depression has continued since diagnosis in 1995 despite adequate antidepressant treatment and various augmentation therapies: one moved to a different hospital because of home relocation in year 1; however, the other 6 patients now receive treatment as outpatients at our department.

3.3. Treatment

The 26 patients in this study received two or more antidepressants and various augmentation therapies. Four patients were treated with ECT, but the efficacy was transient and all patients relapsed (data not shown). Augmentation therapies contributory to full remission were noted and shown in Table 2A and B. In 9 of 13 patients with full remission at the final evaluation, the addition of dopamine receptor agonists (bromocriptine or pergolide) to adequate doses of conventional antidepressants was effective. The combination of lithium and dopamine receptor agonists with antidepressants was effective in one patient. The combination of lithium and L-thyroxine with antidepressants was effective in two patients.

All patients received lithium augmentation trials and 4 (1 bipolar, 3 unipolar according to 1995 diagnosis) of 26 patients (15%) were lithium responders. As the diagnoses were changed after the long-term follow-up, these four patients were three bipolar and one unipolar patients at the final evaluation.

Long-term use of various augmentation therapies, such as lithium, thyroid hormone and dopamine receptor agonists, did not cause any serious side effects or any sequelae. No cases of rapid cycling were observed during the follow-up period. Among the patients in this study, the long-term use of antidepressants clearly did not cause mixed or (hypo)manic episodes.

4. Discussion

In the present study, subsequent to long-term follow-up (mean: 5.7 years, range: 1–7 years), the diagnoses of 5 (24%) of 21 patients with unipolar antidepressant-refractory depression were changed to bipolar disorder. There has been no research conducted on the conversion from unipolar depression to bipolar disorder in antidepressant-refractory depression. However, in non-refractory depression, a similar finding that 70 (12.5%) of 599 unipolar depressed patients were converted to bipolar disorder in a prospective observation period of up to 11 years was reported by Akiskal et al. (1995). In 1995, our patients had been depressed for an average of 5 years, indicating that these unipolar depressed patients were observed for an average of 11 years, a period similar to the study by Akiskal et al. (1995). In comparison to non-refractory depression, there may be more converters from unipolar to bipolar in antidepressant-refractory depression. Recommendation of more intensive use of mood stabilizers might be considered after the completion of future research. Furthermore, the increased tendency for patients diagnosed with unipolar antidepressant-refractory depression to become manic in comparison to non-refractory unipolar depression should be noted. In the end, 10 (38%) of 26 treatment-refractory depressed patients were diagnosed as bipolar at the final evaluation. This relatively high prevalence of bipolar disorder is comparable to the 46% prevalence of bipolar I and II disorders in patients with antidepressant-resistant depression recently observed by Sharma et al. (2005). As antidepressant-refractory depression includes more bipolarity, this suggests that bipolarity plays an important role in the pathophysiology of a subgroup of patients with antidepressant-refractory depression.

Patients diagnosed with depression associated with both unipolar and bipolar mood disorders are believed to be able to completely recover and achieve full remission (Kraepelin, 1913; Weitbrecht, 1973). However, in previous studies, long-term observation until full remission has not been attained. By the 1995 evaluation in the present study, patients with antidepressant-refractory depression had suffered from chronic depression for an average of 5 years, for unipolar depression, and 3.4 years, for bipolar depression. Nevertheless, during the long-term follow-up of the present study, we confirmed that 8 of 10 bipolar depressed patients and 9 of 16 unipolar depressed patients achieved full remission. While full remission of these depressed patients is likely in principle, as observed by Emil Kraepelin (1913), it is clinically important to confirm in naturalistic settings that chronic antidepressant-refractory depression is not a subgroup that is unable to achieve full remission.

During the follow-up period, recurrence occurred in only 2 of 10 remitted unipolar depressed patients and these patients were remitted thereafter again. In bipolar depressed patients, 5 of 10 patients experienced recurrence of depression and 7 of 10 patients experienced hypomanic/manic episodes. Accordingly, as also shown in the 1995 study, bipolar antidepressant-refractory patients had more episodes than unipolar antidepressant-refractory depressed patients. The prevention of such mood episodes by mood stabilizers might be important for these bipolar

antidepressant-refractory depressed patients, although they continue to have chronic episodes of depression for years. However, as half of the bipolar patients had been diagnosed with unipolar depression in 1995 because of a lack of previous manic or hypomanic episodes, they could not receive mood stabilizers until the final diagnosis was made. Therefore, the diagnosis of bipolar depression before the first manic/hypomanic episode is an important issue.

For effective treatment of refractory depression, several limitations of our study must be considered: the choice of augmentation therapies used for our patients was not controlled, and their efficacies were evaluated based on the CGI scale. Nevertheless, we can suggest that lithium, L-thyroxine and dopamine receptor agonists, in combination with antidepressants, are effective treatments for patients with either unipolar or bipolar antidepressant-refractory depression. Meta-analyses based on placebo-controlled double-blind studies have confirmed the efficacy of lithium, but not triiodotyronine, for refractory depression (Aronson et al., 1996; Bauer and Döpfmer, 1999). However, a systematic review indicated that even the evidence for lithium augmentation is very weak (Stimpson et al., 2002). Despite limited evidence, clinicians must pursue effective pharmacological therapies for antidepressant-refractory depression for the benefit of these patients. L-Thyroxine and dopamine receptor agonists have not been investigated by randomized controlled trials for antidepressant-refractory depression; however, their efficacies were reported in open-labeled trials (Inoue et al., 1996b; Bauer et al., 1998). The present study showed that the addition of dopamine receptor agonists was effective in 9 of 13 patients with full remission as the final evaluation, the addition of lithium and a dopamine receptor agonist was effective in 1 patient, and the addition of lithium and L-thyroxine was effective in 2 patients. Accordingly, these augmentation therapies were considered helpful for these remitted patients at the final evaluations in long-term naturalistic observations without any serious side effects.

Interestingly, one of four lithium responders began the follow-up with a bipolar disorder; however, these responders consisted of three bipolar and one unipolar patients by the conclusion of the study. Previous studies have primarily examined the effect of lithium augmentation among unipolar depressed patients. However, with only a small number of bipolar depressed patients, a meta-analysis could not draw conclusion as to whether bipolar patients are more or less likely responders compared with unipolar depressed patients (Bauer and Döpfmer, 1999). Future studies should compare the response rate to lithium augmentation among unipolar and bipolar depressed patients who have been diagnosed based on long-term observation.

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ARTICLE

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Neonatal isolation changes the expression of IGF-IR and IGFBP-2 in the hippocampus in response to adulthood restraint stress

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Abstract

Early adverse experiences are thought to contribute to the development of stress vulnerability, and to increase the onset of stress-related psychiatric disorders in stressful environments in adulthood. One plausible molecular mechanism of stress vulnerability is the modulation of neurotrophic factor signal transduction in the hippocampus by early adversity. In the present study we investigated the influence of neonatal isolation (NI) with or without adulthood single restraint stress (SRS) on the expression of several growth factor-related genes in the rat hippocampus using a cDNA microarray, real-time quantitative PCR, and Western blot. We found that hippocampal insulin-like growth factor-I receptor (IGF-IR) mRNA levels and immunoreactivity, and IGF binding protein-2 (IGFBP-2) mRNA levels were significantly lower in response to SRS in NI rats compared with rats without NI. Immunohistochemical analyses revealed that hippocampal IGF-IR immunoreactivity in the CA1 and CA3 pyramidal cell layers, and in the dentate gyrus granule cell layer of NI rats subjected to SRS was significantly lower compared with rats subjected to SRS. In addition, the hippocampal levels of IGF-IR mRNA were significantly lower in adult rats subjected to NI. These findings indicate that NI down-regulates IGF signal transduction under basal and stressful conditions in later life. Since the activation of IGF signalling plays a role in the development and neuroprotection of the central nervous system, the down-regulation of IGF signal transduction induced by NI may be, at least in part, involved in the development of adulthood stress vulnerability, which in turn precipitates the onset of depression.

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Introduction

Numerous epidemiological studies reveal that early adverse experiences are closely associated with an increased risk of stress-related psychiatric disorders in adulthood, such as major depression and post-traumatic stress disorder (PTSD) (Bifulco et al., 1991; Harris et al., 1986; Widom, 1999). Although the precise mechanism for the precipitation of the onset of stress-related psychiatric disorders remains to be determined, it has been postulated that early life adversity causes the development of stress vulnerability and subsequently induces the onset of psychiatric disorders under stressful environmental conditions in adulthood. For example, various studies using rats and non-human primates have demonstrated that early adverse experience including neonatal isolation (NI), maternal separation, and poor maternal care is associated with enhanced activity of the hypothalamic–pituitary–adrenal (HPA) axis in response to adulthood restraint stress (Liu et al., 1997; McCormick et al., 2002; Meaney et al., 1996; Plotsky and Meaney, 1993), anxiety-like behaviour (Huot et al., 2001; Wigger and Neumann, 1999), or impairment of spatial memory acquisition (Huot et al., 2002) in adulthood.

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In addition to hippocampal dysfunction, morphological changes in the hippocampus may also play an important role in the pathophysiology of depression and PTSD. Clinical studies using MRI-based volumetric analysis showed significant reduction of the hippocampal volume of patients with PTSD or major depression both with (Bremner et al., 2003; Vythilingam et al., 2002) and without an early adverse experience (Bremner et al., 1995, 2000; Sheline et al., 1999). Similarly, hippocampal atrophy in animals in response to stress has also been demonstrated. Prior studies have demonstrated that exposure to stress induces marked neuronal degeneration of hippocampal neurons in vervet monkeys (Uno et al., 1989), atrophy of the apical dendrites of CA3 pyramidal neurons in the rat hippocampus (Watanabe et al., 1992), and a reduction in the proliferation of granule cell precursors in the dentate gyrus (Gould et al., 1997, 1998). Further studies on the mechanism of stress-induced neurobiological changes in the hippocampus proposed that the modulation of neurotrophic factors and their receptors was closely associated with altered hippocampal structure (Molteni et al., 2001; Scaccianoce et al., 2000; Smith et al., 1995; Ueyama et al., 1997). Based on these findings, it is conceivable that early adversity affects the expression of neurotrophic factors and their receptors in the rat hippocampus during maturation, and consequently leads to stress vulnerability in adulthood. Some studies have examined the influence of early adverse experience on the expression of neurotrophic factors, members of the neurotrophin gene family, such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and neurotrophin-3 (NT-3) in rodent hippocampus (Cirulli et al., 2000; Greisen et al., 2005; MacQueen et al., 2003; Roceri et al., 2004; Roceri et al., 2002).

Growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factor (TGF), or insulin-like growth factor (IGF) are also reported to be involved in the development and survival of the central nervous system (CNS) (Cameron et al., 1998). One study recently demonstrated a significant reduction of IGF-II mRNA in the hippocampus with the choroid plexus of adult rats subjected to maternal separation (Kohda et al., 2006), however, the influence of NI on the expression of growth factors, their receptors, or their binding proteins in adult rat hippocampus has not yet been fully elucidated. Furthermore, it is also unknown as to whether adulthood stress can produce a significant change in the expression of growth factors, and their receptors and binding proteins in the hippocampus of rats subjected to early adversity. In this study, we investigated the influence of NI with or without adulthood restraint stress on the expression of several growth factors, and their receptors and binding proteins in the rat hippocampus using a cDNA microarray, real-time quantitative PCR, Western blot, and immunohistochemistry.

Materials and methods

Animals

Pregnant female Sprague–Dawley rats were purchased from Charles River Japan (Yokohama, Japan). The rats were housed individually in a breeding colony at constant room temperature (23±2 °C) and humidity (60%) with a 12 h light/dark cycle (lights on 08:00 to 20:00 hours). Food and water were provided ad libitum. The litters were culled to 12 pups on postnatal day 1 (PN day 1).

NI and single restraint stress (SRS) paradigm

The mothers and pups of the non-isolated group were left undisturbed until weaning (Figure 1). NI was conducted according to the method of Kehoe and Bronzino (1999). Pups were isolated from the dam, nest, and siblings and placed in individual round containers for 1 h per day on PN days 2–9 (Figure 1). All litters were weaned on PN day 21, separated on the basis of sex, and maintained with ad libitum access to food and water. Only the male rats were subjected to the following experimental procedure. On PN day 90, half of both the NI and non-isolated rats were subjected to a SRS for 2 h (Figure 1). The restraint stress was conducted as described previously (Suenaga et al., 2004). Briefly, rats were restrained using a clear polyethylene disposable bag (Asahikasei, Tokyo, Japan). Animals were sacrificed by decapitation after completion of the restraint stress. On PN day 90, NI and non-isolated rats (sham) were sacrificed by decapitation (Figure 1). Two groups of adult rats [one group subjected to repeated NI followed by SRS (NI+SRS); the second group subjected to SRS (SRS)] were used for DNA microarray analysis, Western blot analysis, and immunohistochemistry. Four groups of adult rats [subjected to NI followed by SRS (NI+SRS); NI alone (NI); SRS alone (SRS); or sham treatment (sham)] were used for real-time quantitative PCR. For the measurement of plasma corticosterone levels, both sham and NI rats were subjected to SRS for different durations. A total of 116 rats were used in the study and a different set of rats was used for each of the methods (i.e. DNA microarray, real-time quantitative PCR, Western

Figure 1. Experimental paradigms. (a) Sham treatment: Sham-treated rats were undisturbed until weaning (PN day 21) and were not subjected to a single restraint stress on PN day 90. (b) Neonatal isolation (NI): Pups were isolated from the dam, nest, and siblings for a period of 1 h per day on PN days 2–9, and were not subjected to restraint stress. (c) Single restraint stress (SRS): Pups were undisturbed until weaning and were subjected to SRS for 2 h on PN day 90. (d) NI + SRS: Pups were isolated from the dam, nest, and siblings for a period of 1 h per day on PN days 2–9, and on PN day 90, they were subjected to SRS for 2 h.

blotting, immunohistochemistry, and plasma corticosterone levels). The hippocampus was isolated and used for DNA microarray, real-time quantitative PCR, and Western blot analysis. In this procedure, the lateral choroid plexus was carefully removed from the hippocampus. For immunohistochemistry, brains were removed rapidly, immediately frozen, and stored at −70 °C. All animal procedures were conducted inaccordance with the Guiding Principles on Animal Experimentations in Research Facilities for Laboratory Animal Science Hiroshima University and approved by Hiroshima University Animal Care Committee.

DNA microarray

For the DNA microarray analysis, the ExpressChip™ DNA Microarray System RO1 (Mergen, San Leandro, CA, USA) containing 1152 genes was used. The ExpressChip arrays were pre-spotted with oligonucleotide sequences designed to determine the expression of target genes with the highest specificity. The experiments were carried out according to the manufacturer's protocol. In brief, total RNA was extracted from the hippocampi of five adult rats in each group (NI+SRS, SRS) with an RNAqueous Phenol-free Total RNA Isolation kit (Ambion, Austin, TX, USA), and pooled. After treatment with RNase-free DNase 1 (Takara, Kusatsu, Japan) for the removal of DNA, first-strand cDNA was synthesized using oligo[$(dT)_{24}$ T7promoter]$_{65}$ primer from DNase-treated total RNA, and then double-stranded cDNA was synthesized with T4 DNA polymerase. Biotin-labelled cRNA probes produced from the double-stranded cDNA template were hybridized to microarrays overnight at 30 °C. The arrays were washed, and incubated with streptavidin, anti-streptavidin antibody, and finally, secondary antibody conjugated Cyanine-3 fluorescent dye (Cy3). Signals were detected with a GMS417 array scanner (Affymetrix, Santa Clara, CA, USA), and spot intensity was analysed using ImaGene (BioDiscovery, El Segundo, CA, USA). The relative expression level of each gene was normalized against housekeeping gene controls.

Real-time quantitative PCR

Total RNA was extracted with an RNAqueous Phenol-free Total RNA Isolation kit (Ambion). After treatment with RNase-free DNase I (Takara), real-time quantitative PCR was performed with an ABI PRISM 7700 sequence detection system (PE Applied Biosystems, Foster City, CA, USA) to quantitate relative mRNA levels in samples. Real-time quantitative PCR was performed to amplify insulin-like growth factor-I (IGF-I), insulin-like growth factor-II (IGF-II),

Table 1. Primer and probe sequences used for real time PCR

	Forward primer (5' to 3')	Reverse primer (5' to 3')	Taqman probe (5' [FAM] to 3' [TAMRA])
IGF-I	TCCAGCATTCGGAGGGC	TCACAGCTCCGGAAGCAAC	CCACAGACGGGCATTGTGGATGAG
IGF-II	GCCGTACTTCCGGACGACT	TCCAGGTGTCGAATTTGAAGAA	CCCCAGATACCCCGTGGGCAA
IGF-IR	CGCTCTGGCCGACGAGT	CTGCTGATAGTCGTTGCGGA	AGAAATTTGTGGGCCCGGCATTGAC
IGF-IIR	CGGAATGGAAGCTCGATTATTG	GTGTCGTCCTCACTCTCATCGTAT	TCTCATCCACCGCACTGGTGGTTATGA
IGFBP-2	CAGGTCCTGGAGCGCATC	ATGTTCCAGAGGACCCCGAT	CCACCATGCGCCTTCCGGA

insulin-like growth factor-I receptor (IGF-IR), insulin-like growth factor-II receptor (IGF-IIR), and insulin-like growth factor binding protein-2 (IGFBP-2). The primers and TaqMan hybridization probe were designed using Primer Express software (PE Applied Biosystems). Table 1 shows the sequences and fluorescent dye of PCR primers and TaqMan probes for each molecule. The TaqMan probes, which were designed to hybridize to the PCR products, were labelled with a fluorescent reporter dye at the 5'-end and a quenching dye at the 3'-end. PCR was carried out with TaqMan Universal PCR Master Mix (PE Applied Biosystems). All standards and samples were assayed in triplicate. Each plate contained the same standard. Thermal cycling was initiated with an initial denaturation at 50 °C for 2 min and 95 °C for 10 min. After this initial step, 40 cycles of PCR (heating at 95 °C for 15 s and 60 °C for 1 min) were performed. The PCR assay for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed using TaqMan Rodent GAPDH Control Reagents (PE Applied Biosystems). PCR assays for unknown samples were performed simultaneously with standard samples (rat brain tissue) to construct a standard curve. The relative concentrations of GAPDH and IGF-I, IGF-II, IGF-IR, IGF-IIR, or IGFBP-2 in unknown samples were calculated from this standard curve and the ratio of the relative concentration of IGF-I, IGF-II, IGF-IR, IGF-IIR, or IGFBP-2 was calculated relative to the concentration of GAPDH.

Western blot

Immunoblot analyses for IGF-IR and IGFBP-2 were performed by the method of Cardona and colleagues (2000) with a minor modification. In brief, the rat hippocampus was homogenized on ice with a Polytron homogenizer at top speed (30 000 rpm) in homogenization buffer containing 20 mM Tris–HCl (pH 7.5), 5 mM EDTA, 2 mM DTT, 150 mM NaCl, 0.5% Triton X-100, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 100 μM phenylmethylsulphonyl fluoride. The insoluble material was removed by centrifugation at 10 000 g at 4 °C for 10 min. Protein concentrations were determined with a protein assay kit (Bio-Rad, Hercules, CA, USA). Equal amounts of protein (60 μg) for each group were fractionated using sodium dodecyl sulphate (SDS) gel (7.5% for IGF-IR, and 12.5% for IGFBP-2; Atto, Tokyo, Japan) and transferred to a PVDF membrane (for IGF-IR) or a nitrocellulose membrane (for IGFBP-2) using a semi-dry blotting apparatus (Bio-Rad). Nitrocellulose membrane was incubated with Miser™ Antibody Extender Solution NC (Pierce, Rockford, IL, USA) before blocking. All membranes were blocked at room temperature for 1 h in TBS containing 5% non-fat dry milk and 0.05% Tween-20 (TBST-MLK), and then incubated overnight with anti-IGF-IR β-chain antibody (1:500 dilution; sc-713, Santa Cruz Biotechnology, Santa Cruz, CA, USA) in TBST-MLK, or anti-IGFBP-2 antibody (1:1000 dilution; no. 06-107, Upstate Biotechnology, Lake Placid, NY, USA) in Solution 1 of Can Get Signal™ (Toyobo, Osaka, Japan) as a primary antibody, overnight at 4 °C. The membranes were washed at room temperature in TBST four times for 5 min per wash, and then incubated with horseradish peroxidase-conjugated anti-rabbit IgG antibody (1:2000 dilution for IGF-IR, 1:20000 dilution for IGFBP-2; Zymed, San Francisco, CA, USA) in TBST-MLK (for IGF-IR) or Solution 2 of Can Get Signal™ (for IGFBP-2). IGF-IR blots were detected by a colorimetric method (PerkinElmer, Wellesley, MA, USA), and IGFBP-2 blots were developed using an Enhanced Chemiluminescence (ECL) Western Blotting Detection System (Amersham Pharmacia Biotech, Buckinghamshire, UK). The blots were reprobed with anti-β-actin antibody (Sigma Chemical Co., St. Louis, MO, USA) to ensure equal protein loading. The density of the immunoreactive bands was quantified with Atto Image analysis software (version 4.0 for Macintosh; Atto).

Immunohistochemistry

Freshly frozen coronal brain sections (15 μm) through the hippocampus were cut in a cryostat, thaw-mounted onto slides and fixed with 4%

paraformaldehyde for 5 min. Sections were washed three times with PBST (PBS-0.1% Triton X-100) and pretreated with 10% H_2O_2 in methanol to neutralize the endogenous peroxidase activity. Then, the sections were washed twice in PBST for 10 min. After being blocked in 10% sheep serum in PBST for 60 min, the sections were incubated overnight at 4 °C with anti-IGF-IR monoclonal antibody (1:100 dilution; MAB1123, Chemicon, Temecula, CA, USA) or anti-IGFBP-2 antibody (1:1000 dilution; no. 06-107, Upstate Biotechnology) in 10% sheep serum in PBST. After four 10 min washes in PBST, the sections were incubated at room temperature for 180 min with goat anti-mouse IgG (H + L) HRP conjugate (1:200 dilution for IGF-IR, 1:1000 dilution for IGFBP-2; Zymed) in 10% sheep serum in PBST. The sections were then washed four times in PBST for 10 min per wash and exposed with liquid DAB + substrate chromogen solution (Dako, Carpinteria, CA, USA). The immunohistochemical signal for IGF-IR and IGFBP-2 was detected using a digital video image analyser (Keyence BZ-8000, Osaka, Japan). The mean density of sections from the SRS and NI + SRS groups was measured in the CA1 and CA3 regions, granule cell layer and hilus of the dentate gyrus using the NIH Scion Image analysis program.

Measurement of plasma corticosterone levels

Blood samples were collected before SRS, and 30 min and 2 h after the beginning of SRS in NI and non-NI rats. After centrifugation (500 *g* at 4 °C for 30 min), plasma samples were frozen and stored at −70 °C until the day of analysis. The plasma corticosterone level was determined using a rat corticosterone [^{125}I] assay system (Amersham Pharmacia Biotech).

Statistical analyses

Results were expressed as mean ± S.E.M. The results of real-time quantitative PCR were analysed by two-way analysis of variance (ANOVA) (NI × SRS) followed by Scheffé's test. The levels of plasma corticosterone were analysed by 2 × 3 ANOVA (NI × time after the beginning of SRS) followed by Scheffé's test. For results analysed by ANOVA, the degrees of freedom were presented. For Western blot and immunohistochemical analyses the results of experiments containing two groups of rats were analysed by Mann–Whitney *U* test, and the results of the experiment on IGFBP-2 immunoreactivity at different intervals after the termination of SRS for 2 h was analysed by one-way ANOVA followed by Scheffé's test. Significance was set at $p < 0.05$.

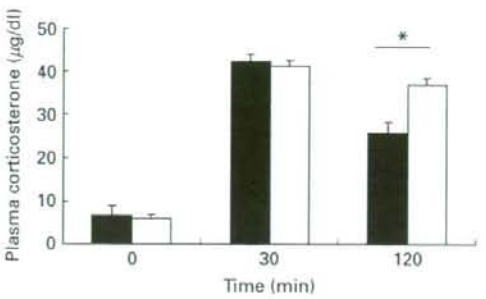

Figure 2. Plasma corticosterone concentrations before (0 min) and after the beginning of single restraint stress (SRS) (30 min, 2 h) in neonatal isolation (NI; □) and non-NI (■) adult rats. The mean ± S.E.M. ($n = 6$) is shown. * $p < 0.01$ compared to non-NI group (2 × 3 ANOVA followed by Scheffé's test).

Results

The influence of NI on the levels of plasma corticosterone

Statistical analysis of the plasma corticosterone levels revealed that there was a significant interaction between NI and restraint time [$F(2,30) = 7.697$, $p = 0.002$]. While there were no differences in the plasma corticosterone levels between NI and non-NI rats before SRS or 30 min after the beginning of SRS, there was the significant difference in the levels of plasma corticosterone between NI and non-NI rats 2 h after the beginning of SRS ($p < 0.01$) (Figure 2).

The influence of NI on the levels of IGF-I, IGF-IR, IGF-II, IGF-IIR, and IGFBP-2 mRNA in response to a SRS in adulthood

Differences in the hippocampal expression of growth factors and related genes between the SRS and NI + SRS groups at PN day 90 were analysed using an ExpressChip™ (Table 2). With a cut-off of 2.0-fold change, the expression of several genes involved in the IGF signal transduction (IGF-I, IGF-IR, IGF-II, IGF-IIR, and IGFBP-2) in the NI + SRS group was different from that in the SRS group. To confirm the difference in the mRNA levels of these genes between the SRS and NI + SRS group, we performed real-time quantitative PCR analysis using a different set of animals from the microarray analysis. For IGF-I mRNA expression, two-way ANOVA showed no significant effect of NI [$F(1,28) = 0.109$, $p = 0.744$], or SRS [$F(1,28) = 1.125$, $p = 0.298$], and no significant interaction between NI and SRS [$F(1,28) = 0.035$, $p = 0.853$] (Figure 3a). For

Table 2. Hippocampal expression of growth factors and related genes

Genbank ID	Gene	Fold change SRS/NI+SRS
U04842	Epidermal growth factor (EGF)	1.1
M37394	EGF receptor	1.1
X14232	Fibroblast growth factor (FGF) 1 (heparin binding)	8.7
M22427	FGF2	1.0
D64085	FGF5	1.0
D14839	FGF9	0.7
D79215	FGF10	1.3
AB004638	FGF18	0.9
S54008	FGF receptor 1, complete cds	1.5
L19109	Heparin binding FGF receptor 2 (intracellular domain) mRNA	1.8
L19104	Heparin binding FGF receptor 2 (extracellular domain) mRNA	2.3
U57715	FGF receptor activating protein (FRAG1) mRNA, complete cds	1.4
D90102	Hepatocyte growth factor	1.0
M15651	Insulin-like growth factor (IGF)-I	2.2
X14834	IGF-II	2.4
M37807	IGF-I receptor	4.1
U59809	IGF-II receptor	3.0
M58634	IGF-binding protein (IGFBP) 1	0.5
J04486	IGFBP2	4.8
M31837	IGFBP3	1.3
M62781	IGFBP5	1.3
M69055	IGFBP6	1.0
M29014	Insulin receptor	0.8
X56551	Keratinocyte growth factor	1.6
L14447	Neurotrophic tyrosine kinase, receptor, type 3 (Ntrk3)	1.3
M36589	Beta-nerve growth factor gene, last exon	1.3
M55291	Neuronal receptor protein-tyrosine kinase (trkB)	1.6
L06238	Platelet-derived growth factor (PDGF) A-chain mRNA	0.6
M63837	PDGF receptor alpha	0.9
M31076	Transforming growth factor (TGF) alpha	1.1
X52498	TGF beta 1	2.0
U03491	TGF beta 3	0.7
M77809	TGF beta receptor III	3.2
M55431	TGF-beta masking protein large subunit, complete cds	1.0
L00981	Tumour necrosis factor (TNF) alpha	1.5
M63122	TNF receptor	1.0
X05137	NGF receptor, fast	1.1
M32167	Vascular endothelial growth factor (VEGF) mRNA, partial cds	0.9
AF014827	VEGF-D mRNA, complete cds	1.9

Total RNA was isolated from the hippocampi of five rats in the NI+SRS and SRS groups, and subjected to the ExpressChip™ (Mergen, San Leandro, CA, USA) followed by analyses with the ImaGene (BioDiscovery).

IGF-IR mRNA expression, there was a significant effect of NI [$F(1,28)=19.972$, $p<0.001$] as well as SRS [$F(1,28)=30.890$, $p<0.001$] but there was no significant interaction between NI and SRS [$F(1,28)=0.062$, $p=0.806$] (Figure 4a). Post-hoc analysis revealed that the level of IGF-IR mRNA ($p<0.05$) in the NI+SRS group was significantly lower than that in the SRS group (Figure 4a). In addition, the level of IGF-IR mRNA in the NI group was significantly lower ($p<0.05$) than that in the sham group (Figure 4a). In the SRS group, the level of IGF-IR mRNA was significantly increased compared to that in the sham group ($p<0.01$) (Figure 4a) . In the NI+SRS group, the level of IGF-IR mRNA was significantly increased compared to that in the NI group ($p<0.01$) (Figure 4a). For IGF-II mRNA expression, two-way ANOVA showed

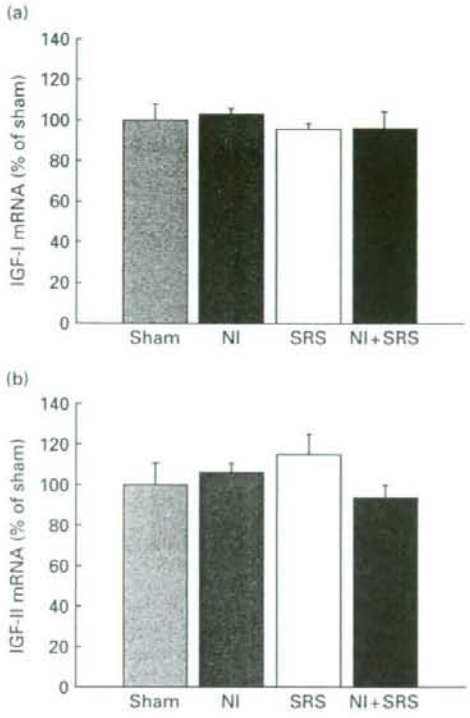

Figure 3. Expression of IGF-I and IGF-II mRNA in the hippocampus of adult rats subjected to sham treatment (sham), neonatal isolation (NI) alone, single restraint stress (for 2 h) on PN day 90 (SRS) alone, and neonatal isolation followed by a single restraint stress (for 2 h) on PN day 90 (NI+SRS). (a) IGF-I mRNA levels were determined by real-time quantitative PCR as described in the Materials and Methods section. Results are expressed as the percentage of sham. The mean ± S.E.M. ($n=8$) is shown. (b) IGF-II mRNA levels were determined by real-time quantitative PCR as described in the Materials and Methods section. Results are expressed as the percentage of sham. The mean ± S.E.M. ($n=6$–8) is shown.

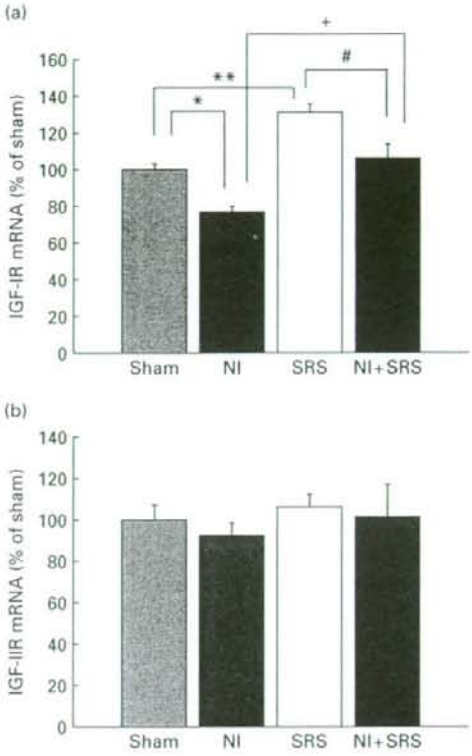

Figure 4. Expression of IGF-IR and IGF-IIR mRNA in the hippocampus of adult rats subjected to sham treatment (sham), neonatal isolation (NI) alone, single restraint stress (for 2 h) on PN day 90 (SRS) alone, and neonatal isolation followed by a single restraint stress (for 2 h) on PN day 90 (NI+SRS). (a) IGF-IR mRNA levels were determined by real-time quantitative PCR as described in the Materials and Methods section. Results are expressed as the percentage of sham. The mean ± S.E.M. ($n=8$) is shown. * $p<0.05$ compared to sham, ** $p<0.01$ compared to sham, # $p<0.05$ compared to SRS, + $p<0.01$ compared to NI (two-way ANOVA followed by Scheffé's test). (b) IGF-IIR mRNA levels were determined by real-time quantitative PCR as described in the Materials and Methods section. Results are expressed as the percentage of sham. The mean ± S.E.M. ($n=8$) is shown.

no significant effect of NI [$F(1,23)=0.444$, $p=0.512$], or SRS [$F(1,23)=0.034$, $p=0.854$], and no significant interaction between NI and SRS [$F(1,23)=2.440$, $p=0.132$] (Figure 3b). For IGF-IIR mRNA expression, two-way ANOVA showed no significant effect of NI [$F(1,28)=0.439$, $p=0.513$], or SRS [$F(1,28)=0.582$, $p=0.452$], and no significant interaction between NI and SRS [$F(1,28)=0.010$, $p=0.921$] (Figure 4b). For IGFBP-2 mRNA expression, two-way ANOVA showed that there was a significant interaction between NI and SRS [$F(1,24)=19.098$, $p<0.001$] (Figure 5). Post-hoc analysis revealed a significant ($p<0.01$) increase in the levels of IGFBP-2 in the SRS group as compared with the other groups (Figure 5).

Hippocampal IGF-IR and IGFBP-2 immunoreactivity in SRS and NI+SRS rats in adulthood

To elucidate whether the significant differences in IGF-IR and IGFBP-2 mRNA expression between the SRS and NI+SRS groups (shown by real-time PCR

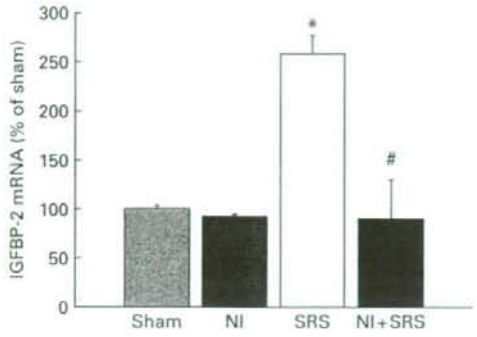

Figure 5. Expression of IGFBP-2 mRNA in the hippocampus of adult rats subjected to sham treatment (sham), neonatal isolation (NI), a single restraint stress (for 2 h) on PN day 90 (SRS), and neonatal isolation followed by a single restraint stress (for 2 h) on PN day 90 (NI+SRS). IGFBP-2 mRNA levels were determined by real-time quantitative PCR as described in the Materials and Methods section. Results are expressed as the percentage of sham. The mean ± S.E.M. ($n=6$–8) is shown. $^{*}p<0.01$ compared to sham, $^{\#}p<0.01$ compared to SRS (two-way ANOVA followed by Scheffé's test).

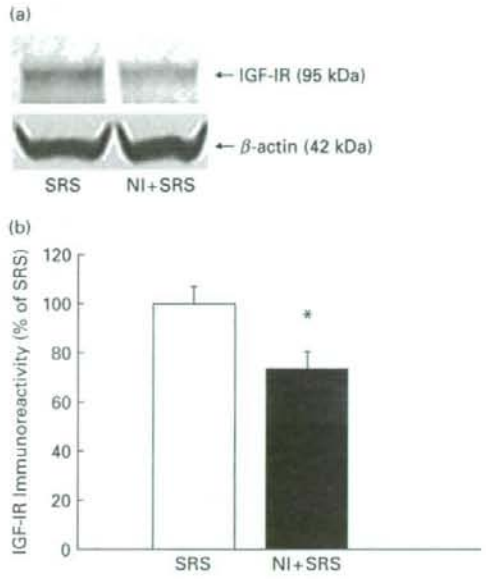

Figure 6. Western blot analysis of IGF-IR expression in the hippocampus of adult rats subjected to a single restraint stress (for 2 h) on PN day 90 (SRS), and neonatal isolation followed by a single restraint stress (for 2 h) on PN day 90 (NI+SRS). Results are expressed as the percentage of SRS. Immunoreactivity was determined by Western blot analysis as described in the Materials and Methods section. (a) Representative immunoblots for IGF-IR, and β-actin as a positive control for loading error, in the rat hippocampus. (b) Comparison of IGF-IR protein levels in the hippocampus of SRS and NI+SRS groups. Results are expressed as the percentage of SRS. The mean ± S.E.M. ($n=6$) is shown. $^{*}p<0.05$ compared to SRS (Mann–Whitney U test).

analysis) affected the protein level of these genes, the levels of IGF-IR and IGFBP-2 protein were measured by Western blotting. Western blot analysis revealed significantly lower IGF-IR immunoreactivity in the NI+SRS group than in the SRS group ($p<0.05$) (Figure 6). However, there was no significant difference in the immunoreactivity of IGFBP-2 between the SRS and NI+SRS groups (Figure 7).

Since no significant difference in IGFBP-2 immunoreactivity was found between the NI and non-NI rats immediately after SRS for 2 h, we determined the levels of hippocampal IGFBP-2 immunoreactivity at different intervals after the termination of SRS for 2 h in adult non-isolated rats. IGFBP-2 immunoreactivity in adult rats sacrificed at 8 h, but not 2 h or 4 h, after the termination of SRS was significantly higher than in rats without SRS ($p<0.05$) (Figure 8).

To identify the hippocampal cell layers in which IGF-IR immunoreactivity in the NI+SRS group was down-regulated, we examined IGF-IR immunoreactivity by immunohistochemistry. Hippocampal IGF-IR immunoreactivity in the CA1 and CA3 pyramidal cell layers and in the granule cell layer and hilus of the dentate gyrus was significantly decreased in NI+SRS rats compared with SRS rats ($p<0.05$) (Figures 9, 10). We also conducted immunohistochemical analysis to determine regional differences in IGFBP-2 immunoreactivity between the SRS and NI+SRS rats (Figure 11). However, hippocampal IGFBP-2 immunoreactivity in the CA1 and CA3 pyramidal cell layers and granule cell layer and hilus of the dentate gyrus did not differ between the two groups (data not shown).

Discussion

Since a stressful environment in later life readily disrupts the homeostasis of neurotransmitters and hormones in individuals with early adversity (Heim et al., 2000; Meaney et al., 2002; Plotsky and Meaney, 1993), and subsequently may induce changes in gene expression in the CNS, early adverse experiences are thought to precipitate stress vulnerability in adulthood. However, the precise molecular mechanism for the development of the stress vulnerability remains to be determined. In this context, we

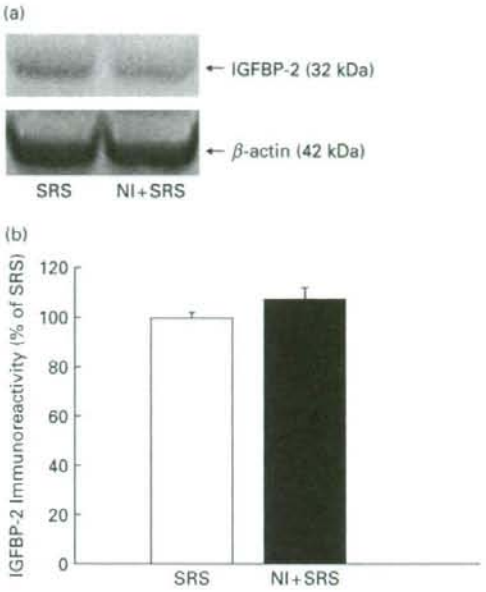

Figure 7. Western blot analysis of IGFBP-2 in the hippocampus of rats subjected to a single restraint stress (for 2 h) on PN day 90 (SRS) alone, and neonatal isolation followed by a single restraint stress (for 2 h) on PN day 90 (NI + SRS). Western blot analysis was performed as described in the Materials and Methods section. (a) Representative immunoblots for IGFBP-2, and β-actin as a positive control for loading error, in the hippocampus are shown. (b) Comparison of IGFBP-2 protein levels in the SRS and NI + SRS groups. Results are expressed as the percentage of SRS. The mean ± S.E.M. ($n = 6$) is shown.

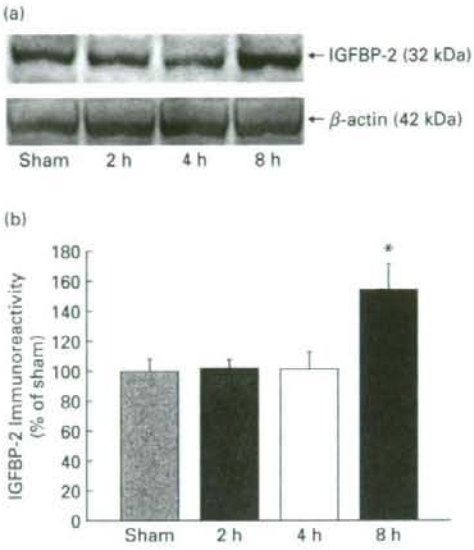

Figure 8. Western blot analysis of IGFBP-2 in the hippocampus of adult rats at three intervals (2 h, 4 h, and 8 h) after the termination of SRS for 2 h. Western blot analysis was performed as described in the Materials and Methods section. (a) Representative immunoblots for IGFBP-2, and β-actin as a positive control for loading error, in the hippocampus are shown. (b) Comparison of IGFBP-2 protein levels at three intervals (2 h, 4 h, and 8 h) after the termination of SRS for 2 h. Results are expressed as the percentage of the sham group. The mean ± S.E.M. ($n = 5$) is shown. * $p < 0.05$ compared to sham (one-way ANOVA followed by Scheffé's test).

examined differences in hippocampal gene expression profiles between rats, with and without NI, in response to adulthood SRS for 2 h. As shown in the present study, SRS for 2 h led to a significant difference in plasma corticosterone levels between SRS and NI + SRS rats, indicating that stress vulnerability induced by NI occurred in this stressful condition. We showed that repeated NI affected IGF-IR mRNA levels and immunoreactivity, and IGFBP-2 mRNA levels in the hippocampus in response to SRS for 2 h in adult rats. In addition, the levels of IGF-IR mRNA in the hippocampus were significantly decreased in adult rats subjected to NI.

Since it is well known that neurotrophic factors play an important role in synaptic formation and cell differentiation during development (Berninger and Poo, 1996; Henderson, 1996), several studies were previously undertaken to examine the influence of maternal separation on the expression of BDNF mRNA in the brain, and to elucidate the mechanism for the development of stress vulnerability in response to early adversity. Almost all studies showed that maternal separation significantly decreased the levels of BDNF in adult rodents (Greisen et al., 2005; MacQueen et al., 2003; Roceri et al., 2002, 2004). In contrast to BDNF, only one study, to our knowledge, has examined the influence of maternal separation on IGF-II mRNA expression in adult rats (Kohda et al., 2006). Kohda and his associates have demonstrated that IGF-II mRNA expression in the hippocampus with the choroid plexus is significantly reduced in adult rats subjected to maternal separation. On the other hand, the result of the present study demonstrates that NI causes no change in the levels of IGF-II mRNA in the hippocampus of adult rats. While the reason for this difference is unclear, the difference in experimental procedures and brain regions may be associated with this discrepancy.

The IGF signalling system consists of IGFs (IGF-I and IGF-II), membrane receptors (IGF-IR and IGF-IIR),

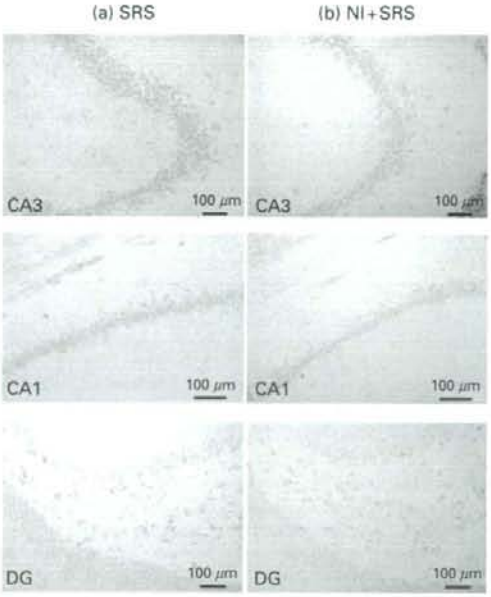

Figure 9. Immunohistochemical analysis of IGF-IR expression in the adult rat hippocampus. (a) IGF-IR immunoreactivity in rats subjected to a single restraint stress (SRS). (b) IGF-IR immunoreactivity in rats subjected to neonatal isolation followed by a single restraint stress (NI + SRS). CA, cornu anmonis; DG, dentate gyrus.

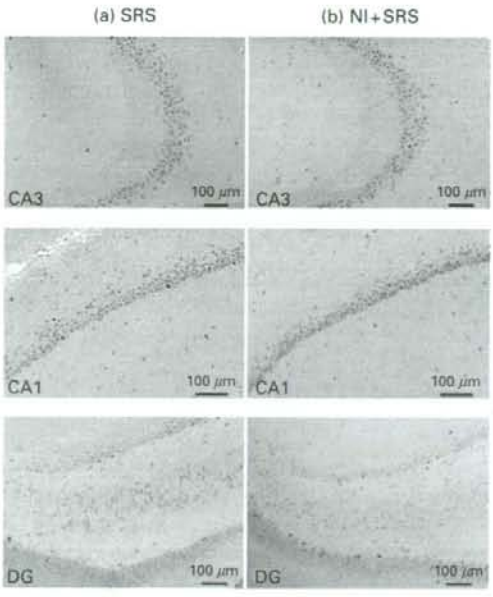

Figure 11. Immunohistochemical analysis of IGFBP-2 expression in the adult rat hippocampus. (a) IGFBP-2 immunoreactivity in rats subjected to a single restraint stress (SRS). (b) IGFBP-2 immunoreactivity in rats subjected to neonatal isolation followed by a single restraint stress (NI + SRS). CA, cornu anmonis; DG, dentate gyrus.

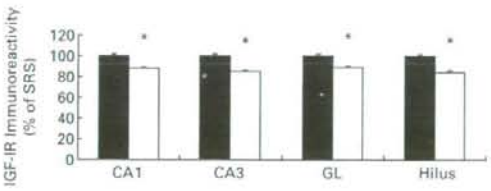

Figure 10. Mean density of IGF-IR immunoreactivity at the CA1 and CA3 pyramidal cell layers, and the granule cell layer and hilus of the dentate gyrus in the hippocampus of single restraint stress (SRS, ■) and neonatal isolation followed by a single restraint stress (NI + SRS, □) rats. Results are expressed as the percentage of SRS. The mean ± S.E.M. ($n = 4$) is shown. * $p < 0.05$ compared to SRS (Mann–Whitney U test). CA, cornu anmonis; GL, granule cell layer.

and IGF binding proteins, and plays a pivotal role in the growth and development of various tissues (LeRoith and Roberts, 2003), including the CNS (D'Ercole et al., 1996; Feldman et al., 1997; Torres-Aleman, 1999). With respect to the function of the IGF system, IGF-IR is the primary mediator of IGF-I action (LeRoith et al., 1995). Transgenic mice lacking IGF-I or IGF-IR show severe brain growth retardation (Baker et al., 1993; Liu et al., 1993). Furthermore, transgenic mice overexpressing IGF-I in the brain show an increase in the total number of neurons and synapses in the dentate gyrus (O'Kusky et al., 2000), while IGF-I −/− mice show a decrease in the number of granule cells in the dentate gyrus (Cheng et al., 2001). Previous studies have shown that peripheral infusion of IGF-I induces neurogenesis in adult rat hippocampus (Aberg et al., 2000), and that IGF-I stimulates proliferation in adult rat hippocampal progenitor cells (Aberg et al., 2003). In this context, it is plausible that the significant decrease in hippocampal IGF-IR expression in response to NI with or without restraint stress may, at least in part, contribute to the disturbance of hippocampal function through the decrease of IGF-I signal transduction.

IGFBPs act as carriers of IGFs, affect the half-lives of IGFs, and modulate the action of IGFs (D'Ercole et al., 1996; Jones and Clemmons, 1995). In the present study, the levels of IGFBP-2 mRNA, but not IGFBP-2 immunoreactivity, immediately after the termination

of adulthood restraint stress for 2 h, were significantly lower in the hippocampus of rats subjected to NI. IGFBP-2 immunoreactivity was significantly increased at 8 h after the termination of SRS in adult rats without NI, and the slower response of IGFBP-2 protein to SRS in the hippocampus might be involved in this difference. Similarly, it was demonstrated that the induction of IGFBP-2 protein was slower than that of IGFBP-2 mRNA in primary astroglial cells in response to IGF-I (Bradshaw and Han, 1993).

It has been reported that the expression of IGFBP-2 and IGF-I mRNA is increased around the site of CNS injury. After hypoxia-ischaemia or cerebral cortical contusion in rats, the expression of IGFBP-2 mRNA as well as IGF-I is induced in the hippocampus (Beilharz et al., 1998; Sandberg Nordqvist et al., 1996). Injection of colchicine into the hippocampus of rats increases the expression of IGFBP-2 mRNA in the hippocampus and damaged cortex (Breese et al., 1996). These findings suggest that not only IGF-I but also IGFBP-2 responds to brain damage and produces neuroprotective effects. It appears that the lack of an increase of IGFBP-2 mRNA expression in response to adulthood restraint stress in rats subjected to NI may be, at least in part, involved in the lack of protection against adulthood stress.

Administration of venlafaxine or fluoxetine for 2 wk has been shown to up-regulate IGF-I protein levels in the hippocampus and to significantly increase the proliferation and survival of progenitor stem cells in the dentate gyrus (Khawaja et al., 2004). Recently, Hoshaw et al. (2005) have shown that central administration of IGF-I or BNDF exerts antidepressant-like effects in the forced swim test, and that the duration of effects is longer than that of traditional antidepressants. We hypothesize that the decrease in hippocampal IGF signal transduction by NI is one plausible mechanism by which early adverse experiences precipitate the onset of depression in adulthood under environmental stress conditions. Further studies examining whether NI with additional adulthood stress affects the activity of the intracellular signal transduction mediated by IGFs and IGF-receptors, are required to verify our hypothesis.

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Statement of Interest

None.

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ORIGINAL PAPER

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Post-stroke affective or apathetic depression and lesion location: left frontal lobe and bilateral basal ganglia

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■ **Abstract** This study was designed to examine the correlation between damage to the basal ganglia or frontal lobe and depression status (both affective and apathetic dimensions) in 243 stroke patients. We assessed the affective dimension in post-stroke depression (PSD) using the Zung Self-rating Depression Scale (SDS) and the apathetic dimension in PSD using the apathy scale (AS). We classified basal ganglia or frontal lobe damage into four groups: no damage, damage to the left side only, damage to the right side only, and damage to both sides. Affective and/or apathetic PSD was found in 126 patients (51.9%). The severity of affective depression (SDS score) was associated with left frontal lobe (but not basal ganglia) damage, and that of apathetic depression (AS score) was related to damage to the bilateral basal ganglia (but not to the frontal lobe). The anatomical correlates of PSD differ depending on the PSD dimension (affective or apathetic) and may explain interstudy differences regarding the association between lesion location and type of PSD.

■ **Key words** basal ganglia · frontal lobe · stroke · apathy · Zung Self-rating Depression Scale

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Introduction

Depression is a common neuropsychiatric consequence of stroke and has been reported to negatively affect functional and cognitive recovery (Alexopoulos et al. 1997; Biringer et al. 2005). Some studies, including those on stroke patients, have demonstrated morphological changes in major depression with respect to the hippocampus, basal ganglia and frontal lobe (Alexopoulos et al. 1997; Bielau et al. 2005; Frodl et al. 2004; Sheline et al. 1996). Therefore, the neuroanatomical model of mood regulation was developed from the observation that lesions in some cortical/subcortical regions resulted in depression (Soares and Mann 1997).

"Depressed mood" is a very sensitive symptom in the diagnosis of depression (affective PSD) using Self-rating Depression (SDS), which is a widely used self-report questionnaire used to measure depression (Kitamura et al. 2004). On the other hand, "loss of interest" is a less sensitive symptom than "depressive mood" using SDS and is thought to be a component of apathy, which is often observed after stroke and is defined as reduced motivation and lack of initiative and exploration (Starkstein et al. 1993; Yamagata et al. 2004).

In the present study, we examined the affective and apathetic dimensions of post-stroke depression (PSD) separately, and evaluated their correlation with basal ganglia or frontal lobe damage in stroke patients.

Subjects and methods

■ Patients

The approval of our institutional ethics committee was obtained for this prospective study. Informed consent was obtained from all patients. Patients included in this study were selected from a consecutive series of 408 patients with hemorrhagic or occlusive stroke, who were diagnosed using computed tomography (CT), and who were admitted to the Nishi-Hiroshima Rehabilitation Hospital less than 3 months after suffering their stroke. Exclusion criteria included (1) history of major psychiatric illness (seven patients); (2) medical illness (four patients) or speech impediment (117 patients) that may affect cognitive function and ability to provide consent; (3) subarachnoid hemorrhage (25 patients); (4) physical disability which precludes cognitive testing (12 patients). The remaining 243 patients were included as subjects in this study.

■ CT findings

The study had a naturalistic design using 243 CT scans. As such a high sample size would not have been possible with MR, the CT technique used is adequate. CT scanning was performed on all patients at admission (with a follow-up CT scan every 1-3 months after admission). Damage to the basal ganglia or frontal lobe (including lacunar infarcts) was defined as a sharply demarcated hypodense lesion with a diameter >5 mm on CT. We classified patients into four groups according to the degree of basal ganglia or frontal lobe damage: no damage, damage to the left side only, damage to the right side only, and damage to the bilateral basal ganglia or frontal lobe. The measurement of the volume of CT-defined LDA was calculated according to the formula $0.5 \times A \times B \times C$; where A and B represent the largest perpendicular diameters and C is the thickness (Montaner et al. 2001).

■ Psychological assessment

We used the Japanese version of the SDS to examine the subjective severity of affective depression (Yamaguchi et al. 1992) and used a Japanese version of the apathy scale (AS) to quantify the apathetic state (Yamagata et al. 2004). We classified the patients into two groups according to their test scores: a non-depressed group (SDS score < 45 points) and a depressed group (SDS score ≥ 45 points), and a non-apathetic group (apathy score < 16 points) and an apathetic group (apathy score ≥ 16 points). The cut-off point was determined on the basis of a previous report on Japanese stroke patients (Yamaguchi et al. 1992).

■ Statistical analyses

Different degrees of basal ganglia or frontal lobe damage (none, left only, right only and bilateral) were compared with SDS or AS scores by one-way analysis of variance (ANOVA) followed by a post-hoc Fisher protected least significant difference test (Fisher PLSD test). Values were considered to be significant at $P < 0.05$. The Stat View 5.0 (SAS Institute, Inc., Cary, NC) statistical package was used for all analyses.

Results

■ Baseline structures and the frequency of PSD in all patients

The subjects consisted of 162 males and 81 females (age: 65.2 ± 11.3, past history of stroke: 27 cases (11.1%), time interval between onset and admission: range 7–90 days, mean 40.7 ± 19.6 days). SDS and AS scores over the cut-off limits were observed in 79 (32.5%) and 98 (40.3%) patients, respectively. Of these, 50 patients (20.6%) showed elevation of both SDS and AS, therefore 126 patients (51.9%) were found to have affective and/or apathetic PSD.

■ The effects of lesion location on affective and/or apathetic PSD

Computed tomography densities of left side and cortical lesions (especially middle cerebral arterial territory damage; e.g., temporal lobe) were found to be greater in speech impaired patients (excluded from this study) than in other patients.

The severity of affective or apathetic PSD was related to CT-defined lesion volume (Mann–Whitney U-test, $P < 0.02$), consistent with a previous report (Nys et al. 2005). But no association could be demonstrated between severity of PSD and lesion location involving supra-or infra-tentorial LDA (Mann–Whitney U-test, $P > 0.2$), so further examinations were made of supratentorial stroke lesions in the frontal lobe and basal ganglia.

The SDS score increased significantly from none, right side only, left side only, to bilateral frontal lobe damage ($P = 0.0450$, ANOVA test) (Fig. 1). Post-hoc testing (Fisher's test) showed a difference between no damage and damage to the left side only ($P = 0.0237$). However, we did not find significant differences in AS scores in relation to frontal lobe damage.

The AS score increased significantly from none, left side only, right side only, to bilateral BG damage ($P = 0.0061$, ANOVA test) (Fig. 1). Post-hoc testing (Fisher's test) showed a difference between no damage and bilateral BG damage ($P = 0.0024$), and between left side only and bilateral BG damage ($P = 0.0075$). However, we did not find significant differences in SDS scores in relation to BG damage.

Discussion

We found that the severity of affective depression (SDS score) was associated with left frontal lobe damage, but not damage to the basal ganglia. Apathetic depression (AS score) was not related to frontal lobe damage but was related to damage to the basal ganglia in both the right and left hemispheres.

Although there is conflicting evidence as to whether the risk of depression after stroke is influenced by the location of the brain lesion, several explanations can be proposed for our findings. Soares and Mann (1997) suggested that functional abnormalities in frontal, subcortical, and limbic structures appear to be part of the pathophysiology of depression. Robinson et al. (1984) and Starkstein et al. (1987) exam-

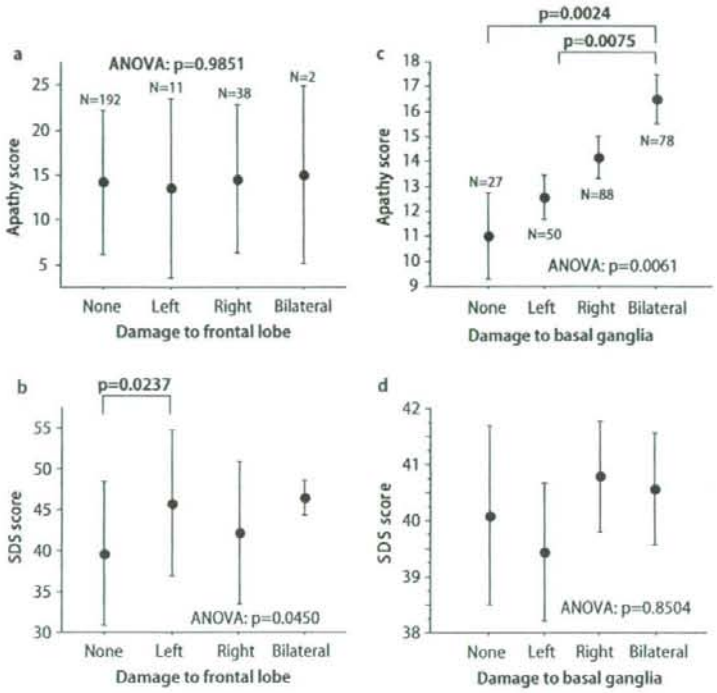

Fig. 1 Differences in apathy score (**A, C**) and SDS score (**B, D**) between patients with no damage, left side only, right side only, and bilateral damage to the frontal lobe (**A, B**) or basal ganglia (**C, D**). The midpoint, top and bottom of each vertical line represent the mean, upper, and lower 95% CI values, respectively. ANOVA of the four frontal lobe or basal ganglia damage subgroups shows significant results for SDS (**B**) or AS (**C**). The Fisher PLSD test also indicates that these parameters can distinguish between some of these SDS or AS subgroups, with the *P*-values given

ined depression in stroke patients using SDS, and their findings suggested a strong correlation between the severity of depression and proximity of the lesion to the frontal pole. Other studies also noted a significant association between strokes affecting the frontal lobe (or anterior part) of the left hemisphere and PSD (Alexopoulos et al. 1997). These reports agree with our findings that "depressed mood (affective PSD)" is associated with the left frontal lobe.

Starkstein et al. (1993) suggested that apathy was significantly associated with lesions involving the posterior limb of the internal capsule. Later, Yamagata et al. (2004) examined the relationship between apathy after subcortical stroke and neural orienting response to novel events using an event-related evoked potential technique, suggesting that apathy after subcortical (include basal ganglia) stroke is intimately linked to dysfunction of the frontal-subcortical system. These findings agree with our data, indicating that damage to basal ganglia leads to dysfunction of the frontal-subcortical system, resulting in apathy after stroke.

Several methodological limitations of this study should be acknowledged. First, patients with severe comprehension deficits and/or severe speech impediments, who had different lesion patterns compared to the other patients, were excluded from the study. Therefore, the results of this study may be biassed. Second, patients enrolled in this study only underwent CT scanning (not MRI). Thus, the presence of lesions that could not be visualized by CT may have influenced our findings. Third, we used a self-report questionnaire to measure the level of depression. Thus, the absence of objective assessment may have influenced our findings.

In summary, this study found that after a stroke there are two separate core symptoms ("depressed mood" or "loss of interest") with different underlying neuroanatomical mechanisms. Therefore, in order to help patients gain independence, future studies should examine whether these different lesion correlates of affective or apathetic PSD, separately or together, may also be reflected in different patterns of treatment response.

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