Light-exercise-induced dopaminergic and noradrenergic stimulation in the dorsal hippocampus: using a rodent physiological exercise model

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Running title: Light exercise activates hippocampus via NA and DA systems

Abbreviations:

5-HT: serotonin

AAS: ascending arousal system

ACTH: adrenocorticotropic hormone

AD: Alzheimer's disease

BDNF: brain-derived neurotrophic factor

BSA: bovine serum albumin

CA: cornu ammonis

DA: dopamine

DAPI: 4',6-diamidino-2-phenylindole

DG: dentate gyrus

DRN: dorsal raphe nucleus

HPLC-ECD: high-performance liquid chromatography with an electrochemical detector

LC: locus coeruleus

LTD: long-term depression

LTP: long-term potentiation

LT: lactate threshold

MCI: mild cognitive impairment

MRN: median raphe nucleus

NA: noradrenaline

PB: phosphate buffer

PBT: phosphate buffer with Triton X-100

PFA: paraformaldehyde

PRP: plasticity-related protein

PVN: paraventricular nucleus

RM: repeated measures

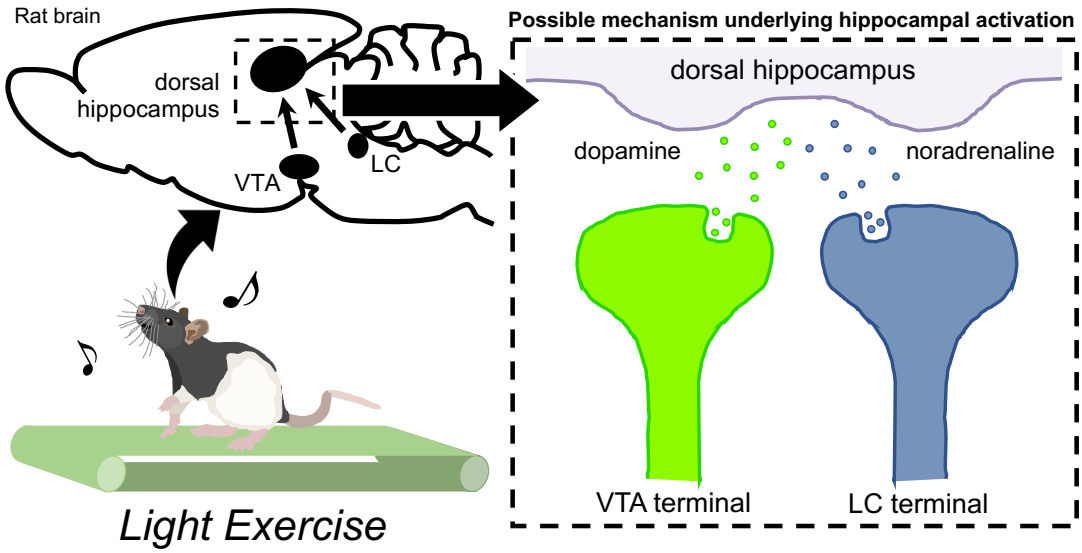
RRF: retrorubral field

SNpc: substantia nigra pars compacta

TH: tyrosine hydroxylase

VTA: ventral tegmental area

Graphical abstract



CONCLUSION

Light exercise could activate the dorsal hippocampus through LC-NA and VTA-DA stimulation.

Our previous research with both animals and humans has demonstrated that even light exercise below the lactate threshold (or 50% $\dot{V}O_{2peak}$) can boost neuronal activity in the dorsal hippocampus and enhance learning and memory. While accumulating evidence highlights the significance of cellular and molecular basis of exercise benefits, the basic circuit mechanism underlying this boost remains largely undetermined. It has been proposed that exercise engages the ascending monoaminergic projections, which facilitates dorsal hippocampus-associated functions. Thus, we examined this hypothesis using our animal exercise model, and found a possible contribution of noradrenergic neurons in the locus coeruleus and dopaminergic neurons in the ventral tegmental area to dorsal hippocampal activation during light exercise, implying a circuit mechanism for light-exercise-induced benefits to the dorsal hippocampus.

Abstract

Growing evidence shows that exercise acts on the dorsal hippocampus and promotes the learning process and memory formation. For decades, these beneficial effects of exercise have been based on a lot of research using demanding and stress-response-inducing exercise at moderate-to-vigorous intensities. In contrast, our translational research with animals and humans has focused on light exercise, which almost anyone can safely perform with minimal stress. Using our special exercise model, which is based on physiological responses such as lactate threshold (LT), we found that even light exercise can boost dorsal hippocampal activity and enhance memory performance. Although the circuit mechanism underlying this boost remains unclear, arousal promotion even with light-intensity exercise implies the involvement of the monoaminergic ascending arousal system, which is crucial for memory enhancement. We thus aimed to examine this hypothesis using our physiological exercise model based on the LT of rats. First, we immunohistochemically assessed the neuronal activation of the dorsal hippocampal sub-regions, as well as tyrosine hydroxylase-positive (TH^+) neurons and serotonin-positive ($5\text{-}HT^+$) neurons in the brainstem monoaminergic nuclei. Second, we monitored the dynamics of monoamine release at the dorsal hippocampus using *in vivo* microdialysis combined with the high-performance liquid chromatography (HPLC) system. We found that even light exercise increased neuronal activity in the dorsal hippocampal sub-regions and induced the release of noradrenaline (NA) and dopamine (DA). Additionally, we found that TH^+ neurons in the locus coeruleus (LC) and the ventral tegmental area (VTA) were activated even by light exercise and were both positively correlated with the dorsal hippocampal activation. Meanwhile, the serotonergic system was not affected by exercise. Overall, these results suggest that light exercise stimulates the dorsal

hippocampal neurons probably through the noradrenergic neurons in the LC and dopaminergic neurons in the VTA. Our findings provide insight into the underlying circuit mechanisms of light-exercise-enhanced memory.

Keywords Light exercise; Dorsal hippocampus; Locus coeruleus; Ventral tegmental area; Noradrenaline; Dopamine

Introduction

There is accumulating evidence showing that physical exercise has beneficial effects on the brain and its functions, including hippocampus-dependent learning and memory (van Praag *et al.* 1999; Erickson *et al.* 2011; Vargas *et al.* 2017; van Dongen *et al.* 2016). The hippocampus is well-known for being involved in several processes related to emotion, stress resilience, and cognitive function. In particular, the dorsal part of the hippocampus plays a key role in the learning process and memory formation (Fanselow and Dong 2010). Physical exercise has been found to increase the activity of the dorsal hippocampal neurons (Oladehin and Waters 2001; Lee *et al.* 2003; Nishijima and Soya 2006), which induces the production of plasticity-related proteins (PRPs) such as the brain-derived neurotrophic factor (BDNF) (Soya *et al.* 2007b; Neeper *et al.* 1995; Vaynman *et al.* 2003). This is, in turn, thought to trigger neuronal and synaptic plasticity and memory enhancement (Vaynman *et al.* 2004; Li *et al.* 2008; van Praag *et al.* 1999; Lee *et al.* 2012).

For decades, the benefits of exercise to learning and memory have been based on evidence using exercise at moderate-to-vigorous intensities (Erickson *et al.* 2011; Vargas *et al.* 2017; van Dongen *et al.* 2016; Pereira *et al.* 2007), which is generally recommended in exercise therapy. However, such demanding exercise can cause physiological stress and reduce exercise adherence (Soya *et al.* 2007a; Ekkekakis *et al.* 2008; Saito and Soya

2004; Hill *et al.* 2008; Perri *et al.* 2002). In contrast, we have focused on light-intensity exercise (light exercise), which can be safely performed with minimum physiological stress, and have revealed its beneficial effects on the hippocampus. We have been employing a rodent treadmill running model based on physiological responses such as lactate threshold (LT). LT is the signpost for exercise-induced stress, which begins with the onset of lactate accumulation in the blood and the accumulation of adrenocorticotropic hormone (ACTH) (Soya *et al.* 2007a; Saito and Soya 2004). This physiological exercise model enables us to define running below the LT as light exercise with minimum physiological stress and running above the LT as vigorous, stress-inducing exercise stimuli. Using this model, we found that even light exercise was sufficient to acutely stimulate hippocampal activity and BDNF induction (Soya *et al.* 2007b), and to promote hippocampal neurogenesis and spatial memory (Inoue *et al.* 2015a; Inoue *et al.* 2015b; Okamoto *et al.* 2012). Conversely, excessive ACTH secretion induced by vigorous exercise cancels out these beneficial effects on hippocampal plasticity and memory due to the stress-induced hippocampal vulnerability (Inoue *et al.* 2015a; Inoue *et al.* 2015b; Okamoto *et al.* 2012; Conrad 2010; Schoenfeld and Gould 2012). Additionally, our research with human participants found that acute light exercise increases hippocampal activity and enhances pattern separation, which is a hippocampus-related function (Suwabe *et al.* 2018). Taken together, our translational research demonstrates the importance of exercise intensity for determining the effect of exercise on the hippocampus and highlights the advantages of light exercise.

However, prior studies, including our own findings, have yet to address the circuit mechanism underlying the benefits of light exercise to the dorsal hippocampus. Classically, it is known that exercise, even light exercise, can promote arousal (Suwabe

144 *et al.* 2018; Kuwamizu *et al.* 2022; McMorris 2016; Cooper 1973; Yamazaki *et al.* 2023),
145 which is thought to originate from the activation of the ascending arousal system (AAS)
146 (Saper *et al.* 2001; Jones 2003). The AAS mainly consists of cholinergic neurons and
147 monoaminergic neurons and sends out widespread projections in order to increase global
148 brain activity. In particular, it has been proposed that exercise can stimulate the release of
149 neuromodulatory factor monoamine in several brain regions, including the dorsal
150 hippocampus (Meeusen *et al.* 2001). Monoamines, including noradrenaline (NA),
151 dopamine (DA), and serotonin (5-HT), are essential for multiple functions such as arousal,
152 attention, reward, motivation, and mood regulation (Carter *et al.* 2010; Bari *et al.* 2020;
153 Saunders *et al.* 2018; Zhu *et al.* 2016; Kim *et al.* 2013; Warden *et al.* 2012; Teissier *et al.*
154 2015). At the dorsal hippocampus, they regulate learning and memory processes by
155 modulating the expression of PRPs and neuronal plasticity, such as neurogenesis and
156 long-term potentiation (LTP) (Palacios-Filardo and Mellor 2019; Poe *et al.* 2020; Wang
157 *et al.* 2010; Mahar *et al.* 2014; Coradazzi *et al.* 2016; Salvi *et al.* 2016). The hippocampal
158 monoamines are mainly synthesized and provided by the specific nuclei located in the
159 pons noradrenergic locus coeruleus (LC), the midbrain dopaminergic nuclei, such as the
160 ventral tegmental area (VTA), the substantia nigra pars compacta (SNpc), and the
161 retrorubral field (RRF), and the midbrain serotonergic raphe nuclei (the dorsal raphe:
162 DRN and the median raphe: MRN) (Gasbarri *et al.* 1996; Schwarz *et al.* 2015; Broussard
163 *et al.* 2016; Muzerelle *et al.* 2016; Luchetti *et al.* 2020). Previous research demonstrates
164 that single bouts of exercise activate brainstem monoaminergic neurons (Morikawa *et al.*
165 2021; Tsai *et al.* 2019; Rodovalho *et al.* 2020) and affect the hippocampal monoamine
166 transmissions (Vargas *et al.* 2017; Goekint *et al.* 2012; Gomez-Merino *et al.* 2001),
167 thereby contributing to the enhancement of hippocampus-related functions (Vargas *et al.*

168 2017; Vargas *et al.* 2020; Morikawa *et al.* 2021). These findings lead us to the hypothesis
169 that the monoamines provided by the brainstem nuclei could be involved in the beneficial
170 effects of light exercise on the dorsal hippocampus.

171 To examine this hypothesis, we employed a treadmill running model based on the
172 LT of rats to investigate the involvement of the brainstem monoaminergic system on
173 dorsal hippocampal activation during light exercise. First, we analyzed the neuronal
174 activation of the dorsal hippocampus and of the brainstem monoaminergic nuclei using
175 immunohistochemistry and then we monitored the dorsal hippocampal monoamine
176 transmission with *in vivo* microdialysis combined with the high-performance liquid
177 chromatography (HPLC) system.

178

179 **Materials and Methods**

180 **Animals**

181 All animal care and experimental procedures were performed in accordance with
182 protocols approved by the University of Tsukuba Animal Experiment Committee (19-378,
183 20-405, and 21-373) based on the NIH Guidelines for the Care and Use of Laboratory
184 Animals (NIH publication, 1996). Male adult Long-Evans rats (n = 72; 380-470 g; 10-12
185 weeks old at the beginning of experiments) from the TH-Cre rat colony (i.e., Cre-negative
186 littermate control of TH-Cre Long-Evans rats) were housed with 2–3 rats in each cage
187 and singly housed after surgery. Rats were kept in a controlled environment at 22 ± 1°C
188 with a 12/12-h light/dark cycle (lights on 7 a.m. to 7 p.m.) and given *ad libitum* access to
189 water and food (MF, Oriental Yeast Co., Ltd., Tokyo, Japan). All experiments were done
190 during the light cycle.

191

192 **Experimental design**

193 Four experiments were performed independently: measurement of LT (Experiment 1: *n* =
194 6), validation of exercise intensity (Experiment 2: *n* = 5-7/group, 3 groups),
195 immunohistochemical analysis of neuronal activation in dorsal hippocampus and
196 brainstem monoaminergic nuclei (Experiment 3: *n* = 6-8/group, 3 groups), and analysis
197 of dorsal hippocampal monoamine release with *in vivo* microdialysis (Experiment 4: *n* =
198 6-7/group, 3 groups). All rats were handled for 1 week before the start of all procedures
199 and habituated to treadmill running (6 days, Table 1). Then, they were randomly allocated
200 to three groups based on LT as follows: 1) sedentary on treadmill (Control: 0 m/min), 2)
201 below-LT light-intensity (low speed) running (Light: 15 m/min), and 3) above-LT
202 vigorous-intensity (high speed) running (Vigorous: 25 m/min). Exercise and control
203 conditions were maintained for 30 minutes.

204

205 **Surgery to insert jugular catheter**

206 In Experiments 1 and 2, 1 day after the habituation to treadmill running periods, the rats
207 were anesthetized with isoflurane, and a silicone catheter was inserted into the jugular
208 vein and fixed with a silk thread suture (37 mm). The external distal end of the catheter
209 was fixed at the nape of the neck.

210

211 **Stereotaxic surgery**

212 In Experiment 4, after a week of handling, all rats were anesthetized with isoflurane and
213 placed in a stereotaxic frame (Model 51900, Muromachi Kikai Co., Ltd., Tokyo, Japan).
214 A guide cannula (AG-6, Eicom, Kyoto, Japan) was implanted above the dorsal
215 hippocampus (AP: -3.6 mm, ML: 3.8 mm, DV: -1.8 mm, 20˚ angle, from bregma). The

216 cannula was secured to the skull with three anchoring screws and dental cement. To
217 prevent occlusion, a dummy cannula (AD-6, Eicom) was inserted into the guide cannula.
218 After the surgery, the rats were housed individually and were allowed to recover for at
219 least 2 days before habituation to treadmill running.
220
221 **Habituation to treadmill running**
222 All rats were habituated to running on a treadmill (KN-73, Natsume Seisakusho Co, Ltd.,
223 Tokyo, Japan) for a total of five sessions over 6 days. The running duration was 30
224 min/day, and the running speed was gradually increased from 5 to 25 m/min with no
225 incline (Nishijima and Soya 2006; Soya *et al.* 2007a). Electrical shock grids were placed
226 at the rear end of the treadmill and provided mild but aversive foot shocks (30 V) to rats
227 to encourage running at the set treadmill speed. To minimize any stress derived from the
228 shocks, electric shocks were limited and a gentle tail touch was given when rats did not
229 start running again. After the habituation period, rats were able to run at the appropriate
230 speeds even when the electric grid was turned off.
231
232 **Treadmill running tests**
233 On the test day, all rats fasted for 2 h before running to obtain stable metabolic conditions.
234 In Experiments 1 and 2, treadmill running tests were conducted 2-3 days after the surgery
235 for inserting the jugular catheter. In Experiment 1, an incremental exercise test was
236 performed to assess the running speed at LT. Rats were forced to run to exhaustion; the
237 running speed was started at 5 m/min and increased by 2.5 m/min every 3 minutes. The
238 speed at which the LT was reached was determined according to the method by Beaver *et*
239 *al.* (Beaver *et al.* 1985). In Experiment 2, a single 30-minute bout of running was

240 conducted to verify the validation of exercise intensity based on the speed at LT as
241 determined in Experiment 1. Rats were randomly subjected to run at 15 m/min (Light) or
242 25 m/min (Vigorous), or they were placed on a treadmill as the at-rest Control for 30
243 minutes. In both experiments, serial blood samples (0.1 ml) were taken at designated time
244 points. Blood lactate and glucose were measured using an automated glucose–lactate
245 analyzer (2300 Stat Plus, Yellow Springs Instruments, OH, USA).
246 In Experiment 3, rats were returned to their home cage after the test, rested for 90 min,
247 and then were transcardially perfused with 0.9% saline, followed by 4%
248 paraformaldehyde (PFA) in a 0.1 M phosphate buffer (PB; pH 7.4) under deep isoflurane
249 anesthesia. The brains were sampled for immunohistochemical analysis.
250 In Experiment 4, a microdialysis probe (2 mm membrane: FX-I-6-02, Eicom) was
251 inserted into the dorsal hippocampus using a guide cannula 1 day before the test. The
252 probe was connected on a treadmill and perfused with Ringer's solution (147 mM NaCl,
253 4 mM KCl, and 2.3 mM CaCl2) at 1.0 μl/min 2-3 h before the start of the test because a
254 stable dialysate monoamine level is usually obtained 2 h after insertion of the probe.
255 Using a fraction collector (EFC-82, Eicom), 15 μl of dialysate was collected every
256 15 minutes and 5 μl of 0.1M PB (pH 3.5) was added to ensure stable monoamine detection
257 using HPLC with an electrochemical detector (HPLC-ECD). At the end of the
258 experiments, rats were transcardially perfused with 0.9% saline, followed by 4% PFA in
259 a 0.1 M PB under deep isoflurane anesthesia and the brain was collected to verify the
260 position of the probe in coronal sections with DAPI (4',6-diamidino-2-phenylindole).
261 Please note that 7 rats in Experiment 4 were excluded from the analysis for the following
262 reasons: failed insertion of the probe into the dorsal hippocampus (1 rat), detachment of
263 the fixed probe during running (3 rats), and failed detection of peaks in chromatography

264 (3 rats).

265

266 **Immunohistochemistry**

267 The brain was post-fixed for 24 h in 4% paraformaldehyde (PFA, 02890-45, Nacalai
268 Tesque, Tokyo, Japan) in a 0.1 M PB and cryoprotected by immersion in a 0.1 M PB
269 containing 20% sucrose for 1 day and 30% sucrose for 2-3 days. Frozen coronal brain
270 sections of 30 μm thickness were cut with a microtome (REM-710, Yamato Kohki
271 Industrial Co., Ltd., Saitama. Japan). Sections were washed with a 0.1M PB and blocked
272 with a 0.1 M PB containing 1% Triton X-100 (35501-15, Nacalai Tesque) (PBT) plus 1%
273 bovine serum albumin (01863-77, BSA; Nacalai Tesque). Then, slices were incubated
274 with the designated primary antibodies in PBT-BSA for 24 h at 4 °C. The primary
275 antibodies used in this study were rabbit polyclonal antibody against c-Fos (1:5,000; Cat#
276 226-003, RRID: AB_2231974, Synaptic Systems, GAU, Germany), mouse monoclonal
277 antibody against NeuN (1:1,000; Cat# MAB377, RRID: AB_2298772, Millipore, MA,
278 USA), mouse monoclonal antibody against tyrosine hydroxylase (1:2,000; Cat# F11:sc-
279 25269, RRID: AB_628422, Santa Cruz, CA, USA), and goat polyclonal antibody against
280 5-HT (1:1,000; Cat# 20079, RRID: AB_572262, Immunostar Inc., WI, USA). After
281 repeated washings with PBT-BSA, sections were incubated in a dark space with
282 designated secondary antibodies diluted in PBT-BSA for >16 h at 4 °C. The secondary
283 antibodies used in this study were Alexa Fluor 488-conjugated goat anti-rabbit IgG
284 (1:5,000; Cat# A-11008, RRID: AB_143165, Invitrogen; Thermo Fisher Scientific Inc.,
285 MA, USA), Alexa Fluor 488-conjugated donkey anti-rabbit IgG (1:5,000; Cat# ab150073,
286 RRID: AB_2636877, abcam, Cambridge, UK), Alexa Fluor 594-conjugated donkey anti-
287 goat IgG (1:1,000; Cat# A-11058, RRID: AB_142540, Invitrogen; Thermo Fisher

288 Scientific Inc., MA, USA), and AMCA AffiniPure donkey anti-mouse IgG (1:1,000; Cat#
289 715-155-150, RRID: AB_2340806, Jackson ImmunoResearch Laboratories, Inc., PA,
290 USA). For DAPI staining to verify the location of microdialysis probes, slices were
291 washed three times in a 0.1 M PB and then incubated with DAPI (1:1,000; 28718-90-3,
292 Dojindo Laboratory, Kumamoto, Japan) in PBT-BSA for 10 minutes in the dark. Slices
293 were washed three times in a 0.1 M PB, mounted on subbed slides, air dried, and
294 coverslipped using a mounting medium.
295

296 **Counts and quantification**

297 All fluorescence z-stacked images were examined on an all-in-one microscope (BZ-X710,
298 Keyence, Osaka, Japan) with a 20× objective lens. Manual counts of neurons were
299 performed by an observer blinded to the group allocations. The number of c-Fos and
300 NeuN double-positive neurons (c-Fos$^+$/NeuN$^+$) in the dorsal hippocampal dentate gyrus
301 (DG), cornu ammonis (CA) 1, CA2, and CA3 sub-regions were counted hemilaterally in
302 4 coronal sections (from Bregma: -3.6, -4.2, -5.2, -5.8). Total neuron numbers were
303 normalized based on NeuN$^+$ region volume (mm^3), which was calculated by the area
304 (mm^2) multiplied by the thickness of sections (30 μm) using a BZ-X analyzer (Keyence).
305 The numbers of c-Fos and TH or 5-HT double-positive neurons (c-Fos$^+$/TH$^+$ or c-Fos$^+$/5-
306 HT$^+$) were bilaterally counted 120 μm apart throughout the LC and 360 μm apart
307 throughout the VTA, SNpc, RRF, DRN, and MRN, all of which are known as
308 monoaminergic nuclei projecting to the dorsal hippocampus.
309

310 **HPLC-ECD for monoamine detection in dorsal hippocampus**

311 Dialysate samples collected from the probe at the dorsal hippocampus were injected into

312 an HPLC-ECD system (HTEC-500; Eicom) using an autosampler (M-510; Eicom) and
313 analyzed for NA, DA, and 5-HT concentration using the HPLC system with an
314 EICOMPAK CAX column (2.0 mm, i.d. × 200 mm; Eicom) and a graft electrode (WE-
315 3G; Eicom) set at 450 mV (vs Ag/AgCl reference electrode). The flow rate and injection
316 volume were set to 250 μl/min and 17 μl, respectively. The mobile phase contained a
317 0.1 M ammonium acetate buffer, 0.05 mg/l sodium sulfate, 50 mg/l EDTA, and methanol
318 (7:3, v/v), with a pH of 6.0. Data analysis was performed using Eicom EPC-710 software.
319 Basal concentrations of the three monoamines (i.e., NA, DA, and 5-HT) were estimated
320 from the averages at four HPLC time points before the beginning of running.
321
322 **Statistical analyses**
323 All data are expressed as mean ± standard error (SEM) and were analyzed using R
324 software (version 4.2.3). Assessment of the normality (Shapiro-Wilk test) of the data,
325 equality of variance (Levene's test), and sphericity (Mendoza's test of sphericity) for
326 repeated measures (RM) were performed. Statistical significance for parametric data was
327 analyzed using one-way analysis of variance (ANOVA) followed by Shaffer's *post-hoc*
328 test, but Welch's ANOVA was performed if equality of variance was not assumed. For the
329 analysis of non-parametric data, the Kruskal-Wallis test with the Mann-Whitney U test
330 adjusted by Holm's post-hoc test was applied. For RM, two-way ANOVA followed by
331 Shaffer's post-hoc test was applied and the degree of freedom was adjusted using Huynh-
332 Feldt-Lecoutre's epsilon for non-spherical data. Pearson's correlation coefficient was
333 used for analyzing the strength of relationships. No tests for outliers were performed.
334 Statistical significance was assumed at $p < 0.05$. No statistical methods were used to
335 predetermine sample size, but the sample sizes were comparable to those reported in

336 previous studies (Soya *et al.* 2007a; Tsai *et al.* 2019; Rodovalho *et al.* 2020; Goekint *et*
337 *al.* 2012; Lee *et al.* 2003).
338
339 **Results**
340 **Measurement of LT and validation of exercise intensity**
341 Initially, we developed a varied-intensity treadmill running model with adult male Long-
342 Evans rats using LT as a signpost for exercise-induced stress. Our analysis revealed that
343 the running speed at the LT of rats was 21.87 m/min ± 1.02 (n = 6; Fig. 1B, C). Thus, we
344 defined 15 m/min, which is below the LT, as the speed for light-intensity running and 25
345 m/min, which is above the LT, as the speed for vigorous-intensity running. The
346 observation of a significant increase in blood lactate concentration after a 30-minute
347 session of vigorous-intensity running (two-way RM ANOVA, Shaffer's *post hoc* test,
348 group and time interaction, $F_{(2, 15)} = 66.036$, $p < 0.001$, Fig 1E) was sufficient to validate
349 the varied-intensity running model based on LT.
350
351 **Light-intensity-running-induced neuronal activation of dorsal hippocampus**
352 We assessed dorsal hippocampal neuronal activation during different intensities of
353 running using c-Fos immunostaining. Representative images in Figure 2B demonstrate
354 that c-Fos expression in dorsal hippocampal sub-regions, including the DG, CA1, and
355 CA3, significantly increased regardless of running speed. Conversely, the CA2 region
356 was not affected by running (DG: Welch's one-way ANOVA, Shaffer's *post hoc* test, $F_{(2,$
357 $18)} = 12.876$, $p < 0.001$, Fig. 2C; CA1: one-way ANOVA, Shaffer's *post hoc* test, $F_{(2, 18)}$
358 $= 9.994$, $p < 0.001$, Fig. 2D; CA2: one-way ANOVA, Shaffer's *post hoc* test, $F_{(2, 18)} =$
359 0.173, $p = 0.843$, Fig. 2E; CA3: one-way ANOVA, Shaffer's *post hoc* test, $F_{(2, 18)} = 11.256$,

360 $p < 0.001$, Fig. 2F).

361

362 **Light-intensity running increased dorsal hippocampal NA and DA release**

363 To assess the monoaminergic neurotransmission to the dorsal hippocampus, we measured
364 extracellular monoamine levels in the dorsal hippocampus using *in vivo* microdialysis and
365 HPLC methods. We found that treadmill running induced a significant increase of both
366 NA and DA levels in the dorsal hippocampus depending on running speed (NA: two-way
367 RM ANOVA, Shaffer's *post hoc* test, group and time interaction, $F_{(7.01, 59.58)} = 5.393$ $p <$
368 0.001; DA: two-way RM ANOVA, Shaffer's *post hoc* test, group and time interaction, F
369 $_{(8.03, 68.23)} = 4.959$, $p < 0.001$, Fig. 3B, C). Notably, DA release was significantly sustained
370 into the post-running time period. Dorsal hippocampal 5-HT levels, on the other hand,
371 were not affected by running (two-way RM ANOVA, Shaffer's *post hoc* test, interaction,
372 $F_{(5.58, 47.46)} = 1.16$, $p = 0.343$, Fig. 3D).

373

374 **Running stimulated TH$^+$ neurons in LC and VTA, but not raphe 5-HT$^+$ neurons**

375 To examine the responsiveness of brainstem monoaminergic nuclei, we performed
376 double-immunostaining of c-Fos$^+$/TH$^+$ neurons for the activation of NA- and DA-
377 producing neurons, and c-Fos$^+$/5-HT$^+$ neurons for the activation of serotonergic neurons,
378 as shown in Figure 4A-C. TH$^+$ neurons in the LC (LCTH) and VTA (VTATH), which are
379 known as the main sources of dorsal hippocampal NA and DA, respectively, were
380 significantly stimulated by both light- and vigorous-intensity running (LC: one-way
381 ANOVA, Shaffer's *post hoc* test, $F_{(2, 18)} = 20.908$, $p < 0.001$, Fig. 4D; VTA: one-way
382 ANOVA, Shaffer's *post hoc* test, $F_{(2, 18)} = 4.992$, $p < 0.05$, Fig. 4E). However, running
383 did not have any impact on dopaminergic neurons in the SNpc or RRF (SNpcTH neurons

384 and RRF^{TH} neurons, respectively), or on serotonergic neurons in the DRN and MRN
385 ($DRN^{5\text{-}HT}$ neurons and $MRN^{5\text{-}HT}$ neurons, respectively) (SNpc: Kruskal-Wallis test,
386 Mann-Whitney U test adjusted by Holm's *post-hoc* test, $X^2 = 4.653$, df = 2, $p = 0.097$, Fig.
387 4F; RRF: Kruskal-Wallis test, Mann-Whitney U test adjusted by Holm's *post-hoc* test, X^2
388 = 4.494, df = 2, $p = 0.106$, Fig. 4G; DRN: one-way ANOVA, Shaffer's *post hoc* test, $F_{(2,}$
389 $_{18)} = 0.832$, $p = 0.454$, Fig. 4H; MRN: one-way ANOVA, Shaffer's *post hoc* test, $F_{(2, 18)}$
390 $= 1.640$, $p = 0.222$, Fig. 4I). Note that TH^+ neurons were also observed in the rostral
391 region of the DRN (DRN^{TH} neurons), which are reported to promote wakefulness through
392 the release of DA (Cho *et al.* 2017). In this study, however, running did not affect the c-
393 Fos expression in DRN^{TH} neurons (Fig. S2A).
394
395 **Neuronal associations between dorsal hippocampus and brainstem monoaminergic**
396 **nuclei**
397 To investigate whether the neuronal activities of brainstem monoaminergic nuclei are
398 associated with dorsal hippocampal activation, we performed Pearson's correlation
399 analysis between c-Fos expression in the brainstem monoaminergic nuclei and in the
400 dorsal hippocampus. A correlation matrix heatmap that summarizes the correlation results
401 is shown in Figure 5. c-Fos expression in LC^{TH} neurons exhibited a significant and strong
402 positive correlation within all examined dorsal hippocampal sub-regions except CA2 (n
403 = 21, DG: $r = 0.672$, $p < 0.001$; CA1: $r = 0.768$, $p < 0.001$; CA2: $r = 0.212$, $p = 0.357$;
404 CA3: $r = 0.700$, $p < 0.001$). Additionally, a significant positive correlation was found
405 between c-Fos expression in VTA^{TH}, $SNpc^{TH}$, and RRF^{TH} neurons and in the dorsal CA1
406 (VTA: $n = 21$, DG: $r = 0.429$, $p = 0.0521$; CA1: $r = 0.566$, $p < 0.01$; CA2: $r = 0.033$, $p =$
407 0.886; CA3: $r = 0.378$, $p = 0.091$; SNpc: $n = 21$, DG: $r = 0.257$, $p = 0.261$; CA1: $r = 0.624$,

408 $p < 0.01$; CA2: $r = 0.198$, $p = 0.389$; CA3: $r = 0.321$, $p = 0.155$; RRF: $n = 21$, DG: $r =$
409 0.251, $p = 0.273$; CA1: $r = 0.713$, $p < 0.001$; CA2: $r = 0.173$, $p = 0.454$; CA3: $r = 0.352$,
410 $p = 0.155$). The higher level of c-Fos expression in $DRN^{5\text{-}HT}$ neurons was significantly
411 correlated with lower levels of DG, CA2, and CA3 c-Fos expression ($n = 21$, DG: $r = -$
412 0.463, $p < 0.05$; CA1: $r = -0.204$, $p = 0.375$; CA2: $r = -0.583$, $p < 0.01$; CA3: $r = -0.573$,
413 $p < 0.01$), but no correlation was found in the c-Fos expression between $MRN^{5\text{-}HT}$ neurons
414 and dorsal hippocampal sub-regions ($n = 21$, DG: $r = -0.275$, $p = 0.228$; CA1: $r = 0.055$,
415 $p = 0.814$; CA2: $r = 0.076$, $p = 0.743$; CA3: $r = -0.307$, $p = 0.176$).
416

417 **Discussion**

418 Our present study provides evidence of possible noradrenergic and dopaminergic
419 stimulation of dorsal hippocampal activation during light exercise. These findings may
420 help to elucidate the neural circuit mechanisms underlying the positive effects of light
421 exercise on dorsal hippocampal plasticity and function. Our results show that even light
422 exercise increases c-Fos expression in the dorsal hippocampal subregions and induces the
423 release of NA and DA at the dorsal hippocampus. Additionally, LC^{TH} neurons and VTA^{TH}
424 neurons were activated even under the light exercise condition, and both were positively
425 correlated with dorsal hippocampal activation. Meanwhile, the serotonergic system was
426 not affected by exercise.

427 In this study, we first established a varied-intensity treadmill running model for male
428 adult Long-Evans rats based on LT as a physiological index of running intensity and stress.
429 The speed at the LT was similar to that in our previous work using male adult Wistar rats
430 (Long-Evans: 21.87 m/min ± 1.02 vs Wistar: 20.7 m/min ± 2.1; Fig. 1B, C; (Soya *et al.*
431 2007a). In addition, a significant increase in blood lactate concentration was observed

following a single bout of vigorous-intensity running, but not with light-intensity running (Fig. 1E), which is consistent with our previous reports (Soya *et al.* 2007a; Saito and Soya 2004). These results confirm the validity of our experimental model (LT-based varied-intensity running) and demonstrate that the two running groups exhibited different metabolic and stress-related responses.

Previous studies have reported that acute varied-intensity treadmill running activates the dorsal hippocampus, including the DG, CA1, and CA3 sub-regions, even at low-speeds (Oladehin and Waters 2001; Lee *et al.* 2003; Soya *et al.* 2007b). Our current results support these findings since both light- and vigorous-intensity running increased c-Fos expression in the dorsal DG, CA1, and CA3 sub-regions (Fig. 2C, D, F). In contrast, the dorsal CA2 region was not activated with running (Fig. 2E). The CA2 is known as a small hippocampal sub-region that modulates social memory and that is stimulated by novel encounters (Chen *et al.* 2020). Since all rats were well familiarized with the experimental conditions and there were no novel or social stimuli, it is reasonable that activity in the CA2 region was not affected. Additionally, we examined the activation of the ventral hippocampus following running and found an increase of c-Fos expression across all sub-regions of the ventral hippocampus in both running groups (Fig. S1). These results suggest that light-intensity running is sufficient to elevate the neuronal activity in the hippocampus across the dorsal to ventral axis, which may help to explain the positive effects of exercise on dorsal-hippocampus-related functions, such as memory, as well as ventral-hippocampus-related anxiolytic and antidepressant effects (Otsuka *et al.* 2016; Inoue *et al.* 2015a; Vargas *et al.* 2017).

Our *in vivo* microdialysis experiment provides the first evidence that even light-intensity running increases the release of NA and DA at the dorsal hippocampus (Fig. 3B,

456 C). Given that NA and DA both perform critical roles in dorsal hippocampal neuronal
457 activity, plasticity, and memory process (Takeuchi *et al.* 2016; Mello-Carpes *et al.* 2016;
458 Coradazzi *et al.* 2016; Salvi *et al.* 2016; Takamura *et al.* 2014), our results suggest that
459 the dorsal hippocampal NA and DA release contributes to the light-exercise-induced
460 hippocampal activation and beneficial effects, such as hippocampal neurogenesis and
461 spatial memory performance (Soya *et al.* 2007b; Inoue *et al.* 2015b; Inoue *et al.* 2015a).
462 Notably, NA and DA have been recognized as important factors in memory consolidation
463 (Takeuchi *et al.* 2016; Mello-Carpes *et al.* 2016), as shown by the synaptic tagging and
464 capture hypothesis (Frey and Morris 1997; Redondo and Morris 2010), raising a
465 hypothesis that a single-bout of light-intensity running could facilitate memory retention
466 through noradrenergic and dopaminergic dorsal hippocampal activation. Intriguingly,
467 prior studies demonstrate that a single session of vigorous-intensity exercise elevates NA
468 and DA concentrations in the hippocampus and promotes novel object recognition, which
469 is a dorsal-hippocampus-related function, through the β adrenergic receptor and
470 dopamine D1 receptor, respectively (Vargas *et al.* 2017; Vargas *et al.* 2020). Although we
471 did not directly examine this in the present study, these findings support our hypothesis.
472 We confirmed that treadmill running, even at light intensity, activates LC^{TH} and
473 VTA^{TH} neurons (Fig. 4D, E). Furthermore, our correlation analysis observed that LC^{TH}
474 activation positively correlates with dorsal hippocampal activation, and the activities of
475 midbrain dopaminergic nuclei, including VTA^{TH}, were positively associated with those
476 of the dorsal CA1 region (Fig. 5). These results suggest that running stimulates the dorsal
477 hippocampal neurons through noradrenergic projection from the LC and dopaminergic
478 projection from the VTA. Although both nuclei are known as major sources of dorsal
479 hippocampal NA and DA (Kempadoo *et al.* 2016; Weitemier and McHugh 2017), recent

480 studies have shown that LC terminals co-release DA and NA to the dorsal hippocampus,
481 and that LC-DA, but not LC-NA, plays a significant role in spatial memory and context
482 learning (Kempadoo *et al.* 2016; Takeuchi *et al.* 2016; Wagatsuma *et al.* 2017;
483 Chowdhury *et al.* 2022). On the other hand, VTA^{TH} neurons have sparse input to the
484 dorsal hippocampus and do not have a significant impact on spatial memory (Kempadoo
485 *et al.* 2016; Takeuchi *et al.* 2016). However, hippocampal DA from VTA^{TH} neurons is still
486 reported to be involved in hippocampus-related functions, such as reward-related spatial
487 memory, aversive memory, and associative memory (McNamara *et al.* 2014; Kramar *et*
488 *al.* 2021; Lee *et al.* 2021; Tsetsenis *et al.* 2021). The significance of LC^{TH} and VTA^{TH}
489 neurons for the dorsal hippocampal functions remains elusive; thus, future studies using
490 viral-tracing techniques and chemogenetic/optogenetic manipulation are needed to
491 investigate the noradrenergic and dopaminergic mechanisms underlying the beneficial
492 effects of light exercise.

493 Unlike the noradrenergic and the dopaminergic systems, 5-HT release was not
494 increased at the dorsal hippocampus, nor were $DRN^{5\text{-}HT}$ or $MRN^{5\text{-}HT}$ neurons found to be
495 stimulated by treadmill running (Fig. 3D; Fig. 4H, I). However, we found a negative
496 correlation between c-Fos expression in the DG, CA2, and CA3 sub-regions and that in
497 $DRN^{5\text{-}HT}$ neurons (Fig. 4). Previous studies have shown that running increases
498 hippocampal neuronal activity and also facilitates hippocampal LTP induction (Miladi-
499 Gorji *et al.* 2014; Radahmadi *et al.* 2016; Farmer *et al.* 2004). Conversely, the
500 hippocampal 5-HT can exert inhibitory effects on basal neural activity and LTP induction
501 (Villani and Johnston 1993; Shakesby *et al.* 2002; Kulla and Manahan-Vaughan 2002).
502 Considering these findings, our results imply that running enhances hippocampal
503 neuronal activity and LTP induction through the inhibitory control of the serotonergic

504 inhibitory projection from $DRN^{5\text{-}HT}$ neurons to the dorsal hippocampus.

505 From a clinical perspective, light exercise, such as yoga, tai-chi, and slow running,
506 is a simple and feasible intervention accessible by most people, including patients with
507 mild cognitive impairment (MCI) and Alzheimer's disease (AD). Recent studies have
508 shown that the hippocampal NA and DA loss and degeneration of LC^{TH} and VTA^{TH}
509 neurons and axons are found in AD model rodents and AD patients (Nobili *et al.* 2017;
510 Sala *et al.* 2021; Francis *et al.* 2012; Rorabaugh *et al.* 2017; Mehla *et al.* 2019). Notably,
511 selective accumulation of amyloid β and tau proteins are observed in LC neurons before
512 the onset or at the early stage of AD symptoms (Matchett *et al.* 2021; Betts *et al.* 2019;
513 Mehla *et al.* 2019; Rorabaugh *et al.* 2017). Light exercise has been reported to have a
514 positive effect on MCI and AD (Yoon *et al.* 2021; Meng *et al.* 2020; Vasconcelos-Filho
515 *et al.* 2021). Therefore, our findings may provide insight into understanding the
516 mechanisms underlying the preventive or therapeutic role of light exercise in cognitive
517 dysfunction conditions, such as AD.

518

519 **Conclusion**

520 In conclusion, our results reveal that even light-intensity running activates the dorsal
521 hippocampus and LC-noradrenergic and VTA-dopaminergic system but that it does not
522 affect the serotonergic system. These findings suggest a possible contribution of LC-
523 noradrenergic and VTA-dopaminergic projections to dorsal hippocampal activation
524 during light exercise, which may provide insight into the circuit mechanism that underlies
525 light-exercise-enhanced learning and memory.

526

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Conflicts of Interest disclosure

The authors declare no competing financial interests.

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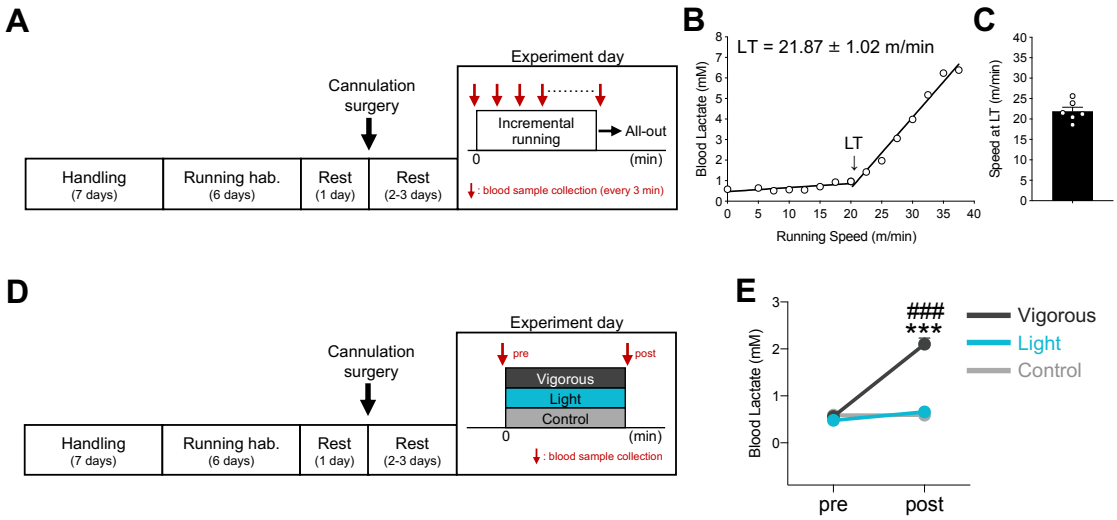
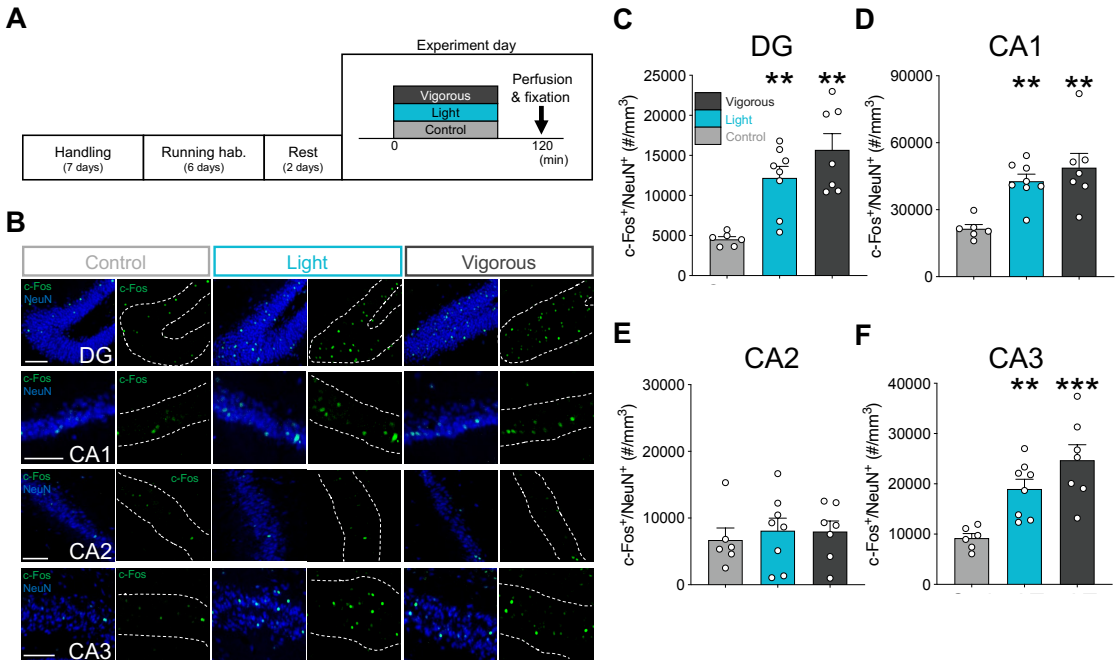


Fig. 1 Measurement of lactate threshold and validation of exercise intensity

(A) Protocol for incremental running test. (B) A representative LT profile from the incremental running test. (C) Speed of rats at LT was 21.87 m/min ± 1.02 (n = 6). (D) Protocol for a single bout of the running test. We defined 15 m/min for light-intensity running and 25 m/min for vigorous-intensity running. (E) Blood lactate concentration was significantly increased only after a 30-minute session of vigorous-intensity running. All data are expressed as mean ± SEM. $^{***}p < 0.001$ vs Control, $^{###}p < 0.001$ vs Light, n = 5-7/group. Control: sedentary control; Light: 15 m/min running; Vigorous: 25 m/min running; hab.: habituation.



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859 **Fig. 2 Running-induced neuronal activation at the dorsal hippocampus during**

860 **varied-intensity treadmill running.**

861 (A) Protocol for a single bout of the running test for immunohistochemical analysis. (B)

862 Representative double-fluorescence images of c-Fos (green) and NeuN (blue) in the

863 DG, CA1, and CA3 regions of the dorsal hippocampus. Scale bars show 100 μm. c-Fos

864 expressions in the DG (C), CA1 (D), CA2 (E), and CA3(F) were significantly increased

865 with running exercise regardless of running speed (light or vigorous). All data are

866 expressed as mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$ vs Control, n = 6-8 / group. Control:

867 sedentary control; Light: 15 m/min running; Vigorous: 25 m/min running; hab.:

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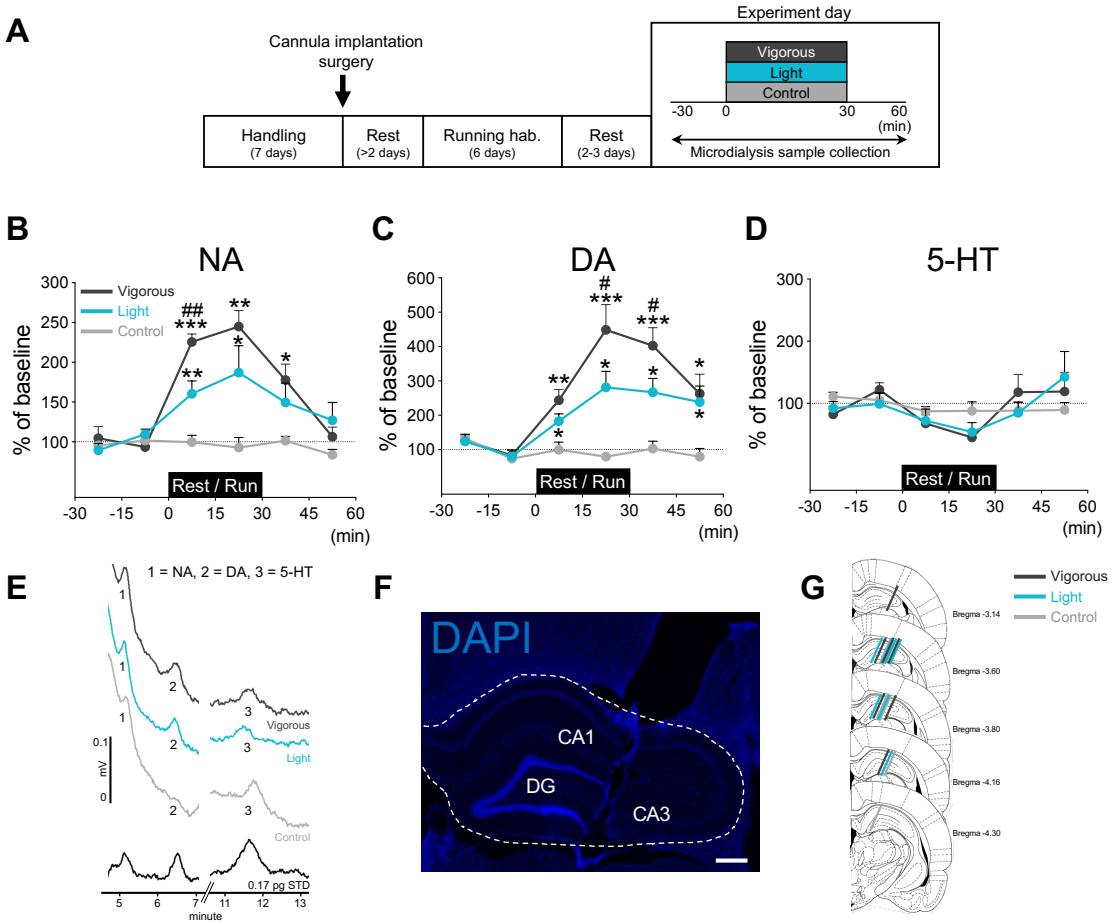

Fig. 3 Running increases dorsal hippocampal NA and DA release, but not 5-HT release

(A) Protocol for a single bout of the running test for dorsal hippocampal monoamine detection with *in vivo* microdialysis. Treadmill running induced a significant increase of both NA (B) and DA (C) levels in the dorsal hippocampus, depending on running speed, but not 5-HT (D). (E) Representative chromatograms showing NA (peak 1), DA (peak 2), and 5-HT (peak 3) from Control (light gray), Light (blue), and Vigorous (dark gray). (F) Representative DAPI fluorescence image of microdialysis probe position in the dorsal hippocampus. Scale bars show 500 μm. (G) Schematic representations indicating the location of the microdialysis probe. Each bar represents the probe position of an

individual rat, and the color of the bar corresponds to the experimental group (Control: light gray, Light: blue, and Vigorous: dark gray). All data are expressed as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs Sed, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs Light, n = 6-7/group. Control: sedentary control; Light: 15 m/min running; Vigorous: 25 m/min running; hab.: habituation.

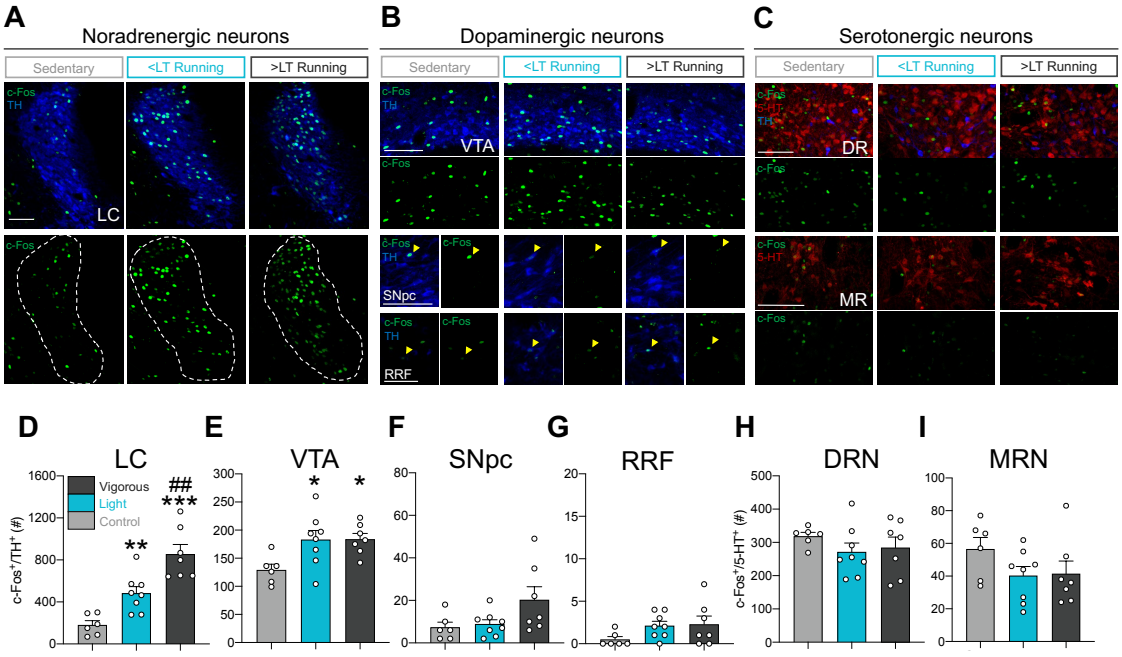

Fig. 4 LCTH and VTATH neurons, but not raphe 5-HT^{+} neurons, stimulated by running

Representative double-fluorescence images of c-Fos (green), TH (blue), and 5-HT (red) neurons in LC (A); VTA, SNpc, and RRF (B); and DRN and MRN (C). Scale bars show 100 μm. Yellow heads show c-Fos^{+}/TH^{+} neurons. LCTH and VTATH neurons were significantly stimulated in both running groups (D, E). However, running did not have an impact on SNpcTH and RRFTH neurons (F, G) or DRN$^{5\text{-}HT}$ and MRN$^{5\text{-}HT}$ neurons (H, I). All data are expressed as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs Control, $^{\#\#}p < 0.01$ vs Light, n = 6-8 / group. Control: sedentary control; Light: 15 m/min running; Vigorous: 25 m/min running.

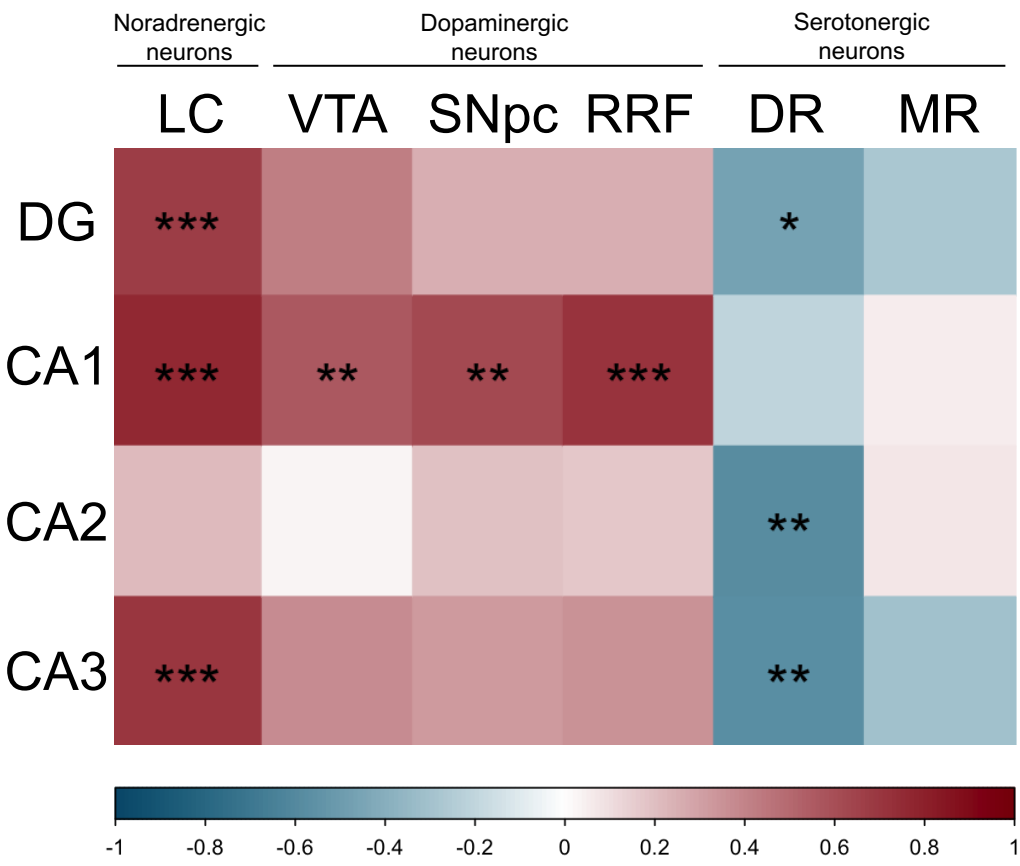

Fig. 5 Neuronal associations between dorsal hippocampus and brainstem monoaminergic nuclei

A correlation matrix heatmap is shown to summarize the correlation analysis between c-Fos expression in dorsal-hippocampal sub-regions and that in LC^{TH}, VTA^{TH}, $SNpc^{TH}$, RRF^{TH}, $DRN^{5\text{-}HT}$, and $MRN^{5\text{-}HT}$ neurons. Colors in the scale show correlation strength. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. $n = 21$.

Table 1 Protocol for habituation to treadmill running

Day	Running speed (duration)
1	Rest (10 min) → 5 m/min (10 min) → 10 m/min (10 min)
2	Rest (5 min) → 5 m/min (10 min) → 10 m/min (10 min) → 15 m/min (10 min)
3	Rest
4	Rest (5 min) → 10 m/min (10 min) → 15 m/min (10 min) → 20 m/min (10 min)
5	Rest (5 min) → 15 m/min (10 min) → 20 m/min (10 min) → 25 m/min (10 min)
6	Rest (5 min) → 15 m/min (10 min) → 20 m/min (10 min) → 25 m/min (10 min)

Supporting information

Light exercise-induced dopaminergic and noradrenergic stimulation in the dorsal hippocampus: using a rodent physiological exercise model

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This PDF file includes:

・Figure S1

・Figure S2

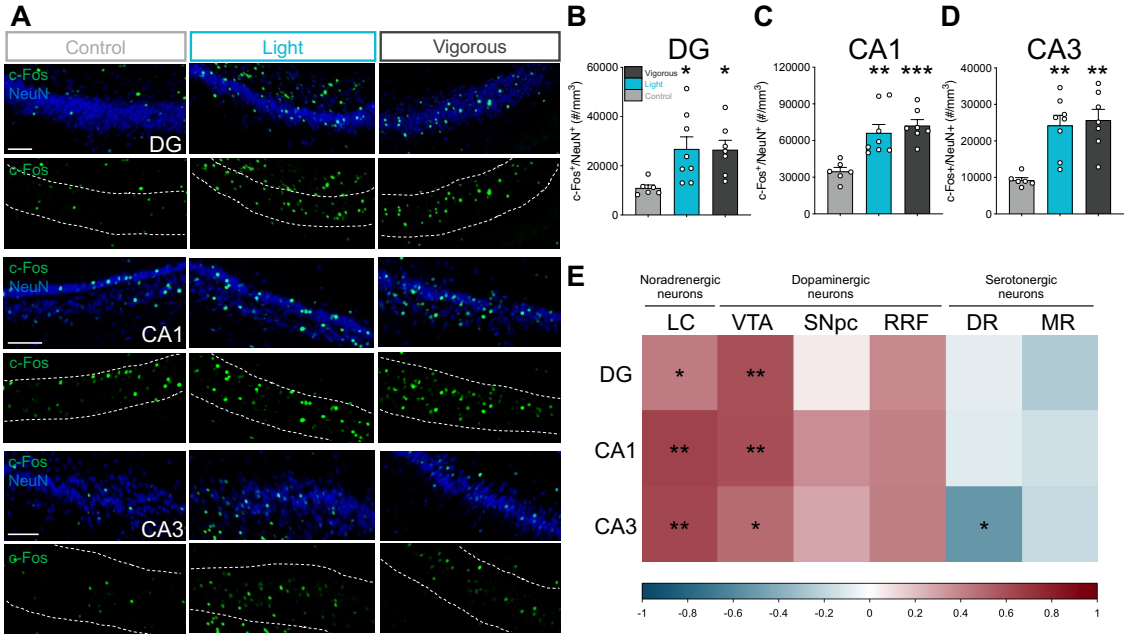

Fig. S1. Running induces neuronal activation of the ventral hippocampus

(A) Representative double-fluorescence images of NeuN (blue) and c-Fos (green) in the DG, CA1, and CA3. Scale bars show 100 µm. c-Fos expression in ventral hippocampal sub-regions, including the DG (B), CA1 (C), and CA3 (D), was also significantly increased regardless of running speed (DG: Kruskal-Wallis test, Mann-Whitney U test adjusted by Holm's *post-hoc* test, $X^2 = 10.245$, df = 2, $p < 0.01$; CA1: one-way ANOVA, Shaffer's *post hoc* test, $F_{(2, 18)} = 11.443$, $p < 0.001$; CA3: Welch's one-way ANOVA, Shaffer's *post hoc* test, $F_{(2, 18)} = 11.758$, $p < 0.001$). (E) A correlation matrix heatmap is shown to summarize the correlation results between c-Fos expression in monoaminergic nuclei and that in ventral hippocampal sub-regions. Colors in the scale show correlation strength. Data were analyzed using Pearson's correlation. c-Fos expression in LC^{TH} and VTA^{-TH} neurons had a significant positive correlation with that in all ventral hippocampal sub-regions (LC: $n = 21$, DG: $r = 0.435$, $p < 0.05$; CA1: $r = 0.658$, $p < 0.01$; CA3: $r = 0.631$, $p < 0.01$; VTA: $n = 21$, DG: $r = 0.599$, $p < 0.05$; CA1: $r = 0.610$, $p < 0.01$; CA3: r

997 = 0.499, $p < 0.05$). Additionally, the higher level of c-Fos expression in $DRN^{5\text{-}HT}$ neurons
998 was significantly correlated with the lower level of ventral CA3 c-Fos expression ($n = 21$;
999 DG: $r = -0.085$, $p = 0.715$; CA1: $r = -0.090$, $p = 0.697$; CA3: $r = -0.522$, $p < 0.05$). No
1000 correlation was found in c-Fos expression between $SNpc^{TH}$, RRF^{TH}, or $MRN^{5\text{-}HT}$ neurons
1001 or in ventral hippocampal sub-regions (SNpc: $n = 21$, DG: $r = 0.064$, $p = 0.783$; CA1: r
1002 $= 0.366$, $p = 0.102$; CA3: $r = 0.286$, $p = 0.208$; RRF: $n = 21$, DG: $r = 0.389$, $p = 0.082$;
1003 CA1: $r = 0.412$, $p = 0.063$; CA3: $r = 0.429$, $p = 0.052$; MRN: $n = 21$, DG: $r = -0.255$, $p =$
1004 0.265; CA1: $r = -0.159$, $p = 0.492$; CA3: $r = -0.181$, $p = 0.429$). All data are expressed as
1005 mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs Control; n = 6-8/group. Control:
1006 sedentary control; Light: 15 m/min running; Vigorous: 25 m/min running.
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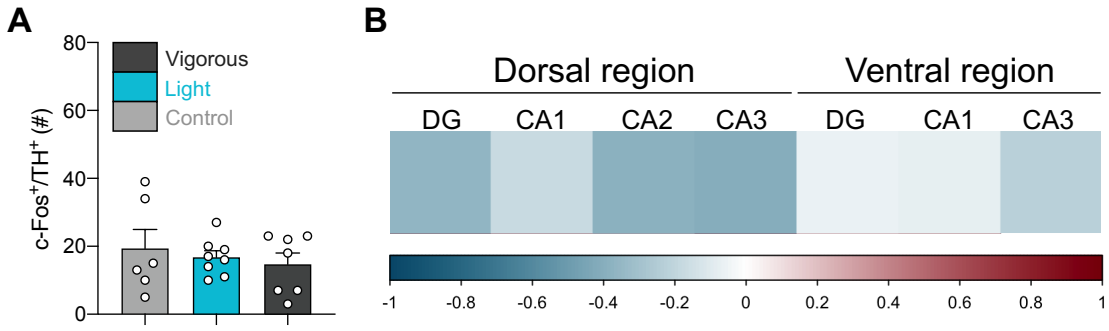

Fig. S2 DRNTH neurons not activated by running and not associated with hippocampal neuronal activation

(A) DRNTH neurons were not activated by running (Welch's one-way ANOVA, Shaffer's *post hoc* test, $F_{(2, 18)} = 0.384$, $p = 0.687$). (B) A correlation matrix heatmap is shown to summarize the correlation results between c-Fos expression in DRNTH neurons and that in hippocampal sub-regions. Colors in the scale show correlation strength. There was no significant correlation between c-Fos expression in hippocampal subregions and c-Fos expression in DRNTH neurons (dorsal DG: $r = -0.364$, $p = 0.105$; dorsal CA1: $r = -0.174$, $p = 0.451$; dorsal CA2: $r = -0.381$, $p = 0.088$; dorsal CA3: $r = -0.409$, $p = 0.066$; ventral DG: $r = -0.063$, $p = 0.786$; ventral CA1: $r = -0.073$, $p = 0.754$; ventral CA3: $r = -0.222$, $p = 0.333$). All data are expressed as mean ± SEM. n = 6-8/group.