Submission to: PNAS

Rapid stimulation of human dentate gyrus function with acute mild exercise

Kazuya Suwabe [1,2]†, Kyeongho Byun [2,3]†, Kazuki Hyodo [1], Zachariah M. Reagh [3], Jared M. Roberts [3], Akira Matsushita [4], Kousaku Saotome [4], Genta Ochi [1], Takemune Fukuie [1], Kenji Suzuki [4], Yoshiyuki Sankai [4], Michael A. Yassa [2,3]*, and Hideaki Soya[1,2]*

Affiliation:

[1] Laboratory of Exercise Biochemistry and Neuroendocrinology; **[2]** Department of Sports Neuroscience, Advanced Research Initiative for Human High Performance (ARIHHP), Faculty of Health and Sport Sciences, University of Tsukuba, Ibaraki 305-8574, Japan

[3] Department of Neurobiology and Behavior, Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA, 92697-3800, USA

[4] Center for Cybernics Research, University of Tsukuba, Ibaraki 305-8574, Japan

† K.S. and K.B. contributed equally to this work.

*To whom correspondence should be addressed:

Hideaki Soya (soya.hideaki.gt@u.tsukuba.ac.jp)

Michael A. Yassa (myassa@uci.edu)

1

www.pnas.org/cgi/doi/10.1073/pnas.1805668115

Supporting information

SI MATERIALS AND METHODS

Cardiorespiratory fitness assessment

At least 48 h prior to the experiments, participants performed a cardiorespiratory fitness assessment test to estimate the appropriate individual exercise load corresponding to very light intensity (30% $\dot{V}O_{2peak}$) using a recumbent ergometer (Strength-ergo 240, Mitsubishi Electric Corporation, Japan). After warming up for 3 min at 30 W, the work rate was increased by 20 W (women: 15 W) per minute in a constant and continuous manner until the subject reached exhaustion. The pedaling rate was maintained at 60 rpm. Exhaled gas was analyzed using a gas analyzer (Aeromonitor AE280S, Minato Medical Science, Japan). Heart rate (HR; Polar RS800CX, Polar, Finland) and Borg's rating of perceived exertion (RPE) (1) was recorded once every minute. $\dot{V}O_{2peak}$ was defined as the value of VO_2 when at least two of the following criteria were satisfied: (1) the respiratory exchange ratio exceeded 1.05, (2) 90% of age-predicted peak HR (220 – age) was achieved, and (3) the RPE score was 19 or 20.

Mood scale

Psychological mood (arousal and pleasure) was measured using the Two-Dimensional Mood Scale (TDMS) before and after the intervention as well as after the study session (2). The TDMS is a psychometric scale comprising eight mood-expressing words describing both pleasure and arousal states (energetic, lively, lethargic, listless, relaxed, calm, irritated, and nervous). Participants were asked to indicate how they were feeling at the time according to a six-point Likert-type scale ranging from 0 = "Not at all" to 5 = "Extremely". Levels of arousal and pleasure at that point were calculated from these TDMS scores (range: -20 to 20).

Saliva sampling and alpha-amylase and cortisol measurements

Saliva was collected in sterile tubes using the “passive drool” method. The saliva samples were immediately placed in a freezer and stored at −80°C for a minimum of 24 h to allow the mucins to precipitate. The samples were then thawed and centrifuged at 4100 rpm for 15 min to extract particulates from the saliva (LC-120, Tomy, Japan). The clear supernatant was transferred to another sterile tube and stored at −80°C until assayed. On the day of the assay, the saliva samples were thawed and centrifuged at 4100 rpm for 15 min before the assay. Alpha-amylase activity levels were determined by enzyme kinetic assay (Salimetrics, State College, PA, USA). Cortisol levels were measured by expanded range enzyme immunoassay (Salimetrics).

Continuous version of the mnemonic discrimination task (Experiment 2)

We adopted a continuous mnemonic discrimination task (3). The paradigm was an explicit three-alternative forced choice task in which the participants were instructed to judge whether the presented item was “old”, “similar”, or “new” by pressing buttons located near the right hand. Each participant completed four functional runs, each comprising 24 similar pairs, 24 identical pairs, and 48 unrelated novel items (foils), which were fully randomized throughout the run (Fig. S1). The presented stimuli (photographs of items) were collected from the same pool as in Experiment 1. The number of trials separating similar and identical pairs was randomly varied between 10 and 40. Each stimulus was presented for 2000 ms with a 500-ms interstimulus interval. The lure discrimination performance was assessed by the same calculation, LDI= p (‘similar’|lure) - p (‘similar’|new), as in Experiment 1. We used the stimulus delivery and experiment control program Presentation (https://www.neurobs.com) to present stimuli and collect behavioral data.

fMRI data preprocessing and cross-participant alignment

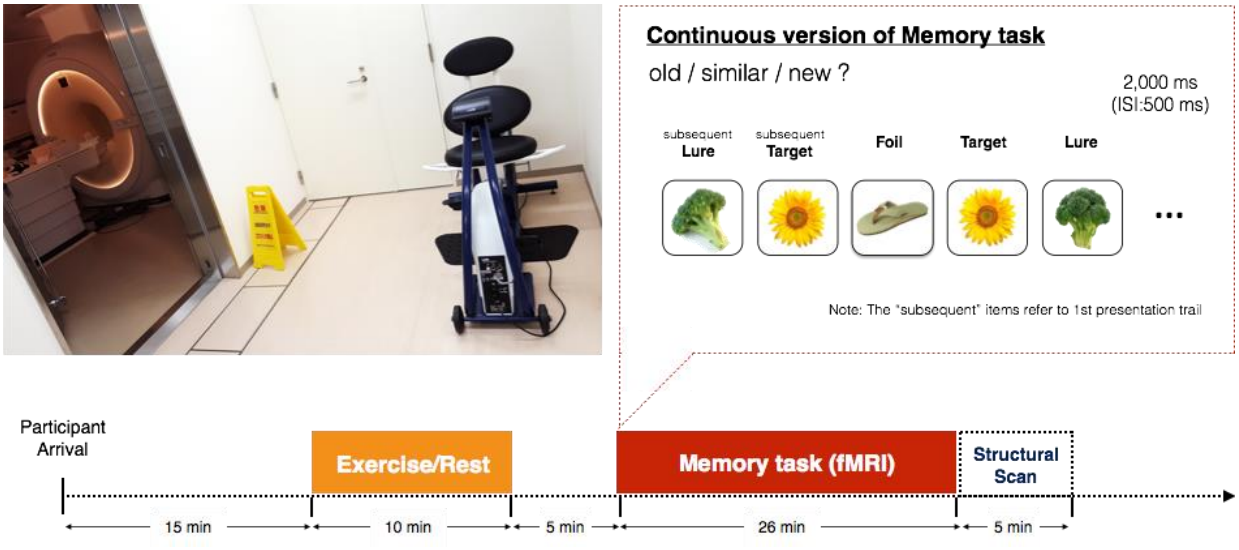
Preprocessing of neuroimaging data and univariate analyses were performed using the Analysis for Functional NeuroImaging (AFNI; https://afni.nimh.nih.gov/) software (4). Images were corrected for slice timing (3dTshift) and subject motion (3dvolreg), as well as global spikes in

signal (3dDespike). The time-points in which a significant motion event occurred (>3° of rotation or 2-mm translation in any direction prior to acquisition), and the previous and subsequent repetition times were censored and removed from further analyses. Additionally, functional images were masked to exclude voxels outside the brain (3dAutomask) and then smoothed (3dmerge) to 2 mm using a Gaussian full-width at half-maximum kernel. Functional images were then coregistered to the structural MRI scan using AFNI's align_epi_anat.py routine. High-resolution structural scans (0.65-mm isotropic) were aligned to an anatomical template based on the entire sample using Advanced Normalization Tools (ANTs) (5), which use a nonlinear registration algorithm called Symmetric Image Normalization (6) to warp each individual participant's MPRAGE structural scan into the template space. The transformation parameters were then applied to the coplanar functional data using an in-house shell script to wrap basic ANT functions (code available upon request). We defined the regions of interest (ROIs) in the medial temporal lobe and hippocampus according to the atlas of (7) and our previous work (8, 9). Hippocampal ROIs included the bilateral combined DG/CA3, CA1, and subiculum subregions. Cortical ROIs included the bilateral temporopolar cortex, entorhinal cortex (EC), perirhinal cortex (PRC), and parahippocampal cortex (PHC). In addition, we used the same template to add the amygdala. ROI masks were resampled to match the resolution of the smoothed fMRI data (2-mm isotropic) and further eroded to exclude partially sampled voxels within and across runs (3dcalc).

References:

1. Borg GA (1982) Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 14(5):377–81.
2. Sakairi Y, Nakatsuka K, Shimizu T (2013) Development of the Two-Dimensional Mood Scale for self-monitoring and self-regulation of momentary mood states. *Jpn Psychol Res* 55(4):338–349.

3. Yassa MA, et al. (2010) High-resolution structural and functional MRI of hippocampal CA3 and dentate gyrus in patients with amnestic Mild Cognitive Impairment. *Neuroimage* 51(3):1242–1252.
4. Cox RW (1996) AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res* 29(3):162–73.
5. Avants B, et al. (2008) Multivariate Analysis of Structural and Diffusion Imaging in Traumatic Brain Injury. *Acad Radiol* 15(11):1360–1375.
6. Klein A, et al. (2009) Evaluation of 14 nonlinear deformation algorithms applied to human brain MRI registration. *Neuroimage* 46(3):786–802.
7. Duvernoy H (1998) *The human hippocampus* (Springer-Verlag).
8. Reagh ZM, Watabe J, Ly M, Murray E, Yassa MA (2014) Dissociated signals in human dentate gyrus and CA3 predict different facets of recognition memory. *J Neurosci* 34(40):13301–13.
9. Yushkevich P a., et al. (2015) Quantitative comparison of 21 protocols for labeling hippocampal subfields and parahippocampal subregions in in vivo MRI: Towards a harmonized segmentation protocol. *Neuroimage*:1–16.



93

94 **Fig. S1.**

95 Mnemonic discrimination task and experimental procedure (Experiment 2).

96

97

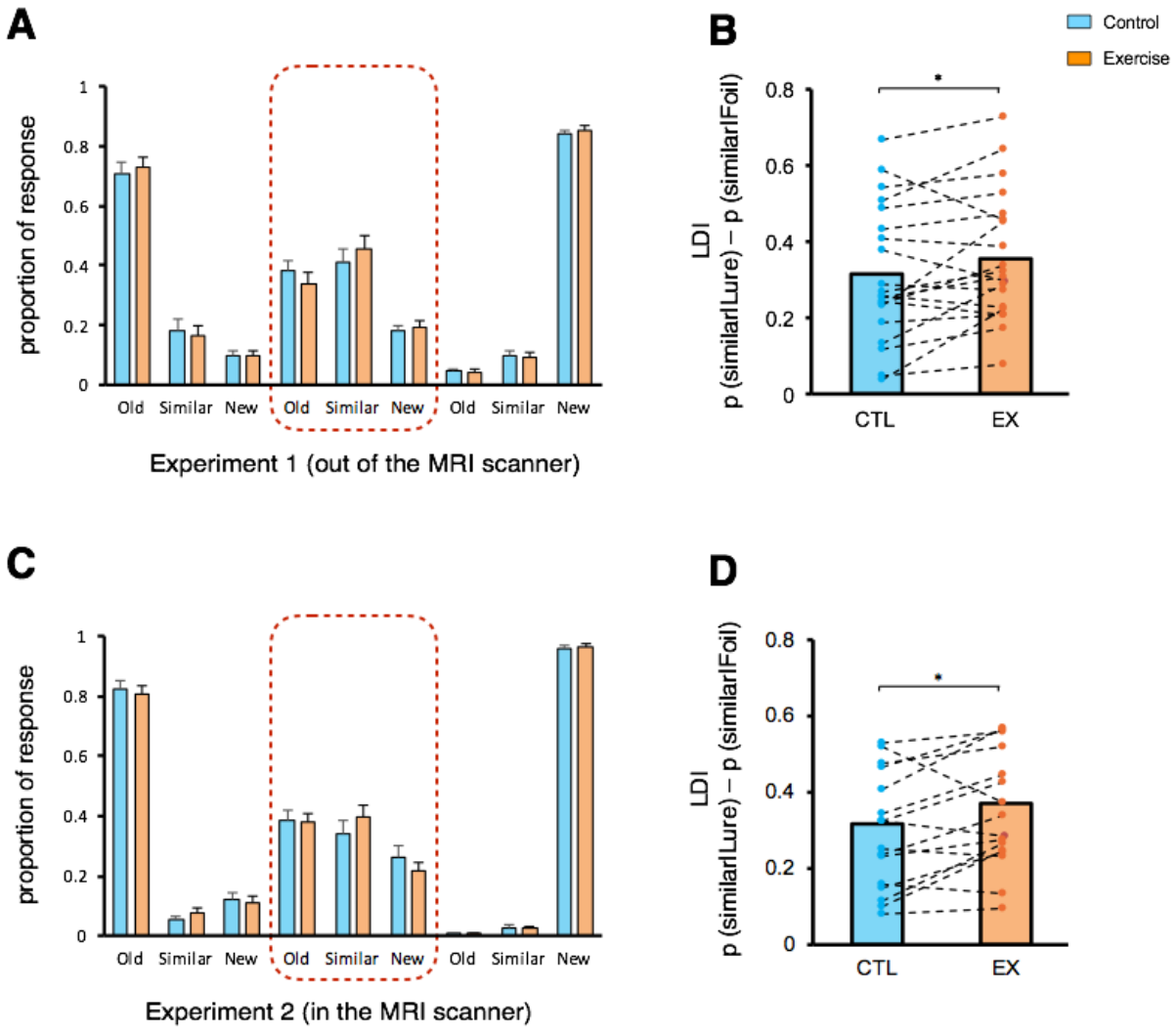


Fig. S2.

Overall task performance and behavioral improvement with mild exercise.

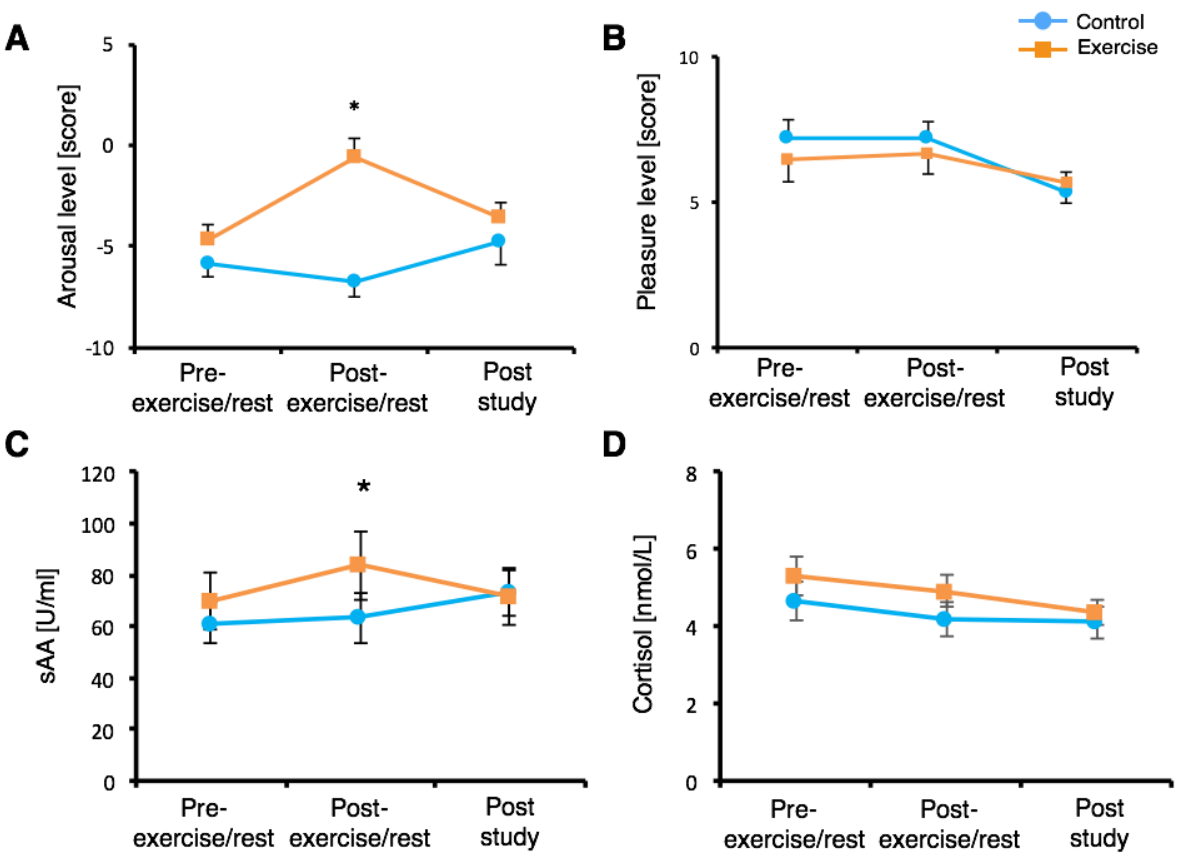


Fig. S3.

Mood (arousal, pleasure), salivary alpha-amylase, and cortisol resposes.

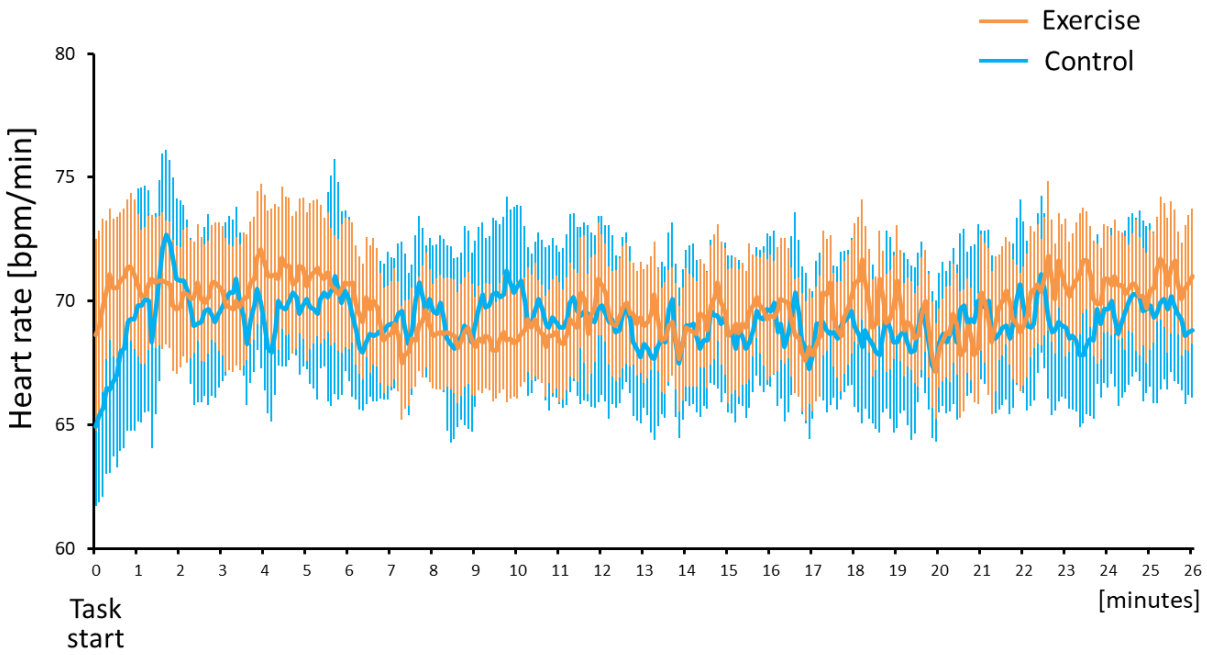


Fig. S4.

Heart rate response during functional imaging (mean ± SE).

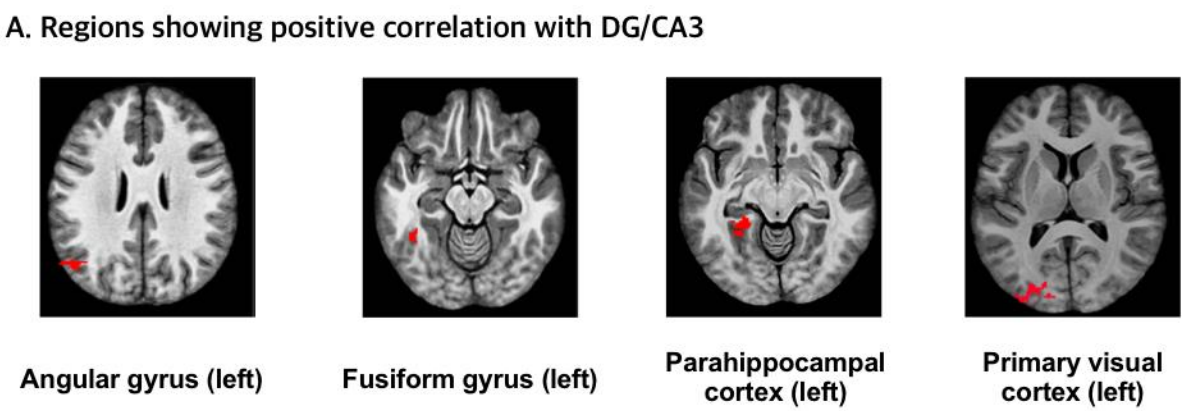


Fig. S5.

Results of the PPI analysis

Table S1.

Participant demographic and physiological characteristics.

Note: BMI=Body Mass Index; BDI=Beck Depression Inventory; VO2peak=peak oxygen uptake; HR=heart rate; RAVLT= Rey Auditory Verbal Learning Test; RPE=rating of perceived exertion; WR=work road. Values are mean (SD).

Measure	Experiment 1 (Behavioral)			Experiment 2 (fMRI)		
	All	Men	Women	All	Men	Women
Sample Size	20	12	8	16	4	12
Age[yr]	20.6(1.73)	20.8(1.52)	20.0(2.08)	21.1(2.00)	20.8(2.06)	21.3(2.05)
Height [cm]	166.7(6.42)	170.4(4.55)	160.0(2.81)	164.2(9.12)	176.1(8.03)	160.3(5.25)
Weight [kg]	59.7(13.78)	64.9(14.55)	49.8(2.33)	55.4(7.74)	64.7(7.10)	52.3(5.12)
BMI [kg/m^2]	21.3(3.88)	22.3(4.51)	19.5(0.92)	20.5(1.56)	20.8(0.62)	20.4(1.78)
BDI-2	7.3(5.33)	8.8(5.86)	4.9(3.48)	4.4(3.88)	7.3(3.77)	3.5(3.58)
RAVLT (Total learning)	56.4(6.24)	55.5(5.65)	57.9(7.29)	56.9(5.97)	52.3(5.32)	58.4(5.52)
RAVLT (Immediate recall)	12.5(1.92)	12.7(1.74)	12.1(2.27)	12.2(1.72)	11.8(2.22)	12.3(1.61)
RAVLT (Delayed recall)	12.0(2.30)	12.1(1.87)	11.9(3.02)	11.9(1.73)	11.8(2.22)	12.0(1.65)
Graded exercise test						
VO_{2peak} [ml/kg/min]	40.5(7.60)	42.5(8.40)	36.8(4.18)	37.9(8.21)	50.0(3.55)	33.8(4.09)
HR_{peak} [bpm]	175.5(13.40)	176.0(11.43)	174.6(17.25)	172.2(10.22)	173.0(7.39)	171.9(11.28)
RPE_{peak}	19.2(1.39)	19.5(0.66)	18.4(2.07)	19.4(0.89)	19.8(0.50)	19.3(0.97)
WR_{peak}	202.3(48.39)	229.7(34.77)	151.4(18.74)	179.3(50.47)	258.8(23.23)	152.8(16.18)
Exercise condition						
HR [bpm]	100.3(6.93)	99.4(7.30)	101.9(6.40)	102.0(8.64)	106.4(9.13)	100.5(8.34)
RPE	10.4(2.11)	10.2(1.88)	10.6(2.64)	10.6(1.31)	11.3(0.96)	10.3(1.37)
WR	46.4(17.04)	50.3(15.65)	31.8(7.15)	38.4(15.59)	62.5(2.65)	30.4(6.97)