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ORIGINAL ARTICLE

Brain-derived neurotrophic factor predominantly regulates the expression of synapse-related genes in the striatum: Insights from *in vitro* transcriptomics

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Abstract

Aim: The striatum, a main component of the basal ganglia, is a critical part of the motor and reward systems of the brain. It consists of GABAergic and cholinergic neurons and receives projections of dopaminergic, glutamatergic, and serotonergic neurons from other brain regions. Brain-derived neurotrophic factor (BDNF) plays multiple roles in the central nervous system, and striatal BDNF has been suggested to be involved in psychiatric and neurodegenerative disorders. However, the transcriptomic impact of BDNF on the striatum remains largely unknown. In the present study, we performed transcriptomic profiling of striatal cells stimulated with BDNF to identify enriched gene sets (GSs) and their novel target genes *in vitro*.

Methods: We carried out RNA sequencing (RNA-Seq) of messenger RNA extracted from primary dissociated cultures of rat striatum stimulated with BDNF and conducted Generally Applicable Gene-set Enrichment (GAGE) analysis on 10599 genes. Significant differentially expressed genes (DEGs) were determined by differential expression analysis for sequence count data 2 (DESeq2).

Results: GAGE analysis identified significantly enriched GSs that included GSs related to regulation and dysregulation of synaptic functions, such as synaptic vesicle cycle and addiction to nicotine and morphine, respectively. It also detected GSs related to various types of synapses, including not only GABAergic and cholinergic synapses but also dopaminergic and glutamatergic synapses. DESeq2 revealed 72 significant DEGs, among which the highest significance was observed in the *apolipoprotein L domain containing 1* (*Apold1*).

Conclusions: The present study indicates that BDNF predominantly regulates the expression of synaptic-function-related genes and that BDNF promotes synaptogenesis in various subtypes of neurons in the developing striatum. *Apold1* may represent a unique target gene of BDNF in the striatum.

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KEYWORDS
Apold1, BDNF, DESeq2, GAGE, RNA-Seq, striatum

1 | INTRODUCTION

The striatum is a main component of the subcortical basal ganglia and is a critical component of the motor and reward systems. It consists of various neuronal types. Medium spiny neurons (MSNs) are the major constituent of the striatum, representing 90%-95% of the neuronal population and are GABAergic inhibitory neurons.[1,2] MSNs have two characteristic types: dopamine D1 receptor (D1R)-expressing MSNs and D2R-expressing MSNs. Subpopulations of MSNs express both D1R and D2R. Striatal cholinergic interneurons (CINs) represent 5%-10% of the population.[1,2] There are also many types of GABAergic interneurons (GINs), including parvalbumin-positive, somatostatinergic, and calretinin-positive interneurons. The striatum receives projections of cortical pyramidal glutamatergic neurons, midbrain substantia nigra (SN), dopaminergic neurons, and serotonergic neurons in the midbrain suture nucleus. Striatal dysfunction is known to be associated with psychiatric disorders including drug addiction and schizophrenia,[3–5] and neurodegenerative diseases such as Huntington's disease (HD) and Parkinson's disease (PD).

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family of growth factors, plays a pivotal role in the central nervous system, including cell survival and development.[6,7] In the developing mouse brain, BDNF protein expression is detected in some areas, including the piriform cortex and hippocampus (HIPP), but is not detectable in the striatum.[8,9] BDNF is anterogradely transported from the cerebral cortex (CTX), and SN is the source of BDNF in the striatum.[9–11] The receptor for BDNF, tropomyosin receptor kinase B (TrkB), is expressed in both MSNs and striatal interneurons,[12,13] while astrocytes predominately express the truncated TrkB (TrkB.T1) receptor.[14] CTX-specific knock-out of BDNF leads to a reduction in size in the striatum that displays morphological abnormalities of MSNs, such as shrunken cell somas, thinner dendrites, fewer dendritic spines, and loss of neuronal cells.[8] BDNF has been shown to regulate the size of the striatum by promoting the survival of immature neurons.[9,15] Accumulating evidence indicates that BDNF in the striatum and its associated regions is involved in psychiatric and neurodegenerative disorders. BDNF in the nucleus accumbens (NAc) is indicated to be involved in nicotine abstinence.[16] BDNF plays a role as a negative modulator of morphine action in the NAc.[17] In a mouse model of alcohol addiction, the BDNF pathway in the dorsolateral striatum controls the level of ethanol self-administration.[18] The dopamine D1-D2 receptor heteromer signaling induces upregulation of BDNF,[19] and the molecular interaction between dopamine D1 and D2 receptors is significantly reduced in the postmortem striatum of patients with schizophrenia.[20] In a mouse model of HD, it was demonstrated that increasing striatal BDNF levels rescued HD-related abnormalities.[11] BDNF levels decrease in the nigrostriatal dopaminergic pathway in PD patients and animal models of PD.[21]

In the present study, we carried out RNA sequencing (RNA-Seq) to better understand the transcriptomic effects of BDNF on striatal cells. We performed Generally Applicable Gene-set Enrichment (GAGE) analysis to identify the enriched gene sets (GSs) and differential expression analysis to identify significant differentially expressed genes (DEGs). To the best of our knowledge, this is the first study to directly and comprehensively examine the transcriptomic profiles induced by BDNF in the striatum.

2 | METHODS

2.1 | Materials

SD rats (Slc:SD; RRID: RGD_12910483) were obtained from NIPPON SLC. All efforts were made to minimize animal suffering and the number of animals used for the study. Animals were housed, handled, and bred according to the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions by the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan. Recombinant human BDNF was kindly provided by Sumitomo Dainippon Pharma and was dissolved in a 2% bovine serum albumin (BSA)/phosphate-buffered saline (PBS). The final solvent concentration in the culture medium was 0.1%. Anti-microtubule-associated protein-2 (anti-MAP2; 1:400; Sigma-Aldrich), anti-dopamine- and cAMP-regulated phosphoprotein, 32 kDa (anti-DARPP-32; 1:30; Santa Cruz Biotechnology), and anti-glial fibrillary acidic protein (GFAP; 1:400; Merck Millipore) for immunocytochemistry were used. Anti-rabbit and anti-mouse IgG secondary antibodies conjugated to Alexa Fluor 488 (1:400) or 546 (1:400) were purchased from Molecular Probes.

2.2 | Cell culture

Primary cultures of dissociated striatal neurons were prepared from rats (12 animals, both sexes) on embryonic day 20 (E20). The animals were exposed to isoflurane in the air until they became unconscious. The anesthetized state of the rats was confirmed by the disappearance of the eyelid reflex, corneal reflex, and loss of muscular tone. The animals were then immediately killed by cervical dislocation, followed by decapitation to ensure minimal suffering of the animals. The neurons were cultured in a medium consisting of 5% heat-inactivated fetal bovine serum (FBS), 5% heat-inactivated horse serum, and 90% DMEM at a final density of 1×10^6 cells on six-well plates or 1×10^5 cells in an 8-well chamber slide coated with polyethyleneimine (PEI) for the real-time quantitative polymerase chain reaction (RT-qPCR) assay. After 1 day *in vitro* (DIV1), the

culture medium was changed to serum-free Neurobasal Medium (Gibco) containing B27 supplement (Gibco). Cultures were incubated in a medium containing 1 μmol/L cytosine arabinoside (AraC) to suppress glial growth after DIV3. At DIV6, BDNF was applied with a final concentration of 10 ng/mL.[22] Cells were lysed in RLT butter (RNeasy mini kit, Qiagen) for NGS library preparation at DIV 8, after 48 hours of BDNF incubation.[23–25] Striatal cells at DIV6 were fixed with 4% paraformaldehyde for immunostaining.

2.3 | Immunocytochemistry

Immunocytochemical analysis was performed as previously reported.[26] Briefly, striatal cultures were fixed with 4% paraformaldehyde for 30 min at room temperature (RT). The cells were permeabilized with PBS containing 0.2% Triton-X for 2 min and blocked in 5% bovine serum albumin in PBS (PBSB) for 15 min at RT. Primary antibodies against MAP2, DARPP-32, and GFAP were diluted with PBSB and applied to the cells that were then incubated overnight at 4°C, and the cells were visualized using secondary antibodies conjugated to Alexa Fluor dyes (see section 2.1 for details). 4′, 6-Diamidino-2-phenylindole (DAPI) was used for nuclear staining. Fluorescent images were obtained using a CQ1 confocal quantitative image cytometer (Yokogawa).

2.4 | RNA sequencing and data analysis

Total RNA was extracted from striatal cultures using the RNeasy mini kit (Qiagen) and quantified using a NanoDrop 1000 (Thermo Fisher Scientific). Messenger RNA was purified from 1 μg of total RNA using the NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs). NGS libraries were generated using the NEBNext Ultra II RNA Library Prep (New England Biolabs) and NEBNext Multiplex Oligos for Illumina (New England Biolabs) according to the manufacturer's instructions. Libraries were sequenced on a HiSeq2500 (Illumina) with a configuration of 2 × 150 bp (GENEWIZ). After demultiplexing, fastx_toolkit was used to trim the sequences and remove short and/or low-quality reads. Reads were aligned using RSEM/bowtie2, and the data were summarized by R. Gene clustering, principal component analysis (PCA), and GS enrichment analysis were carried out using the iDEP.91 platform (integrated differential expression and pathway analysis ver. 0.91).[27] To identify enriched GSs, GAGE analysis[28] was conducted on iDEP.91. Two-group comparisons were carried out using differential expression analysis for sequence count data 2 (DESeq2)[29] and the empirical analysis of DGE in R (edgeR)[30] on iDEP.91.

3 | RESULTS AND DISCUSSION

First, we analyzed the cell population of our striatal cultures by immunocytochemical analysis using antibodies against microtubule-associated protein 2 (MAP2, a neuronal cell marker) and GFAP (astrocyte marker). DAPI staining was performed to determine the number of viable cells. Representative images are shown in Figure 1A. Cellular populations of MAP2- and GFAP-positive cells were 69.3 ± 4.2% and 27.5 ± 4.0%, respectively (Figure 1B). We categorized the cells that were not positive for neither MAP2- nor GFAP antibodies as "others" whose cellular population was 3.2 ± 0.4%. The cultures were also stained with DAPI and antibodies against dopamine- and cAMP-regulated phosphoproteins, 32 kDa (DARPP-32, medium spiny neuron marker), and MAP2 (Figure 1C). In our culture system, nearly all MAP2-positive cells were DARPP-32-positive, indicating that the majority of neuronal cells were MSNs.

The culture of DIV6 was incubated in the presence or absence of BDNF for 48 hours. This is to focus on chronic changes, but not acute temporal ones, in gene expression. Then, total RNA was extracted at DIV8. Library preparation and RNA sequencing were performed using GENEWIZ. Libraries were subjected to 150-bp paired-end sequencing on the HiSeq 2500 platform. iDEP.91 was used to analyze the RNA-Seq dataset. The distribution of the transformed data was assessed, and the boxplot and density plot are presented in Figure 2A,B, respectively. The variation among replicates was small. *K*-mean clustering analysis ($K = 3$) was then carried out for 10599 genes that displayed transcripts per million (TPM) values above 10 in more than three out of six samples (three experimental groups and three control groups). The results of hierarchical clustering analysis are shown in Figure 2C. Cluster A contained 4010 genes, and Clusters B and C included 916 and 5673 genes, respectively. BDNF-stimulated samples in Cluster A predominantly contained down-regulated genes, while control samples in Cluster A predominantly contained up-regulated genes. In contrast, BDNF-treated samples in Cluster B predominantly contained highly up-regulated genes, while those in Cluster C also predominantly contained up-regulated genes. PCA was then conducted on 10599 genes. The PCA plot of the first and second principal components is shown in Figure 3A. There is a distinctive difference between the BDNF-treated and control samples, along with the first-principal component that explains 38% of the variance. Together with the heatmap presented in Figure 2C, these observations indicate a substantial difference in the genes induced by BDNF in the striatum. The principal components and their related categories are shown in Figure 3B. The plot shows the five most important components resulting from PCA. The color of each cell represents the direction of change (red: positive, blue: negative) and the magnitude of each GS category contribution (row) to each principal component (column).

To identify highly enriched GSs in the BDNF-stimulated striatum, we carried out the GAGE analysis[28] using Kyoto Encyclopedia of Genes and Genomes (KEGG) as a reference database (KEGG-GAGE) for 10599 genes. The top-ranked GSs/pathways are listed in Table 1. Nine out of 12 GSs/pathways showing significant enrichment were related to the regulation of synaptic function. "Synaptic vesicle cycle" showed the highest significance (adjusted *P*-value: 1.1×10^{-3}, number of genes: 47), and "Long-term potentiation" also significant (adjusted *P*-value: 3.6×10^{-2}, number of genes: 45). There are GSs/

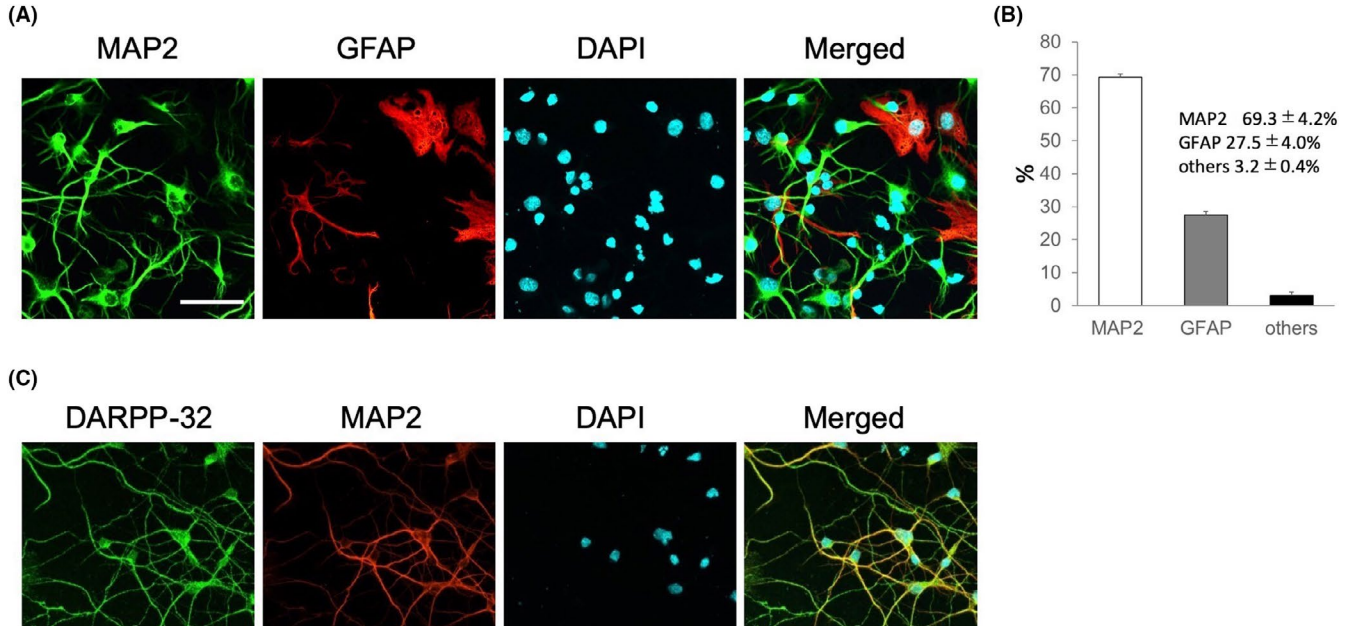

FIGURE 1 Immunocytochemical analysis for characterization of primary cultures of dissociated striatal neurons. A, Rat striatum neurons were stained with DAPI and antibodies against MAP2 (neuronal cell marker, green) and GFAP (astrocytes marker, red). Scale bar indicates 50 μm. B, Quantification of cell types in the striatal culture. C, Cultures were stained with DAPI and antibodies against DARPP-32 (medium spiny neuron marker, green) and MAP2 (red)

pathways of disorders related to dysregulation of synaptic function, such as "nicotine addiction" (adjusted *P*-value: 2.6×10^{-3}, number of genes: 16) and "morphine addiction" (adjusted *P*-value: 2.6×10^{-3}, number of genes: 50). This is consistent with previous studies reporting the involvement of striatal BDNF in substance addiction.[16,17,31] KEGG-GAGE analysis also detected GSs related to various synapse types: "GABAergic synapse" (adjusted *P*-value: 1.1×10^{-3}, number of genes: 54), "Dopaminergic synapse" (adjusted *P*-value: 2.6×10^{-3}, number of genes: 90), "Glutamatergic synapse" (adjusted *P*-value: 4.3×10^{-3}, number of genes: 63), "Cholinergic synapse" (adjusted *P*-value: 1.9×10^{-2}, number of genes: 61). The genes were mapped into KEGG pathway diagrams of GABAergic, dopaminergic, glutamatergic, and cholinergic synapses (Supplemental Figures 1-4). The majority of the genes indicated on the pathway diagrams were up-regulated in the synapse types.

Next, we sought to identify the genes that exhibited distinctive changes in expression levels upon BDNF treatment. The DESeq2 method detected 59 upregulated significant DEGs in BDNF-treated groups ($\log_2$ fold-change [$\log_2$ FC] ≥ 1, $-\log_{10}$ false discovery rate [$-\log_{10}$ FDR] >1) and 13 downregulated significant DEGs in BDNF-treated groups ($\log_2$ FC ≤ −1, $-\log_{10}$ FDR > 1) (Figure 4A,B). *K*-means clustering was able to group 72 significant DEGs into two clusters (Figure 4C). The highest enrichment was detected in the GS of "GABAergic synapse" (adjusted *P*-value: 4.4×10^{-5}, number of genes: 5) from the KEGG database, which is consistent with the results obtained from 10599 genes. The second highest enrichment was found in the GS of "regulation of neurotransmitter" (adjusted *P*-value: 3.0×10^{-4}, number of genes: 8) from Gene Ontology (GO). Top-10 ranked GSs are shown in Table 2. It is noted that seven out of 10 GSs are synapse-related.

Finally, we assessed DEGs induced by BDNF. The highest significance was detected in *apolipoprotein L domain containing 1* (*Apold1*; $\log_2$ FC = 3.57, $P = 1.64 \times 10^{-5}$), and the second and third significances were found in *secretogranin II* (*Scg2*; $\log_2$ FC = 2.51, $P = 4.38 \times 10^{-5}$) and *glutamate decarboxylase 2* (*Gad2*; $\log_2$ FC = 1.62, $P = 2.08 \times 10^{-4}$), respectively. The list of top-ranked significant DEGs is presented in Table 3. Differential expression analysis was also conducted using edgeR.[30] The list of top-ranked significant DEGs detected by edgeR is presented in Table S1. The highest significance was found in *calbindin* (*Calb1*; $\log_2$ FC = 1.42, $P = 1.10 \times 10^{-79}$, FDR = 3.62×10^{-75}) and consistent with the results obtained using DESeq2, *Apold1* and *Scg2* showed the second ($\log_2$ FC = 3.67, $P = 3.42 \times 10^{-79}$, FDR = 5.63×10^{-75}) and third ($\log_2$ FC = 2.54, $P = 3.42 \times 10^{-77}$, FDR = 3.75×10^{-73}) significances, respectively.

Thus, in GAGE analyses, top-ranked GSs are up-regulated ones, suggesting that BDNF mainly promotes, but not suppresses striatal cellular functions via expressing its target genes in the developing stratum. We identified BDNF-induced GS enrichments that predominantly include GSs related to regulation and dysregulation of synaptic functions and various types of synapses (Table 1, Figures S1-S4) in primary cultures of rat striatal neurons at DIV8. Presumably consistent with our observations, in cultured mouse striatal neurons, synaptic structures are formed at the period of DIV9-10,[32] and it is expected that the expression of genes related to synaptogenesis is initiated. In the present study, BDNF displayed significant transcriptomic effects on these genes. Previous studies

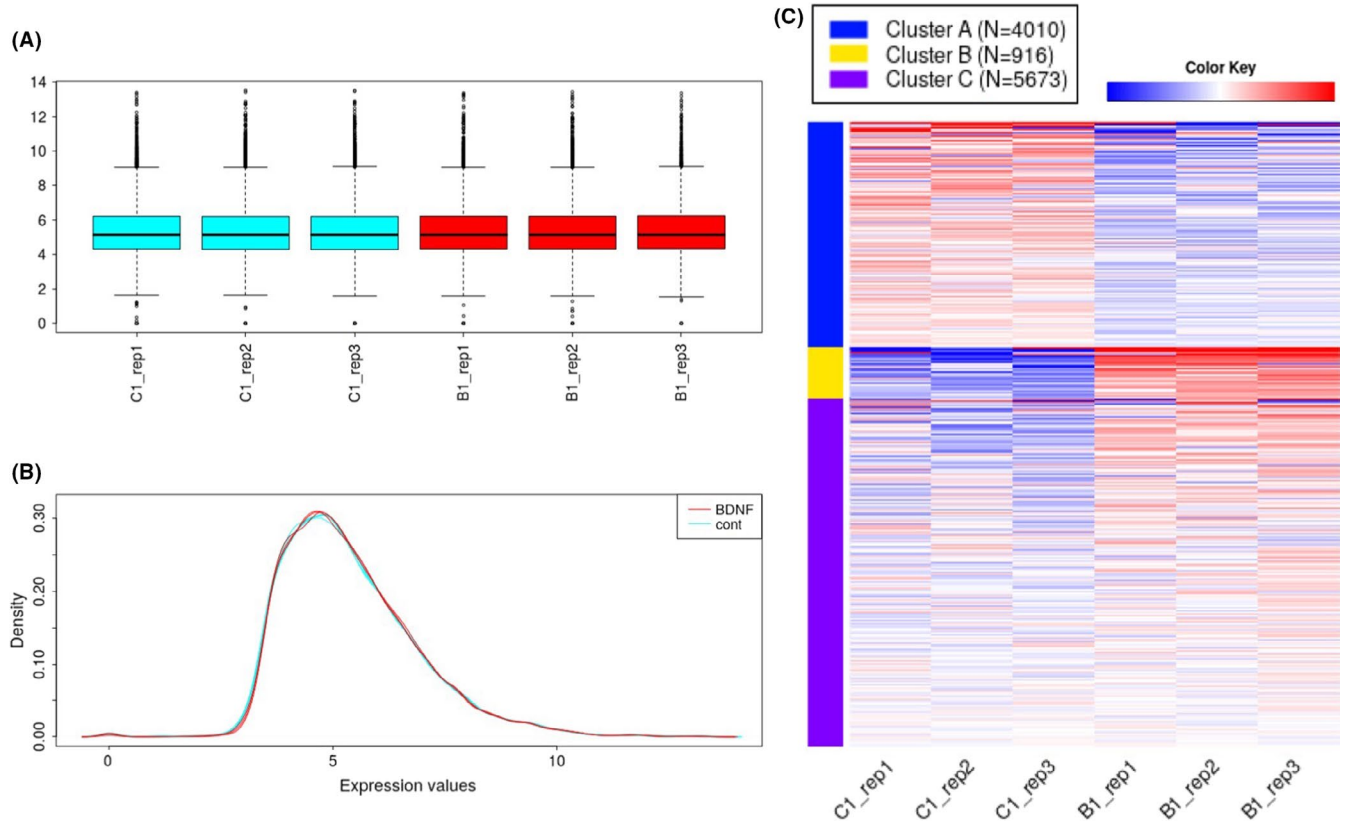

FIGURE 2 Diagnostic plots for read-counts data and bioinformatics analysis of mRNA expression patterns in brain-derived neurotrophic factor (BDNF)-stimulated and nonstimulated groups by RNA-Seq. A, Boxplot of transformed data drawn using the ggplot2 data visualization package. B, Density plot for the distribution of transformed data. C, Heatmap of *K*-means clustering ($K = 3$) of gene expression profiles for 10599 genes that display Transcripts Per Million (TPM) values above 10 in more than half of the samples. Key color scale: maximum values are shown in red, minimum in blue

have consistently demonstrated that BDNF regulates the synaptogenesis of glutamatergic excitatory synapses.[33,34] In the striatum, BDNF-induced upregulation of synaptic function-related genes was detected in various synapse subtypes, including GABAergic and cholinergic inhibitory synapses as well as dopaminergic and glutamatergic excitatory synapses. Given that the cell bodies of glutamate- and dopamine-releasing neurons are located in the CTX and midbrain SN, respectively, but not in the striatum, these neurons are likely not to be included in striatal cultures. Therefore, it is expected that the cells showing enrichment in synapse type-related GSs are striatal cells, such as MSNs, CINs, GINs, and astrocytes. The present study indicates that BDNF promotes synaptogenesis and synaptic maturation of striatal neurons with a broad specificity to synapse subtypes and that BDNF may induce the generation and maturation of postsynaptic terminals of dopaminergic and glutamatergic synapses, presumably in the absence of presynaptic terminals of neurons projected from other areas.

We identified 72 significant DEGs induced by BDNF, among which there are genes that were previously detected. For example, *Scg2*, a member of the chromogranin/secretogranin family of neuroendocrine secretory proteins showed the second highest significance among the BDNF-induced DEGs in the striatum. It has been reported that *Scg2* is up-regulated in rodent HIPP cultures upon BDNF stimulation.[35] *Gad2*, which encodes the GAD65 protein, exhibited the third highest significance among the DEGs and is known to be up-regulated by BDNF in mouse cortical interneurons.[36] Among the top-ranked significant DEGs, the majority of DEGs, including *Scg2* and *Gad1*, were either significantly ($P < .01$) upregulated or downregulated in primary human neural progenitor cells during differentiation induced by substances, including BDNF and neurotrophin-3 (NT-3) (Table 3).[37,38] Some of the top-ranked significant DEGs, such as *Scg2* and *protein tyrosine phosphatase nonreceptor type 5* (*Ptpn5*), also showed significant changes in expression levels in cerebellar granule neuron progenitors.[39] Together, these observations suggest that significant DEGs represent an underlying mechanism of striatal maturation. Among the BDNF-induced significant DEGs, there were genes whose sensitivity to BDNF was newly identified. *Apold1* showed the highest significance, and to the best of our knowledge, this is the first study to detect significant upregulation of *Apold1* induced by BDNF. It represents a potential unique target gene of BDNF in the striatum. *Apold1*, also termed as *vascular early response gene protein* (*VERGE*), is known to play a role in the regulation of endothelial cell signaling and is suggested to be involved in the regulation of blood-brain permeability.[40] The GTEx RNA expression database indicates that *Apold1* is widely expressed in various brain regions, including the NAc.[41] A single-cell RNA-Seq study of mouse

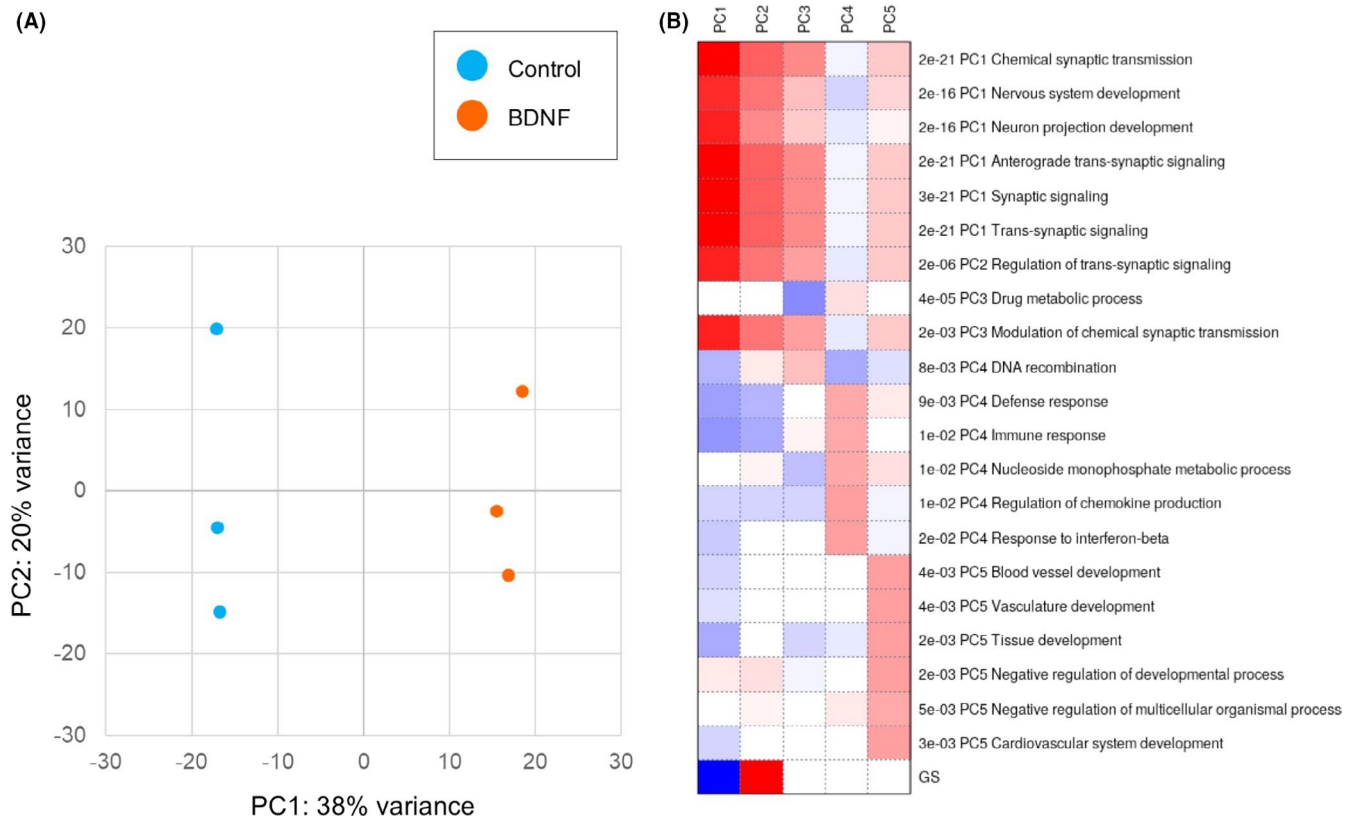

FIGURE 3 A, Principal component analysis (PCA) for differentially expressed genes (DEGs) induced by BDNF. B, The five most important components are resulting from PCA on the DEGs. The color of each cell represents the sign (red positive, blue negative) and magnitude of the contribution of each gene set (GS; row) to each principal component (PC; column). The darkest colored cell at the bottom raw represents the minimum (blue) and maximum (red), respectively

TABLE 1 Top 12 ranked gene sets/pathways detected by generally applicable gene-set enrichment (GAGE) analysis on 10599 genes

Gene set/pathway	Direction	Statistic	# of genes	Adjusted *P* value
Synaptic vesicle cycle[a]	Up-regulated	4.6810	47	1.1E-03
GABAergic synapse[a]	Up-regulated	4.5212	54	1.1E-03
Nicotine addiction[a]	Up-regulated	4.6078	16	2.6E-03
Morphine addiction[a]	Up-regulated	4.0233	50	2.6E-03
Dopaminergic synapse[a]	Up-regulated	3.9697	90	2.6E-03
Retrograde endocannabinoid signaling[a]	Up-regulated	3.9293	100	2.6E-03
Glutamatergic synapse[a]	Up-regulated	3.7972	63	4.3E-03
Circadian entrainment	Up-regulated	3.5641	53	9.1E-03
Insulin secretion	Up-regulated	3.2772	38	1.9E-02
Cholinergic synapse[a]	Up-regulated	3.2367	61	1.9E-02
Oxytocin signaling pathway	Up-regulated	3.2053	85	1.9E-02
Long-term potentiation[a]	Up-regulated	3.0122	45	3.6E-02

Note: KEGG is used as the reference database.

[a]Synaptic function-related gene set/pathways

CTX and HIPP revealed that *Apold1* is dominantly expressed in vascular endothelial cells and smooth muscle cells but is also expressed in both neuronal and glial cells.[42] Given that the population of endothelial cells is expected to be less than 3.2 ± 0.4% in our striatal culture, *Apold1* detected in the present study is likely to be expressed in neuronal cells and/or astrocytes. Upregulation of *Apold1* is seen in mouse HIPP neurons upon stimulation with kainic acid, an agonist of kainate glutamate receptors.[43] A transcriptomic study of human postmortem brains indicated that *Apold1* is up-regulated in the striatum of patients with schizophrenia.[44] Upregulation of *Apold1* is

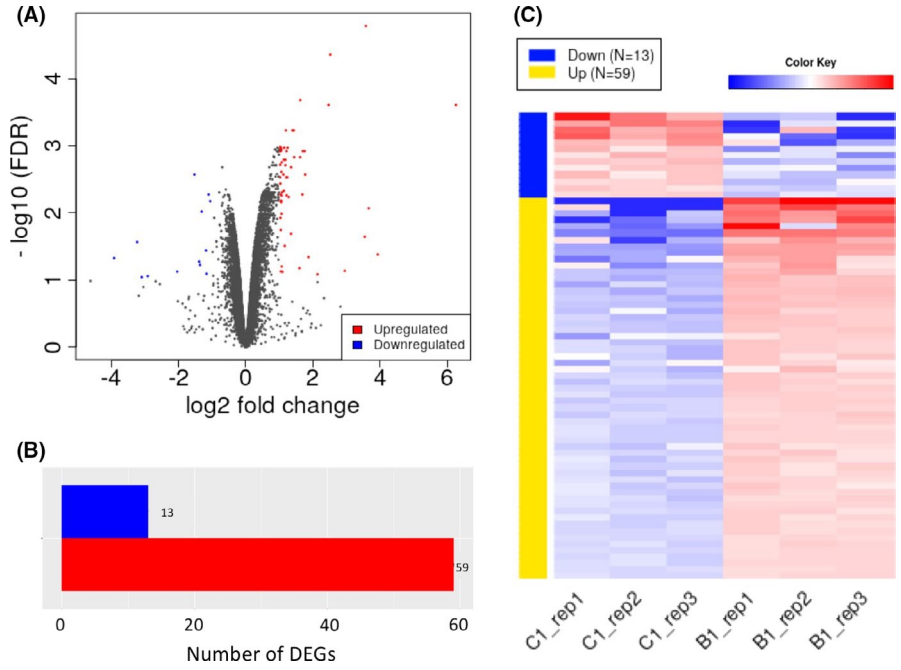

FIGURE 4 Identification of significant DEGs induced by BDNF in striatum cultures. A, Volcano plot of RNA-Seq results of BDNF-stimulated versus control striatal cultures. Differential gene expression with $\log_2$-normalized fold changes (FC) in BDNF-treated samples (n = 3) or control samples (n = 3) plotted versus -log10 FDR. Red dots: upregulated significant DEGs in BDNF-treated groups ($\log_2$ FC ≥1, $-\log_{10}$ FDR >1), blue dots: downregulated significant DEGs in BDNF-treated groups ($\log_2$ FC ≤ −1, $-\log_{10}$ FDR >1). B, The number of significant DEGs. Red: upregulation, Blue: downregulation C, Heatmap of DEG clustering. The significant DEGs were grouped into two clusters by *K*-means clustering. The number of significant DEGs in each cluster is indicated. Key color scale: maximum values are shown in red, minimum in blue. Down: downregulation, Up: upregulation

TABLE 2 Top 10 ranked gene sets/pathways detected by Generally Applicable Gene-set Enrichment (GAGE) analysis for 72 significant DEGs induced by BDNF in striatal cultures

Gene set/pathway	Reference database	Direction	# of Genes	Adjusted *P*-value
GABAergic synapse[a]	KEGG	Up-regulated	5	4.4E-05
Regulation of neurotransmitter levels[a]	GO	Up-regulated	8	4.3E-04
Cerebellar granular layer maturation	GO	Up-regulated	2	9.5E-04
Receptor localization to synapse[a]	GO	Up-regulated	4	9.5E-04
Taurine and hypotaurine metabolism	KEGG	Up-regulated	2	1.1E-03
Clustering of voltage-gated potassium channels[a]	GO	Up-regulated	2	1.8E-03
Protein transport within plasma membrane	GO	Up-regulated	3	2.0E-03
Neurotransmitter transport[a]	GO	Up-regulated	6	2.0E-03
Exocytic insertion of neurotransmitter receptor to plasma membrane[a]	GO	Up-regulated	2	2.5E-03
Anterograde trans-synaptic signaling[a]	GO	Up-regulated	8	2.5E-03

[a]Synaptic function-related gene set/pathways.

detected in the NAc in patients with alcohol dependence[45] and the caudate nucleus of patients with HD.[46]

Our study suggests that BDNF predominantly promotes synaptogenesis in various neuronal subtypes in the developing striatum. The highly enriched GSs and the significant DEGs induced by BDNF may represent the underlying mechanisms of striatal circuit maturation, and potentially, therapeutic targets for substance addiction. There are, however, limitations in the present study. In particular, striatal tissues of littermates from a single pregnant rat were used for RNA-seq in triplicate, and there could be the possibility that non-specific gene expressions are detected together with specific ones. We assessed at a single time point (48 hours) so that we might fail to identify chronic gene expression changes that are terminated before 48 hours. In addition, cell-type specificities for the highly

TABLE 3 The top-ranked significant differentially expressed genes ($P < .01$) obtained by Differential Expression analysis for Sequence count data 2 (DESeq2)

Ensembl ID	Gene Symbol	Log_2 FC	Adjusted *P* value	Gene expression induced by BDNF detected in previous studies ($P < .01$)
ENSRNOG00000007830	Apold1	3.57	1.64E-05	
ENSRNOG00000015055	Scg2	2.51	4.38E-05	**Human iPSC-derived primary neural progenitor cells**,[38] ***Primary human neural progenitor cells***,[37] **Rat hippocampus**,[35] *Mouse cerebellar granule neuron progenitors*[39]
ENSRNOG00000018200	Gad2	1.62	2.08E-04	**Human iPSC-derived primary neural progenitor cells**,[38] **Primary human neural progenitor cells**,[37] **Mouse cortical interneuron**[36]
ENSRNOG00000051286	LOC100911516	6.26	2.47E-04	
ENSRNOG00000011921	Dusp4	2.46	2.47E-04	***Primary human neural progenitor cells*** ,[37] **Mouse cerebellar granule neuron progenitors**[39]
ENSRNOG00000016977	Calb2	1.43	5.92E-04	**Human iPSC-derived primary neural progenitor cells**,[38] **Primary human neural progenitor cells**[37]
ENSRNOG00000060407	Cacna1i	1.39	5.92E-04	**Human iPSC-derived primary neural progenitor cells**[38]
ENSRNOG00000006037	Snap25	1.19	5.92E-04	**Human iPSC-derived primary neural progenitor cells**,[38] **Primary human neural progenitor cells**[37]
ENSRNOG00000005345	Vsnl1	1.24	1.07E-03	**Human iPSC-derived primary neural progenitor cells**,[38] *Primary human neural progenitor cells*,[37] **Mouse cerebellar granule neuron progenitors**[39]
ENSRNOG00000012290	Gchfr	1.75	1.20E-03	**Human iPSC-derived primary neural progenitor cells**,[38] *Primary human neural progenitor cells*[37]
ENSRNOG00000013981	Ptpn5	1.70	1.20E-03	**Mouse cerebellar granule neuron progenitors**[39]
ENSRNOG00000013290	Nrip3	1.21	1.20E-03	**Mouse cerebellar granule neuron progenitors**,[39] **Primary human neural progenitor cells**[37]
ENSRNOG00000032328	Diras2	1.62	1.48E-03	**Human iPSC-derived primary neural progenitor cells**,[38] **Primary human neural progenitor cells**[37]
ENSRNOG00000046947	Ak5	1.44	1.48E-03	**Human iPSC-derived primary neural progenitor cells**,[38] **Primary human neural progenitor cells**[37]
ENSRNOG00000007907	Tmem178a	1.18	1.62E-03	**Primary human neural progenitor cells**[37]
ENSRNOG00000014232	P2ry1	1.18	1.96E-03	**Human iPSC-derived primary neural progenitor cells**,[38] **Primary human neural progenitor cells**[37]
ENSRNOG00000025889	Gnas	1.35	2.09E-03	*Primary human neural progenitor cells*[37]
ENSRNOG00000018018	LOC10091195	1.77	2.69E-03	
ENSRNOG00000018018	Kcnip2	1.77	2.69E-03	
ENSRNOG00000058793	Vma21	−1.52	2.69E-03	**Primary human neural progenitor cells**[37]
ENSRNOG00000058793	LOC103689964	−1.52	2.69E-03	
ENSRNOG00000058793	LOC100912478	−1.52	2.69E-03	
ENSRNOG00000003288	Cacng5	1.24	2.95E-03	*Primary human neural progenitor cells*[37]
ENSRNOG00000008203	Synpr	1.20	2.95E-03	**Human iPSC-derived primary neural progenitor cells**,[38] **Primary human neural progenitor cells**[37]
ENSRNOG00000005148	Scrt2	1.68	5.32E-03	**Primary human neural progenitor cells**[37]

(Continues)

TABLE 3 (Continued)

Ensembl ID	Gene Symbol	Log_2 FC	Adjusted *P* value	Gene expression induced by BDNF detected in previous studies ($P < .01$)
ENSRNOG00000017444	Nrsn1	1.21	5.49E-03	**Human iPSC-derived primary neural progenitor cells,[38] Primary human neural progenitor cells[37]**
ENSRNOG00000016628	Scgn	1.28	5.81E-03	**Human iPSC-derived primary neural progenitor cells,[38] Primary human neural progenitor cells[37]**
ENSRNOG00000050450	Kcnip2	3.66	8.56E-03	**Mouse cerebellar granule neuron progenitors[39]**
ENSRNOG00000050450	LOC100911951	3.66	8.56E-03	
ENSRNOG00000057755	Ndst2	−1.31	9.57E-03	
ENSRNOG00000057755	NEWGENE_1304700	−1.31	9.57E-03	

Abbreviation: Log_2 FC, log_2 fold-change.

Grey-shading: upregulation, no shading: downregulation, bold: upregulation, ***bold and italic***: up-regulation and downregulation (in multi-data set), *italic*: downregulation.

enriched GSs and the significant DEGs remain unclear. Further biochemical and physiological studies are required to reveal the cross talk between signaling cascades downstream of BDNF and the molecular functions of its target genes both during and after striatal development.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

HK, NA, and SS conceived and designed the experiments. AK, JO, KO, and SS performed the experiments. HK TM, MS, HM, NA, and SS analyzed the data. HK, HM, NA, and SS wrote the paper.

ANIMAL STUDIES

All animal experiments were conducted in strict accordance with the protocols set out by the Institutional Animal Care and Use Committee of Kagawa University.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in Figures S1-S4 and Table S1 of this article.

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