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

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

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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

IGF-I: insulin-like growth factor I

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

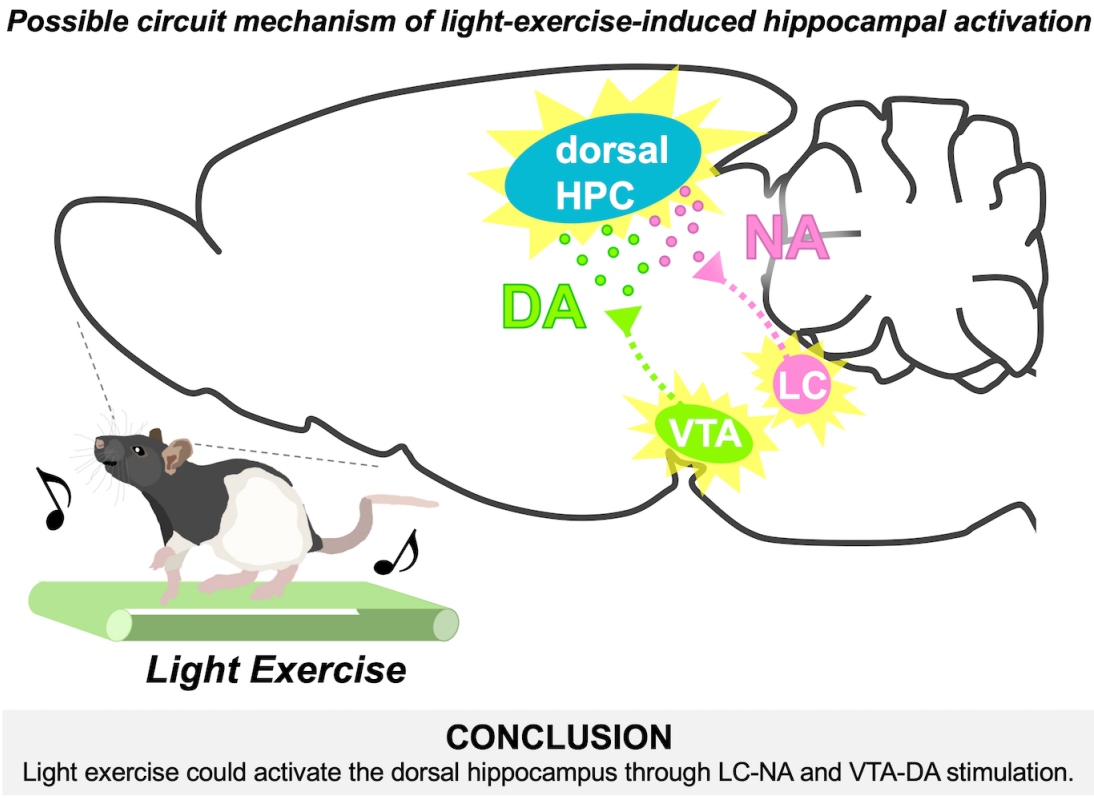
RRF: retrorubral field

SNpc: substantia nigra pars compacta

TH: tyrosine hydroxylase

VTA: ventral tegmental area

Graphical abstract



Our previous research with animals and humans has demonstrated that even light exercise can boost neuronal activity in the dorsal hippocampus and improve memory. While the mechanism underlying this remains undetermined, recent studies suggest the involvement of the ascending monoaminergic system. Here, we examined this hypothesis 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-enhanced memory.

(80 / 80 words)

Abstract

Exercise activates the dorsal hippocampus, which triggers the synaptic and cellar plasticity and ultimately promotes memory formation. For decades, these benefits have been explored 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 below the lactate threshold (LT), which almost anyone can safely perform with minimal stress, and found that even light exercise can stimulate hippocampal activity and enhance memory performance. Although the circuit mechanism of this boost remains unclear, arousal promotion even with light exercise implies the involvement of the ascending monoaminergic system, which is essential to modulate hippocampal activity and impact memory. To examine this hypothesis, we employed our physiological exercise model based on the LT of rats that can be applied to human and immunohistochemically assessed the neuronal activation of the dorsal hippocampal sub-regions and brainstem monoaminergic neurons. Also, we monitored the dynamics of monoamine release at the dorsal hippocampus using *in vivo* microdialysis. We found that even light exercise increased neuronal activity in the dorsal hippocampal sub-regions and induced noradrenaline and dopamine release. Furthermore, we found that tyrosine hydroxylase-positive 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. In conclusion, our findings demonstrate that light exercise stimulates hippocampal neurons, possibly through the LC-noradrenergic and/or VTA-dopaminergic neurons. This sheds light on the circuit mechanisms responsible for hippocampal neural activation during exercise, consequently enhancing memory function. (250/250 words)

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

1 Introduction

There is accumulating evidence showing that physical exercise has beneficial effects on the brain and its functions, including hippocampus-dependent learning and memory.[1–4] 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.[5] Physical exercise has been found to stimulate the activity of the dorsal hippocampal neurons.[6–8] This subsequently triggers the production of plasticity-related proteins (PRPs) such as the brain-derived neurotrophic factor (BDNF) in the hippocampus.[9–11] Also, this activation increases cerebral blood flow[8,12] and facilitates the entrance of bioactive hormones "exerkines" secreted from peripheral organs, such as the insulin-like growth factor I (IGF-I), cathepsin B, and irisin, across the blood-brain barrier into the hippocampus.[13–18] In turn, these boosts ultimately contribute to cellar and synaptic plasticity and memory enhancement.[1,15,16,19–21]

For decades, the benefits of exercise to learning and memory have been based on evidence using exercise at moderate-to-vigorous intensities,[2–4,22] which is generally recommended in exercise therapy. However, such demanding exercise can cause physiological stress and reduce exercise adherence.[23–27] Besides, while the chronic intervention of voluntary wheel running is widely used in animal research,[1,15,16,18] this exercise model provides less information about effective exercise intensity, frequency, and duration for memory enhancement. Moreover, voluntary running rodents run at a relatively high intensity (45 m/min) and exhibit intermittent running patterns in the dark cycle,[28] suggesting that wheel running is not an appropriate translational exercise model for human exercise. We thus 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).[23,25] 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,[9] and to promote hippocampal neurogenesis and spatial memory.[29–31] 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.[29–33] Additionally, our research with human participants and functional magnetic resonance imaging (fMRI) found that acute light exercise increases hippocampal activity and enhances pattern separation, which is a hippocampus-related function.[34] 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 dorsal hippocampal activation by light exercise and its beneficial effect on memory performance. Classically, it is known that exercise can promote arousal,[35–37] which is thought to originate from the activation of the ascending arousal system (AAS).[38,39] This is supported by our recent studies showing that arousal promotion can occur by exercise even at very light-intensity accompanied by pupil dilation,[34,40–43] which reflects the activity of AAS.[44–48] The AAS sends out widespread projections in order to increase global brain activity, including the dorsal hippocampus, which largely overlaps the monoaminergic neuromodulatory system. Monoamines like noradrenaline

155 (NA), dopamine (DA), and serotonin (5-HT) are essential for multiple functions such as
156 arousal, attention, reward, motivation, and mood regulation.[49–55] At the dorsal
157 hippocampus, monoaminergic stimulation facilitates learning and memory processes by
158 regulating the expression of PRPs and synaptic and cellar plasticity, such as long-term
159 potentiation (LTP) and neurogenesis.[56–61] The hippocampal monoamines are mainly
160 synthesized and provided by the specific nuclei located in the pons noradrenergic locus
161 coeruleus (LC),[62–66] the midbrain dopaminergic nuclei, such as the ventral tegmental area
162 (VTA) and the substantia nigra pars compacta (SNpc),[67–71] and the midbrain serotonergic
163 raphe nuclei (the dorsal raphe: DRN and the median raphe: MRN).[72,73] Previous research
164 demonstrate that single bouts of exercise activate these brainstem monoaminergic
165 neurons[74–76] and affect the hippocampal monoamine transmissions,[3,77,78] thereby
166 contributing to the enhancement of hippocampus-related functions.[3,74,79] These findings
167 lead us to the hypothesis that the monoamines provided by the brainstem nuclei could be
168 involved in the light exercise-induced dorsal hippocampal activation, as the neural basis
169 of memory enhancement.

170 To test this hypothesis, the involvement of the brainstem monoaminergic system in
171 dorsal hippocampal activation during light exercise was investigated using a rat LT-based
172 treadmill running model that can be applied to humans. We analyzed the neuronal
173 activation of the dorsal hippocampus and of the brainstem monoaminergic neurons using
174 immunohistochemistry. Also, we monitored the dorsal hippocampal monoamine
175 transmission with *in vivo* microdialysis combined with the high-performance liquid
176 chromatography (HPLC) system.

177

178 **2 Materials and Methods**

179 **2.1 Animals**

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

190

191 **2.2 Experimental design**

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

203

204 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)

205

206 **2.3 Surgery to insert jugular catheter**

207 In Experiments 1 and 2, 1 day after the habituation to treadmill running periods, the rats
208 were anesthetized with isoflurane, and a silicone catheter was inserted into the jugular
209 vein and fixed with a silk thread suture (37 mm). The external distal end of the catheter
210 was fixed at the nape of the neck. After the surgery, the rats were injected with antibiotics
211 (Mycillin Sol; Meiji Seika, Tokyo, Japan). Then, they were housed individually and were
212 allowed to recover for 2 or 3 days before the treadmill running test.

213

214 **2.4 Stereotaxic surgery**

215 In Experiment 4, after a week of handling, all rats were anesthetized with isoflurane and
216 placed in a stereotaxic frame (Model 51900, Muromachi Kikai Co., Ltd., Tokyo, Japan).
217 A guide cannula (AG-6, Eicom, Kyoto, Japan) was implanted above the dorsal
218 hippocampus (AP: -3.6 mm, ML: 3.8 mm, DV: -1.8 mm, 20˚ angle, from bregma). The
219 cannula was secured to the skull with three anchoring screws and dental cement. To
220 prevent occlusion, a dummy cannula (AD-6, Eicom) was inserted into the guide cannula.
221 After the surgery, the rats were injected with antibiotics (Mycillin Sol; Meiji Seika, Tokyo,
222 Japan). Then, they were housed individually and were allowed to recover for at least 2

223 days before habituation to treadmill running.

224

225 **2.5 Habituation to treadmill running**

226 All rats were habituated to running on a treadmill (KN-73, Natsume Seisakusho Co, Ltd.,
227 Tokyo, Japan) for a total of five sessions over 6 days. The running duration was 30
228 min/day, and the running speed was gradually increased from 5 to 25 m/min with no
229 incline [8,23]. Electrical shock grids were placed at the rear end of the treadmill and provided
230 mild but aversive foot shocks (30 V) to rats to encourage running at the set treadmill
231 speed. To minimize any stress derived from the shocks, electric shocks were limited and
232 a gentle tail touch was given when rats did not start running again. After the habituation
233 period, rats were able to run at the appropriate speeds even when the electric grid was
234 turned off.

235

236 **2.6 Treadmill running tests**

237 On the test day, all rats fasted for 2 h before running to obtain stable metabolic conditions.
238 In Experiments 1 and 2, treadmill running tests were conducted 2-3 days after the surgery
239 for inserting the jugular catheter. In Experiment 1, an incremental exercise test was
240 performed to assess the running speed at LT. Rats were forced to run to exhaustion; the
241 running speed was started at 5 m/min and increased by 2.5 m/min every 3 minutes. The
242 speed at which the LT was reached was determined according to the method by Beaver *et*
243 *al.*.[80] In Experiment 2, a single 30-minute bout of running was conducted to verify the
244 validation of exercise intensity based on the speed at LT as determined in Experiment 1.
245 Rats were randomly subjected to run at 15 m/min (Light) or 25 m/min (Vigorous), or they
246 were placed on a treadmill as the at-rest Control for 30 minutes. In both experiments,

247 serial blood samples (0.1 ml) were taken at designated time points. Blood lactate and
248 glucose were measured using an automated glucose–lactate analyzer (2300 Stat Plus,
249 Yellow Springs Instruments, OH, USA).
250 In Experiment 3, rats were returned to their home cage after the test, rested for 90 min,
251 and then were transcardially perfused with 0.9% saline, followed by 4%
252 paraformaldehyde (PFA) in a 0.1 M phosphate buffer (PB; pH 7.4) under deep isoflurane
253 anesthesia. The brains were sampled for immunohistochemical analysis.
254 In Experiment 4, a microdialysis probe (2 mm membrane: FX-I-6-02, Eicom) was
255 inserted into the dorsal hippocampus using a guide cannula 1 day before the test. The
256 probe was connected to the microsyringe pump (ESP-32, Eicom) using a Teflon tubing
257 (JT-10, Eicom) and perfused with Ringer's solution (147 mM NaCl, 4 mM KCl, and
258 2.3 mM CaCl2) at 1.0 μl/min 2-3 h before the start of the test because a stable dialysate
259 monoamine level is usually obtained 2 h after insertion of the probe. Using a fraction
260 collector (EFC-82, Eicom), 15 μl of dialysate was collected every 15 minutes and 5 μl of
261 0.1M PB (pH 3.5) was added to ensure stable monoamine detection using HPLC with an
262 electrochemical detector (HPLC-ECD). At the end of the experiments, rats were
263 transcardially perfused with 0.9% saline, followed by 4% PFA in a 0.1 M PB under deep
264 isoflurane anesthesia and the brain was collected to verify the position of the probe in
265 coronal sections with DAPI (4',6-diamidino-2-phenylindole). Please note that 7 rats in
266 Experiment 4 were excluded from the analysis for the following reasons: failed insertion
267 of the probe into the dorsal hippocampus (1 rat), detachment of the fixed probe during
268 running (3 rats), and failed detection of peaks in chromatography (3 rats).
269
270 **2.7 Immunohistochemistry**

271 The brain was post-fixed for 24 h in 4% paraformaldehyde (PFA, 02890-45, Nacalai
272 Tesque, Tokyo, Japan) in a 0.1 M PB and cryoprotected by immersion in a 0.1 M PB
273 containing 20% sucrose for 1 day and 30% sucrose for 2-3 days. Frozen coronal brain
274 sections of 30 μm thickness were cut with a microtome (REM-710, Yamato Kohki
275 Industrial Co., Ltd., Saitama. Japan). Sections were washed with a 0.1M PB and blocked
276 with a 0.1 M PB containing 1% Triton X-100 (35501-15, Nacalai Tesque) (PBT) plus 1%
277 bovine serum albumin (01863-77, BSA; Nacalai Tesque). Then, slices were incubated
278 with the designated primary antibodies in PBT-BSA for 24 h at 4 °C. The primary
279 antibodies used in this study were rabbit polyclonal antibody against c-Fos (1:5,000; Cat#
280 226-003, RRID: AB_2231974, Synaptic Systems, GAU, Germany), mouse monoclonal
281 antibody against NeuN (1:1,000; Cat# MAB377, RRID: AB_2298772, Millipore, MA,
282 USA), mouse monoclonal antibody against tyrosine hydroxylase (1:2,000; Cat# F11:sc-
283 25269, RRID: AB_628422, Santa Cruz, CA, USA), and goat polyclonal antibody against
284 5-HT (1:1,000; Cat# 20079, RRID: AB_572262, Immunostar Inc., WI, USA). After
285 repeated washings with PBT-BSA, sections were incubated in a dark space with
286 designated secondary antibodies diluted in PBT-BSA for >16 h at 4 °C. The secondary
287 antibodies used in this study were Alexa Fluor 488-conjugated goat anti-rabbit IgG
288 (1:5,000; Cat# A-11008, RRID: AB_143165, Invitrogen; Thermo Fisher Scientific Inc.,
289 MA, USA), Alexa Fluor 488-conjugated donkey anti-rabbit IgG (1:5,000; Cat# ab150073,
290 RRID: AB_2636877, abcam, Cambridge, UK), Alexa Fluor 594-conjugated donkey anti-
291 goat IgG (1:1,000; Cat# A-11058, RRID: AB_142540, Invitrogen; Thermo Fisher
292 Scientific Inc., MA, USA), and AMCA AffiniPure donkey anti-mouse IgG (1:1,000; Cat#
293 715-155-150, RRID: AB_2340806, Jackson ImmunoResearch Laboratories, Inc., PA,
294 USA). For DAPI staining to verify the location of microdialysis probes, slices were

295 washed three times in a 0.1 M PB and then incubated with DAPI (1:1,000; 28718-90-3,
296 Dojindo Laboratory, Kumamoto, Japan) in PBT-BSA for 10 minutes in the dark. Slices
297 were washed three times in a 0.1 M PB, mounted on subbed slides, air dried, and
298 coverslipped using a mounting medium.
299
300 **2.8 Counts and quantification**
301 All fluorescence z-stacked images were examined on an all-in-one microscope (BZ-X710,
302 Keyence, Osaka, Japan) with a 20× objective lens. Manual counts of neurons were
303 performed by an observer blinded to the group allocations. The number of c-Fos and
304 NeuN double-positive neurons (c-Fos$^+$/NeuN$^+$) in the dorsal hippocampal dentate gyrus
305 (DG), cornu ammonis (CA) 1, CA2, and CA3 sub-regions were counted hemilaterally in
306 4 coronal sections (from Bregma -3.60 mm, -4.16 mm, -5.20 mm, -5.80 mm). Total
307 neuron numbers were normalized based on NeuN$^+$ region volume (mm^3), which was
308 calculated by the area (mm^2) multiplied by the thickness of sections (30 μm) using a BZ-
309 X analyzer software (https://www.keyence.co.jp/products/microscope/fluorescence-
310 microscope/bz-x700/models/bz-h3a/, version 1.4.1.1, RRID:SCR_017375). The numbers
311 of c-Fos and TH or 5-HT double-positive neurons (c-Fos$^+$/TH$^+$ or c-Fos$^+$/5-HT$^+$) were
312 bilaterally counted 120 μm apart throughout the LC (around Bregma -9.16 mm ~ 10.52
313 mm, 10 ~ 16 coronal sections) and 360 μm apart throughout the VTA, SNpc (around
314 Bregma -4.80 mm ~ -6.72 mm, 5 coronal sections), DRN, and MRN (around Bregma -
315 7.30 mm ~ -8.80 mm, 5 coronal sections), all of which are known as monoaminergic
316 nuclei projecting to the dorsal hippocampus.
317
318 **2.9 HPLC-ECD for monoamine detection in the dorsal hippocampus**

319 Dialysate samples collected from the probe at the dorsal hippocampus were injected into
320 an HPLC-ECD system (HTEC-500; Eicom) using an autosampler (M-510; Eicom) and
321 analyzed for NA, DA, and 5-HT concentration using the HPLC system with an
322 EICOMPAK CAX column (2.0 mm, i.d. × 200 mm; Eicom) and a graft electrode (WE-
323 3G; Eicom) set at 450 mV (vs Ag/AgCl reference electrode). The flow rate and injection
324 volume were set to 250 μl/min and 17 μl, respectively. The mobile phase contained a
325 0.1 M ammonium acetate buffer, 0.05 mg/l sodium sulfate, 50 mg/l EDTA, and methanol
326 (7:3, v/v), with a pH of 6.0. Data analysis was performed using Eicom EPC-710 software.
327 Basal concentrations of the three monoamines (i.e., NA, DA, and 5-HT) were estimated
328 from the averages at four HPLC time points before the beginning of running.
329
330 **2.10 Statistical analyses**
331 All data are expressed as mean ± standard error (SEM) and figures are generated using
332 GraphPad Prism (https://www.graphpad.com/, version 8, RRID:SCR_002798, GraphPad
333 Software, San Diego, CA, USA). All data were analyzed using R software (https://www.r-
334 project.org/, version 4.2.3, RRID:SCR_001905). Assessment of the normality (Shapiro-
335 Wilk test) of the data, equality of variance (Levene's test), and sphericity (Mendoza's test
336 of sphericity) for repeated measures (RM) were performed. Statistical significance for
337 parametric data was analyzed using one-way analysis of variance (ANOVA) followed by
338 Shaffer's *post-hoc* test, but Welch's ANOVA was performed if equality of variance was
339 not assumed. For the analysis of non-parametric data, the Kruskal-Wallis test with the
340 Mann-Whitney U test adjusted by Holm's post-hoc test was applied. For RM, two-way
341 ANOVA followed by Shaffer's post-hoc test was applied and the degree of freedom was
342 adjusted using Huynh-Feldt-Lecoutre's epsilon for non-spherical data. Pearson's

correlation coefficient was used for analyzing the strength of relationships. No tests for outliers were performed. Statistical significance was assumed at $p < 0.05$. No statistical methods were used to predetermine sample size, but the sample sizes were comparable to those reported in previous studies.[7,23,75–77]

3 Results

3.1 Measurement of LT and validation of exercise intensity

Initially, we developed a varied-intensity treadmill running model with adult male Long-Evans rats using LT as a signpost for exercise-induced stress. Our analysis revealed that the running speed at the LT of rats was 21.87 m/min ± 1.02 ($n = 6$; Fig. 1B, C). Thus, we defined 15 m/min, which is below the LT, as the speed for light-intensity running and 25 m/min, which is above the LT, as the speed for vigorous-intensity running. The observation of a significant increase in blood lactate concentration after a 30-minute session of vigorous-intensity running (two-way RM ANOVA, Shaffer's *post hoc* test, group and time interaction, $F_{(2, 15)} = 66.036$, $p < 0.001$, Fig 1E) was sufficient to validate the varied-intensity running model based on LT.

3.2 Light-intensity-running-induced neuronal activation of dorsal hippocampus

We assessed dorsal hippocampal neuronal activation during different intensities of running using c-Fos immunostaining. Representative images in Figure 2B demonstrate that c-Fos expression in dorsal hippocampal sub-regions, including the DG, CA1, and CA3, significantly increased regardless of running speed. Conversely, the CA2 region was not affected by running (DG: Welch's one-way ANOVA, Shaffer's *post hoc* test, $F_{(2, 18)} = 12.876$, $p < 0.001$, Fig. 2C; CA1: one-way ANOVA, Shaffer's *post hoc* test, $F_{(2, 18)}$

367 = 9.994, $p < 0.001$, Fig. 2D; CA2: one-way ANOVA, Shaffer's *post hoc* test, $F_{(2, 18)}$ =
368 0.173, $p = 0.843$, Fig. 2E; CA3: one-way ANOVA, Shaffer's *post hoc* test, $F_{(2, 18)} = 11.256$,
369 $p < 0.001$, Fig. 2F).
370

371 **3.3 Light-intensity running increased dorsal hippocampal NA and DA release**

372 To assess the monoaminergic neurotransmission to the dorsal hippocampus, we measured
373 extracellular monoamine levels in the dorsal hippocampus using *in vivo* microdialysis and
374 HPLC methods. We found that treadmill running induced a significant increase of both
375 NA and DA levels in the dorsal hippocampus depending on running speed (NA: two-way
376 RM ANOVA, Shaffer's *post hoc* test, group, $F_{(2, 17)} = 11.545$, $p < 0.001$, time, $F_{(3.5, 59.58)}$
377 = 16.236, $p < 0.001$, group and time interaction, $F_{(7.01, 59.58)} = 5.393$, $p < 0.001$; DA: two-
378 way RM ANOVA, Shaffer's *post hoc* test, group, $F_{(2, 17)} = 18.720$, $p < 0.001$, time, $F_{(4.01,}$
379 $_{68.23)}$ = 14.019, $p < 0.001$, group and time interaction, $F_{(8.03, 68.23)} = 4.959$, $p < 0.001$, Fig.
380 3E, F). Notably, DA release was significantly sustained into the post-running time period.
381 Dorsal hippocampal 5-HT levels, on the other hand, were not affected by running (two-
382 way RM ANOVA, Shaffer's *post hoc* test, group, $F_{(2, 17)} = 0.046$, $p = 0.955$, time, $F_{(2.79,}$
383 $_{47.46)}$ = 16.236, $p < 0.05$, group and time interaction, $F_{(5.58, 47.46)} = 1.16$, $p = 0.343$, Fig.
384 3G).
385

386 **3.4 Running stimulated TH$^+$ neurons in LC and VTA, but not raphe 5-HT$^+$ neurons**

387 To examine the responsiveness of brainstem monoaminergic nuclei, we performed
388 double-immunostaining of c-Fos$^+$/TH$^+$ neurons for the activation of NA- and DA-
389 producing neurons, and c-Fos$^+$/5-HT$^+$ neurons for the activation of serotonergic neurons,
390 as shown in Figure 4A-C. TH$^+$ neurons in the LC (LCTH) and VTA (VTATH), which are

391 known as the main sources of dorsal hippocampal NA and DA, respectively, were
392 significantly stimulated by both light- and vigorous-intensity running (LC: one-way
393 ANOVA, Shaffer's *post hoc* test, $F_{(2, 18)} = 20.908$, $p < 0.001$, Fig. 4D; VTA: one-way
394 ANOVA, Shaffer's *post hoc* test, $F_{(2, 18)} = 4.992$, $p < 0.05$, Fig. 4E). However, running
395 did not have any impact on dopaminergic neurons in the SNpc ($SNpc^{TH}$ neurons), or on
396 serotonergic neurons in the DRN and MRN ($DRN^{5\text{-}HT}$ neurons and $MRN^{5\text{-}HT}$ neurons,
397 respectively) (SNpc: Kruskal-Wallis test, Mann-Whitney U test adjusted by Holm's *post-*
398 *hoc* test, $X^2 = 4.653$, df = 2, $p = 0.097$, Fig. 4F; DRN: one-way ANOVA, Shaffer's *post*
399 *hoc* test, $F_{(2, 18)} = 0.832$, $p = 0.454$, Fig. 4G; MRN: one-way ANOVA, Shaffer's *post hoc*
400 test, $F_{(2, 18)} = 1.640$, $p = 0.222$, Fig. 4H). Note that TH^+ neurons in the retrorubral field
401 (RRF) is also reported to have projections to the hippocampus,[81] which were not activated
402 by running (Fig. S2B). Additionally, TH^+ neurons were also observed in the rostral region
403 of the DRN (DRN^{TH} neurons), which are reported to contribute to wakefulness by
404 salience stimuli, opioid addiction, and pain modulation through the release of DA.[82] In
405 this study, however, running did not affect the c-Fos expression in DRN^{TH} neurons (Fig.
406 S2D).
407

408 **3.5 Neuronal associations between dorsal hippocampus and brainstem**
409 **monoaminergic nuclei**

410 To explore the potential association between light-exercise-induced neuronal activities in
411 brainstem monoaminergic nuclei and dorsal hippocampal activation, we conducted
412 Pearson's correlation analysis using the dataset from the control group and the light-
413 intensity running group. This analysis assessed the correlation between c-Fos expression
414 in the brainstem monoaminergic nuclei and the dorsal hippocampus. A correlation matrix

415 heatmap that summarizes the correlation results is shown in Figure 5. c-Fos expression in
416 LC^{TH} neurons exhibited a significant and positive correlation within all examined dorsal
417 hippocampal sub-regions except CA2 (n = 14, DG: r = 0.607, p < 0.05, Fig 5B; CA1: r =
418 0.691, p < 0.01, Fig 5C; CA2: r = 0.132, p = 0.652; CA3: r = 0.643, p < 0.05, Fig 5D).
419 Additionally, a significant positive correlation was found between c-Fos expression in
420 VTA^{TH} neurons and in the dorsal CA1 (VTA: n = 14, DG: r = 0.482, p = 0.0816; CA1: r
421 = 0.570, p < 0.05, Fig 5E; CA2: r = 0.004, p = 0.989; CA3: r = 0.391, p = 0.167). The
422 higher level of c-Fos expression in $DRN^{5\text{-}HT}$ neurons was significantly correlated with
423 lower levels of CA2 and CA3 c-Fos expression (n = 14, DG: r = -0.532, p = 0.0502; CA1:
424 r = -0.506, p = 0.0649; CA2: r = -0.675, p < 0.01, Fig 5F; CA3: r = -0.704, p < 0.01, Fig
425 5G). However, no correlation was found in the c-Fos expression between $SNpc^{TH}$ or
426 $MRN^{5\text{-}HT}$ neurons and dorsal hippocampal sub-regions (SNpc: n = 14, DG: r = -0.0362,
427 p = 0.902; CA1: r = 0.196, p = 0.502; CA2: r = -0.0477, p = 0.871; CA3: r = 0.0374, p =
428 0.899; MRN: n = 14, DG: r = -0.252, p = 0.385; CA1: r = -0.207, p = 0.478; CA2: r =
429 0.0693, p = 0.814; CA3: r = -0.402, p = 0.155).
430

431 **4. Discussion**

432 To probe the neural circuitry responsible for activating the dorsal hippocampus during
433 light exercise and its subsequent contribution to enhanced memory functions, this study
434 examined the involvement of the ascending monoaminergic system—a critical
435 neuromodulatory mechanism for memory—in the activation of the dorsal hippocampus
436 induced by light exercise. Employing a rat exercise model based on the LT, we assessed
437 neuronal activity in the dorsal hippocampus and brainstem monoaminergic neurons and
438 observed real-time dynamics of monoamine release in the dorsal hippocampus using *in*

439 *vivo* microdialysis. The results demonstrate that even light exercise led to increased c-Fos
440 expression in specific subregions of the dorsal hippocampus and triggered the release of
441 both NA and DA within this brain region. Furthermore, the activity of LC^{TH} neurons and
442 VTA^{TH} neurons elevated during exercise even at the light-intensity, and both were
443 positively correlated with the activation of the dorsal hippocampus. In contrast, the
444 serotonergic system did not appear to be influenced by exercise. These findings imply
445 LC-noradrenergic and VTA-dopaminergic contributions to dorsal hippocampal activation
446 during light exercise (Fig. 6), offering insights into the circuitry behind light-exercise-
447 enhanced learning and memory.

448 Here, we first established a varied-intensity treadmill running model for male adult
449 Long-Evans rats based on LT as a physiological index of running intensity and stress. The
450 speed at the LT was similar to that in our previous work using male adult Wistar rats
451 (Long-Evans: 21.87 m/min ± 1.02 vs Wistar: 20.7 m/min ± 2.1; Fig. 1B, C).[23] In addition,
452 a significant increase in blood lactate concentration was observed following a single bout
453 of vigorous-intensity running, but not with light-intensity running (Fig. 1E), which is
454 consistent with our previous reports.[23,25] These results confirm the validity of our
455 experimental model (LT-based varied-intensity running) and demonstrate that the two
456 running groups exhibited different metabolic and stress-related responses.

457 Previous studies have reported that acute varied-intensity treadmill running activates
458 the dorsal hippocampus, including the DG, CA1, and CA3 sub-regions, even at low-
459 speeds.[6,7,9] Our current results support these findings since both light- and vigorous-
460 intensity running increased c-Fos expression in the dorsal DG, CA1, and CA3 sub-regions
461 (Fig. 2C, D, F). In contrast, the dorsal CA2 region was not activated with running (Fig.
462 2E). The CA2 is known as a small hippocampal sub-region that modulates social memory

463 and that is stimulated by novel encounters.[83] Since all rats were well familiarized with the
464 experimental conditions and there were no novel or social stimuli, it is reasonable that
465 activity in the CA2 region was not affected. Additionally, we examined the activation of
466 the ventral hippocampus following running and found an increase of c-Fos expression
467 across all sub-regions of the ventral hippocampus in both running groups (Fig. S1). These
468 results suggest that light-intensity running is sufficient to elevate the neuronal activity in
469 the hippocampus across the dorsal-to-ventral axis, which may help to explain the positive
470 effects of exercise on dorsal-hippocampus-related functions, such as memory, as well as
471 ventral-hippocampus-related anxiolytic and antidepressant effects.[3,29,84]

472 Our *in vivo* microdialysis experiment provides the first evidence that even light-
473 intensity running increases the release of NA and DA at the dorsal hippocampus (Fig. 3E,
474 F). Given that NA and DA both perform critical roles in dorsal hippocampal neuronal
475 activity, plasticity, and memory process,[60,61,63,64,85–87] our results suggest that the dorsal
476 hippocampal NA and DA release contributes to the light-exercise-induced hippocampal
477 activation and beneficial effects, such as hippocampal neurogenesis and spatial memory
478 performance.[9,29,30] Notably, NA and DA have been recognized as important factors in
479 memory consolidation,[63,86] as shown by the synaptic tagging and capture hypothesis,[88,89]
480 raising a hypothesis that a single-bout of light-intensity running could facilitate memory
481 retention through noradrenergic and dopaminergic dorsal hippocampal activation.
482 Intriguingly, prior studies demonstrate that a single session of vigorous-intensity exercise
483 elevates NA and DA concentrations in the hippocampus and promotes novel object
484 recognition, which is a dorsal-hippocampus-related function, through the β adrenergic
485 receptor and dopamine D1 receptor, respectively.[3,79] Although we did not directly
486 examine this in the present study, these findings support our hypothesis.

487 We confirmed that treadmill running, even at light intensity, activates LC^{TH} and
488 VTA^{TH} neurons (Fig. 4D, E). Furthermore, our correlation analysis observed that LC^{TH}
489 activation positively correlates with dorsal hippocampal activation, and the activities of
490 VTA^{TH} were positively associated with those of the dorsal CA1 region (Fig. 5). These
491 results suggest that running stimulates the dorsal hippocampal neurons through
492 noradrenergic projection from the LC and dopaminergic projection from the VTA. While
493 our study didn't investigate the projections from light-exercise-activated TH^{+} neurons in
494 the LC and the VTA to the dorsal hippocampus, interestingly, a recent study with *in vivo*
495 two-photon calcium imaging provides robust support for our hypothesis, which
496 demonstrates an elevation in the activities of both the LC and the VTA axons within the
497 dorsal CA1 in head-fixed mice during voluntary running.[90] Both nuclei are known as
498 major sources of dorsal hippocampal NA and DA,[64,65,91] but recent studies have shown
499 that LC terminals co-release DA and NA to the dorsal hippocampus, and that LC-DA, but
500 not LC-NA, plays a significant role in spatial memory and learning of novel context.[63–
501 66,85,92] On the other hand, VTA^{TH} neurons have sparse input to the dorsal hippocampus
502 and do not have a significant impact on spatial memory.[63,64] However, hippocampal DA
503 from VTA^{TH} neurons is still reported to be involved in hippocampus-related functions,
504 such as spatial memory and aversive memory.[70,71,93] The significance of LC^{TH} and VTA^{TH}
505 neurons for the dorsal hippocampal functions remains elusive; thus, future studies using
506 viral-tracing techniques and chemogenetic/optogenetic manipulation are needed to
507 investigate the noradrenergic and dopaminergic mechanisms underlying the beneficial
508 effects of light exercise.

509 Unlike the noradrenergic and the dopaminergic systems, 5-HT release was not
510 increased at the dorsal hippocampus, nor were $DRN^{5\text{-}HT}$ or $MRN^{5\text{-}HT}$ neurons found to be

511 stimulated by treadmill running (Fig. 3G; Fig. 4G, H). However, we found a negative
512 correlation between c-Fos expression in the DG, CA2, and CA3 sub-regions and that in
513 $DRN^{5\text{-}HT}$ neurons (Fig. 5F, G). Previous studies have shown that running increases
514 hippocampal neuronal activity and also facilitates hippocampal LTP induction.[94–96]
515 Conversely, the hippocampal 5-HT can exert inhibitory effects on basal neural activity
516 and LTP induction.[97–99] Considering these findings, our results imply that running
517 enhances hippocampal neuronal activity and LTP induction through the inhibitory control
518 of the serotonergic inhibitory projection from $DRN^{5\text{-}HT}$ neurons to the dorsal
519 hippocampus.

520 From a clinical perspective, light exercise, such as yoga, tai-chi, and slow running,
521 is a simple and feasible intervention accessible by most people, including patients with
522 mild cognitive impairment (MCI) and Alzheimer's disease (AD). Recent studies have
523 shown that the hippocampal NA and DA loss and degeneration of LC^{TH} and VTA^{TH}
524 neurons and axons are found in AD model rodents and AD patients.[100–104] Notably,
525 selective accumulation of amyloid β and tau proteins are observed in LC neurons before
526 the onset or at the early stage of AD symptoms.[103–107] Light exercise has been reported to
527 have a positive effect on MCI and AD.[108–110] Our findings using a translational animal
528 exercise model, therefore, could provide insight into understanding the mechanisms
529 underlying the preventive or therapeutic role of light exercise in cognitive dysfunction
530 conditions, such as AD.

531

532 **5. Conclusion**

533 Collectively, our findings using a translational exercise model revealed that even light-
534 intensity running can stimulate the dorsal hippocampal neuronal activity and the release

of NA and DA probably from LC and VTA, respectively but that it does not affect the serotonergic system. These findings suggest a possible contribution of LC-noradrenergic and VTA-dopaminergic projections to dorsal hippocampal activation during light exercise (Fig. 6), which may provide insight into the circuit mechanism that underlies light-exercise-enhanced learning and memory.

Author Contributions

Taichi Hiraga, Toshiaki Hata, Shingo Soya, Kanako Takahashi, Masahiro Okamoto, and Hideaki Soya conceived and designed the experiments; Joshua P. Johansen provided the experimental animals; Taichi Hiraga and Kanako Takahashi performed the treadmill running experiments for LT measurement and validation of exercise intensity; Taichi Hiraga performed the treadmill running experiments for immunohistochemistry and microdialysis; Toshiaki Hata and Ryo Shimoda performed experimental assistances; Taichi Hiraga and Ryo Shimoda analyzed the data; Taichi Hiraga wrote the original manuscript, and all authors were involved in reviewing and editing the manuscript.

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Disclosures

The authors declare no competing financial interests.

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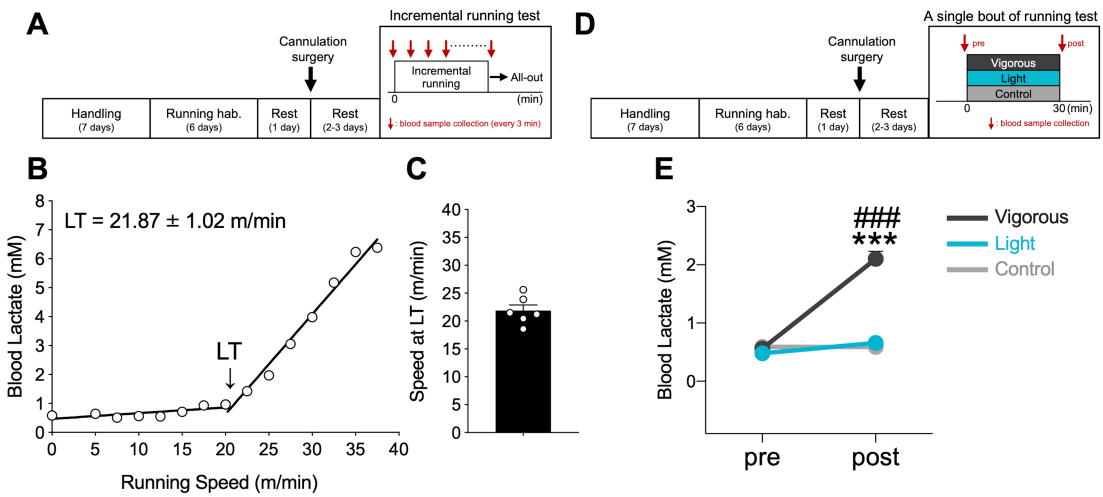


Figure 1

Measurement of lactate threshold and validation of exercise intensity.

(A) Experimental design for incremental running test. (B) A representative LT profile from the incremental running test. (C) Speed of rats at LT was 21.87 ± 1.02 m/min (n = 6). (D) Experimental design 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.

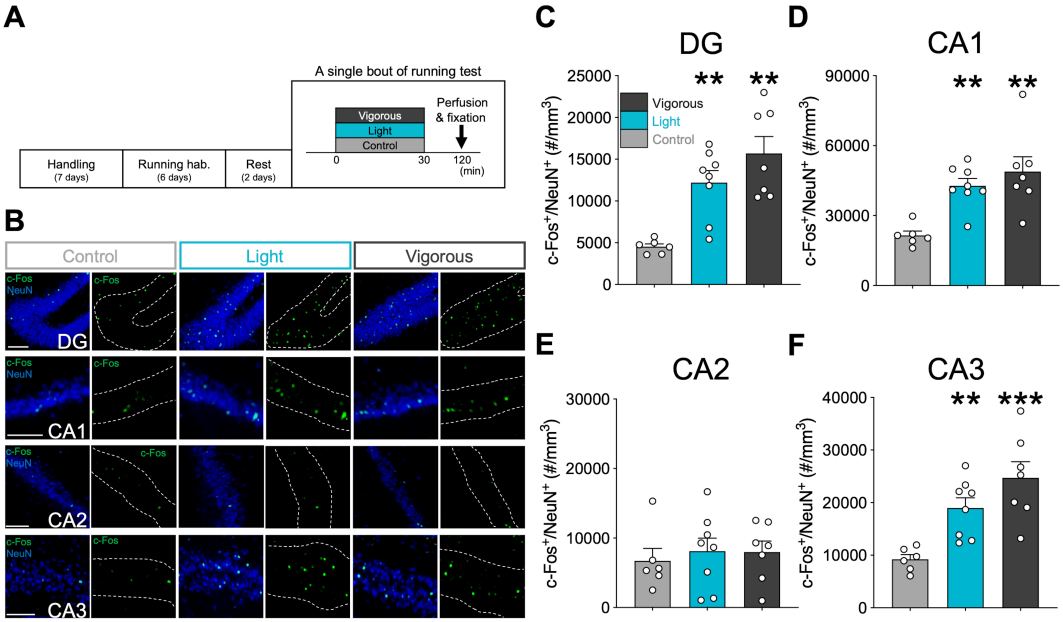


Figure 2

Running-induced neuronal activation at the dorsal hippocampus during varied-intensity treadmill running.

(A) Experimental design for a single bout of the running test for immunohistochemical analysis. (B) Representative double-fluorescence images of c-Fos (green) and NeuN (blue) in the DG, CA1, and CA3 regions of the dorsal hippocampus. Scale bars show 100 µm. c-Fos expressions in the DG (C), CA1 (D), CA2 (E), and CA3(F) were significantly increased with running exercise regardless of running speed (light or vigorous). All data are expressed as mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$ vs Control, n = 6-8 / group. Control: sedentary control; Light: 15 m/min running; Vigorous: 25 m/min running; hab.: habituation.

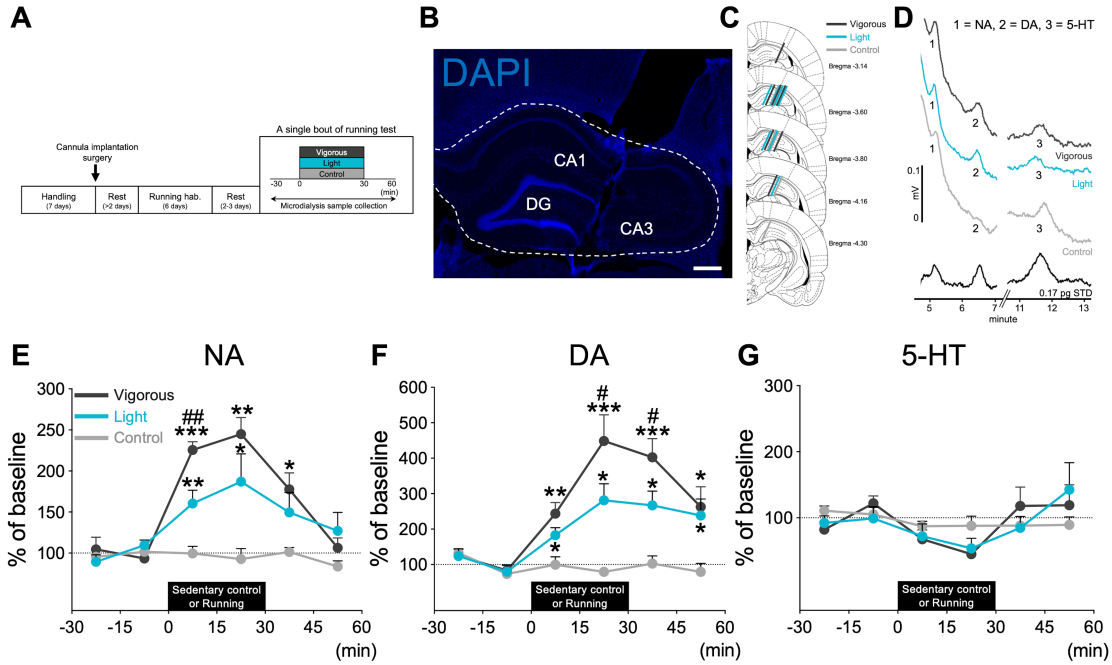
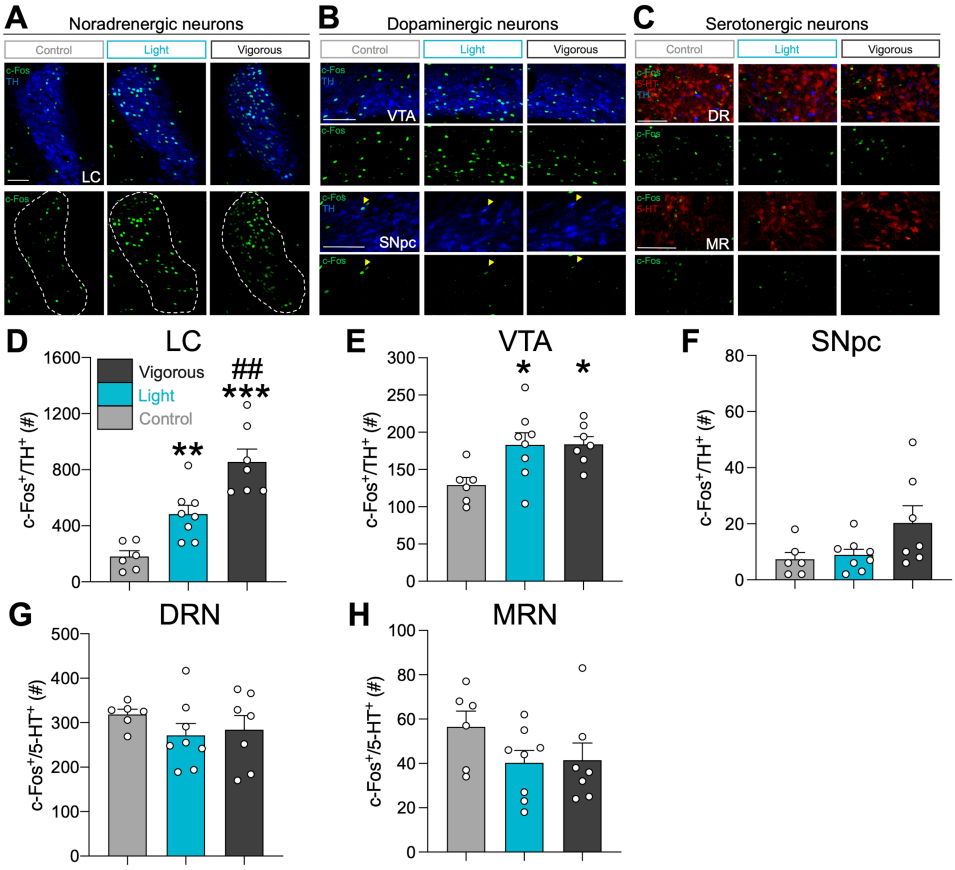


Figure 3

Running increases dorsal hippocampal NA and DA release, but not 5-HT release. (A) Experimental design for a single bout of the running test for dorsal hippocampal monoamine detection with *in vivo* microdialysis. (B) Representative DAPI fluorescence image of microdialysis probe position in the dorsal hippocampus. Scale bars show 500 µm. (C) 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). (D) Representative chromatograms showing NA (peak 1), DA (peak 2), and 5-HT (peak 3) from Control (light gray), Light (blue), and Vigorous (dark gray). Treadmill running induced a significant increase of both NA (E) and DA (F) levels in

the dorsal hippocampus, depending on running speed, but not 5-HT (G). 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.



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1008 **Figure 4**

1009 **Running stimulates LCTH and VTATH neurons, but not raphe 5-HT^{+} neurons.**

1010 Representative double-fluorescence images of c-Fos (green), TH (blue), and 5-HT (red)

1011 neurons in LC (A); VTA and SNpc (B); and DRN and MRN (C). Scale bars show 100

1012 µm. Yellow heads show c-Fos^{+}/TH^{+} neurons. LCTH and VTATH neurons were

1013 significantly stimulated in both running groups (D, E). However, running did not have

1014 an impact on SNpcTH, DRN$^{5\text{-}HT}$, and MRN$^{5\text{-}HT}$ neurons (F, G, H). All data are expressed

1015 as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs Control, $^{\#\#}p < 0.01$ vs Light, n =

1016 6-8 / group. Control: sedentary control; Light: 15 m/min running; Vigorous: 25 m/min

1017 running.

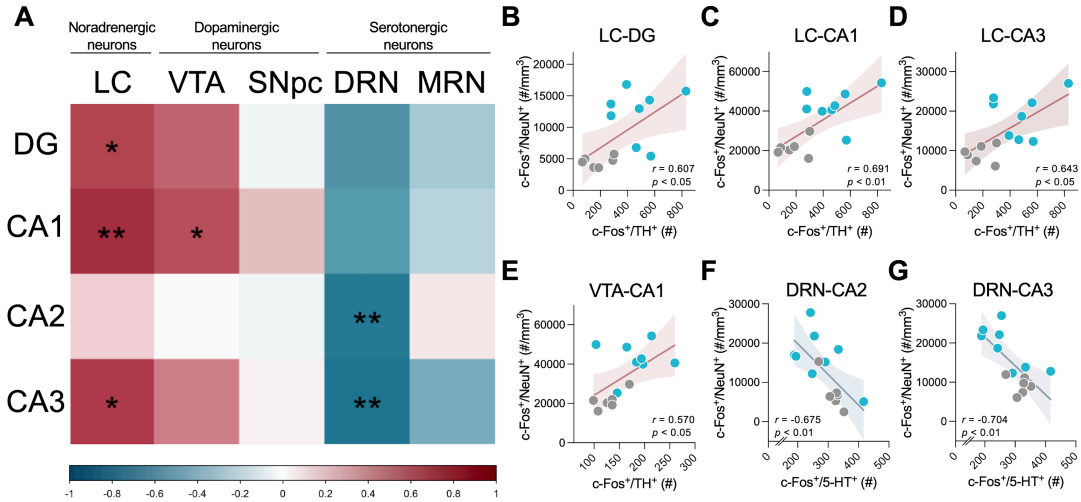
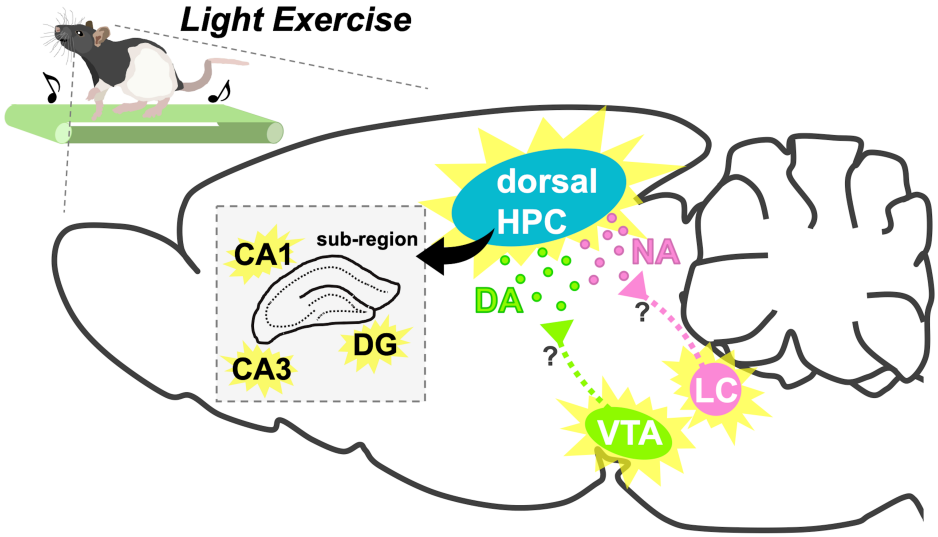


Figure 5

Neuronal associations between dorsal hippocampus and brainstem monoaminergic nuclei.

(A) 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}, $SNpc^{TH}$, $DRN^{5\text{-}HT}$, and $MRN^{5\text{-}HT}$ neurons. Colors in the scale show correlation strength. c-Fos expression in LC^{TH} neurons exhibited a significant and positive correlation within the dorsal DG (B), CA1 (C), and CA3 (D). A significant positive correlation was also found between c-Fos expression in VTA^{TH} neurons and in the dorsal CA1 (E). c-Fos expression in $DRN^{5\text{-}HT}$ neurons was significantly and negatively correlated with that in CA2 (F) and CA3 (G). The colored line represents linear regression, and the colored band represents 95% confidence bands. $^{*}p < 0.05$, $^{**}p < 0.01$. $n = 14$.



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1035 **Figure 6**

1036 **Schematic diagram showing possible circuit mechanism for light-exercise-induced**

1037 **dorsal hippocampal activation.**

1038 Light exercise activates TH^{+} neurons at the LC and the VTA. At the dorsal

1039 hippocampus, subsequently, axon terminals originating from the LC^{TH} and the VTA^{TH}

1040 increase the release of NA and DA, respectively. These, in turn, upregulate the neuronal

1041 activity of the dorsal hippocampal DG, CA1, and CA3 sub-regions. HPC, hippocampus;

1042 DG, dentate gyrus; NA, noradrenaline; DA, dopamine; LC, locus coeruleus; VTA,

1043 ventral tegmental area.

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Supplemental data

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

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This PDF file includes:

- Figure S1 Running induces neuronal activation of the ventral hippocampus
- Figure S2 RRF^{TH} neurons and DRN^{TH} neurons are not activated by running.

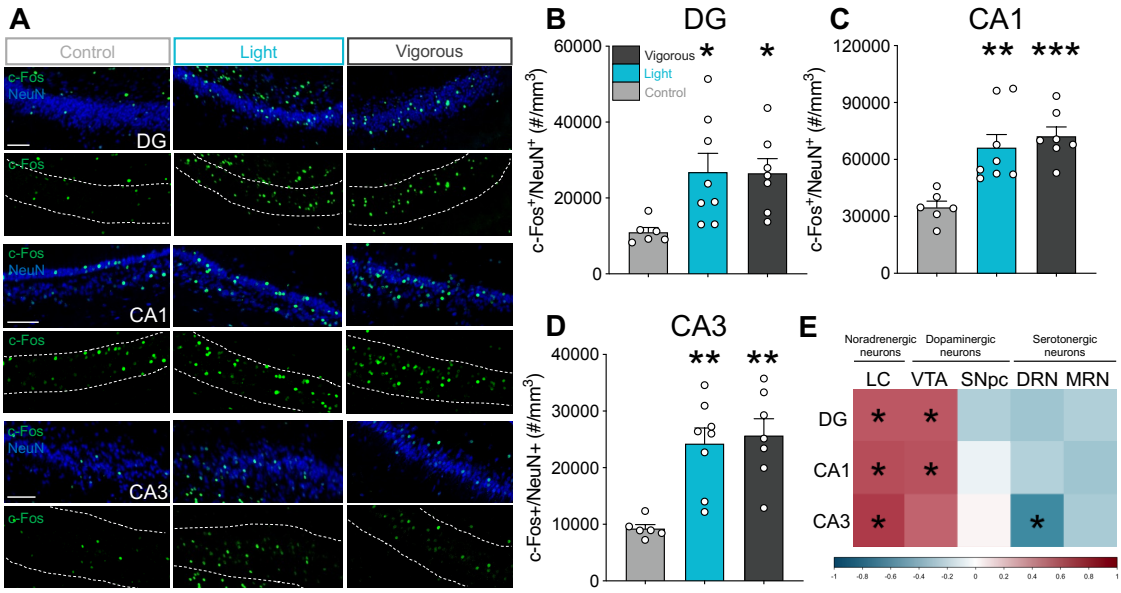

Figure S1

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 the LC^{TH} had a significant positive correlation with that in all ventral hippocampal sub-regions ($n = 14$, DG: $r = 0.543$, $p < 0.05$; CA1: $r = 0.587$, $p < 0.05$; CA3: $r = 0.654$, $p < 0.05$). Additionally, a significant positive correlation was found between c-Fos expression in the

VTATH neurons and in the ventral DG and CA1 (n = 14, DG: r = 0.550, p < 0.05; CA1: r = 0.564, p < 0.01; CA3: r = 0.507, p = 0.064). The higher level of c-Fos expression in the DRN$^{5\text{-}HT}$ neurons was significantly correlated with the lower level of ventral CA3 c-Fos expression (n = 14; DG: r = -0.085, p = 0.715; CA1: r = -0.090, p = 0.697; CA3: r = -0.522, p < 0.05). No correlation was found between c-Fos expression in SNpcTH or MRN$^{5\text{-}HT}$ neurons and in ventral hippocampal sub-regions (SNpc: n = 14, DG: r = -0.234, p = 0.422; CA1: r = -0.051, p = 0.864; CA3: r = 0.039, p = 0.895; MRN: n = 14, DG: r = -0.238, p = 0.412; CA1: r = -0.283, p = 0.328; CA3: r = -0.253, p = 0.382). All data are expressed as mean ± SEM. $^{*}p$ < 0.05, $^{**}p$ < 0.01, $^{***}p$ < 0.001 vs Control; n = 6-8/group. Control: sedentary control; Light: 15 m/min running; Vigorous: 25 m/min running.

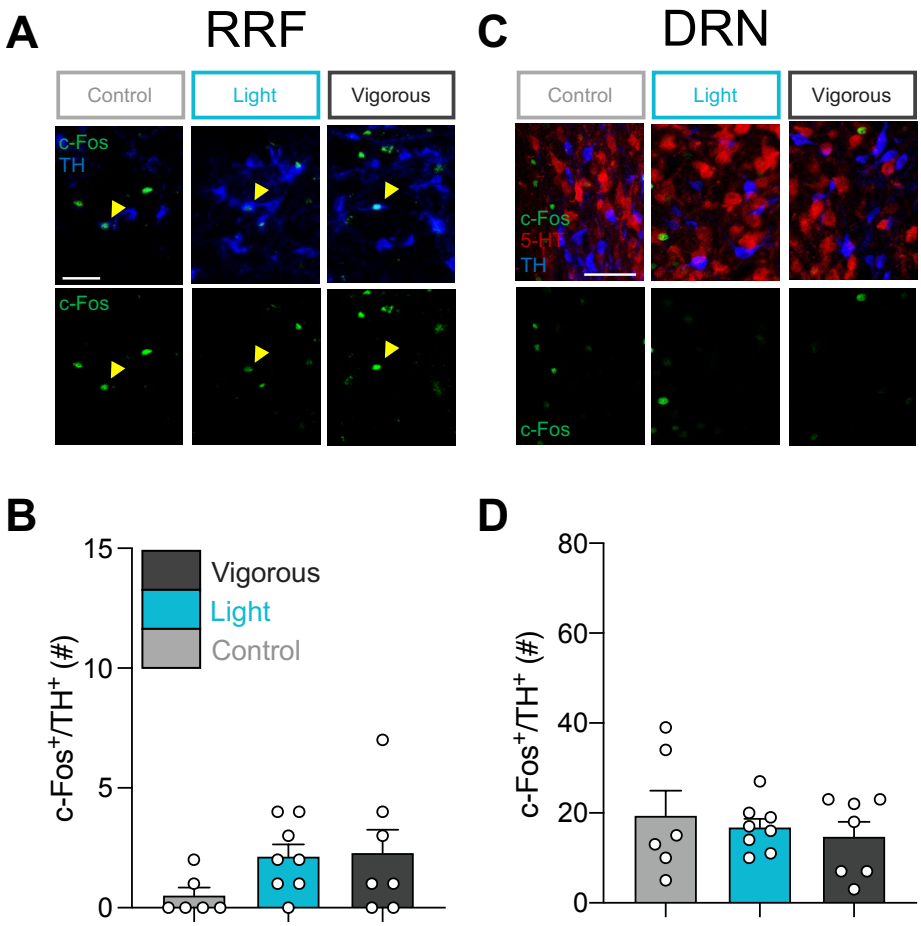

Figure S2

RRFTH neurons and DRNTH neurons are not activated by running.

(A) Representative double-fluorescence images of c-Fos (green) and TH (blue) neurons in the RRF. Scale bars show 100 µm. Yellow heads show c-Fos^{+}/TH^{+} neurons. (B) RRFTH neurons were not activated by running (Kruskal-Wallis test, Mann-Whitney U test adjusted by Holm's *post-hoc* test, $X^2 = 4.494$, df = 2, $p = 0.106$). (C) Representative triple-fluorescence images of c-Fos (green), TH (blue), and 5-HT (red) neurons in the DRN. Scale bars show 50 µm. (D) DRNTH neurons were not activated by running (Welch's one-way ANOVA, Shaffer's *post hoc* test, $F_{(2, 18)} = 0.384$, $p = 0.687$). All data are expressed as mean ± SEM. n = 6-8/group. Control: sedentary control; Light: 15 m/min running; Vigorous: 25 m/min running.