

# From Gut to Brain: Alteration in Inflammation Markers in the Brain of Dextran Sodium Sulfate-induced Colitis Model Mice

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**Objective:** Neuropsychiatric manifestations like depression and cognitive dysfunction commonly occur in inflammatory bowel disease (IBD). In the context of the brain-gut axis model, colitis can lead to alteration of brain function in a bottom-up manner. Here, the changes in the response of the hypothalamic-pituitary-adrenal axis and inflammation-related markers in the brain in colitis were studied.

**Methods:** Dextran sodium sulfate (DSS) was used to generate a mouse model of colitis. Mice were treated with DSS for 3 or 7 days and sacrificed. We analyzed the gene expression of brain-derived neurotrophic factor (BDNF), cyclooxygenase 2 (COX-2), and glial fibrillary acidic protein (GFAP), and the expression of GFAP, in the hippocampus, hypothalamus, and amygdala. Additionally, the levels of C-reactive protein (CRP) and serum cortisol/corticosterone were measured.

**Results:** Alteration of inflammatory-related markers varied depending on the brain region and exposure time. In the hippocampus, COX-2 mRNA, GFAP mRNA, and GFAP expression were upregulated during exposure to DSS. However, in the hypothalamus, COX-2 mRNA was upregulated only 3 days after treatment. In the amygdala, BDNF and COX-2 mRNAs were downregulated. CRP and corticosterone expression increased with DSS treatment at day 7.

**Conclusion:** IBD could lead to neuroinflammation in a bottom-up manner, and this effect varied according to brain region. Stress-related hormones and serum inflammatory markers, such as CRP, were upregulated from the third day of DSS treatment. Therefore, early and active intervention is required to prevent psychological and behavioral changes caused by IBD, and region-specific studies can help understand the precise mechanisms by which IBD affects the brain.

**KEY WORDS:** Inflammatory bowel disease; Dextran sodium sulfate; Neuroinflammation; Cyclooxygenase 2; Glial fibrillary acidic protein; Brain-derived neurotrophic factor.

## INTRODUCTION

Inflammatory bowel disease (IBD), consisting of Crohn's disease and ulcerative colitis, is a disease causing gastrointestinal tract symptoms such as abdominal pain, vomiting, diarrhea, weight loss, anemia, and anal bleeding.<sup>1)</sup> Millions of people in the US and Europe experience IBD, and the prevalence of IBD is increasing in southern Europe, Asia, and developing countries, which previously

had a low incidence.<sup>2)</sup> As approximately 30% of IBD patients develop symptoms before the age of 21 years, the illness permanently affects the quality of life of the patient.<sup>3-5)</sup> Even though IBD is a gastrointestinal disease, several studies have shown the presence of neuropsychiatric manifestations, such as depression and cognitive dysfunction.<sup>6-8)</sup> The prevalence of depressive symptoms is higher in the active phase of IBD.<sup>9)</sup>

The exact mechanism by which depression or cognitive dysfunction occur in patients with IBD, is unknown. However, recent animal studies on the brain-gut axis have provided a direction for understanding the abovementioned neuropsychiatric conditions. These studies revealed that a harmful environment in the gut leads to behavioral deviation, representing depressive and anxiety symptoms, in a bottom-up manner.<sup>10-14)</sup> The authors sug-

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gest that activation of the hypothalamic-pituitary-adrenal (HPA) axis and immune system, caused by the deviation of the intestinal environment, leads to alteration of the central nervous system. Indeed, previous studies show glucocorticoids, which are increased by activation of the HPA axis, can damage the hippocampus and inhibit neurogenesis.<sup>15-18)</sup> Glucocorticoids are also involved in the immune system and can trigger the inflammation process.<sup>19)</sup>

Although previous studies in animal models of colitis have addressed stress-related changes in hormones, such as corticosterone, and various gene expression patterns in the brain, there are still some unexplored avenues.<sup>20,21)</sup> For instance, it is necessary to observe changes in HPA axis-related hormones other than corticosterone, as well as histopathological changes in the brain. It is also important to observe effects in the early stage, i.e. 3 days after dextran sodium sulfate (DSS) treatment, because acute stress also affects the brain and body.<sup>22,23)</sup>

Recent studies on brain-gut interactions have revealed changes brain function initiated in a bottom-up manner by gut inflammation. These changes in the central nervous system are expected to be due to HPA axis activation, changes in the immune system, and the autonomic nervous system, caused by alterations in the intestinal environment, which lead to neuropsychiatric dysfunction and behavioral deviation.<sup>24,25)</sup> However, in an animal model of colitis, it is necessary to analyze inflammation-related markers in the brain. In this study, we investigated whether chemically-induced colitis could cause changes in the HPA axis response and in the brain at both 3 days, the early stage, and 7 days DSS treatment.

DSS is a chemical that induces IBD-like colitis in animals. To cross the gut-blood barrier and the blood-brain barrier by transmembrane diffusion, a molecule needs to have a high degree of lipid solubility and low molecular weight. DSS is a large, negatively charged molecule (molecular weight 36 to 50 kDa), and thus does not circulate on a systemic level or reach the central nervous system due to its molecular characteristics. Thus, we can exclude any potential systemic effects and focus on colitis itself. We designed this study to identify the expression of inflammatory markers, glial fibrillary acidic protein (GFAP), and cyclooxygenase 2 (COX-2), as well as brain-derived neurotrophic factor (BDNF) in the hippocampus, hypothalamus, and amygdala. We also observed

changes in levels of cortisol, corticosterone, and C-reactive protein (CRP) in the serum, confirming systemic inflammation and changes in the HPA axis.

## METHODS

### Samples

Frozen serum samples and brain paraffin blocks from IBD model mice were received from Laboratory Animal Resources Bank (LAREB, Daegu, Korea). According to information from LAREB, experiments were conducted with C57BL/6J mice (male, 7-8 week). They were purchased from Jung-Ang Laboratory Animal Company (Seoul, Korea). All animals were housed with the maintained in temperature, humidity and light controlled animal rooms (24±2°C, 50±20% relative humidity, 12/12 hour light/dark cycle with lights on at 07:00) and had *ad-libitum* access to food and water. To induce colitis for IBD mice model, the mice were treated with 3% (wt/vol) DSS (MP Biomedicals, Santa Ana, CA, USA) in drinking water for 3 days and 7 days. The animals were sacrificed at 3 days and 7 days, the blood collection was conducted from abdominal vein with 32-gauge syringe at morning time (10:00-11:00). To minimize pain and stress, the mice were anesthetized with 3% isoflurane (Hana Pharm., Seoul, Korea). All methods were reviewed and approved by the Animal Care and Use Committee at Pusan National University (IACUC No. PNUH 2016-096). The serum was stored at -90°C for 1 year and brain paraffin blocks were stored at room temperature (21°C-25°C) for 1 year. Samples were divided into 3 groups (n=6 for the control group, n=6 for the 3 day DSS treatment group, and n=6 for the 7 day DSS treatment group).

### Serum Analysis

CRP was measured using a biochemistry analyzer (TBA-120FR; Toshiba Medical System Co., Tochigi, Japan) according to the manufacturer's instructions.

### Histopathology

Tissue sections from the brain paraffin blocks were cut to a thickness of 4 µm, and hematoxylin and eosin (H&E) staining was performed using H&E stainer (DAKO CoverStainer; Agilent, Santa Clara, CA, USA). Additionally, tissue sections were stained immunohistochemically using the labelled polymer DAKO EnVision™+ System-HRP

(Agilent) according to the manufacturer's instructions. Brain sections were stained with anti-GFAP (ab7260; Abcam, Cambridge, MA, USA) primary antibody. After staining, all areas of the brain that were scanned with the slide scanner (Pannoramic SCAN II; 3DHISTECH Kft., Budapest, Hungary) and images were captured in the hypothalamus, the hippocampus and the amygdala by slide viewer (CaseViewer; 3DHISTECH Kft.). GFAP in hypothalamus, hippocampus, and amygdala was quantified (ImageJ software; NIH, Bethesda, MD, USA) and expressed as a percentage of tissue area, as previously described.<sup>26)</sup>

#### Enzyme-linked Immunosorbent Assay (ELISA)

Serum separated from whole blood was used to measure stress levels by ELISA. The concentration of cortisol and corticosterone were determined in a 96-well plate using a cortisol ELISA Kit (FineTest, Wuhan, China) and corticosterone ELISA Kit (Enzo Life Sciences, Ann Arbor, MI, USA), respectively, in accordance with the manufacturer's instructions. Both standard and diluted samples were allowed to react with their antibody and conjugate in a pre-coated plate. Plates were then washed with washing buffer several times. Samples were then incubated with substrate, and a stop solution was added to the wells. The absorbance was read on a microplate reader at a wavelength of 450 nm or 405 nm.

#### RNA Extraction and Real Time Polymerase Chain Reaction (PCR) Analysis

Total RNA was isolated using an RNeasy Mini Kit and RNase-Free DNase Set (QIAGEN, Hilden, Germany) according to the manufacturer's instructions in each tissue (hypothalamus, hippocampus, and amygdala). The first-strand cDNA synthesis was performed from 1 µg of extracted RNA using SuperScript III (Invitrogen, Groningen, Netherlands). To identify expression of the neuroinflam-

mation-related marker genes (*BDNF*, *COX-2*, *GFAP*), real-time PCR from each PCR mixture, containing synthesized cDNA, 2× SYBR Green I Master, 10 pmole of forward-reverse primer, and up to 20 µl of PCR grade water, was performed using the LightCycler 480 system (Roche Diagnostics, Mannheim, Germany). The sequences of the designed primers are shown in Table 1, and *Gapdh* was used as a control gene. The conditions of the real-time PCR were as follows: pre-incubation at 95°C for 5 minutes, followed by 35 cycles of amplification at 95°C for 10 seconds, 55°C for 10 seconds, and 72°C for 15 seconds. Data were analyzed with LightCycler 480 Software, and normalization and relative quantification followed the  $2^{-\Delta\Delta Ct}$  method. The sequences of the primers used are shown in Table 1.

#### Statistical Analysis

All values are presented as the mean±standard error of mean. Statistical analyses were performed using Student's *t* test. The value of statistical significance was set at  $p < 0.05$ .

## RESULTS

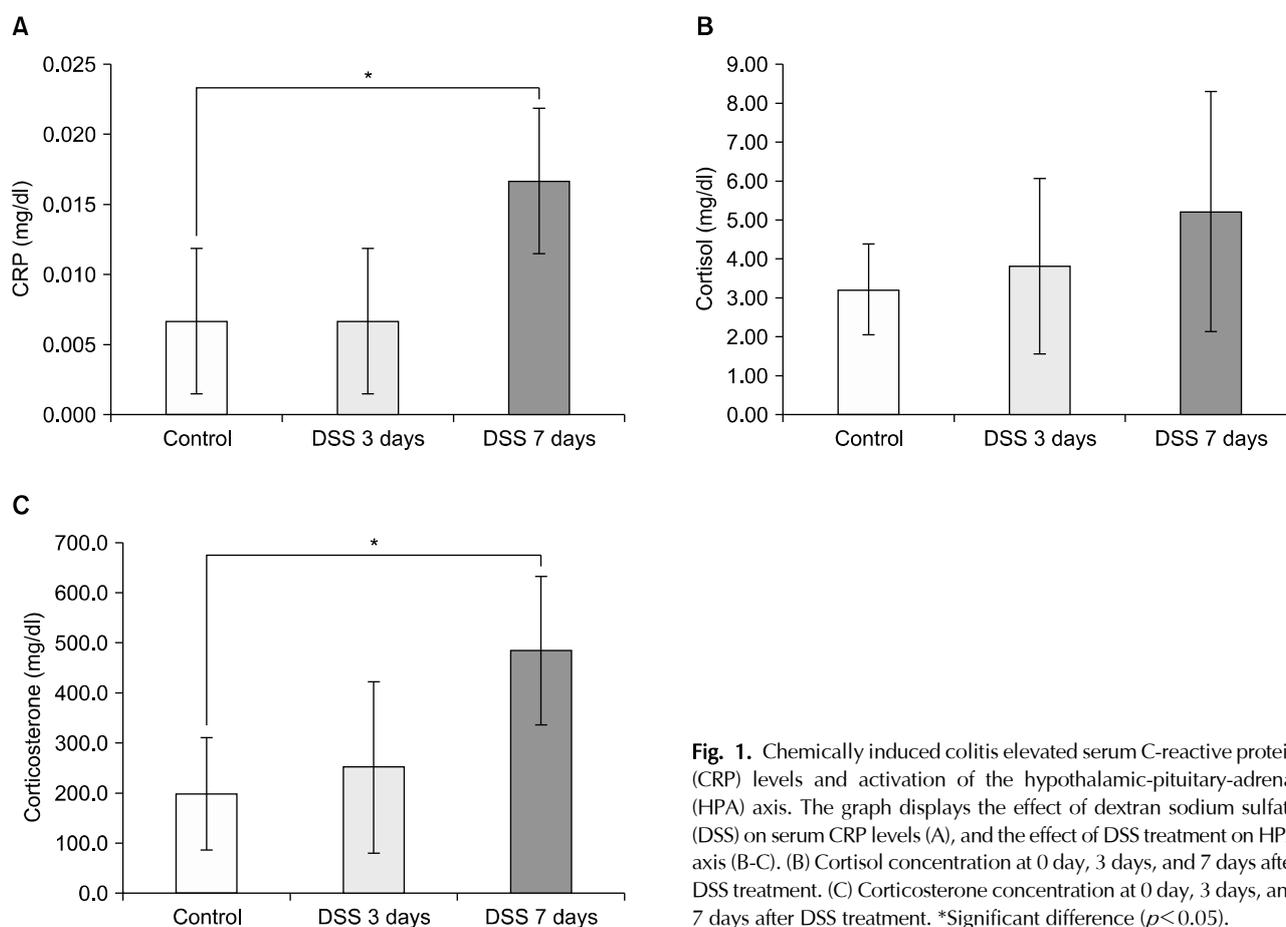
#### Effects of DSS-induced Colitis on CRP Levels in Serum and the HPA Axis

We first measured C-reactive protein (CRP) levels in peripheral blood 3 times to ascertain inflammation induced by DSS in mice. Before treating the mice with DSS, we sampled blood as a control. At 3 days and 7 days DSS treatment, we obtained peripheral blood from the mice to confirm increased CRP level induced by DSS treatment. There was no significant difference in CRP levels between the control group and the 3 days DSS treatment group (Fig. 1A; control=0.007±0.005 mg/dl, DSS 3 days=0.007±0.005 mg/dl,  $p=0.500$ ). However, CRP levels of mice increased significantly after 7 days of DSS treatment

**Table 1.** Primer sequences of the neuroinflammation-related marker genes for real-time PCR

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>BDNF</i>	AGCTGAGCGTGTGTGACAGT	GCAGCCTTCCTTGGTGTAAAC
<i>COX-2</i>	CAGACAACATAAACTGCCGCTT	GATACACCTCTCCACCAATGACC
<i>GFAP</i>	TCTATGAGGAGGAAGTTCCGAGA	TGCAAACCTTAGACCGATACCA
<i>GAPDH</i>	GTGGTGAAGCAGGCATCTG	AGCCGTATTTCATTGCATACCA

PCR, polymerase chain reaction; BDNF, brain-derived neurotrophic factor; COX-2, cyclooxygenase 2; GFAP, glial fibrillary acidic protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.



**Fig. 1.** Chemically induced colitis elevated serum C-reactive protein (CRP) levels and activation of the hypothalamic-pituitary-adrenal (HPA) axis. The graph displays the effect of dextran sodium sulfate (DSS) on serum CRP levels (A), and the effect of DSS treatment on HPA axis (B-C). (B) Cortisol concentration at 0 day, 3 days, and 7 days after DSS treatment. (C) Corticosterone concentration at 0 day, 3 days, and 7 days after DSS treatment. \*Significant difference ( $p < 0.05$ ).

(Fig. 1A; DSS 7 days =  $0.017 \pm 0.005$  mg/dl,  $p = 0.003$ ). Peripheral blood levels of cortisol and corticosterone were then measured to assess the effect of DSS on the HPA axis. Even though cortisol levels in disease induced model mice were increased after dosing them with DSS, it was not statistically significant (Fig. 1B; control =  $3.21 \pm 1.16$  ng/ml, DSS 3 days =  $3.81 \pm 2.24$  ng/ml,  $p = 0.570$ ; DSS 7 days =  $5.21 \pm 3.07$  ng/ml,  $p = 0.180$ ). In the case of corticosterone, there was a significant increase after 7 days DSS treatment in mice compared to the control group (Fig. 1C; control =  $198.34 \pm 112.10$  ng/ml, DSS 3 days =  $251.3 \pm 171.83$  ng/ml,  $p = 0.866$ ; DSS 7 days =  $485.20 \pm 148.78$  ng/ml,  $p = 0.013$ ).

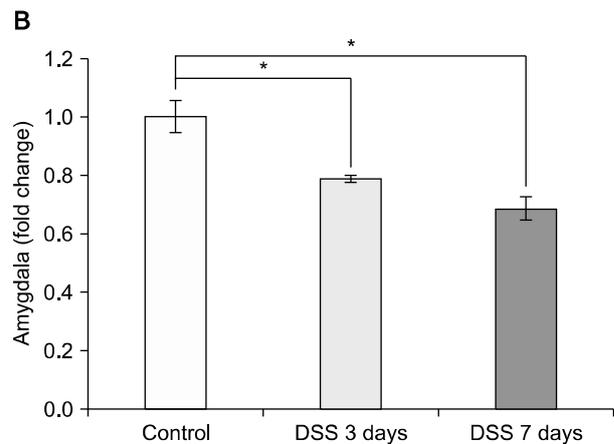
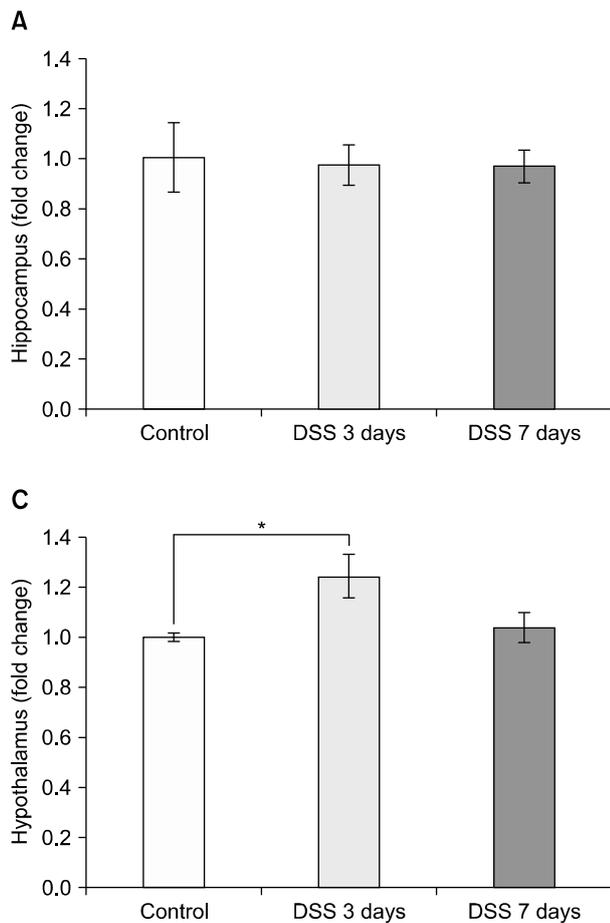
#### Effects of DSS-induced Colitis on BDNF mRNA Expression in Different Limbic Structures

We checked mRNA expression levels of BDNF in the hippocampus, amygdala, and hypothalamus. There was no significant decrease in BDNF mRNA level in the hippocampus at any of the time points tested (Fig. 2A; DSS 3

days =  $0.975 \pm 0.079$ , DSS 7 days =  $0.970 \pm 0.064$  relative to control), but there was a significant decrease of BDNF mRNA in the amygdala at both 3 and 7 days compared to control (Fig. 2B; DSS 3 days =  $0.788 \pm 0.013$  relative to control,  $p = 0.017$ ; DSS 7 days =  $0.685 \pm 0.041$  relative to control,  $p = 0.002$ ). In the hypothalamus, BDNF mRNA levels were increased significantly at 3 days DSS treatment (Fig. 2C; DSS 3 days =  $1.245 \pm 0.088$  relative to control,  $p = 0.036$ ; DSS 7 days =  $1.039 \pm 0.059$  relative to control,  $p = 0.378$ ).

#### Effects of DSS-induced Colitis on COX-2 and GFAP mRNA Expression in Different Limbic Structures

We then assessed changes in COX-2 mRNA levels in different parts of the limbic system, such as the hippocampus, the amygdala and the hypothalamus of the colitis model mice by relative quantitative PCR to compare relative changes of mRNA expression levels. COX-2 levels in the hippocampus significantly increased after DSS treatment at both 3 and 7 days compared to control (Fig. 3A;



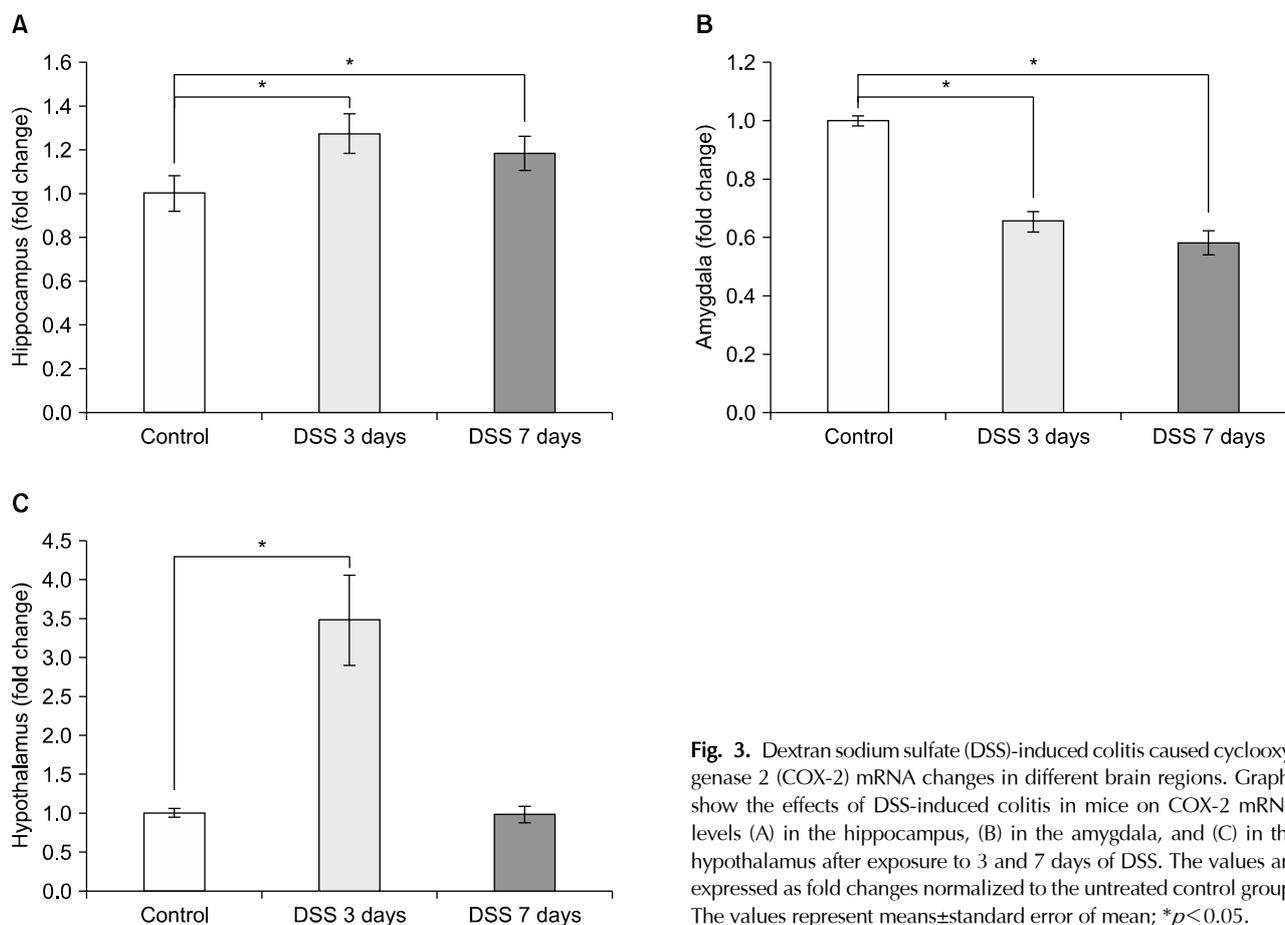
**Fig. 2.** Dextran sodium sulfate (DSS)-induced colitis led to brain-derived neurotrophic factor (BDNF) mRNA changes in different brain regions. Graphs show the effects of DSS-induced colitis in mice on BDNF mRNA levels (A) in the hippocampus, (B) in the amygdala, and (C) in the hypothalamus after exposure to 3 and 7 days of DSS. The values are expressed as fold changes normalized to the untreated control group. The values represent mean $\pm$ standard error of mean; \* $p$ <0.05.

DSS 3 days=1.273 $\pm$ 0.090 relative to control,  $p$ =0.018; DSS 7 days=1.183 $\pm$ 0.078 relative to control,  $p$ =0.049). In contrast to the increase in COX-2 levels seen in the hippocampus, COX-2 level in the amygdala decreased compared to controls after dosing mice with DSS (Fig. 3B; DSS 3 days=0.654 $\pm$ 0.036 relative to control,  $p$ =0.0007; DSS 7 days=0.582 $\pm$ 0.041 relative to control,  $p$ =0.0008). In the hypothalamus, COX-2 mRNA increased significantly after 3 days DSS treatment (Fig. 3C; DSS 3 days=3.481 $\pm$ 0.579 relative to control,  $p$ =0.017; DSS 7 days=0.985 $\pm$ 0.102 relative to control,  $p$ =0.825). GFAP levels in hippocampus were significantly increased by DSS-induced colitis at both 3 and 7 days after DSS treatment (Fig. 4A; DSS 3 days=1.351 $\pm$ 0.046 relative to control,  $p$ =0.024, DSS 7 days=1.198 $\pm$ 0.033 relative to control,  $p$ =0.047). However, the group showed no significant change in GFAP levels in the amygdala (Fig. 4B; DSS 3 days=0.989 $\pm$ 0.032 relative to control; DSS 7 days=1.003 $\pm$ 0.028 relative to control) and hypothalamus (Fig. 4C; DSS 3 days=1.085 $\pm$ 0.063 relative to control, DSS 7 days=1.007 $\pm$ 0.030 relative to con-

trol).

#### Pathological Analysis of GFAP Changes in the Hippocampus, Amygdala, and Hypothalamus in a DSS-induced Colitis Animal Model

Finally, we confirmed pathological changes with H&E staining and the expression of GFAP in each part of the limbic system using immunohistochemistry (IHC). In histopathological analysis, there were no significant differences in DSS-treated mice at 3 or 7 days by H&E staining compared with control. To confirm the expression of GFAP, IHC was conducted in different brain regions: the hippocampus, the amygdala, and the hypothalamus. Representative photomicrographs are shown (Fig. 5). GFAP expression in the hippocampus was significantly increased (Fig. 6A; control=8.595 $\pm$ 0.722%, DSS 3 days=13.856 $\pm$ 2.639%,  $p$ =0.032; DSS 7 days=12.531 $\pm$ 2.055%,  $p$ =0.033). GFAP expression levels in amygdala and hypothalamus were not significant changed during DSS exposure (Fig. 6B; control=1.822 $\pm$ 0.374%, DSS 3 days=



**Fig. 3.** Dextran sodium sulfate (DSS)-induced colitis caused cyclooxygenase 2 (COX-2) mRNA changes in different brain regions. Graphs show the effects of DSS-induced colitis in mice on COX-2 mRNA levels (A) in the hippocampus, (B) in the amygdala, and (C) in the hypothalamus after exposure to 3 and 7 days of DSS. The values are expressed as fold changes normalized to the untreated control group. The values represent means  $\pm$  standard error of mean; \* $p < 0.05$ .

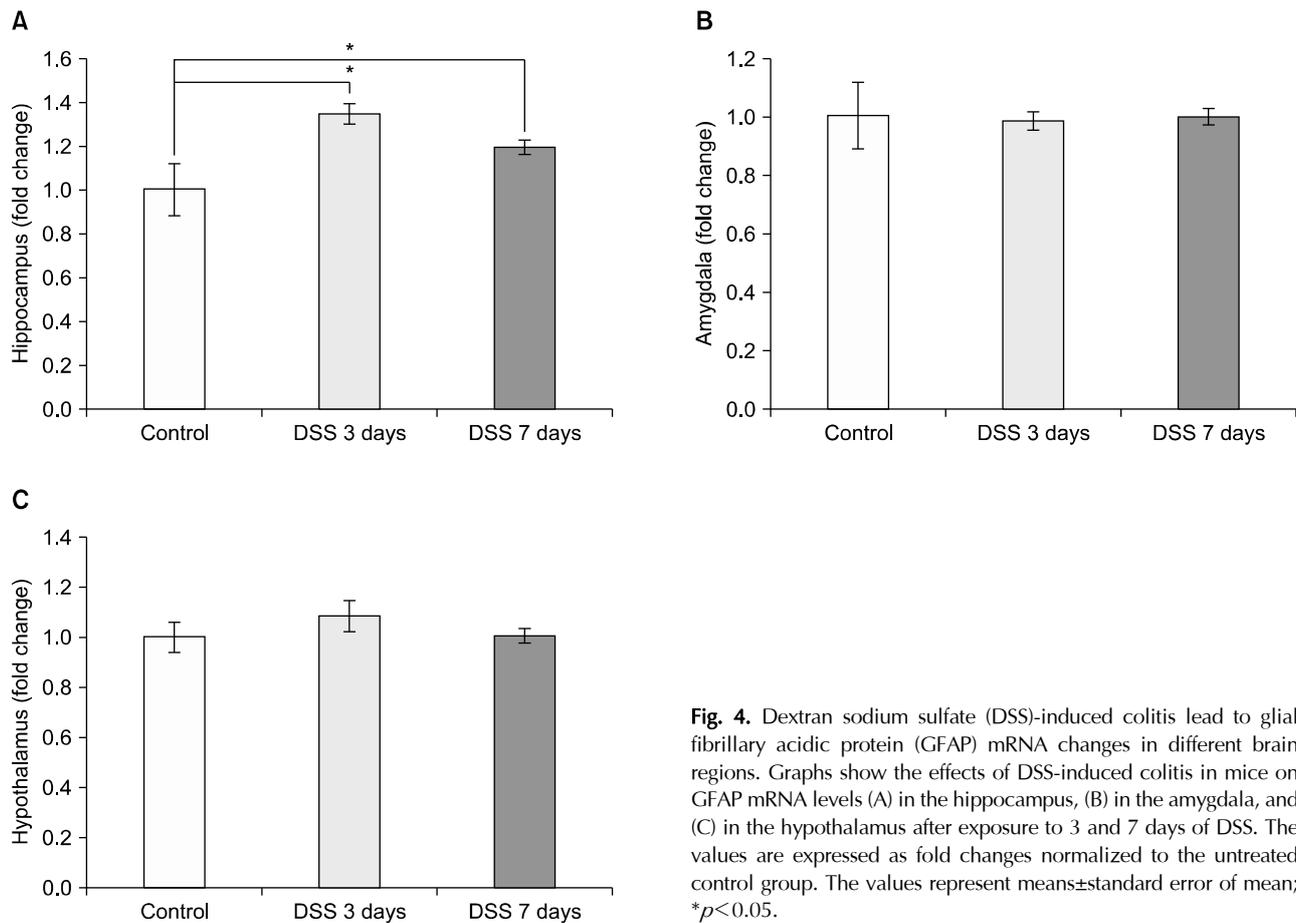
2.118 $\pm$ 0.777%, DSS 7 days=3.306 $\pm$ 1.970%; Fig. 6C: control=2.382 $\pm$ 0.522%, DSS 3 days=1.999 $\pm$ 0.245%, DSS 7 days=2.162 $\pm$ 0.606%).

## DISCUSSION

The aim of this experiment was to examine whether experimental colitis affects the brain, the changes that occur according to brain region, and how colitis-induced inflammation changes in each brain region. Previous studies have focused on chronic colitis and its effects on the hippocampus<sup>27</sup> and gene expression patterns in certain brain regions<sup>20</sup>; however, these studies are not sufficient in their understanding of the early stage effects of colitis. We attempted to determine whether IBD could affect the central nervous system of IBD disease model mice by identifying changes in mRNA and protein expression associated with inflammation and neuroplasticity in the brain.

In our research, the changes of mRNA expression var-

ied depending on the region in the limbic system investigated. At first, COX-2 mRNA, GFAP mRNA, and GFAP expression were upregulated in the hippocampus. COX-2 and GFAP are well-known inflammatory markers in conditions of neuroinflammation and neurodegenerative disease. Cyclooxygenase is a prostaglandin-endoperoxide synthase that forms thromboxane and prostanoids, such as prostaglandins. During inflammatory states in the brain, activated COX-2 can induce production and release of prostaglandin E2 (PGE2).<sup>28</sup> In chemically-induced Alzheimer's disease animal model, COX-2 and PGE2 levels in the hippocampus increased simultaneously. This research demonstrated that etoricoxib, a COX-2 inhibitor, could induce recovery from memory impairments, neurodegeneration, and neuroinflammation in the hippocampus, and reduce levels of COX-2 and PGE2.<sup>29</sup> GFAP has been also used as marker for reactivity of astrocytes and inflammation in the brain for a long time.<sup>30-32</sup> When astrocytes are activated in various neurodegenerative diseases, they increase the expression of GFAP, which is



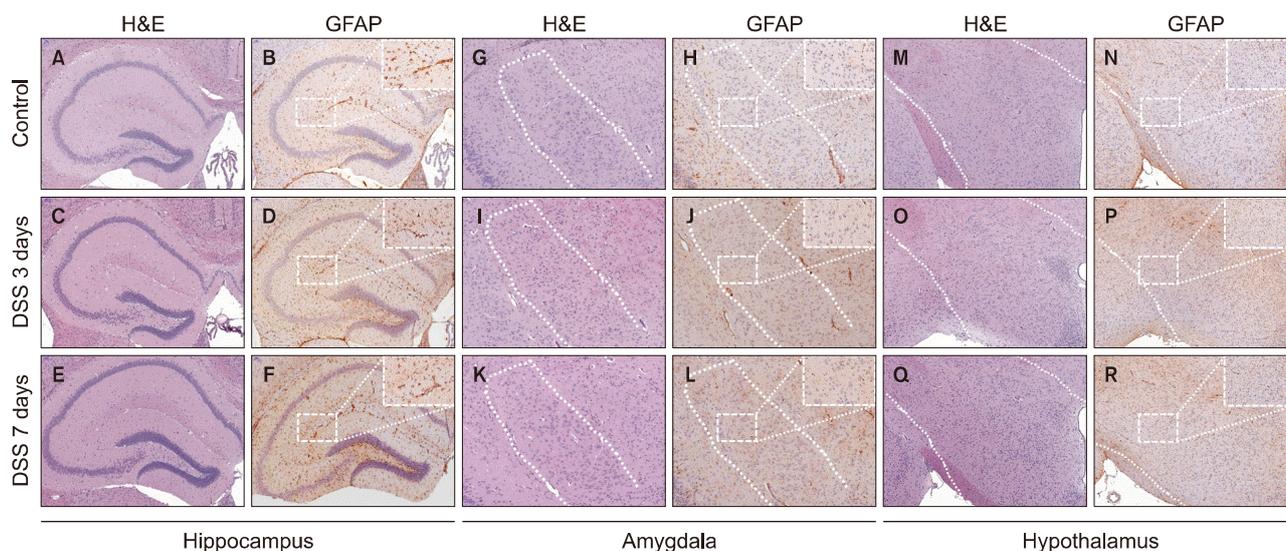
**Fig. 4.** Dextran sodium sulfate (DSS)-induced colitis lead to glial fibrillary acidic protein (GFAP) mRNA changes in different brain regions. Graphs show the effects of DSS-induced colitis in mice on GFAP mRNA levels (A) in the hippocampus, (B) in the amygdala, and (C) in the hypothalamus after exposure to 3 and 7 days of DSS. The values are expressed as fold changes normalized to the untreated control group. The values represent means  $\pm$  standard error of mean; \* $p < 0.05$ .

marker for astrogliosis.<sup>33-35</sup> In our research, a significant increase of COX-2 mRNA, and GFAP mRNA and protein were observed at early stage colitis, 3-day DSS-treatment, and these changes preceded the significant increase of CRP and glucocorticoids. These results suggest that inflammatory changes in the hippocampus appear early.

In the hypothalamus, BDNF and COX-2 were upregulated 3 days after DSS treatment, but the levels of BDNF and COX-2 measured 7 days after DSS treatment were reduced to that of the control group. Both the hippocampus and hypothalamus showed elevated COX-2 mRNA level 3 days after DSS treatment, but upregulated COX-2 mRNA expression levels in the hypothalamus had nearly normalized to that of the control group 7 days after DSS treatment. Additionally, there was no increase in GFAP mRNA or GFAP expression after DSS treatment. These results indicate that the hippocampus was more susceptible than the hypothalamus to DSS-induced colitis and the progression rate of hippocampal inflammation is more rapid than that of the hypothalamus, resulting in ex-

pression of GFAP before glucocorticoids can sufficiently inhibit expression of GFAP and COX-2. Glucocorticoids have a role in alleviating the progression of inflammation, and several studies have shown that glucocorticoids reduce expression of BDNF, COX-2, and GFAP in the brain.<sup>36-41</sup> Thus, elevated activity of the HPA axis affects activation of expression of BDNF and COX-2 in hypothalamus. We also observed that COX-2 and GFAP expression in the 7 day DSS treatment group was decreased in the hippocampus compared to the 3 day DSS treatment group.

The amygdala has important roles in processing sensory input and affective emotion. Even though we evoked systemic inflammation and altered the HPA axis, unlike other limbic structures, significant increases of neuro-inflammatory markers in the amygdala, such as COX-2, and GFAP, were not observed. DSS-induced colitis caused downregulation of BDNF mRNA expression and COX-2 mRNA expression in the amygdala. BDNF is a factor related to neurogenesis and neural plasticity.<sup>42,43</sup>



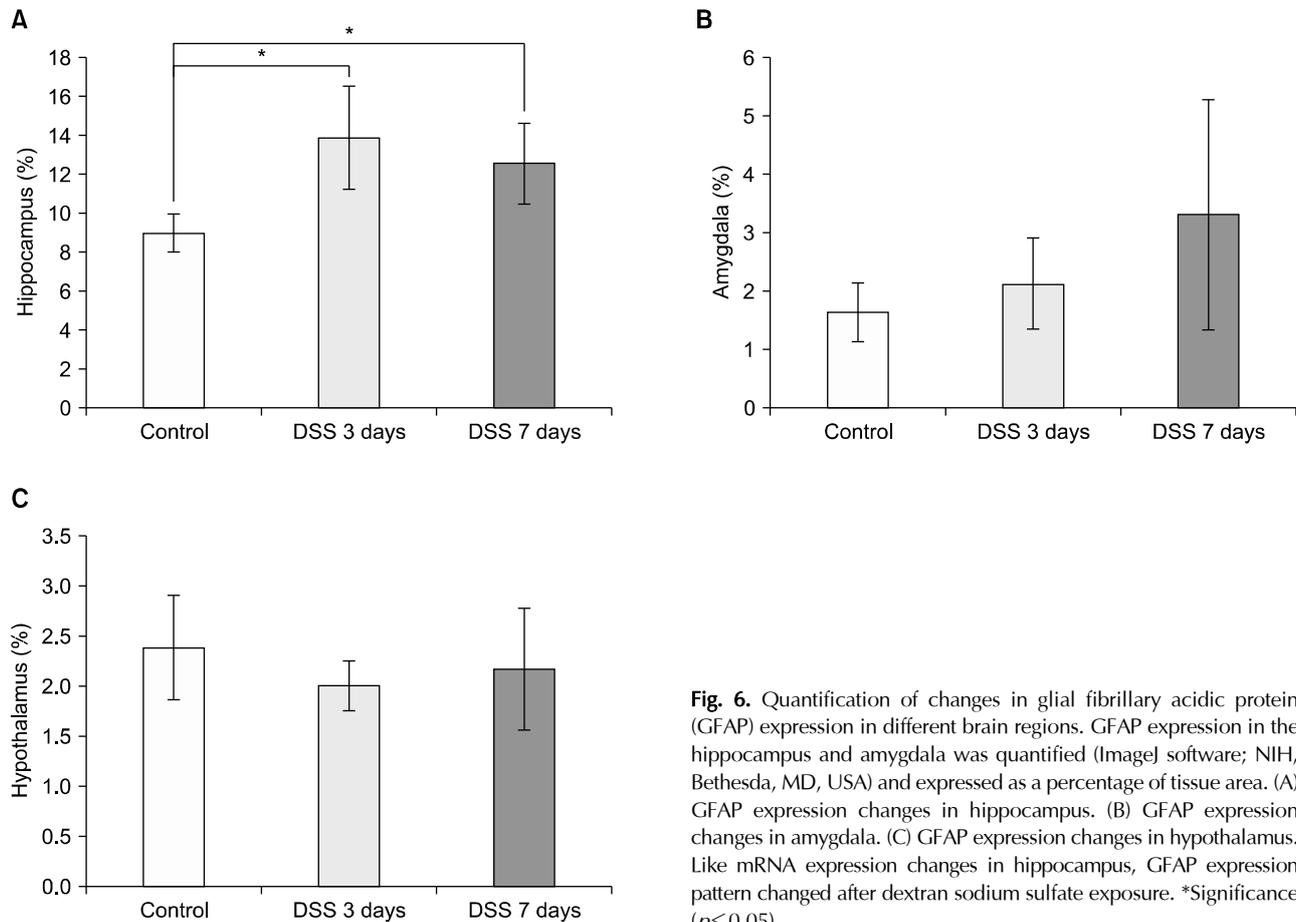
**Fig. 5.** Glial fibrillary acidic protein (GFAP) expression patterns in the hippocampus, amygdala and hypothalamus after exposure to dextran sodium sulfate (DSS). These are representative photomicrographs of the hippocampus (A-F), the amygdala (G-L) and the hypothalamus (M-R) from our study. Representative histology of the hippocampus was stained with hematoxylin and eosin (H&E) at untreated (control) (A), 3 days (C), and 7 days (E) after exposed to DSS. GFAP expression in hippocampus tissue was increased compared to (B) control group after exposed to DSS at (D) 3 days, and (F) 7 days, as shown by immunohistochemistry (IHC). Representative histology of the amygdala was also stained with H&E at (G) untreated (control), (I) 3 days, and (K) 7 days after exposed to DSS. IHC of GFAP expression in the amygdala showed no significant difference between the (H) control group, (J) the 3-day treatment group, and (L) the 7-day treatment group. (M) Hypothalamus tissue stained with H&E at untreated (control), (O) 3 days, and (Q) 7 days after exposed to DSS. IHC of GFAP expression in the hypothalamus did not show any significant difference between (N) the control group, (P) the 3-day treatment group, and (R) the 7-day treatment group.

BDNF is known to have different expression patterns in response to stress depending on the brain region. Previous studies have shown that the expression pattern of the BDNF gene in the hypothalamus on the 7th day of DSS administration is not different from that of normal mice.<sup>21)</sup> However, in our results, the expression of BDNF in the hypothalamus after 3 days of DSS treatment was significantly increased. This expression pattern of BDNF can be regarded as a major clue to when the stress period begins.

In stress research, the stress period can be a major factor. Recent studies have reported that neurogenesis of the hippocampus is activated by acute stress.<sup>44)</sup> In addition, the expression pattern of BDNF responds differently in the brain region depending on the type and severity of stress. When exposed to restraint stress, for 10 days chronically, an increase in BDNF expression is reported in the amygdala region as observed in psychological stress.<sup>45)</sup> In contrast, no difference or decrease in BDNF expression has been observed in the hippocampus.<sup>45)</sup> In this experiment, inflammation-mediated stress is thought to differ from that of psychological stress in the brain region.

It has long been proposed that a deficiency of BDNF

may lead to psychiatric diseases, especially major depression.<sup>46)</sup> Systematic review and meta-analysis of the relationship between BDNF protein levels and anxiety disorders show that the BDNF levels of patients with anxiety disorders were lower than those of control groups.<sup>47)</sup> Postmortem study showed that BDNF levels in the amygdala of women who were diagnosed with major depressive disorder (MDD) was lower than that of the control group.<sup>48)</sup> In a meta-analysis, unmedicated patients with major depression had a significant decrease in amygdala volume, while the amygdala volume of the antidepressant medicated group was significantly increased.<sup>49)</sup> Hypometabolism in the amygdala was observed in a patient with treatment-resistant depression.<sup>50)</sup> In a functional magnetic resonance imaging study, there was a reduced blood oxygen level-dependent signal in the amygdala of patients with ulcerative colitis observed relative to control.<sup>51)</sup> In summary, it seems that BDNF deficiency in the amygdala is correlated with hypometabolism, volume reduction in depression, and IBD. In contrast to the upregulation of COX-2 in the hippocampus and hypothalamus, there was downregulation of COX-2 in the amygdala. In our study, we have discussed the role of COX-2 in neuro-



**Fig. 6.** Quantification of changes in glial fibrillary acidic protein (GFAP) expression in different brain regions. GFAP expression in the hippocampus and amygdala was quantified (ImageJ software; NIH, Bethesda, MD, USA) and expressed as a percentage of tissue area. (A) GFAP expression changes in hippocampus. (B) GFAP expression changes in amygdala. (C) GFAP expression changes in hypothalamus. Like mRNA expression changes in hippocampus, GFAP expression pattern changed after dextran sodium sulfate exposure. \*Significance ( $p < 0.05$ ).

inflammation in the hippocampus and amygdala, but COX-2 also has other roles in brain. Even if inflammation does not occur in the mammalian brain, COX-2 is constitutively expressed in normal physiological conditions, and it has been proposed that COX-2 activity affects synaptic activity and consolidation in the brain.<sup>40,52,53</sup> One study found that the activity of COX-2 determines long-term potentiation in the lateral nucleus of the amygdala using a selective COX-2 inhibitor and COX-2 knockout mice.<sup>54</sup> Combining our results with previous studies, DSS-colitis affects neural plasticity and neurogenesis in the amygdala by reducing expression of BDNF and COX-2.

Compared with previous studies, our study has yielded some significant findings. Previous studies have focused on whether, and how, external stress affects the HPA axis, and the progression and prognosis of IBD to demonstrate the relationship between psychiatric problems and IBD. It is imperative that we understand how IBD itself affects the

brain to further clarify the bidirectional gut-brain interaction, and the relationship between inflammatory disease and central nervous systems, but there is insufficient research to describe how inflammation in the gut affects the brain. We observed changes in the limbic system at 3 days DSS-treatment, or the early stage, and these changes occurred before glucocorticoids and CRP increased significantly. Unlike in other studies, we harvested samples twice, at 3 days and 7 days after DSS treatment, taking into account the importance of the acute stress response. We found changes in COX-2 mRNA, BDNF mRNA, and GFAP mRNA and protein expression patterns 3 days after DDS treatment. Contrary to the claims of the glucocorticoid vulnerability hypothesis, that damage to the hippocampus is caused by overexpressed glucocorticoid in chronic stress,<sup>19</sup> this finding suggests that changes in the disease model occur at a very early stage, before changes in the HPA axis, through a pathway independent of glucocorticoid. Therefore, active, early intervention is

necessary to prevent psychiatric disease because IBD can affect the central nervous systems sooner than we previously expected. Our study also showed that the pattern of inflammation varies depending on the location within the limbic system. A previous study has shown that lipopolysaccharide in the mouse brain can lead to region-specific neuroinflammation.<sup>55)</sup> In this study, we only present patterns of inflammation in the limbic system in an IBD animal model. In the future, we should examine whether the brain changes in the IBD disease model are the same as those in other inflammatory diseases, or whether these patterns in the brain are universally altered, and how inflammation of peripheral tissue affects the brain bidirectionally.

In addition, histopathological evaluation with H&E staining revealed no significant findings; however, the expression of GFAP started to increase significantly compared to the control group in the hippocampus of DSS-treated mice at 3 days, which is representative of the early stage of colitis. Considering this finding, further studies on the early response in IBD-related disease are necessary.

We conclude that the changes in each region of brain caused by DSS-induced colitis were not consistent, and varied depending on location. Unlike inflammation in peripheral tissues, changes in the brain caused by experimental colitis appear to proceed in a planned and orderly fashion, depending on the brain area. Further research is needed to determine whether regional differences are due to differences in susceptibility to systemic inflammation or differences in regional ability to adapt to colitis. Our study suggests that DSS-induced colitis itself could cause neuronal changes in the limbic system at an early stage. It has also been shown that the vulnerability to IBD can vary depending on the region within the limbic system. In this experiment, we did not elucidate the mechanism by which IBD affects the brain. However, as further research on the interaction of the brain with IBD is underway, we hope the mechanism will soon be determined. We believe that early intervention will alleviate the comorbidity of IBD and psychiatric illness and improve the quality of life of patients with IBD.

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