocampal neurogenesis. In: Neurogenesis and Neural Plasticity.
471 Springer Berlin Heidelberg, Berlin, Heidelberg, pp 189–210
472 Voss MW, Vivar C, Kramer AF, van Praag H (2013) Bridging animal and human models of
473 exercise-induced brain plasticity. Trends Cogn Sci 17:525–544

Wang X-Q, Wang G-W (2016) Effects of treadmill exercise intensity on spatial working memory and long-term memory in rats. Life Sci 149:96–103

Wu Y, Deng F, Wang J, et al (2020) Intensity-dependent effects of consecutive treadmill exercise on spatial learning and memory through the p-CREB/BDNF/NMDAR signaling in hippocampus. Behav Brain Res 386:112599

Zhao X, van Praag H (2020) Steps towards standardized quantification of adult neurogenesis. Nat Commun 11:4275

Figure

Fig. 1 Timeline of the experiment

Starting with a 5-day exercise acclimatization period, mice were offered a 3-day rest period. Following the rest period, a 37-day exercise was initiated, which included a range of low and high-intensity aerobic and resistance exercises. Within the first ten days of exercise, four mice from each group were administered 5-bromo-2`-deoxyuridine (BrdU) intraperitoneally, a step essential for future histological evaluation. During the final week of the exercise, all groups underwent cognitive performance assessments of the different exercise modes and intensities. Forty-eight hours after the end of the exercise, brain tissue samples were taken from each of the mice.

Figure. 2 The effects of different modes and intensities of exercise on cognitive performance in mice

(A) Spontaneous alternations in the Y-maze, (B) Forced alternations in the Y-maze, (C) Escape latency during the training trial of the Barnes maze, (D) Search errors during the training trial of the Barnes maze, (E) Distance traveled during the training trial of the Barnes maze, (F) Time spent in the target quadrant during the probe trial of the Barnes maze. Data is represented as the mean ± SEM derived from 8 mice per group. Statistical significance was evaluated using one-way ANOVA followed by Tukey's HSD post hoc test.

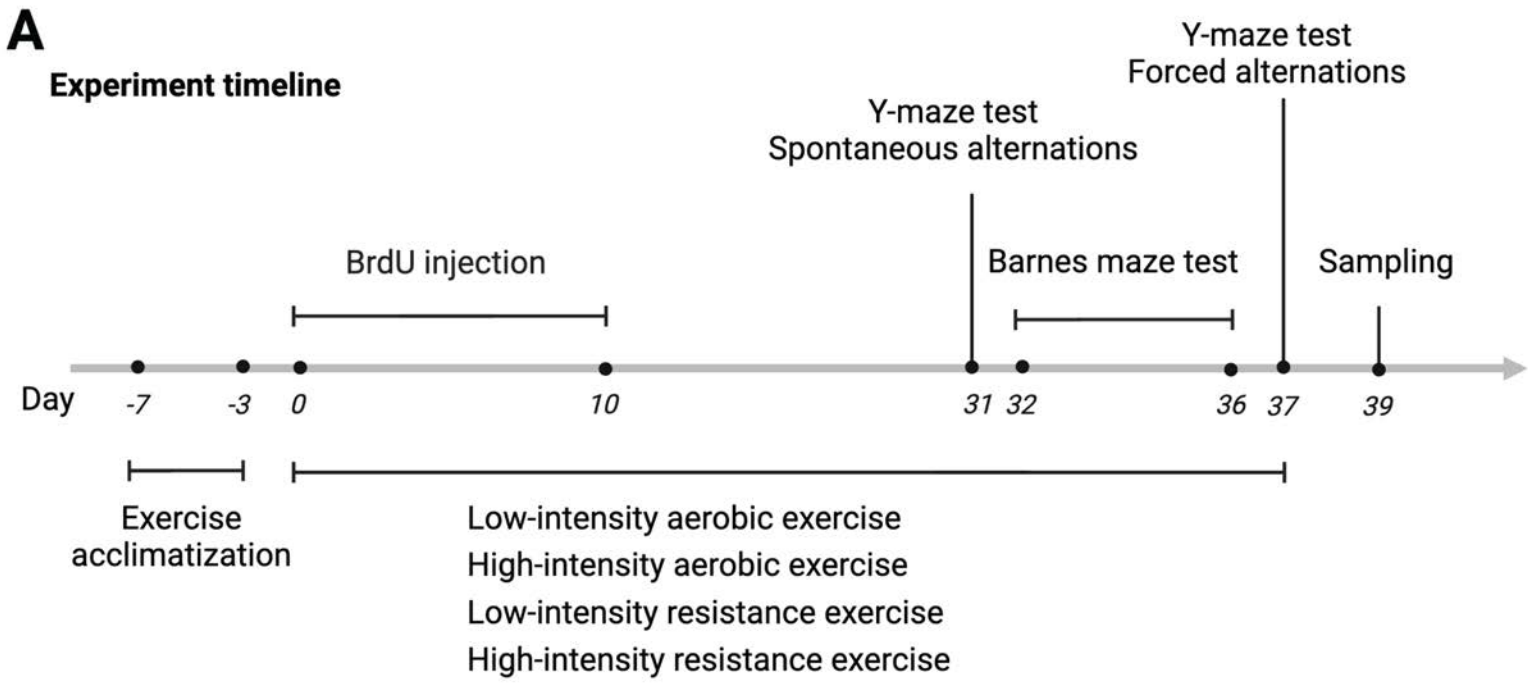
Figure. 3 The effects of different modes and intensities of exercise on adult hippocampal neurogenesis in mice

(A) Immunofluorescence staining illustrating BrdU (arrows) and NeuN (arrowheads) in the dentate gyrus of the adult mouse hippocampus. (B) Quantification of cells expressing both BrdU/ NeuN (double positive), indicative of adult neurogenesis. Data are represented as mean ± SEM, derived from 4 mice per group. Statistical significance was determined by one-way ANOVA followed by Tukey's HSD post hoc test; $^{**}P < 0.01$.

Figure. 4 The effects of different modes and intensities of exercise on the protein levels of synaptophysin and PSD-95 in the hippocampus of mice

(A) Representative western blot bands and densitometric quantification of synaptophysin levels normalized to GAPDH. (B) Representative western blotting bands and densitometric quantification of PSD-95 levels, normalized to GAPDH. Data are expressed as mean ± SEM from 4 mice per group. Statistical significance was determined using one-way ANOVA followed by Tukey's HSD post hoc test.

Fig. 1


A
Experiment timeline
Y-maze test
Forced alternations
Y-maze test
Spontaneous alternations
BrdU injection
Barnes maze test
Sampling
Day
-7
-3
0
10
31
32
36
37
39
Exercise
acclimatization
Low-intensity aerobic exercise
High-intensity aerobic exercise
Low-intensity resistance exercise
High-intensity resistance exercise

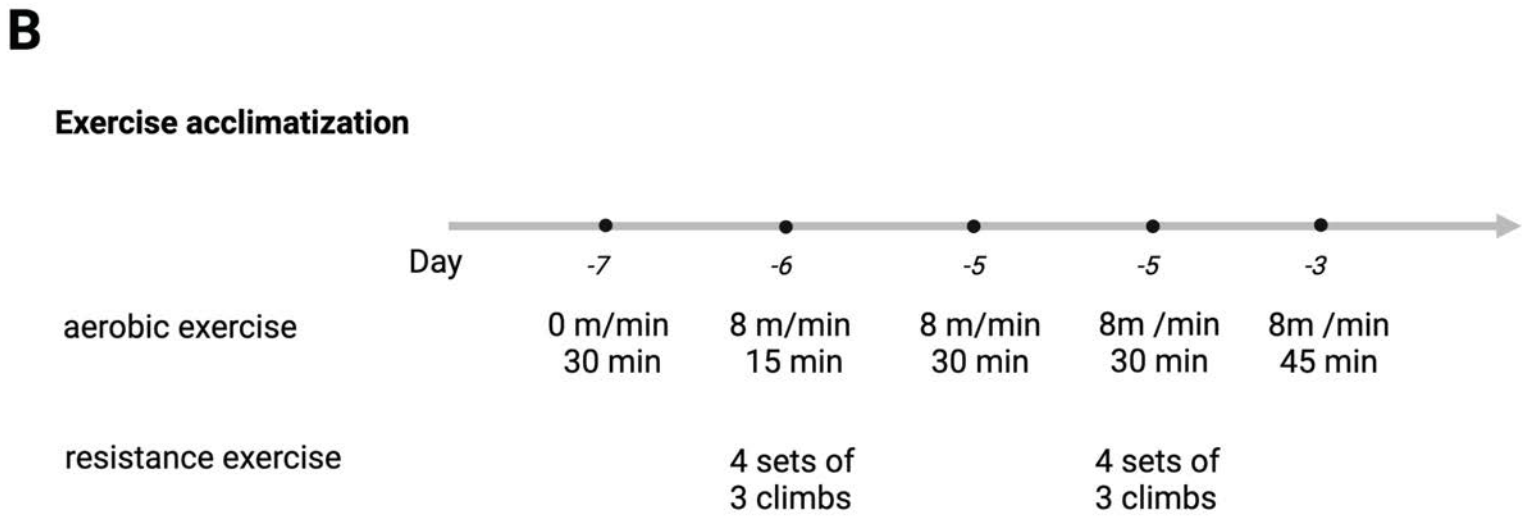

B
Exercise acclimatization
Day
-7
-6
-5
-5
-3
aerobic exercise
0 m/min
30 min
8 m/min
15 min
8 m/min
30 min
8m /min
30 min
8m /min
45 min
resistance exercise
4 sets of
3 climbs
4 sets of
3 climbs

Fig. 2

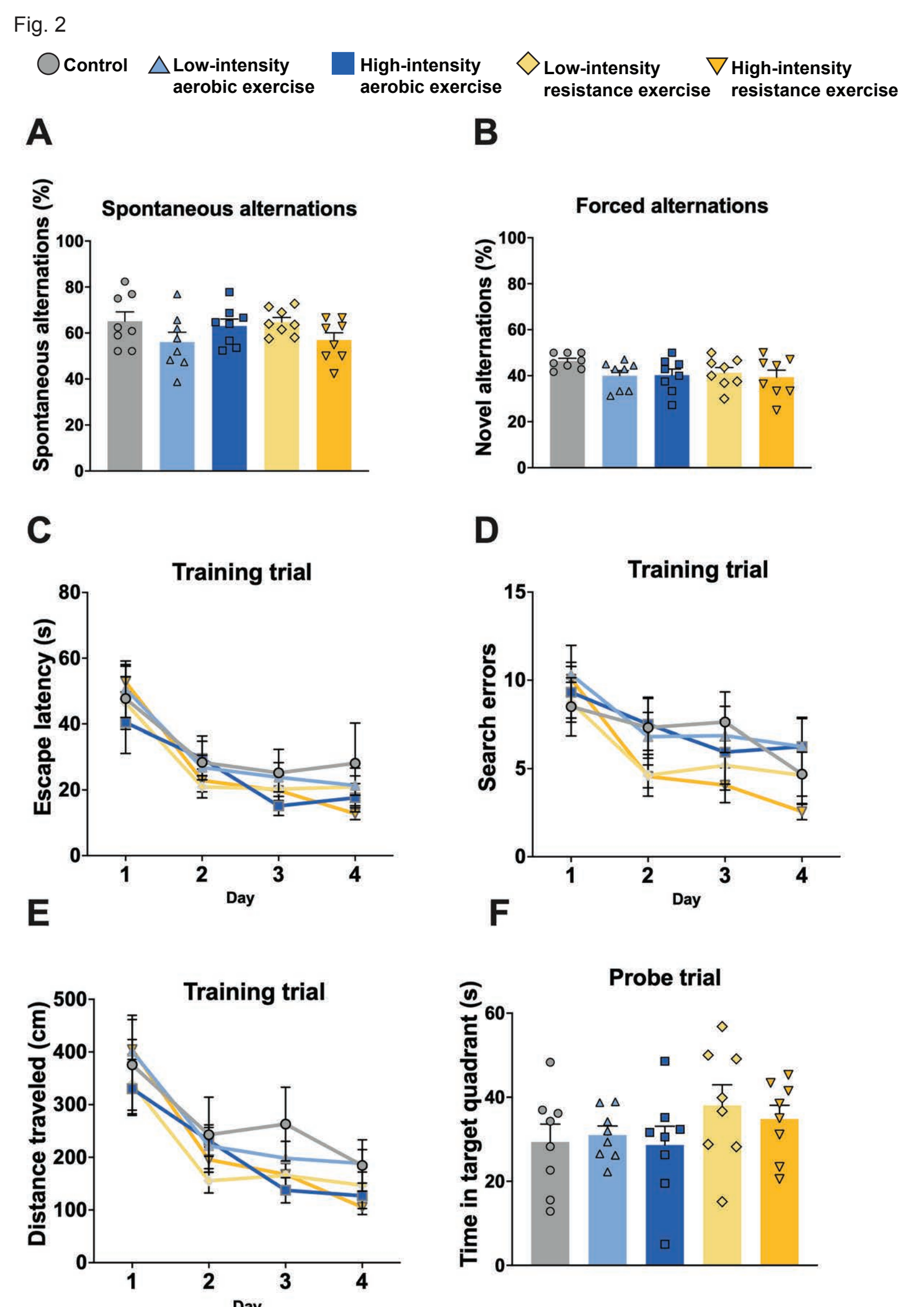
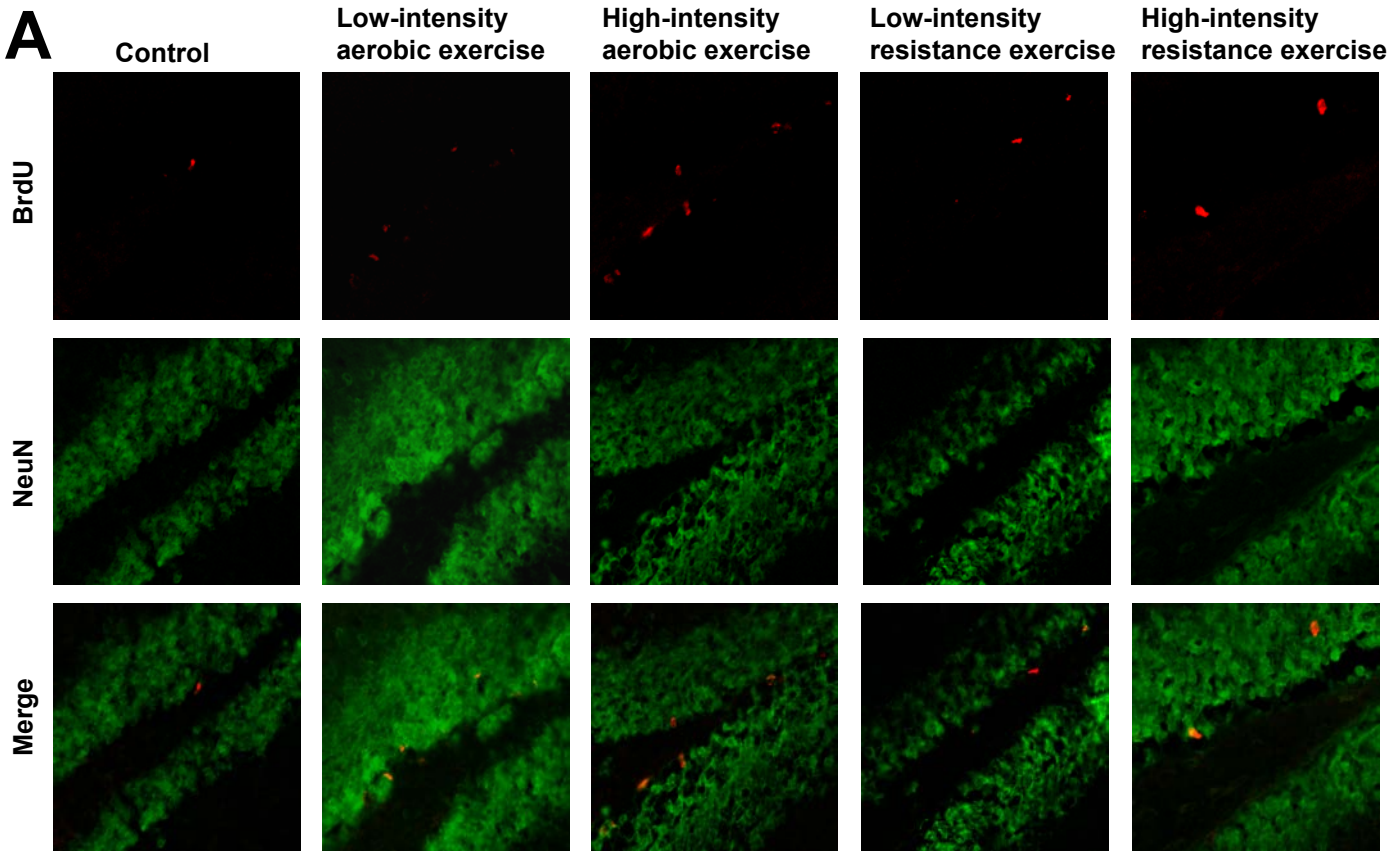

Control
Low-intensity aerobic exercise
High-intensity aerobic exercise
Low-intensity resistance exercise
High-intensity resistance exercise
A
Spontaneous alternations
Spontaneous alternations (%)
B
Forced alternations
Novel alternations (%)
C
Training trial
Escape latency (s)
Day
D
Training trial
Search errors
Day
E
Training trial
Distance traveled (cm)
Day
F
Probe trial
Time in target quadrant (s)

Fig. 3


A
Control
Low-intensity aerobic exercise
High-intensity aerobic exercise
Low-intensity resistance exercise
High-intensity resistance exercise
BrdU
NeuN
Merge

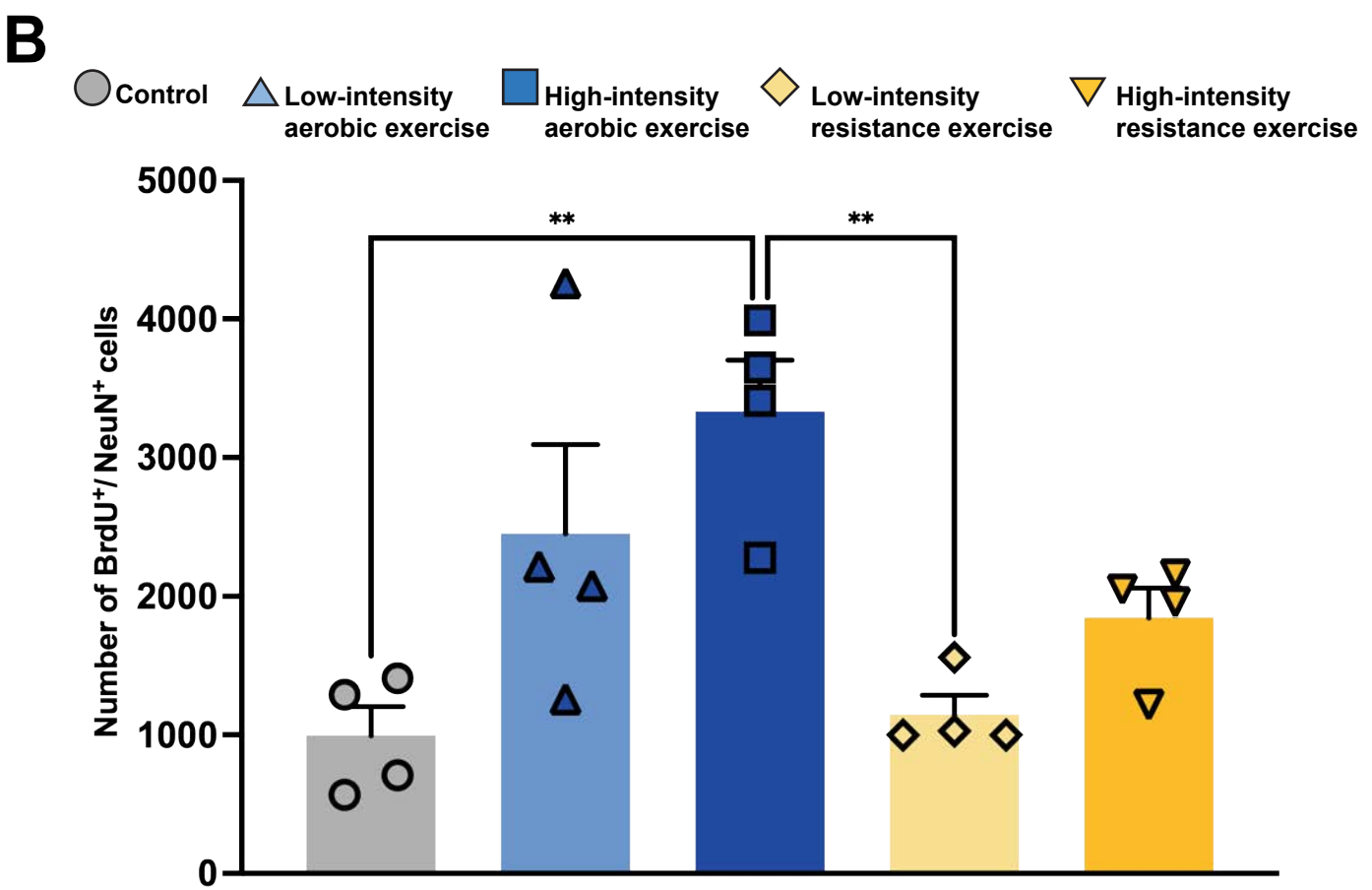

B
Control
Low-intensity aerobic exercise
High-intensity aerobic exercise
Low-intensity resistance exercise
High-intensity resistance exercise
Number of BrdU+/NeuN+ cells
5000
4000
3000
2000
1000
0
**
**

Fig. 4

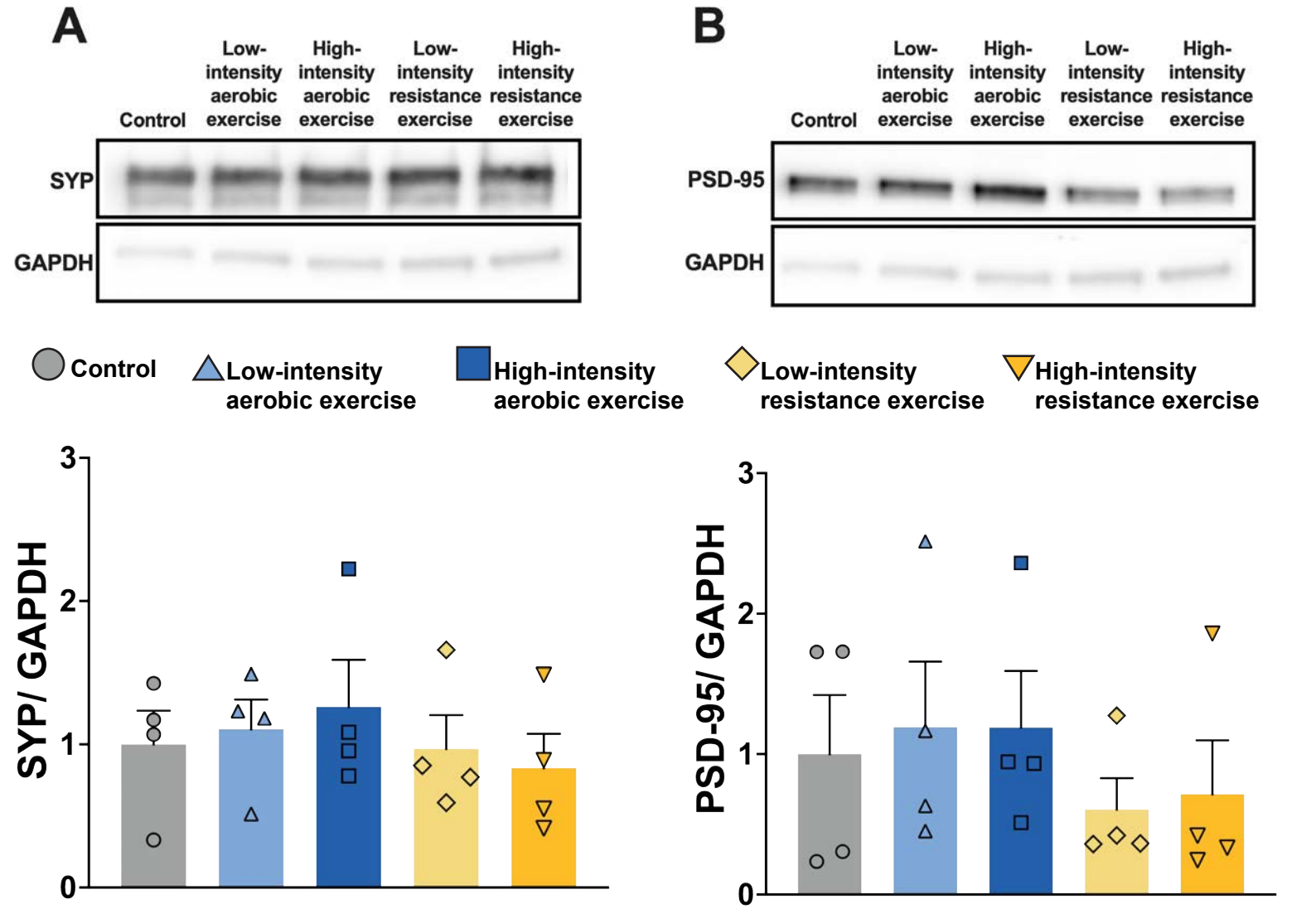

A
Control
Low-intensity aerobic exercise
High-intensity aerobic exercise
Low-intensity resistance exercise
High-intensity resistance exercise
SYP
GAPDH
B
Control
Low-intensity aerobic exercise
High-intensity aerobic exercise
Low-intensity resistance exercise
High-intensity resistance exercise
PSD-95
GAPDH
Control
Low-intensity aerobic exercise
High-intensity aerobic exercise
Low-intensity resistance exercise
High-intensity resistance exercise
SYP/ GAPDH
0
1
2
3
PSD-95/ GAPDH
0
1
2
3

Fig. 2

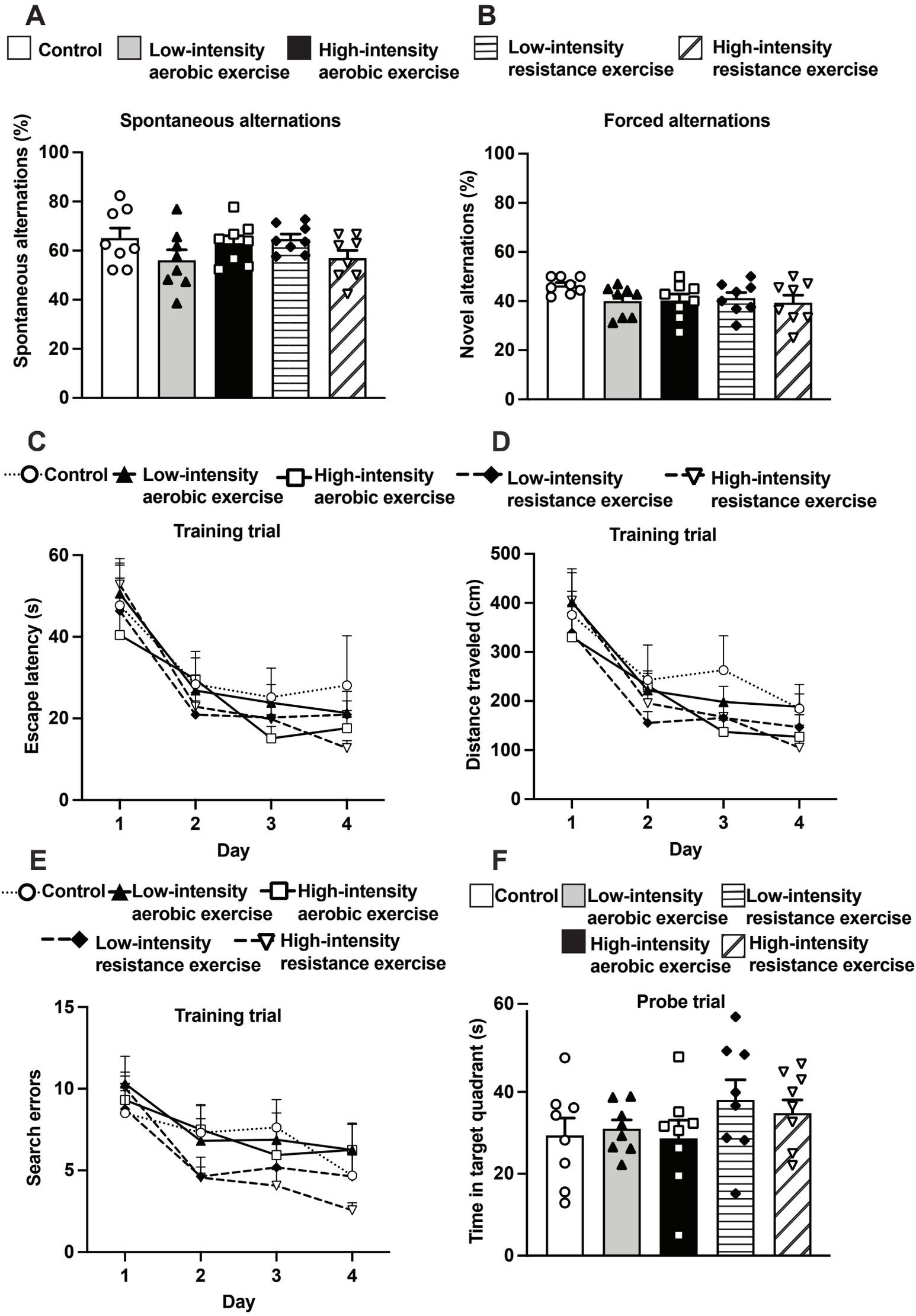
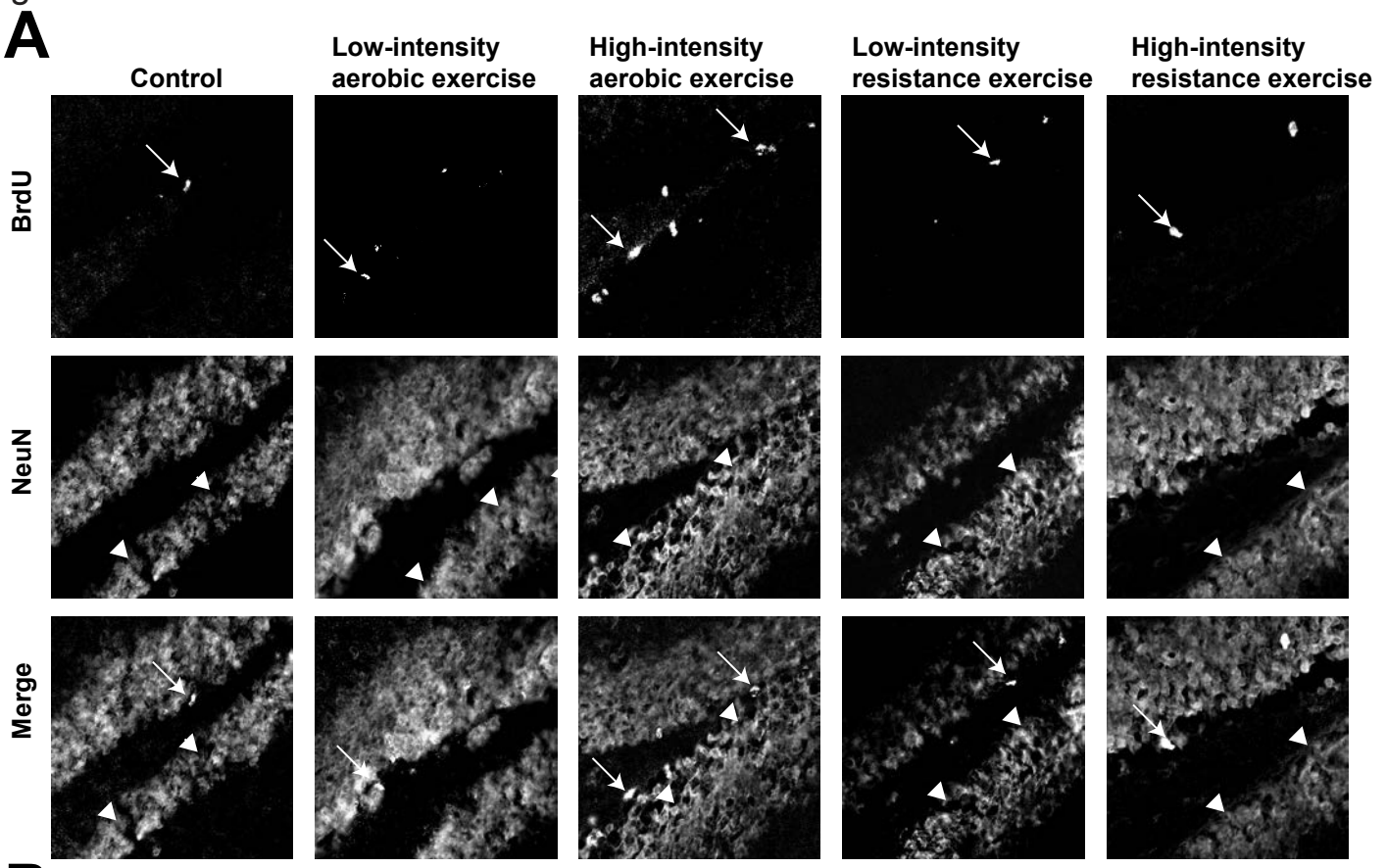

A
Control
Low-intensity aerobic exercise
High-intensity aerobic exercise
B
Low-intensity resistance exercise
High-intensity resistance exercise
Spontaneous alternations
Spontaneous alternations (%)
Forced alternations
Novel alternations (%)
C
D
Training trial
Escape latency (s)
Distance traveled (cm)
Day
E
F
Search errors
Probe trial
Time in target quadrant (s)

Fig. 3


A
Control
Low-intensity aerobic exercise
High-intensity aerobic exercise
Low-intensity resistance exercise
High-intensity resistance exercise
BrdU
NeuN
Merge

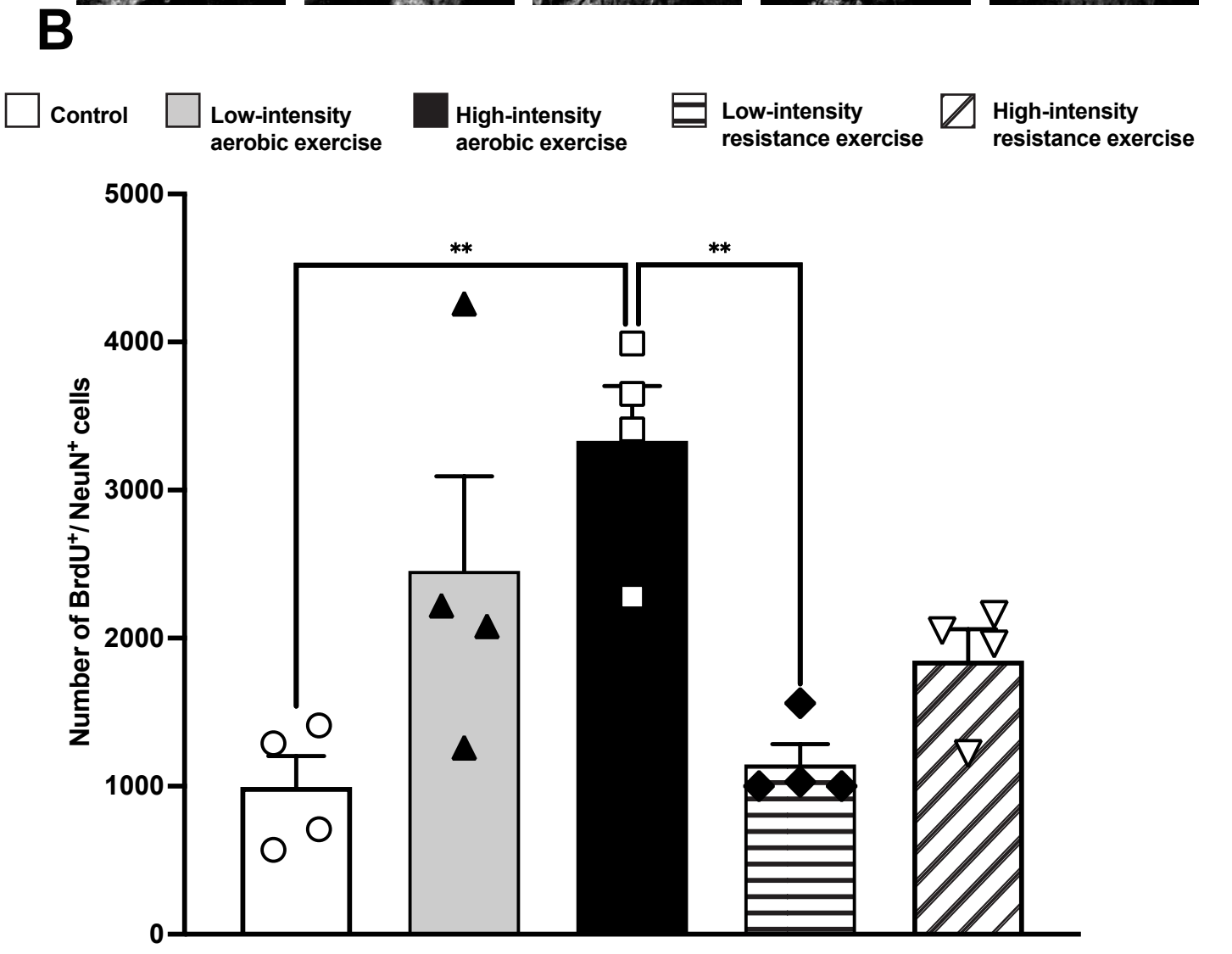
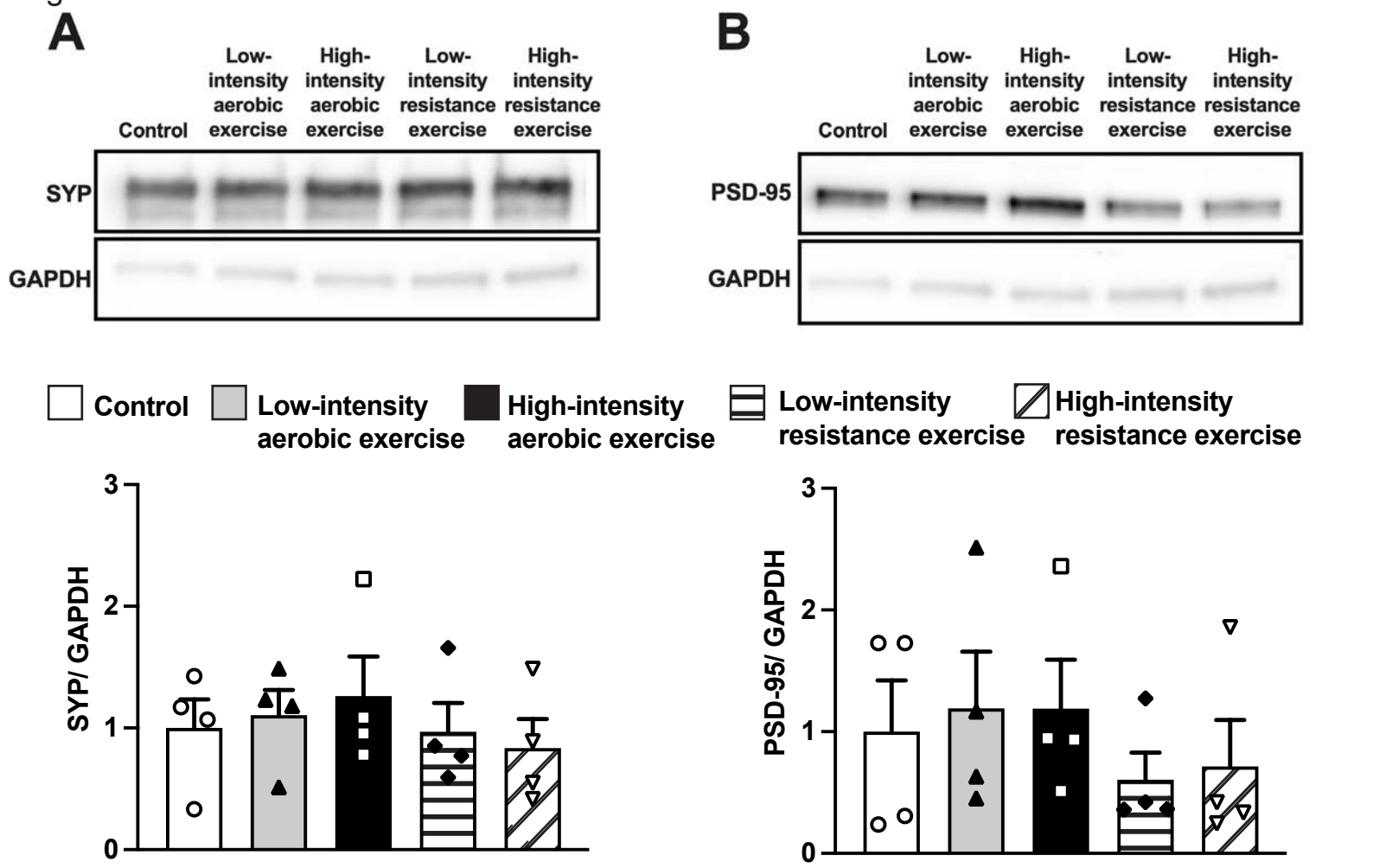

B
Control
Low-intensity aerobic exercise
High-intensity aerobic exercise
Low-intensity resistance exercise
High-intensity resistance exercise
Number of BrdU+/NeuN+ cells
5000
4000
3000
2000
1000
0
**
**

Fig. 4


A
Control
Low-intensity aerobic exercise
High-intensity aerobic exercise
Low-intensity resistance exercise
High-intensity resistance exercise
SYP
GAPDH
B
Control
Low-intensity aerobic exercise
High-intensity aerobic exercise
Low-intensity resistance exercise
High-intensity resistance exercise
PSD-95
GAPDH
Control
Low-intensity aerobic exercise
High-intensity aerobic exercise
Low-intensity resistance exercise
High-intensity resistance exercise
SYP/ GAPDH
0
1
2
3
PSD-95/ GAPDH
0
1
2
3