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MICRO REPORT

Bidirectional effects of voluntary exercise on the expression of *Bdnf* isoforms in the hippocampus of Hatano rat strains displaying different activity levels

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Abstract

Brain-derived neurotrophic factor has functional mRNA isoforms, whose expression is assumed to mediate the beneficial effects of exercise in neuropsychiatric disorders. This study aims to reveal the mechanism of intensity-dependent effects of voluntary exercise, focusing on the expression of *Bdnf* mRNA isoforms in Hatano rats. Animals with different voluntary activity were housed in cages with a locked or unlocked wheel for 5 weeks. The expression levels of *Bdnf* isoforms and the corresponding coding sequences (CDS) were measured in the hippocampus using real-time polymerase chain reaction (PCR). We found that exercise increased the expression of *Bdnf* isoform containing exon 1 in the high-intensity-running strain and decreased the expressions of *Bdnf* exon 1, 3, 6, 7, 8, and 9a in mild-intensity-running animal. The expression of *Bdnf* CDS was increased by exercise in both strains. These results suggest that expressions of *Bdnf* isoforms depend on the intensities of voluntary exercise, but the involvement of subjects' genetic background could not be excluded. Our finding also implies that the bidirectional effects of exercise may not be mediated via the final product of *Bdnf*.

KEYWORDS
brain-derived neurotrophic factor, exercise, Hatano rats

1 | INTRODUCTION

Brain-derived neurotrophic factor (BDNF), a protein in the neurotrophin family, plays an important role in neural development.[1,2] The involvement of BDNF is also suggested in the pathology of neuropsychiatric disorders.[3–5] The *Bdnf* gene comprises noncoding exons (1–9a) and a coding exon (9), generating mRNA isoforms by various combinations of the exons.[6]

Shuichi Chiba and Hikaru Asano contributed equally to the work.

Exercise alters the expression levels of *Bdnf*[7,8] in the hippocampus and relieves the symptoms of neuropsychiatric diseases, such as depressive and anxiety disorders.[9,10] The positive effects of exercise are suggested to be mediated through *Bdnf* expression.[11,12] The beneficial effects of exercise depend on its intensity.[13] Because Maynard et al.[14] suggested that each *Bdnf* isoform plays a different role in physiological processes, we hypothesized that the changes in the expression patterns of *Bdnf* isoforms lead to the intensity-dependent effects of exercise.

Hatano rats were descended from the Sprague–Dawley, and selective breeding based on performance in the shuttle box active avoidance task generated inbred high- and low-avoidance animals (HAA and LAA). Earlier, we reported a significant difference in the amount of voluntary wheel running (WR): the number of revolutions of the wheel in Hatano LAA is one-quarter the number of revolutions in HAA.[15,16] In addition, a stronger anxiolytic-like effect of the exercise was found in LAA than in HAA,[16] which makes Hatano strains a useful model to study the effects of exercise. Here, we used HAA and LAA to elucidate the effects of exercise on the *Bdnf* isoform expressions.

2 | METHODS

2.1 | Animals

Three-week-old Hatano rats (Hatano Research Institute, Hadano, Kanagawa) were introduced into our laboratory and randomly classified into the control (C) or WR groups ($n = 9$, each) at 4 weeks of age. Three animals were housed in each cage (30 × 53 × 32 cm) equipped with either an unlocked running wheel (25.5 × 15.5 × 30 cm) or a locked one, with free access to food and water under a 12-h:12-h light/dark cycle (light on: 0800-2000) and controlled room temperature (24°C ± 2°C) and humidity (50% ± 5%).

2.2 | Sampling and real-time PCR

Nine-week-old animals were sacrificed by decapitation without anesthesia, and their bilateral hippocampi were removed on ice and stored at −80°C. Total RNA was extracted using TRIzol (Thermo Fisher Scientific, Massachusetts, USA) while homogenizing the samples using Minilys (Bertin Instruments, Montigny-le-Bretonneux, France). The concentration and purity of the extracted RNA were measured using a spectrophotometer (Model DS-11, Denovix, Delaware, USA). Reverse transcription was performed using the PrimeScript RT reagent Kit (Takra Bio, Shiga, Japan) only if RNA concentration reached 100 ng/μl or higher and if the A260/A280 ratio was between 1.7 and 2.1. Real-time polymerase chain reaction (PCR) (the ΔΔCt method) was performed using SYBR Premix Ex Taq master mix (Takara Bio) and Quant Studio5 Real-time PCR System (Thermo Fisher Scientific). Table 1 lists the details of primers used in this study.

TABLE 1 Primers used for real-time PCR

Bdnf isoforms/Gene	Forward	Reverse
Exon 1	5′-TGTTGGGGAGA CGAGATTTT-3′	5′-CGTGGACG TTTGCT TCTTTC-3′
Exon 2	5′-TTCGGCTCACA CTGAGATCG-3′	5′-CAGTATAC CAACCCGGA GCTT-3′
Exon 3	5′-CTGAGACTGCG CTCCACTC-3′	5′-GTGGACGT TTGCTT CTTTCA-3′
Exon 4	5′-GAGCAGCTGCC TTGATGTTT-3′	5′-GTGGACGT TTGCTT CTTTCA-3′
Exon 5	5′-AAACCATAACC CCGCACACT-3′	5′-CTTCCCGC ACCACA GAGCTA-3′
Exon 6	5′-GATGAGACCGG GTTCCCTCA-3′	5′-TTGTTGTC ACGCTC CTGGTC-3′
Exon 7	5′-ACTGTCAC CTGCTTTCT AGGG-3′	5′-GAGTTCCG CAGACC CTTTCA-3′
Exon 8	5′-GTGCTCAG GCTAATCCT CGTT-3′	5′-CTTTCTCC TGGGATGCA CAGT-3′
Exon 9a	5′-ACGGCGTGAAC AGAGATCAT-3′	5′-ACGGTTTC TAAGCAAGT GACG-3′
Bdnf CDS	5′-GTGACAGT ATTAGCGAG TGGG-3′	5′-GGGTAGTT CGGCAT TGC-3′
Impdh2	5′-TCAAGCCA AGASCCTCA TCGA-3′	5′-AGCGACGG GCATAC TCAGA-3′

Abbreviations: CDS, coding sequence; Impdh2, inosine-5′-monophosphate dehydrogenase 2; PCR, polymerase chain reaction.

2.3 | Data analysis

The estimated concentration of each target was divided by inosine-5′-monophosphate dehydrogenase 2 (an internal control). Data were analyzed using two-way analysis of variance (ANOVA) with strain and exercise as fixed factors, followed by Holm's post-hoc comparisons. Statistical significance was defined as $p < 0.05$.

3 | RESULTS

Real-time PCR revealed that WR increased the expression of some *Bdnf* isoforms in HAA, whereas WR had the opposite effect in LAA. Table 2 summarizes the results from ANOVA. Strain differences were found in the expression of *Bdnf* isoforms containing exons 1–4 and 6; however, this finding was weakened by significant interactions between strain and exercise found for all *Bdnf* exons, except exon 4. Exercise significantly increased *Bdnf*

TABLE 2 Summary of results from factorial two-way ANOVA for *Bdnf* isoforms and the coding sequence (CDS) expression in the hippocampus

	Strain		Exercise		Interactions	
mRNA isoforms	*F*	*p*	*F*	*P*	*F*	*p*
Exon 1	11.93	0.003*	1.13	0.298	27.16	0.001*
Exon 2	13.36	0.002*	0.17	0.681	8.14	0.010*
Exon 3	6.37	0.019*	6.35	0.019*	22.03	0.001*
Exon 4	32.24	0.001*	0.26	0.528	3.25	0.084
Exon 5	0.13	0.727	0.01	0.957	10.71	0.004*
Exon 6	7.81	0.011*	7.85	0.010*	15.23	0.001*
Exon 7	0.76	0.392	0.01	0.994	15.03	0.001*
Exon 8	0.83	0.371	0.07	0.790	16.67	0.001*
Exon 9a	1.02	0.322	0.31	0.587	11.36	0.003*
CDS	8.71	0.007*	49.12	0.001*	0.44	0.513

Note: Degree of freedom (DF) is 1 (factors) and 24 (residuals) in all analysis except for the isoform containing exon 2 where the DF of residuals is 23 due to a missing value caused by technical errors.
*$p < 0.05$.

exon 1 expression in HAA (Figure 1A), whereas it significantly reduced the expression of isoforms containing exons 1, 3, 6–8, and 9a in LAA (Figure 1A,C,F–I, respectively). Expression levels of the *Bdnf* coding sequence (CDS) was increased by exercise, without fully erasing a significant difference between the strains (Figure 2).

4 | DISCUSSION

Our results raise the possibility that high- and mild-intensity voluntary exercises exert the opposite influence on the expression of *Bdnf* isoforms, especially *Bdnf* isoforms containing exon 1, although we cannot rule out the involvement of different genetic backgrounds. The measured bidirectional changes in *Bdnf* mRNA isoform expression may mediate the different effects of exercise. Our previous study showed that an exercise-induced reduction of anxiety-like behavior was found only in the LAA strain.[16] Thus, the downregulation of *Bdnf* isoforms may mediate the anxiolytic-like effect of exercise in LAA. The results lend partial support to our working hypothesis – intensity-dependent exercise effects mediated by different *Bdnf* isoform expression patterns – with the caveat that we did not detect conspicuous isoform-specific differences between the strains.

Whereas voluntary running induced the above opposite changes in the expression of mRNA isoforms in the two strains, it upregulated *Bdnf* CDS expression in HAA and LAA. These results suggest that the sum of mRNA isoforms does not necessarily predict the quantity of the final BDNF protein product and transcripts are modified by unknown mechanisms. Because the anxiolytic-like effect of exercise was not consistent with exercise-induced *Bdnf* CDS expression, BDNF proteins may not be the mediators of the anxiolytic-like effect, leaving open the possibility that *Bdnf* isoforms may mediate it via other targets (Figure 3). The functional connections between the effects of exercise and *Bdnf* isoform expression should be clarified by investigating the function of each transcript using genetically engineered animals expressing targeted exons, assuming each *Bdnf* mRNA isoform has a different function.[14] One candidate is a change in the expression level of transcript containing exon 3 because Alme et al.[17] reported the downregulation of this transcript after administration of an antidepressant fluoxetine, which is also used as an anxiolytic. However, the transcription of *Bdnf* mRNA isoforms has diverse differences among the antidepressants and electroconvulsive seizure treatment protocols as well as its time courses[18]; therefore, prudent examination is required.

Another important finding of this study is that the baseline (i.e., in control environment) expression levels of *Bdnf* mRNA isoforms as well as CDS were higher in LAA than HAA. To the best of our knowledge and findings from the literature review, these data are new, which may explain the behavioral differences between strains such as voluntary exercise[15,16] and anxiety.[16] Future research on the abovementioned functional connections is expected to elucidate the mechanism underlying the diverse impacts of exercise in health and disease especially anxiety disorders, through which the development of novel therapies will be facilitated for psychiatric diseases.

AUTHOR CONTRIBUTIONS

HA conducted animal and PCR studies. HA and SC wrote the manuscript. SC, SM, TH, SK, RO, and MK discussed results and helped draft the manuscript. MK supervised all aspect of the study. All authors have read and approved to submit the final manuscript.

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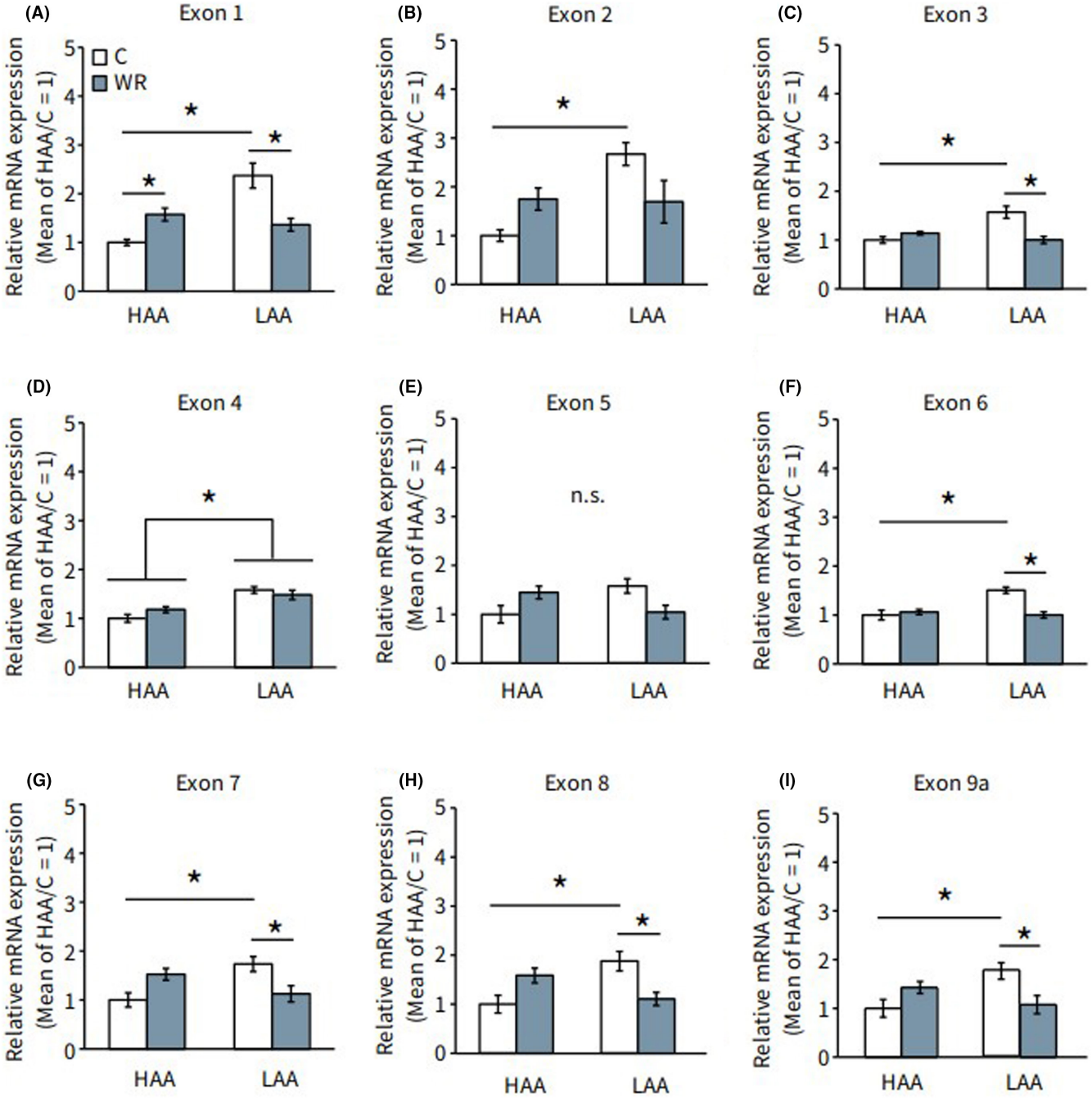

FIGURE 1 Effects of wheel running (WR) on the expression of *Bdnf* isoforms in the hippocampus. (A) In high-intensity voluntary running animals (HAA), WR leads to selective increases in the expression of isoform containing exon 1. Alternatively, in mild-intensity voluntary running animals (LAA), WR led to reductions in the expressions of *Bdnf* exons 1, 3, 6–8, and 9a (A, C, F–I, respectively). Additionally, a significant effect of strain was observed in *Bdnf* exon 4 expression (D), while the strain difference of *Bdnf* exon 2 (B) was only observable in control (C) environment. (E) No significant differences (n.s.) was found in Bdnf exon 5 expression. The measured expression levels were divided by the mean value for the HAA in the C environment to report relative expression levels. Bars represent the group means; whickers, ± standard error of mean. $^*p < 0.05$, $n = 6–8$

CONFLICT OF INTEREST

The authors have no conflicts of interests to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the Supplementary Material of this article.

ETHICAL APPROVAL

Approval of the research protocol by an institutional reviewer board: N/A.

Informed consent: N/A.

Registry and the registration no. of the study/trial: N/A.

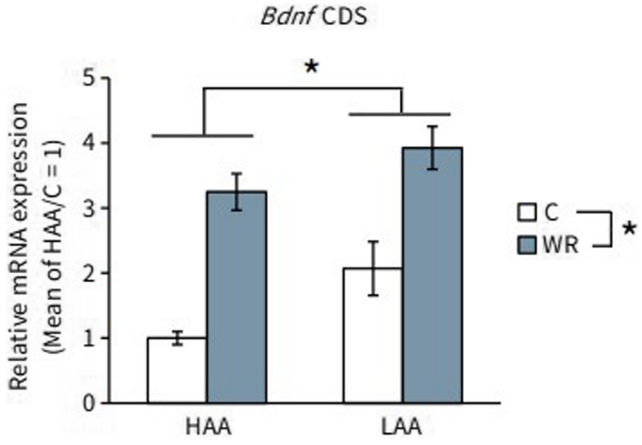

FIGURE 2 Effects of strain and WR on the expression of *Bdnf* CDS in the hippocampus. The levels of *Bdnf* CDS expression were lower in the high-intensity voluntary running animals (HAA) than in the mild-intensity voluntary running animals (LAA). WR significantly enhanced the expression of *Bdnf* CDS in both strains. Plotted expression values are relative to the mean value of HAA in the control (C) environment. Bars represent the group means; whickers, ± standard error of mean. $^{*}p < 0.05$, $n = 6–8$

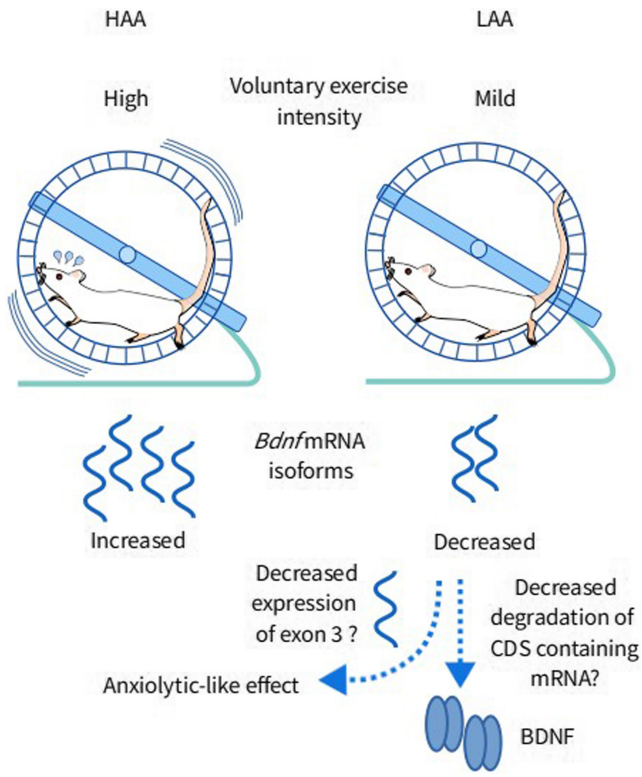

FIGURE 3 The summary and hypothesis of the current study. BDNF, brain-derived neurotrophic factor; CDS, coding sequence; HAA, Hatano high-intensity voluntary running animals; LAA, Hatano mild-intensity voluntary running animals

Animal studies: The experimental procedures in this study were approved by the Animal Care and Use Committee of Meiji University (IACU12-0010).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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