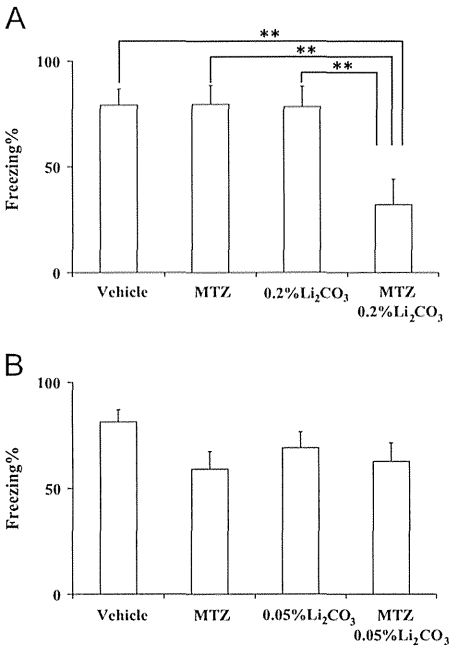

Fig. 3. Effect of subchronic lithium treatment (A, 0.2% Li_2CO_3 in diet; B, 0.05% Li_2CO_3 in diet) on mirtazapine (MTZ, 10 mg/kg)-induced inhibition of the expression of contextual conditioned freezing. Li_2CO_3 was administered p.o. in the diet for 7 days after the footshock. Mirtazapine (10 mg/kg) was administered intraperitoneally 30 min before testing. Data are represented as the mean ± S.E.M of freezing scored for a 5-min observation period. Behavior was sampled in 10-s intervals. $^{**}P < 0.01$. N=8 per group.

Table 1
The effect of the combination of mirtazapine (MTZ; 10 mg/kg) and 0.2% Li_2CO_3 on spontaneous motor activity.

Drug treatment	Motor activity (counts/5 min)
Vehicle–0% Li_2CO_3	83.4 ± 43.3
MTZ (10)–0% Li_2CO_3	139.1 ± 22.2
Vehicle–0.2% Li_2CO_3	34.9 ± 22.7
MTZ (10)–0.2% Li_2CO_3	78.7 ± 34.7

Two-way ANOVA showed no significant effect of mirtazapine, 0.2% Li_2CO_3 or interaction. All the data are represented as the mean ± S.E.M. $N=8$ per group.

et al., 1999). Taken together, these data suggest that the increasing effect of lithium on the anxiolytic-like effect of acute mirtazapine may also be associated with the enhancement of 5-HT neurotransmission.

Much evidence indicates that 5-HT_{1A} receptors play a key role in the serotonergic mechanism associated with the etiology of stress-related disorders (Popova and Naumenko, 2013). The effect of mirtazapine on the serotonergic system is reportedly mainly dependent on 5-HT_{1A} receptor function (Rauggi et al., 2005) because mirtazapine blocks 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptors (de Boer, 1996). Furthermore, our earlier study showed that the anxiolytic-like effect of the systemic administration of mirtazapine on contextual conditioned fear was inhibited by the co-administration of a selective 5-HT_{1A} receptor antagonist (Kakui et al., 2009), which demonstrated the significant involvement of postsynaptic 5-HT_{1A} receptors in the anxiolytic-like effect of mirtazapine. Blier et al. (1987) has reported that lithium treatment enhanced the sensitivity of postsynaptic 5-HT_{1A} receptors. Haddjeri et al. (2000) demonstrated that the addition of lithium to antidepressant drugs induced a greater enhancement of the tonic activation of postsynaptic 5-HT_{1A} receptors in the rat dorsal hippocampus than any drug given alone. Our previous behavioral study also showed that the inhibitory effect of MKC-242, a selective 5-HT_{1A} receptor agonist, on contextual conditioned freezing was enhanced by subchronic 0.2% Li_2CO_3 treatment (Muraki et al., 1999). Subchronic lithium-induced enhancement of the anxiolytic-like effect of mirtazapine may also be mediated by the enhancement of postsynaptic 5-HT_{1A} receptor function.

Mirtazapine is extensively metabolized principally in the liver by the cytochrome P450 (CYP) isoenzymes CYP3A4, CYP2D6 and CYP1A2 (Holm and Markham, 1999). Lithium is purely renally excreted with no hepatic component and lacks any inhibitory or inductive capabilities (Sandson et al., 2005). Hence, based on the individual pharmacokinetic characteristics of each drug, drug interaction due to pharmacokinetic interactions between lithium and mirtazapine is unlikely to occur. Indeed, a clinical study reported that there is no pharmacokinetic interaction between lithium and mirtazapine (Sitsen et al., 2000). Therefore, a pharmacodynamic mechanism is likely involved in the increase of the effects of mirtazapine by the addition of lithium.

In conclusion, in the present study, we investigated the combined effect of subchronic lithium treatment and acute mirtazapine treatment in the rat contextual conditioned fear stress model. Subchronic 0.2% Li_2CO_3 treatment significantly enhanced the anxiolytic-like effect of mirtazapine on contextual conditioned fear, similar to how subchronic lithium enhanced the anxiolytic-like effect of the SSRI citalopram (Muraki et al., 1999). Our results provide further evidence for the lithium augmentation of mirtazapine, and this augmentation therapy may be demonstrated to be useful in the treatment of anxiety disorders.

Acknowledgments

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References

An, Y., Inoue, T., Kitaichi, Y., Izumi, T., Nakagawa, S., Song, N., Chen, C., Li, X., Koyama, T., Kusumi, I, 2013. Anxiolytic-like effect of mirtazapine mediates its effect in the median raphe nucleus. Eur. J. Pharmacol. 720, 192–197.
Bauer, M., Adli, M., Bschor, T., Pilhatsch, M., Pfennig, A., Sasse, J., Schmid, R., Lewitzka, U., 2010. Lithium's emerging role in the treatment of refractory major depressive episodes: augmentation of antidepressants. Neuropsychobiology 62, 36–42.
Blier, P., de Montigny, C., Tardif, D, 1987. Short-term lithium treatment enhances responsiveness of postsynaptic 5-HT_{1A} receptors without altering 5-HT autoreceptor sensitivity: an electrophysiological study in the rat brain. Synapse 1, 225–232.
Bruijn, J.A., Moleman, P., Mulder, P.G., van den Broek, W.W., 1998. Comparison of 2 treatment strategies for depressed inpatients: imipramine and lithium addition or mirtazapine and lithium addition. J. Clin. Psychiatry 59, 657–663.
Chenu, F., Bourin, M., 2006. Potentiation of antidepressant-like activity with lithium: mechanism involved. Curr. Drug Targets 7, 159–163.
Davidson, J.R., Weisler, R.H., Butterfield, M.I., Casat, C.D., Connor, K.M., Barnett, S., van Meter, S., 2003. Mirtazapine vs. placebo in posttraumatic stress disorder: a pilot trial. Biol. Psychiatry 53, 188–191.
De Boer, T., 1996. The pharmacologic profile of mirtazapine. J. Clin. Psychiatry 57 (4), S19–S25 (Suppl).
Eroglu, L., Hizal, A., 1987. Antidepressant action of lithium in behavioral despair test. Pol. J. Pharmacol. Pharm. 39, 667–673.
Gambi, F., De Berardis, D., Campanella, D., Carano, A., Sepede, G., Salini, G., Mezzano, D., Cicconetti, A., Penna, L., Salerno, R.M., Ferro, F.M., 2005. Mirtazapine treatment of generalized anxiety disorder: a fixed dose, open label study. J. Psychopharmacol. 19, 483–487.
Graeff, F.G., Guimarães, F.S., De Andrade, T.G., Deakin, J.F., 1996. Role of 5-HT in stress, anxiety, and depression. Pharmacol. Biochem. Behav. 54, 129–141.
Haddjeri, N., Szabo, S.T., de Montigny, C., Blier, P, 2000. Increased tonic activation of rat forebrain 5-HT1A receptors by lithium addition to antidepressant treatments. Neuropsychopharmacology 22, 346–356.
Holm, K.J., Markham, A., 1999. Mirtazapine: a review of its use in major depression. Drugs 57, 607–631.

Inoue, T., Kitaichi, Y., Koyama, T., 2011. SSRIs and conditioned fear. Prog. Neuropsychopharmacol. Biol. Psychiatry 35, 1810–1819.
Kakui, N., Yokoyama, F., Yamauchi, M., Kitamura, K., Imanishi, T., Inoue, T., Koyama, T., 2009. Anxiolytic-like profile of mirtazapine in rat conditioned fear stress model: functional significance of 5-hydroxytryptamine1A receptor and alpha1-adrenergic receptor. Pharmacol. Biochem. Behav. 92, 393–398.
Kitaichi, Y., Inoue, T., Nakagawa, S., Izumi, T., Koyama, T., 2004. Effect of co-administration of lithium and reboxetine on extracellular monoamine concentrations in rats. Eur. J. Pharmacol. 489, 187–191.
Kitaichi, Y., Inoue, T., Nakagawa, S., Izumi, T., Koyama, T., 2006. Effect of co-administration of subchronic lithium pretreatment and acute MAO inhibitors on extracellular monoamine levels and the expression of contextual conditioned fear in rats. Eur. J. Pharmacol. 532, 236–245.
Kornstein, S.G., Schneider, R.K., 2001. Clinical features of treatment-resistant depression. J. Clin. Psychiatry 62 (Suppl 16), S18–S25.
Muraki, I., Inoue, T., Hashimoto, S., Izumi, T., Ito, K., Koyama, T., 2001. Effect of subchronic lithium treatment on citalopram-induced increases in extracellular concentrations of serotonin in the medial prefrontal cortex. J. Neurochem. 76, 490–497.
Muraki, I., Inoue, T., Hashimoto, S., Izumi, T., Ito, K., Ohmori, T., Koyama, T., 1999. Effect of subchronic lithium carbonate treatment on anxiolytic-like effect of citalopram and MKC-242 in conditioned fear stress in the rat. Eur. J. Pharmacol. 383, 223–229.
Ohmori, T., Abekawa, T., Muraki, A., Koyama, T., 1994. Competitive and noncompetitive NMDA antagonists block sensitization to methamphetamine. Pharmacol. Biochem. Behav. 48, 587–591.
Popova, N.K., Naumenko, V.S., 2013. 5-HT_{1A} receptor as a key player in the brain 5-HT system. Rev. Neurosci. 24, 191–204.
Price, L.H., Charney, D.S., Delgado, P.L., Heninger, G.R., 1990. Lithium and serotonin function: implications for the serotonin hypothesis of depression. Psychopharmacology 100, 3–12.
Rauggi, R., Cassanelli, A., Raone, A., Tagliamonte, A., Gambarana, C., 2005. Study of mirtazapine antidepressant effects in rats. Int. J. Neuropsychopharmacol. 8, 369–379.
Sandson, N.B., Armstrong, S.C., Cozza, K.L., 2005. An overview of psychotropic drug-drug interactions. Psychosomatics 46, 464–494.
Sitsen, J., Voortman, G., Timmer, C., 2000. Pharmacokinetics of mirtazapine and lithium in healthy male subjects. J. Psychopharmacol. 14, 172–176.
Suppes, T., Marangell, L.B., Bernstein, I.H., Kelly, D.I., Fischer, E.G., Zboyan, H.A., Snow, D.E., Martinez, M., Al Jurdi, R., Shivakumar, G., Sureddi, S., Gonzalez, R., 2008. A single blind comparison of lithium and lamotrigine for the treatment of bipolar II depression. J. Affect. Disord. 111, 334–343.
Thomsen, K., Olesen, O.V., 1974. Long-term lithium administration to rats. Lithium and sodium dosage and administration, avoidance of intoxication, polyuric control rats. Int. Pharmacopsychiatry 9, 118–124.
Wegener, G., Bandpey, Z., Heiberg, I.L., Mork, A., Rosenberg, R., 2003. Increased extracellular serotonin level in rat hippocampus induced by chronic citalopram is augmented by subchronic lithium: neurochemical and behavioural studies in the rat. Psychopharmacology 166, 188–194.
Yamauchi, M., Imanishi, T., Koyama, T., 2012. A combination of mirtazapine and milnacipran augments the extracellular levels of monoamines in the rat brain. Neuropharmacology 62, 2278–2287.
Zamorski, M.A., Albucher, R.C., 2002. What to do when SSRIs fail: eight strategies for optimizing treatment of panic disorder. Am. Fam. Physician 66, 1477–1484.

RESEARCH ARTICLE

International Journal of Geriatric Psychiatry

Delayed atrophy in posterior cingulate cortex and apathy after stroke

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Objective: A few studies have been performed on chronic structural changes after stroke. The primary purpose of the present study was to investigate regional cortical volume changes after the onset of stroke and to examine how the cortical volume changes affected neuropsychiatric symptoms.

Methods: Participants were 20 stroke patients and 14 control subjects. T1-MRI was performed twice, once at the subacute stage and again 6 months later, and whole brain voxel-based morphometric (VBM) analysis was used to detect significant cortical gray matter volume changes in patients. We also assessed the correlation between changes in cortical volumes and changes in neuropsychiatric symptoms during the 6 months following a stroke.

Results: In the present study, we found significant volume reductions in the anterior part of the posterior cingulate cortex (PCC) over the 6 months following a stroke by exploratory VBM analysis. We also found that the amount of volume change was significantly correlated with the change in apathy-scale scores during the 6 months poststroke.

Conclusions: The present study suggests that delayed atrophic change is evident in the PCC 6 months after a stroke. There was greater apathetic change in the stroke patients with the larger volume reductions. The delayed atrophy of the PCC may reflect degeneration secondary to neuronal loss due to stroke. Such degeneration might have impaired control of goal-directed behavior, leading to the observed increase in apathy. Copyright © 2014 John Wiley & Sons, Ltd.

Key words: stroke; apathy; magnetic resonance imaging; voxel-based morphometric analysis; posterior cingulate cortex
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Introduction

Stroke is one of the leading contributors to disease burden. A worldwide study in 2010 showed that stroke was the second most prevalent cause of death, representing an estimated 11.1% of all deaths (Lozano *et al.*, 2012). In respect to morbidity, stroke was found to be the fourth leading cause of lost DALYs (disability-adjusted life years) globally in nonpediatric populations (Mukherjee and Patil, 2011). Further, neuropsychiatric symptoms following stroke, such as cognitive impairment, depression and apathy, are associated with excess disability, cognitive impairment, and mortality in stroke patients (Hackett *et al.*, 2005; Pendlebury and Rothwell, 2009; van Dalen *et al.*, 2013).

Evidence concerning stroke has been compiled and is being applied to clinical practice. However, only a few studies on structural changes in the chronic stage

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exist. A recent study reported atrophic change in regions anatomically remote from the ischemic lesions (Kraemer *et al.*, 2004), which may reflect degeneration secondary to neuronal loss, possibly Wallerian degeneration. This may mean that degenerative cortical changes appear after an ischemic attack, and that this cortical damage produces a biological vulnerability to neuropsychiatric symptoms after a stroke. However, to our knowledge, there has been no studies that focused on where and how the degenerative cortical changes occurred and whether these affected neuropsychiatric symptoms after a stroke.

The primary purpose of the present study was to investigate regional cortical volume changes after the onset of stroke and to examine how they affected any neuropsychiatric symptoms developing in the patient after the stroke. T1-MRI was performed twice, once at the stage of subacute stroke and again 6 months after the stroke, and whole brain voxel-based morphometric (VBM) analysis was used to quantify cortical gray matter (GM) volume changes in patients during the 6-month period following the stroke. We also assessed the correlation between changes in cortical volumes and any changes in neuropsychiatric symptoms.

We hypothesized that regions, such as cingulate cortex, which have unusually extensive connectivity with other areas, would show especially large degenerative cortical changes in stroke due to simultaneous loss of connections from many spatially distinct sites individually affected by the stroke. The regions of degenerative cortical change might relate to the network abnormalities underlying poststroke depression/apathy, which is a common and serious emotional symptom following stroke.

Methods

Subjects

After the study had been completely described to the subjects, and before enrollment, written informed consent was obtained. The study was approved by the Medical Ethics Committee of the National Cerebral and Cardiovascular Center of Japan. The patients were of Japanese ethnicity and were recruited from the neurology unit of the National Cerebral and Cardiovascular Center hospital. These patients had initially been hospitalized for treatment of acute ischemic stroke.

Stroke is diagnosed by neurologists according to the World Health Organization (WHO) criteria. After the assessment, a group of psychiatrists and neurologists reviewed the data and reached a consensus regarding the presence or absence of psychiatric disease, including dementia, according to DSM-IV criteria. Patients were included if they met the following criteria: (i) a focal lesion of either the right or left hemisphere on MRI; (ii) absence of other neurologic, neurotoxic, or metabolic conditions; (iii) modest ischemic insult (modified Rankin scale ≤ 4) with absence of a significant verbal comprehension deficit; and (iv) occurrence of stroke 10–28 days before the first examinations. Exclusion criteria were the following: (i) transient ischemic attack, cerebral hemorrhage, subdural hematoma, or subarachnoid hemorrhage; (ii) history of a central nervous system disease, such as tumor, trauma, hydrocephalus, Parkinson's disease, etc.; and (iii) any pre-stroke history of depression/apathy. Twenty stroke patients who fulfilled the criteria and completed the series of examinations were included in this study. Fourteen control subjects were recruited who completed a series of examinations for the 6-month follow-up study. Exclusion criterion for the control subjects was a history or present diagnosis of any DSM-IV axis-I or neurological illness.

MRI examinations were conducted twice for all patients and control subjects, once at the subacute stage (10–28 days after onset) and again at the chronic stage (6 months after onset). The lesion location was established from MRI data, and its volume was calculated from a volume of interest manually delineated on the lesion. There were no changes in medication usage between baseline and follow-up, and no patient or control was on antidepressant treatment during the examinations. All patients were subjected to a neurological examination [modified Rankin scale, (mRS) (Brott *et al.*, 1989); National Institutes of Health Stroke Scale, NIHSS (Goldstein and Samsa, 1997)] on the day of the MRI scan. All patients and control subjects were administered a series of standardized, quantitative measurements of depressive symptoms [Zung Self-rating Depression Scale, SDS (Zung, 1965); Apathy scale (Starkstein *et al.*, 1993); and mini-mental state examination (for cognitive function), MMSE (Folstein *et al.*, 1975)] on the day of the MRI scan.

MRI data acquisition

All MRI examinations were performed using a 3.0-Tesla whole-body scanner (Signa Excite HD V12M4; GE Healthcare, Milwaukee, WI, USA) with an 8-channel phased-array brain coil. High-resolution three-dimensional T1-weighted images were acquired using a spoiled gradient-recalled sequence ($TR = 12.8$ ms, $TE = 2.6$ ms, flip angle = 8°, $FOV = 256$ mm; 188 sections in the sagittal plane; acquisition matrix, 256×256; acquired

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resolution, $1 \times 1 \times 1$ mm). T2-weighted images were obtained using a fast-spin echo ($TR = 4{,}800$ ms; $TE = 101$ ms; echo train length (ETL) = 8; $FOV = 256$ mm; 74 slices in the transverse plane; acquisition matrix, 160×160, acquired resolution, $1 \times 1 \times 2$ mm).

Image processing

Image preprocessing and statistical analyses were carried out using SPM8 software (Wellcome Department of Imaging, Neuroscience Group, London, UK; http://www.fil.ion.ucl.ac.uk/spm), and VBM was carried out using the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm.html) with default parameters. Images were bias-corrected, tissue classified, and registered using linear (12-parameter affine) and nonlinear transformations (warping), within a unified model (Ashburner and Friston, 2005). Subsequently, analyses were performed on GM segments, which were multiplied by the nonlinear components derived from the normalization matrix in order to preserve actual GM values locally (modulated GM volumes). Finally, the modulated volume was smoothed with a Gaussian kernel of 5 mm full-width at half-maximum. The voxel size of the final images was $1.5 \times 1.5 \times 1.5$ mm.

Voxel-wise GM differences before and after a 6-month period beginning shortly after the stroke were examined using paired t-tests. To avoid possible edge effects between different tissue types, we excluded all voxels with GM values of less than 0.2 (absolute threshold masking). As this was a hypothesis-led analysis, we applied a liberal threshold of $p < 0.001$ with an extent of 25 voxels across the whole brain.

Spherical volumes of interest (VOIs) were determined from regions where a significant volume change over the 6-month period was found in patients. The centers of the spherical VOIs were determined from the Montreal Neurological Institute coordinate with peak t-value. The radius of the spherical VOI was determined in accordance with size of the clusters revealed by the analysis. The regional volume was calculated by averaging the values for all voxels within the spherical VOIs.

Statistical analysis

To identify demographic variables distinguishing patients and controls, group differences in demographic characteristics were examined by unpaired t-test and Pearson χ^2-test. To identify changes in neuropsychiatric symptoms and to confirm the SPM8 results on changes in cortical volumes during the first 6 months after a stroke, the psychometric scores and gray matter volumes of spherical VOIs in patients and controls over 6 months were examined by paired t-test. The group differences in changes in the volumes of spherical VOIs over 6 months were examined with repeated-measures analysis of variance.

To examine the relationship between the fractional volume change of VOIs where a significant volume change was found in patients [(volume at 2nd test - volume at 1st test)/volume at 1st test] and the fractional change of depression/apathy scale scores [(scores at 2nd test - scores at 1st test)/scores at 1st test] in patients and controls, we performed Pearson's correlation analysis. Bonferroni correction was applied to avoid type I errors because of the multiplicity of statistical analyses. All statistical tests were 2-tailed and reported at $p < 0.05$. Statistical analysis of the data was performed using SPSS for Windows 21.0 (IBM Japan Inc., Tokyo, Japan).

Results

Demographic and clinical data

Table 1 summarizes the demographic and clinical characteristics of the study subjects. Patients did not differ significantly from controls in age, sex, education, or MMSE scores. On the psychometric scales, patients had worse scores on SDS and apathy scales when compared with controls. Moreover, a history of hypertension was significantly more prevalent in patients than in controls. As shown by the mRS/NIHSS scores, patients showed some disability due to stroke at the time of the initial examination. All of the patients were receiving anticoagulant and/or antiplatelet medication. The mean total volume of infarction was 1.8 ± 1.2 mL.

The locations of the patients' infarctions were restricted to subcortical regions, including the basal ganglia (50.0%), subcortical white matter (40.0%), and thalamus (10.0%). This is because our studies focused on degenerative cortical gray matter changes remote from the primary ischemic lesions therefore, including cases of cortical infarction would make interpretation of the results difficult. In 13 of the patients, the infarction was located in the left hemisphere.

Changes in psychometric scores and regional gray matter volumes over 6 months

As shown in Table 2, we found significant improvement in mRS score, NIHSS score, and MMSE score, while there was no significant change in depression or apathy scale scores overall in patients during the 6 months following the stroke. Two patients at the 1st and one patient

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Table 1 Demographic characteristics of patients and control subjects at baseline

Characteristic	Stroke patients ($n=20$)	Control subjects ($n=14$)	t or χ^2	p
Age (y)	69.2 ± 8.5	72.4 ± 3.0	$t=-1.53$	0.14
Female sex (n, %)	4 (20.0)	6 (42.9)	$\chi^2=2.07$	0.15
Education (years)	12.5 ± 3.5	12.3 ± 2.7	$t=0.15$	0.88
MMSE score	27.5 ± 3.4	29.1 ± 1.3	$t=-1.99$	0.06
SDS	27.8 ± 6.1	21.7 ± 1.9	$t=4.17$	<0.001***
Apathy score	9.4 ± 4.0	5.4 ± 4.0	$t=2.84$	0.008**
History of disease, No (%)				
Diabetes mellitus	3 (15.0)	0 (0.0)	$\chi^2=2.30$	0.13
Hyperlipidemia	3 (15.0)	0 (0.0)	$\chi^2=2.30$	0.13
Hypertension	14 (70.0)	1 (0.1)	$\chi^2=13.2$	<0.001**
mRS score	2.1 ± 0.8	—		
NIHSS score	2.5 ± 1.8	—		
Volume of acute infarcts (mL)	1.8 ± 1.2	—		
Acute infarcts (n, %) in:				
Basal ganglia	10 (50.0)	—		
Subcortical white matter	8 (40.0)	—		
Thalamus	2 (10.0)	—		
Laterality of acute hemisphere infarcts				
Left hemisphere (n, %)	13 (65.0)	—		

MMSE, Mini-Mental State examination; SDS, Zung Self-rating Depression Scale; mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale.
Data are mean ± sd.
*, $p<0.05$;
**, $p<0.01$;
***, $p<0.001$.

Table 2 Changes in psychometry scores and PCC volume over 6 months in patients ($n=20$) and controls ($n=14$)

	10–28 days after stroke	6 months after first exam	paired t-test (t)	p
Patients				
mRS score	2.1 ± 0.8	1.6 ± 0.6	3.68	0.002**
NIHSS score	2.5 ± 1.8	1.0 ± 0.8	4.41	<0.001***
MMSE score	27.5 ± 3.5	28.9 ± 2.1	−2.32	0.03*
SDS	27.8 ± 6.1	28.1 ± 9.8	−0.17	0.87
Apathy score	9.4 ± 4.0	9.4 ± 4.3	−0.05	0.96
Volume of PCC	0.40 ± 0.07	0.38 ± 0.06	5.60	<0.001***
Controls				
MMSE score	29.1 ± 1.3	29.6 ± 0.5	−1.53	0.15
SDS	21.7 ± 1.9	22.2 ± 2.4	−1.20	0.25
Apathy score	5.4 ± 4.0	5.1 ± 3.5	0.64	0.53
Volume of PCC	0.43 ± 0.06	0.43 ± 0.06	−0.44	0.67

mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale; MMSE, Mini-Mental State examination; SDS, Zung Self-rating Depression Scale; PCC, posterior cingulate cortex.
Data are mean ± sd.
*, $p<0.05$;
**, $p<0.01$;
***, $p<0.001$.

at the 2nd examination had clinically relevant depression (SDS score >=40), while three patients at the 1st and two at the 2nd examination had clinically relevant apathy (apathy scale score >=14). We found no significant changes in MMSE, depression scale, or apathy scores in control subjects over the 6-month study.

Voxel-based analysis revealed a significant reduction in volume of the anterior part of the PCC in the patients 6 months after the stroke [$(x, y, z)=(-3, -10, 33)$, cluster voxel size = 38, $T=4.77$] (Figure 1). The radius of the spherical VOI was determined to be 3 mm, so that the volume of this size of VOI

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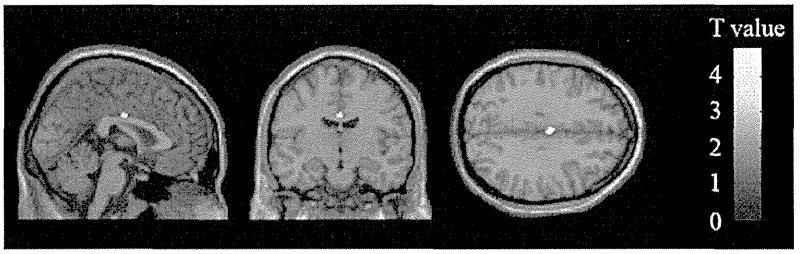

Figure 1 Gray matter volume changes in stroke patients over 6 months, by voxel-based analysis. Images are presented in radiological orientation. Detected areas exceed an uncorrected p-value of 0.001 in 25 or more contiguous voxels. These statistical parametric mapping projections are then superimposed on representative transaxial ($z = 33$), sagittal ($x = -3$), and coronal ($y = -10$) magnetic resonance images.

(113.04 mm^3) almost fit the volume of the cluster (128.25 mm^3). We found a significant reduction of volume of spherical VOIs in the PCC in patients ($p < 0.001$), but not in controls by paired t-test (Table 2; Figure 2). There was no significant difference in VOI volumes between patients and controls at the first examination by unpaired t-test ($t = 1.20$, $p = 0.24$), but the difference became significant after 6 months ($t = 2.36$, $p = 0.024$). We found a significant group effect on the raw volume change in PCC over 6 months by repeated-measures analysis of variance (group-by-volume interaction, $F_{1,32} = 14.2$; $p < 0.001$).

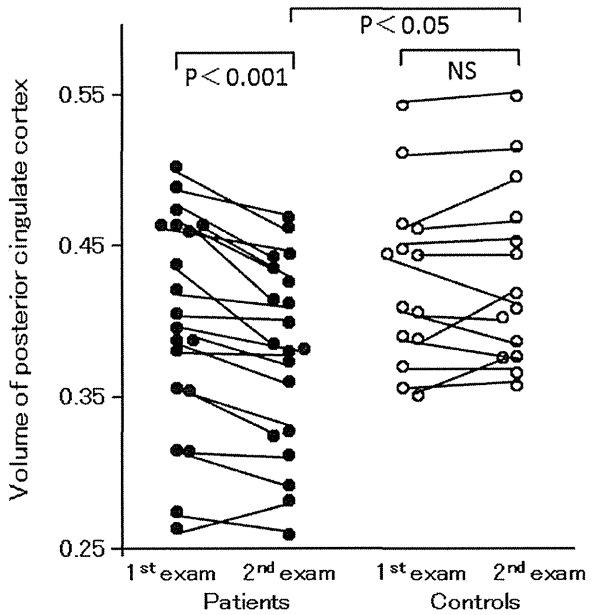

Figure 2 Scatterplots showing volume changes in cingulate cortex over 6 months in patients and controls. We placed spherical VOIs (3-mm radius) on the region where we found significant reduction in volume by voxel-based analysis over 6 months in the patient group. Examining these voxels, we find a significant reduction of gray matter volume in patients ($p < 0.001$) but not in controls by paired t-test. We find a significant group difference in volume only at the 2nd examination.

Relation between volume and apathy scale

We found a significant negative relationship between the fractional change of scored apathy and that of volume change in the PCC VOIs in patients ($r = -0.58$, $p = 0.007$), but not in controls ($r = -0.29$, $p = 0.32$) (Figure 3). When we considered the confounding effects of age, sex, laterality of the infarction, and acute stroke size as covariates in a partial correlation analysis, the above negative relationship remained significant in patients ($r = -0.51$, $p = 0.04$). We found no significant relationships between the fractional change of SDS scores and those of any VOIs in patients or controls.

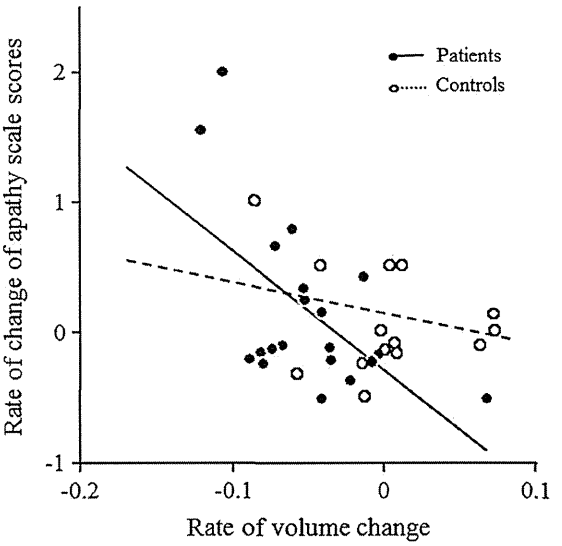

Figure 3 Scatterplots showing the relation between volume change in cingulate cortex and apathy score change over 6 months in patients and controls. A significant correlation is observed between VOI volume change and apathy score change over 6 months in patients ($r = -0.58$, $p = 0.007$), but not in controls ($r = -0.29$, $p = 0.32$) ($y = -9.1 \times x - 0.3$ for patients, $y = -2.6 \times x - 0.1$ for controls). The correlation in patients is still significant after partial correlation analysis with age, sex, laterality of the infarction, and acute stroke size as covariates ($r = -0.51$, $p = 0.04$).

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When we divided the patients into two groups whose volume reduction at PCC was less than ($n = 9$) or greater than ($n = 11$) the average (difference in fractional volume change = −0.05), we found a significant difference in the fractional change of scored apathy between the groups (less-than-average group, change = −0.17; greater-than-average group, change = 0.43; $t = 2.41$; $p = 0.03$). When we considered the confounding effects of age, sex, laterality of the infarction, and acute stroke size as covariates in a one-way analysis of covariance the change in the apathy scores between groups showed a trend that did not reach significance ($F_{1, 14} = 3.2, p < 0.1$).

Discussion

In the present study, we found a significant volume reduction in the anterior part of the PCC over the 6 months following a stroke, by exploratory VBM analysis. Furthermore, the fractional volume change was observed to be negatively correlated with the apathy scale scores during the 6 months after the stroke. The reduced volume of PCC due to the 6-month interval was associated with increased apathy scores. Our findings indicate that the neuronal changes in PCC after stroke are one of the factors that affect the degree of poststroke apathy.

In the patients, delayed atrophy was observed in a part of the PCC anatomically remote from the respective subcortical infarct site. This finding may reflect degeneration secondary to neuronal loss, possibly due to Wallerian degeneration, which is a degeneration of distal parts of nerve axons after injury of the proximal axon or cell body (Thomalla *et al.*, 2004). Axonal degeneration, in turn, leads to the death of postsynaptic cell bodies (Raff *et al.*, 2002) and should result in a secondary volume reduction in the part of the brain constituting the projection target of the lost axons. From an anatomical perspective, the PCC has dense structural connections to many other brain regions, suggesting a role as a structural hub (Hagmann *et al.*, 2008). Its volume reduction seen here may reflect the simultaneous loss of multiple afferent projections due to stroke in spatially different sites.

We found more apathetic change in the group of stroke patients with larger volume reductions in PCC. A recent meta-analysis showed that apathy occurred in almost every third patient after a stroke (van Dalen *et al.*, 2013). Because poststroke apathy can have a negative effect on the rehabilitation of activities of daily living or quality of life (Samus *et al.*, 2005; Hama *et al.*, 2007), poststroke apathy has attracted considerable attention. Marin has described apathy as a neuropsychiatric syndrome characterized by diminished goal-directed overt behavior, diminished goal-directed cognition, and diminished emotional concomitants of goal-directed behavior (Marin, 1991).

How is the volume reduction of PCC related to the increase in apathy of stroke patients? The PCC is a hub within the brain, connecting networks that function together to support complex behavior (Hagmann *et al.*, 2008). Leech *et al.* (2011) reported that the PCC is subdivided into dorsal and ventral parts differing in the regions to which they functionally connect. The dorsal part of the PCC is consistent with the region showing the volume reduction in stroke patients in the present study. Anatomically, the dorsal part of the PCC has connectivity with the ventral medial prefrontal cortex, part of the default mode network (DMN), and frontal and parietal regions involved in the cognitive control network (CCN). Abnormalities have been identified in CCN and DMN during episodes of late-life depression that have often been characterized as apathy (Alexopoulos *et al.*, 2012), and the PCC was suggested to have an important role in the regulation of these two networks necessary for controlling efficient goal-directed behavior (Leech *et al.*, 2011). We speculate that the degeneration of the PCC following a stroke might impair the function of control of goal-directed behavior, leading to increased apathy scores.

Our study has some limitations. First, we could not find volume reductions in any other regions of the brain than the PCC. However, the sample size in our study was not large enough to reveal moderate-sized differences between groups. Further study with increased numbers of subjects will be necessary for drawing any definitive conclusions. Second, we could not find associations between cognitive scales and the volume reduction at PCC. We assessed cognitive deficits using MMSE scores, but this test is not highly sensitive to differences between persons with normal and higher performances; thus, the possible presence of a ceiling effect must be considered. Extensive neuropsychological testing is needed for the assessment of cognitive dysfunction. Finally, we were not able to control precisely for important variables, such as social support, premorbid personality, and prior medication histories. Further analysis in light of these points is needed to confirm our present findings.

Conclusion

The present study suggests that delayed atrophic change in the PCC is evident 6 months after a stroke. We also found that the fractional volume change during the 6 months following a stroke was negatively correlated with apathy scale scores. The larger the volume reduction in PCC, the

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greater was the increase in apathy-scale scores. The delayed atrophy of part of the PCC seen here may reflect degeneration secondary to neuronal loss due to stroke. Damage in this area may damage control of goal-directed behavior, and it is plausible that such a defect would appear clinically as greater apathy. Knowledge of secondary brain degeneration in stroke patients and its impact on the adverse outcome of development of apathy could provide clues for recognizing new therapeutic targets.

Conflict of interest

None declared.

Key points

- We found significant volume reduction in the anterior part of the PCC over 6 months after the incidence of stroke in patients by exploratory VBM analysis.
- We also found that the rate of volume change was significantly correlated with the apathy scale scores during 6 months post-stroke.
- The delayed atrophy of the PCC may reflect degeneration secondary to neuronal loss due to stroke, and it might deteriorate the function of controlling goal-directed behavior related to apathetic change.

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References

Alexopoulos GS, Hoptman MJ, Kanellopoulos D, *et al.* 2012. Functional connectivity in the cognitive control network and the default mode network in late-life depression. *J Affect Disord* **139**: 56–65.
Ashburner J, Friston KJ. 2005. Unified segmentation. *Neuroimage* **26**: 839–851.
Brott T, Adams HPJ, Olinger CP, *et al.* 1989. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke* **20**: 864–870.
Folstein MF, Folstein SE, McHugh PR. 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* **12**: 189–198.
Goldstein LB, Samsa GP. 1997. Reliability of the National Institutes of Health Stroke Scale. Extension to non-neurologists in the context of a clinical trial. *Stroke* **28**: 307–310.
Hackett ML, Yapa C, Parag V, *et al.* 2005. Frequency of depression after stroke: a systematic review of observational studies. *Stroke* **36**: 1330–1340.
Hagmann P, Cammoun L, Gigandet X, *et al.* 2008. Mapping the structural core of human cerebral cortex. *PLoS Biol* **6**: 1479–1493.
Hama S, Yamashita H, Shigenobu M, *et al.* 2007. Depression or apathy and functional recovery after stroke. *Int J Geriatr Psychiatry* **22**: 1046–1051.
Kraemer M, Schormann T, Hagemann G, *et al.* 2004. Delayed shrinkage of the brain after ischemic stroke: preliminary observations with voxel-guided morphometry. *J Neuroimaging* **14**: 265–272.
Leech R, Kamourieh S, Beckmann CF, *et al.* 2011. Fractionating the default mode network: distinct contributions of the ventral and dorsal posterior cingulate cortex to cognitive control. *J Neurosci* **31**: 3217–3224.
Lozano R, Naghavi M, Foreman K, *et al.* 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380**: 2095–2128.
Marin RS. 1991. Apathy: a neuropsychiatric syndrome. *J Neuropsychiatry Clin Neurosci* **3**: 243–254.
Mukherjee D, Patil CG. 2011. Epidemiology and the global burden of stroke. *World Neurosurg* **76**: S85–S90.
Pendlebury ST, Rothwell PM. 2009. Prevalence, incidence, and factors associated with pre-stroke and post-stroke dementia: a systematic review and meta-analysis. *Lancet Neurol* **8**: 1006–1018.
Raff MC, Whitmore AV, Finn JT. 2002. Axonal self-destruction and neurodegeneration. *Science* **296**: 868–871.
Samus QM, Rosenblatt A, Steele C, *et al.* 2005. The association of neuropsychiatric symptoms and environment with quality of life in assisted living residents with dementia. *Gerontologist* **45**: 19–26.
Starkstein SE, Fedoroff JP, Price TR, *et al.* 1993. Apathy following cerebrovascular lesions. *Stroke* **24**: 1625–1630.
Thomalla G, Glauche V, Koch MA, *et al.* 2004. Diffusion tensor imaging detects early Wallerian degeneration of the pyramidal tract after ischemic stroke. *Neuroimage* **22**: 1767–1774.
van Dalen JW, Moll van Charante EP, Nederkoorn PJ, *et al.* 2013. Poststroke apathy. *Stroke* **44**: 851–860.
Zung WW. 1965. A self-rating depression scale. *Arch Gen Psychiatry* **12**: 63–70.

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ORIGINAL ARTICLE

Microstructural abnormality in white matter, regulatory T lymphocytes, and depressive symptoms after stroke

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Key words: *diffusion tensor imaging, fractional anisotropy (FA), magnetic resonance imaging (MRI), post-stroke depression, regulatory T lymphocytes (T_{reg}), stroke.*

Abstract

Background: The purpose of the present study was to investigate the existence of microstructure abnormalities in the white matter circuit in stroke patients and its relationship to depressive episodes. To target the prevention of depression, we also investigated the relationship between lymphocyte subsets and cerebral abnormalities in patients.

Methods: Participants included 18 patients with acute ischemic stroke and 22 healthy control subjects. Diffusion tensor imaging was performed. Whole-brain voxel-based analysis was used to compare fractional anisotropy (FA) between groups. Blood samples were obtained, and the lymphocyte subsets were evaluated using flow cytometry. Follow-up examinations were conducted on 12 patients at 6 months.

Results: FA was decreased in the bilateral anterior limb of the internal capsule in stroke patients. At the 6-month follow-up examination, there was a significant increase in FA, which was associated with a lower depression scale score. Patients showed a decreased percentage of circulated regulatory T lymphocytes, and the degree of reduction was related to the decrease in the FA value in the internal capsule.

Conclusions: FA reductions in the anterior limb of the internal capsule cause abnormality in the frontal-subcortical circuits and confer a biological vulnerability, which in combination with environmental stressors results in the onset of depression. Our findings also demonstrated the possibility of preventing post-stroke depression by targeting the role of regulatory T lymphocytes in brain tissue repair and regeneration after stroke.

INTRODUCTION

Stroke is the third leading cause of death and the most frequent cause of permanent disability in adults worldwide.[1] Depression is common and serious emotional symptom following stroke and is associated with excess disability, cognitive impairment, and mortality.[2] Despite considerable advances in understanding the pathophysiology of cerebral ischemia, therapeutic options for stroke and its related emotional symptoms are still limited. Inflammatory mechanisms activated after brain ischemia represent a key target of current translational cerebrovascular research. Stroke induces profound local inflammatory response involving various types of immune cells that transmigrate across the activated blood–brain barrier to invade the brain.[3]

In attempts to target the prevention of cerebral damage due to stroke, several factors related to inflammation have received considerable attention.[4–6] T lymphocytes are especially central to the development of a sustained inflammatory response in brain injury after a stroke. T cells are sources of pro-inflammatory cytokines and cytotoxic substances, such as reactive oxygen species, in the brain after a stroke; these likely contribute to neuronal death and poor outcomes. However, recent evidence has

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indicated a novel role of T cells in promoting brain tissue repair and regeneration in the weeks and months after a stroke.[7] The role of T lymphocytes in ischemic stroke is complex and remains poorly understood. More research is needed to gain a greater understanding of which T-cell subpopulations produce and prevent damage after a stroke.

The primary aim of the present study was to elucidate the microstructural abnormalities in the white matter circuit in stroke patients, as well as their relationship with depressive symptoms after a stroke. When abnormalities were found and a statistical association with post-stroke depressive symptoms was demonstrated, we investigated their relationship with circulating T lymphocytes. For identification of the microstructural abnormalities in stroke patients, diffusion tensor imaging was performed, and whole-brain voxel-based analysis was used to compare fractional anisotropy (FA) between acute ischemic stroke patients and healthy control subjects. Furthermore, we examined which circulating T-cell subpopulations showed differences in stroke patients when compared to healthy subjects and how such T-cell subpopulations are associated with microstructural abnormalities in the white matter of patients.

METHODS

Subjects

After the study was described to subjects, written informed consent was obtained. The study was approved by the medical ethics committee of the National Cerebral and Cardiovascular Center of Japan. The patients, all of whom were of Japanese ethnicity, were recruited from the neurology unit of the National Cerebral and Cardiovascular Center Hospital. These patients had initially been hospitalized for treatment of acute ischemic stroke.

Stroke was diagnosed by neurologists according to the World Health Organization criteria (1989). After the assessment, a group of psychiatrists and neurologists reviewed the data and reached a consensus regarding the presence or absence of psychiatric disease, including dementia according to the Diagnostic and Statistical Manual, 4th edition criteria. Patients were included if they met the following criteria: (i) a focal lesion of either the right or left hemisphere on magnetic resonance imaging (MRI); (ii) absence of other neurologic, neurotoxic, or metabolic conditions; (iii) modest ischemic insult (modified Rankin scale ≤4) with absence of a significant verbal comprehension deficit; and (iv) occurrence of stroke 10–28 days before the examinations. Exclusion criteria included the following: (i) transient ischemic attack, cerebral haemorrhage, subdural haematoma, or subarachnoid haemorrhage; (ii) history of a central nervous system disease such as tumour, trauma, hydrocephalus or Parkinson's disease; and (iii) pre-stroke history of depression. Eighteen subjects met the criteria and participated in this study.

Twenty-two healthy control subjects were recruited locally for this study based on their response to a poster seeking subjects. Exclusion criteria for healthy subjects were a history or present diagnosis of any Diagnostic and Statistical Manual, 4th edition axis I or neurological illness. Major characteristics of this cohort are summarized in Table 1.

All patients were subjected to a neurological examination using the modified Rankin scale (mRS) and the National Institutes of Health Stroke Scale (NIHSS) on the day of the MRI scan.[8,9] A quantitative measurement of cognitive function, the Mini-Mental State Examination (MMSE), and of depressive symptoms, the Hamilton Rating Scale for Depression (HAM-D), was carried out in patients and control subjects. MRI were conducted for all study subjects.[10,11]

At 6 months, follow-up MRI were conducted for 12 of the 18 patients. There were no changes in medication use between baseline and follow-up, and no patients were on antidepressant treatment during the examinations. All patients were subjected to a series of quantitative measurements of depressive symptoms (HAM-D), cognitive function (MMSE), and neurological examination (mRS, NIHSS) on the day of the follow-up MRI scan.[8–11]

Data acquisition of MRI

All MRI examinations were performed with a 3-Tesla whole-body scanner (Signa Excite HD V12M4; GE Healthcare, Milwaukee, WI, USA) with an eight-channel phased-array brain coil. Diffusion tensor images were acquired with a locally modified single-shot echo-planar imaging sequence that used parallel acquisition at a reduction factor of 2 in the axial plane. Imaging parameters were as follows: repetition time = 17 s; echo time = 72 ms; b value = 0, 1000 mm^2/s, acquisition matrix, 128 × 128; field of view, 256 mm; section thickness, 2.0 mm; no intersection gap; 74 sections. The reconstruction matrix was the same as

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Table 1 Demographic characteristics of patients and healthy control subjects

Characteristic	Stroke patients (n = 18)	Healthy control subjects (n = 22)	t or χ^2	P-value
Age (years)	70.0 ± 6.7	67.2 ± 5.5	t = 1.46	0.15
Female sex (n)	4 (22.2%)	8 (36.3%)	χ^2 = 0.94	0.33
MMSE score	28.4 ± 1.9	29.3 ± 1.0	t = 1.98	0.06
HAM-D score	2.4 ± 2.4	1.0 ± 1.5	t = 2.20	0.03*
History of disease (n)				
Diabetes mellitus	5 (27.8%)	2 (9.1%)	χ^2_1 = 2.40	0.12
Hyperlipidaemia	5 (27.8%)	1 (4.5%)	χ^2_1 = 4.19	0.04*
Hypertension	14 (77.8%)	5 (22.7%)	χ^2_1 = 12.0	<0.01**
mRS score	1.9 ± 0.7	—		
NIHSS score	2.8 ± 0.9	—		
Anticoagulant or anti-platelet medication (n)				
Warfarin	3 (16.7%)			
Acetylsalicylic acid	13 (72.2%)			
Clopidogrel sulfate	2 (11.1%)			
Cilostazol	3 (16.7%)			
Acute infarcts	1.2 ± 0.5	—		
Volume of acute infarcts (mL)	1.6 ± 0.9	—		
Location of acute infarcts (n)				
Basal ganglia	11 (61.1%)	—	0.611	
Subcortical white matter	6 (33.3%)			
Thalamus	1 (5.6%)	—	0.056	
Laterality of hemisphere infarcts				
Left hemisphere (n)	9 (50.0%)	—		

Data are mean ± SD. *P < 0.05, **P < 0.01.
HAM-D, Hamilton Rating Scale for Depression; MMSE, Mini-Mental State Examination; mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale.

the acquisition matrix, and 2 × 2 × 2 mm isotropic voxel data were obtained. Motion-probing gradient was applied in 55 directions, the number of images was 4144, and the acquisition time was 15 min 52 s.

To reduce blurring and signal loss arising from field inhomogeneity, we used an automated high-order shimming method based on spiral acquisitions before acquiring diffusion tensor imaging scans.[12] To correct for motion and distortion from eddy current and B_0 inhomogeneity, FMRIB software (FMRIB Center, Department of Clinical Neurology, University of Oxford, Oxford, UK; http://www.fmrib.ox.ac.uk/fsl/fslwiki/) was used. B_0 field mapping data were also acquired with the echo time shift (2.237 ms) method based on two gradient echo sequences.

High-resolution 3-D, T_1-weighted images were acquired with a spoiled gradient-recalled sequence (repetition time = 12.8 ms, echo time = 2.6 ms, flip angle = 8, field of view, 256 mm; 188 sections in the sagittal plane; acquisition matrix, 256 × 256; acquired resolution, 1 × 1 × 1 mm). T_2-weighted images were obtained with a fast-spin echo (repetition time = 4800 ms; echo time = 101 ms; echo train length = 8; field of view = 256 mm; 74 slices in the transverse plane; acquisition matrix, 160 × 160, acquired resolution, 1 × 1 × 2 mm).

Image processing

Fractional anisotropy (FA) maps and three eigenvalues (λ1, λ2, and λ3) were generated from each individual with FMRIB software. First, brain tissue was extracted using the Brain Extraction Tool. Brain maps for each of the 55 directions were eddy corrected, subsequent to which FA values were calculated at each voxel with the FSL FMRIB Diffusion Toolbox.

Image pre-processing and statistical analysis were carried out using SPM8 software (Wellcome Department of Imaging Neuroscience, London, UK). Each subject's echo planar image was spatially normalized to the Montreal Neurological Institute echo planar image template using parameters determined from the normalization of the image with a b value of 0 mm^2/s and the echo planar image template in SPM8. Images were resampled with a final voxel size of 2 × 2 × 2 mm^3. Normalized maps were spatially smoothed using an isotropic Gaussian filter (8-mm full-width at half-maximum).

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Voxel-based analysis

Voxel-based analysis was performed using SPM8 software. FA maps were compared between patients and healthy subjects with ANCOVA, with age and sex as covariates of no interest. Statistical inferences were made with a voxel-level threshold of $P < 0.001$, uncorrected, with a minimum cluster size of 100 voxels. The regional FA value was calculated by averaging the FA values for all voxels within the volume of interest corresponding to the cluster composed of significant contiguous voxels. The same volumes of interest were applied to λ_1–λ_3 images, and λ_1–λ_3 values were extracted. Axial (λ_1) and radial diffusivity ($(\lambda_2 + \lambda_3)/2$) were compared.

Flow cytometric analysis of lymphocyte subsets in peripheral blood

Blood samples (5 mL) were obtained from all of the patients and healthy control subjects at the initial examination. The samples were collected into tubes containing sodium heparin. Peripheral blood mononuclear cells (PBMC) were isolated using a Ficoll density gradient (Ficoll-Paque PLUS; GE Healthcare Bio-Sciences AB, Uppsala, Sweden) according to the manufacturer's protocol. PBMC were washed twice with phosphate-buffered saline containing 1% foetal calf serum and 2-mM EDTA.

To identify helper T cells (CD3+ & CD4+), cytotoxic T cells (CD3+ & CD8+), B cells (CD19+) and natural killer cells (CD16+ or CD56+), we incubated the PBMC with fluorescein isothiocyanate-conjugated anti-human CD3 (Beckman Coulter, Orange Country, CA, USA), phycoerythrin-cyanin (PC)5-conjugated anti-human CD4 (Beckman Coulter), PC7-conjugated anti-human CD8 (Beckman Coulter), phycoerythrin-conjugated anti-human CD19 (Beckman Coulter), PC5-conjugated anti-human CD16 (Beckman Coulter), and/or phycoerythrin-conjugated anti-human CD56 (Beckman Coulter) at 4°C for 20 min. To identify regulatory T lymphocytes (T_{reg}) (CD4+, CD25+ & FOXP3+), we incubated the PBMC with fluorescein isothiocyanate-conjugated anti-human CD4 (Beckman Coulter) and PC5-conjugated anti-human CD25 (Beckman Coulter) at 4°C for 20 min. After surface staining, PBMC were fixed, followed by permeabilization and staining with phycoerythrin-conjugated anti-human FOXP3 (Becton Dickinson, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. As negative controls, fluorochrome-conjugated non-specific isotype-matched antibodies (Beckman Coulter) were used. Stained cells were analyzed using a FC500 cytometer and CXP software (Beckman Coulter). Percentages of cells stained with a particular antibody are reported after subtraction of the percentage of cells stained with the relevant negative isotype control antibodies.

Statistical analysis

Group differences in demographic characteristics between patients and healthy controls were examined by unpaired *t*-test and Pearson's χ^2 test. To examine the group differences in FA values and axial/radial diffusivity in volume of interest shown in the voxel-based analysis, we performed ANCOVA with age and sex as covariates.

Paired *t*-tests were performed 6 months after the initial examinations to determine changes in patients' mRS, NIHSS, MMSE, and HAM-D scores and FA values. We performed Pearson's correlation analysis to examine the relationship between FA values and depressive symptoms at the first assessment and at the assessment performed 6 months later. To examine the relationship between the change in depression scale scores and the ratio of the FA values (FA values at second vs initial examination) in patients, we performed Pearson's correlation analysis.

To examine whether the ratio of the FA values was related to the change in depression scale scores (HAM-D scores at second examination minus initial examination), we performed multiple regression analysis after adjustment for age and gender. The change in depression scale scores was the dependent variable, and the ratio of the FA values was the independent variable.

Additionally, we performed ANCOVA with age and sex as covariates to examine the differences in the numbers of helper T cells, cytotoxic T cells, regulatory T cells, B cells, and natural killer cells between patients and healthy control subjects. For the cells showing significant differences between groups, we examined the correlation between FA values and cell number by Spearman's correlation analysis. To determine whether FA values were related to cell number, we performed multiple regression analysis with the FA values as dependent variable and cell number as independent variables, after adjustment for age and gender.

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All statistical tests were two-tailed and reported at $P < 0.05$. Bonferroni correction was applied to avoid type I errors due to the multiplicity of statistical analyses. Statistical analysis of the data was performed using SPSS for Windows 19.0 (IBM Japan Inc., Tokyo, Japan).

RESULTS

Demographic and clinical data

Table 1 summarizes the demographic and clinical characteristics of the study subjects. Patients did not differ significantly from healthy control subjects with regard to age, sex, and MMSE scores. As to the history of disease, the ratio of the history of hyperlipidaemia and hypertension was significantly higher in the patients than in the healthy controls. mRS and NIHSS score, anticoagulant or anti-platelet medication, and the location and volume of the infarction among the patients are also shown in Table 1. Patients showed some disability from the stroke at the time of the examination. All of the patients took anticoagulant and/or anti-platelet medicine. Infarctions were located in the basal ganglia (61.1%), subcortical white matter (33.3%), and thalamus (5.6%). There was no significant laterality of hemisphere infarcts.

Comparisons of FA values between groups

In the voxel-based analysis of FA values, the patient and healthy control groups differed in white matter FA values in the left and right anterior limbs of the internal capsule (left anterior limb of internal capsule: $(x, y, z) = (-26, 12, 18)$, cluster voxel size = 831, T value = 5.20; right anterior limb of internal capsule: $(x, y, z) = (26, 16, 4)$, cluster voxel size = 487, T value = 5.24) (Fig. 1a). Figure 1b shows the scatter diagrams of the FA values of the anterior limb of the internal capsule. Table 2 shows the quantification of the differences in FA values and radial/axial diffusivity in these affected regions. These regions revealed decreased axial diffusivity but no change in radial diffusivity.

No patients had lesions in the location of the anterior limbs of the internal capsule. Using Pearson's correlation analysis, we found no significant relationship ($P > 0.05$) between the FA value in the anterior limb of the internal capsule and the volume of infarcts or the severity of stroke shown with mRS and NIHSS scores.

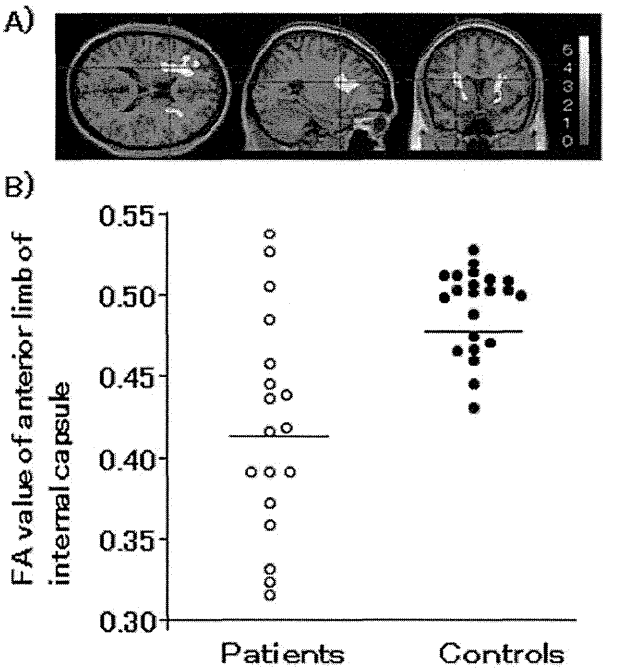

Figure 1 (a) White matter fractional anisotropy (FA) differences in voxel-based analysis comparisons between stroke patients ($n = 18$) and control subjects ($n = 22$). Images are presented in radiological orientation. Statistical parametric mapping projections were superimposed on a representative magnetic resonance image ($x = -26$, $y = 12$, $z = 18$). Patients showed reduced FA in the right and left anterior limbs of the internal capsule. Statistical inferences were made with a voxel-level statistical threshold ($P < 0.001$), uncorrected, with a minimum cluster size of 100 voxels. (b) Scatter plots of FA values in the region of FA reduction of patients and control subjects. Patients' FA values were lower than those of healthy subjects in the bilateral anterior limb of the internal capsule ($P < 0.01$).

Change in FA values of patients after 6 months

Patients showed significantly increased FA values in the anterior limb of the internal capsule 6 months after the infarction (Table 3, Fig. 2a).

There were no significant changes in MMSE and HAM-D scores for either group 6 months after the initial examination (Table 3). There was no significant relationship between FA values and depressive symptoms at the first assessment and at the assessment performed 6 months later. However, we found a significant negative correlation between the increased ratio of the FA values and the change in the scores of depression scales of HAM-D at follow-up 6 months later ($r = -0.67$, $P = 0.02$) (Fig. 2b).

When multiple regression analysis was used to evaluate whether the increased ratio of FA values was

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Table 2 Differences in values of FA and axial/radial diffusivity in VOI between patients and healthy control subjects

FA and axial/radial diffusivity	Stroke patients (n = 18)	Healthy control subjects (n = 22)	ANCOVA†	
			$F_{1,36}$	P-value
Left anterior limb of internal capsule				
FA	0.41 ± 0.08	0.48 ± 0.03	16.4	<0.001**
Axial diffusivity (×10^{-3})	4.16 ± 0.32	4.37 ± 0.30	4.24	0.05*
Radial diffusivity (×10^{-3})	3.96 ± 0.30	4.04 ± 0.29	0.48	0.49
Right anterior limb of internal capsule				
FA	0.43 ± 0.06	0.50 ± 0.03	23	<0.001**
Axial diffusivity (×10^{-3})	4.14 ± 0.33	4.35 ± 0.30	4.03	0.05
Radial diffusivity (×10^{-3})	3.93 ± 0.31	4.01 ± 0.30	0.46	0.50
Bilateral anterior limb of internal capsule				
FA	0.42 ± 0.07	0.49 ± 0.03	20.6	<0.001**
Axial diffusivity (×10^{-3})	4.15 ± 0.32	4.36 ± 0.30	4.15	0.05*
Radial diffusivity (×10^{-3})	3.95 ± 0.30	4.02 ± 0.29	0.47	0.50

†Age and gender are entered as covariates. Data are mean ± SD. *P < 0.05, **P < 0.01.
FA, fractional anisotropy; VOI, volume of interest.

Table 3 Change in psychometry scores, FA values, and axial/radial diffusivity over 6 months in patients (n = 12)

	10–28 days after stroke	6 months after first exam	Paired t-test	P-value
Patients				
mRS score	1.9 ± 0.5	1.6 ± 0.5	t_{11} = 2.35	0.04*
NIHSS score	2.8 ± 1.0	1.8 ± 0.7	t_{11} = 4.00	0.002**
MMSE score	29.0 ± 1.5	29.7 ± 0.5	t_{11} = 1.54	0.15
HAM-D score	3.7 ± 2.9	2.3 ± 3.0	t_{11} = 1.13	0.28
Anterior limb of internal capsule				
FA	0.40 ± 0.06	0.43 ± 0.06	t_{11} = 2.26	0.04*
Axial diffusivity (×10^{-3})	4.22 ± 0.30	4.13 ± 0.2	t_{11} = 0.74	0.48
Radial diffusivity (×10^{-3})	4.01 ± 0.26	3.87 ± 0.25	t_{11} = 1.50	0.16

Data are mean ± sd. * P < 0.05, ** P < 0.01.
FA, fractional anisotropy; HAM-D, Hamilton Rating Scale for Depression; MMSE, Mini-Mental State Examination; mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale.

related to the change in depression scale scores (HAM-D) after 6 months, the ratio of FA values was found to be negatively related to the change in the HAM-D scores (β = –0.46, P = 0.04).

Lymphocyte subsets and their relation to FA values in patients

Patients showed significantly decreased numbers of T_{reg} compared with healthy controls (Table 4, Fig. 3a). We also found a significant positive relationship between the level of circulating T_{reg} and the FA value in the anterior limb of the internal capsule in the patients (r = 0.50, P = 0.04) (Fig. 3b). There was no significant relationship between the level of circulating T_{reg} and the HAM-D scores.

When multiple regression analysis was used to evaluate whether the level of circulating T_{reg} was related to the FA value in the anterior limb of the

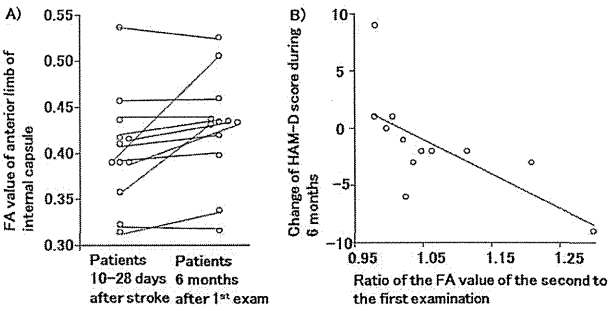

Figure 2 (a) Scatter plots of fractional anisotropy (FA) values in the region of FA reduction among stroke patients (n = 12) at 10–28 days after the stroke and at the 6-month follow-up. A significant FA increase was observed in the patients at the 6-month follow-up (P < 0.05). (b) Scatter plots showing the relationship between the ratio of the FA values of the second to the first examination and the change in depression scale scores among patients (n = 12). Significant correlations were observed between the ratio of the FA values of the second to the first examination and the changes in depression scale scores (r = –0.67, P = 0.02). HAM-D, Hamilton Rating Scale for Depression.

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Table 4 Differences in percentage of lymphocytes in the circulation between patients and healthy control subjects

			ANCOVA†	
	Stroke patients (n = 18)	Healthy control subjects (n = 22)	$F_{1,36}$	P-value
Helper T lymphocyte	67.2 ± 15.1	61.5 ± 12.0	1.00	0.32
Cytotoxic T lymphocyte	27.9 ± 13.5	33.2 ± 10.6	0.92	0.35
B lymphocyte	17.7 ± 8.3	12.2 ± 7.8	5.42	0.03
NK cell	21.6 ± 11.3	27.5 ± 10.4	1.78	0.19
Regulatory T lymphocyte	2.1 ± 1.6	3.8 ± 2.3	7.89	0.008*

†Age and sex are entered as covariates. Data are mean ± SD. *Significant after correction for multiple statistical tests to avoid type I errors (P < 0.01 (0.05/5)). NK, natural killer.

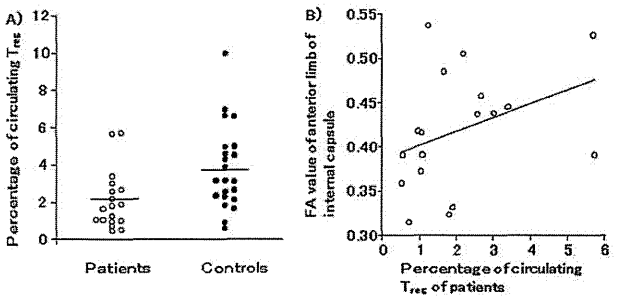

Figure 3 (a) Scatter diagrams showing the differences in circulating T_{reg} between patients and controls. A significant difference in the percentage of T_{reg} was observed between groups ($F_{1,36}$ = 7.89, P = 0.008). (b) The relationship between the percentage of circulating T_{reg} and FA values of the anterior limb of the internal capsule in patients. A significant correlation was observed between the percentage of T_{reg} and FA values (r = 0.50, P = 0.04). FA, fractional anisotropy; T_{reg}, regulatory T lymphocytes.

internal capsule, the T_{reg} level was found to be positively related to the FA values (β = 0.59, P = 0.02).

DISCUSSION

Our findings showed that stroke patients had lower FA in the bilateral anterior limb of the internal capsule relative to healthy control subjects. Six months after initial assessment, a significant increase in FA was noted, and it revealed an association with a reduction in depression scale scores. Our findings are not the result of direct neuronal damage caused by the infarction located on the internal capsule, as no patients had a lesion in this location. Also, there was no direct relationship between the FA value in this region and the volume of infarcts or the severity of stroke.

Reduced FA level was associated with decreased axial diffusivity. Axonal damage leads to a marked decrease in axial diffusivity, while demyelination leads to an increase in radial diffusivity.[13] Therefore, our finding was not a result of demyelination but of gross reduction in axonal number and/or size, possibly reflecting Wallerian degeneration secondary to neuronal loss due to stroke.[14] From an anatomical perspective, the anterior limb of the internal capsule represents the intercept point in the course of the frontal-subcortical circuits,[15] and it has extensive connectivity with the cortical and subcortical areas. Its reduced FA may reflect the conjunctive focus of degeneration due to stroke in the spatially different sites of cortical and subcortical areas.

The frontal-striatal-thalamic-cortical circuits, connected by the anterior limb of the internal capsule, play an important role in behavioural regulation,[16] and based on MRI, microstructural change of the anterior limb of the internal capsule is related to the severity of depressive symptoms in adults with major depressive disorder.[17] Degeneration in this region may relate to a loss of white matter integrity of these neural circuits,[18] and this abnormality might trigger the onset of negative mood change. Our findings on the association between the change in FA values of the internal capsule and depression scale scores might reflect an association between axonal damage of the internal capsule and depressive mood in stroke patients.

Our findings demonstrate that patients had reduced amounts of circulating T_{reg}, with the degree of reduction being related to the decrease in FA value in the internal capsule. This may indicate that a decrease in T_{reg} is related to the axonal damage of the internal capsule in stroke patients. Our findings showed no direct relationship between T_{reg} level and depression scale scores, but T_{reg} may indirectly affect post-stroke depressive symptoms via its effect on the cerebral damage.

An ischemic stroke caused T lymphocytes to become activated, infiltrate the brain, and then function as sources of pro-inflammatory cytokines and cytotoxic substances.[19–21] However, not all T-cell

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subtypes are detrimental to acute stroke outcome, and recent evidence indicates a novel role of T cells in promoting brain tissue repair and regeneration. T_{reg} cell is an important T-cell subtype, and it supports brain tissue repair and regeneration.[22] T_{reg} cells act to limit the immune response by releasing transforming growth factor-β and interleukin-10,[23] and they have also been reported to be required for neurogenesis.[24] Infarct volume and neuronal dysfunction were significantly increased in mice treated with an anti-CD25 monoclonal antibody to neutralize T_{reg} compared with controls.[23] Furthermore, this protection was observed only 7 days after a modest ischemic insult.[23] These findings of the brain-protecting and outcome-improving effects of T_{reg} were also confirmed by Li *et al.* using post-stroke T_{reg} cell therapy.[25]

One possible explanation for our results of T_{reg} is that people with lower circulated T_{reg} are more likely to develop stroke and tend to have severe axonal damage after stroke. Another possibility is that the reduction in circulated T_{reg} after stroke might be induced by the consumption of T_{reg} to repair cerebral neuronal injuries, including axonal damage. Our findings of lower circulated T_{reg} are based on cross-sectional data, which provide limited ability to infer which explanation is right. In any case, our results are consistent with previous reports of the brain-protecting and outcome-improving effects of T_{reg}. In principle, our findings showed the possibility of improving stroke outcome by targeting the role of T_{reg} in protecting brain tissue damage after a stroke.

Ren *et al.* and Kleinschnitz *et al.* respectively demonstrated no role or an opposite role for T_{reg} in exacerbating brain injury early after transient ischemia.[26,27] The animal model they used was different from that of the Liesz study in several aspects, including the duration of ischemia and methods for T_{reg} depletion. Furthermore, the late stage effect of T_{reg} depletion was not addressed in their studies. In our study, stroke patients were predominantly of modest ischemic insult, and their circulating lymphocytes were studied after 10–28 days. There is a possibility that the differences in the severity and stage of the ischemic insult caused different results regarding the role of T_{reg} in their studies and ours.

Our study has some limitations. First, patients with significant comprehension deficits were excluded because clinical verbal interviews could not be conducted. Second, all of the patients took anticoagulant or anti-platelet medicine. Specifically, 13 patients took acetylsalicylic acid, which has an anti-inflammatory effect, and this may have affected our results. However, the extent to which our findings relate to medication remain uncertain. Further analysis, inclusive of considerations of these points, is needed to confirm our present findings.

In conclusion, the present study suggests that FA reduction in the bilateral anterior limb of the internal capsule is evident in stroke patients. This regional damage relates to abnormality of neuroanatomical pathways in frontal-subcortical circuits and renders a biological vulnerability, which then gives rise to the onset of depressive symptoms. Our findings also demonstrate that patients have reduced amounts of circulating T_{reg}, with the degree of reduction being related to the decrease in FA value in the internal capsule. T_{reg} cells might have a role in improving post-stroke white matter tissue damage by limiting the immune response and promoting neurogenesis.

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REFERENCES

1 Donnan GA, Fisher M, Macleod M, Davis SM. Stroke. *Lancet* 2008; **371**: 1612–1623.
2 Whyte EM, Mulsant BH. Post stroke depression: epidemiology, pathophysiology, and biological treatment. *Biol Psychiatry* 2002; **52**: 253–264.
3 Magnus T, Wiendl H, Kleinschnitz C. Immune mechanisms of stroke. *Curr Opin Neurol* 2012; **25**: 334–340.
4 Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. *Nat Med* 2011; **17**: 796–808.
5 Macrez R, Ali C, Toutirais O *et al.* Stroke and the immune system: from pathophysiology to new therapeutic strategies. *Lancet Neurol* 2011; **10**: 471–480.
6 Craft TK, DeVries AC. Role of IL-1 in poststroke depressive-like behavior in mice. *Biol Psychiatry* 2006; **60**: 812–818.
7 Brait VH, Arumugam TV, Drummond GR, Sobey CG. Importance of T lymphocytes in brain injury, immunodeficiency, and recovery after cerebral ischemia. *J Cereb Blood Flow Metab* 2012; **32**: 598–611.
8 Brott T, Adams HPJ, Olinger CP *et al.* Measurements of acute cerebral infarction: a clinical examination scale. *Stroke* 1989; **20**: 864–870.
9 Goldstein LB, Samsa GP. Reliability of the National Institutes of Health Stroke Scale. Extension to non-neurologists in the context of a clinical trial. *Stroke* 1997; **28**: 307–310.
10 Folstein MF, Folstein SE, McHugh PR. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; **12**: 189–198.

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11 Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry* 1960; **23**: 56–62.
12 Kim DH, Adalsteinsson E, Glover GH, Spielman DM. Regularized higher-order *in vivo* shimming. *Magn Reson Med* 2002; **48**: 715–722.
13 Song SK, Yoshino J, Le TQ *et al*. Demyelination increases radial diffusivity in corpus callosum of mouse brain. *Neuroimage* 2005; **26**: 132–140.
14 Thomalla G, Glauche V, Koch MA *et al*. Diffusion tensor imaging detects early Wallerian degeneration of the pyramidal tract after ischemic stroke. *Neuroimage* 2004; **22**: 1767–1774.
15 Axer H, Keyserlingk DG. Mapping of fiber orientation in human internal capsule by means of polarized light and confocal scanning laser microscopy. *J Neurosci Methods* 2000; **94**: 165–175.
16 Duran FL, Hoexter MQ, Valente AAJ *et al*. Association between symptom severity and internal capsule volume in obsessive-compulsive disorder. *Neurosci Lett* 2009; **452**: 68–71.
17 Zou K, Huang X, Li T *et al*. Alterations of white matter integrity in adults with major depressive disorder: a magnetic resonance imaging study. *J Psychiatry Neurosci* 2008; **33**: 525–530.
18 Budde MD, Kim JH, Liang HF *et al*. Toward accurate diagnosis of white matter pathology using diffusion tensor imaging. *Magn Reson Med* 2007; **57**: 688–695.
19 Jander S, Kraemer M, Schroeter M *et al*. Lymphocytic infiltration and expression of intercellular adhesion molecule-1 in photochemically induced ischemia of the rat cortex. *J Cereb Blood Flow Metab* 1995; **15**: 42–51.
20 Yilmaz G, Arumugam TV, Stokes KY, Granger DN. Role of T lymphocytes and interferon-gamma in ischemic stroke. *Circulation* 2006; **113**: 2105–2112.
21 Brait VH, Jackman KA, Walduck AK *et al*. Mechanisms contributing to cerebral infarct size after stroke: gender, reperfusion, T lymphocytes, and Nox2-derived superoxide. *J Cereb Blood Flow Metab* 2010; **30**: 1306–1317.
22 Zouggari Y, Ait-Oufella H, Waeckel L *et al*. Regulatory T cells modulate postischemic neovascularization. *Circulation* 2009; **120**: 1415–1425.
23 Liesz A, Suri-Payer E, Veltkamp C *et al*. Regulatory T cells are key cerebroprotective immunomodulators in acute experimental stroke. *Nat Med* 2009; **15**: 192–199.
24 Saino O, Taguchi A, Nakagomi T *et al*. Immunodeficiency reduces neural stem/progenitor cell apoptosis and enhances neurogenesis in the cerebral cortex after stroke. *J Neurosci Res* 2010; **88**: 2385–2397.
25 Li P, Gan Y, Sun BL *et al*. Adoptive regulatory T-cell therapy protects against cerebral ischemia. *Ann Neurol* 2013; **74**: 458–471.
26 Ren X, Akiyoshi K, Vandenbark AA *et al*. CD4+FoxP3+ regulatory T-cells in cerebral ischemic stroke. *Metab Brain Dis* 2011; **26**: 87–90.
27 Kleinschnitz C, Kraft P, Dreykluft A *et al*. Regulatory T cells are strong promoters of acute ischemic stroke in mice by inducing dysfunction of the cerebral microvasculature. *Blood* 2013; **121**: 679–691.

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Microstructural abnormalities in white matter and their effect on depressive symptoms after stroke

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ABSTRACT

The aim of the study was to investigate the existence of microstructural abnormalities in the white matter of the brain in stroke patients, as well as the relationship between these microstructural abnormalities and changes in depressive symptoms over 6 months. Participants were 29 acute ischemic stroke patients and 37 healthy control subjects. Depressive symptoms were assessed in all subjects using the Hamilton Rating Scale for Depression and the Zung Self-rating Depression Scale. Whole brain voxel-based analysis was used to compare diffusion tensor imaging measures of Fractional Anisotropy (FA) between the groups. Six-month follow-up examinations were conducted. Patients showed significantly lower white matter FA values in the left and right anterior limbs of the internal capsule, and 6 months after the stroke they showed significantly increased FA values in these regions. We found a significant negative correlation between the increased ratio of the FA values and the change in depression scale scores at 6-month follow-up. Regional white matter damage may reflect abnormalities in neuroanatomical pathways related to the pathophysiology of depression.

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1. Introduction

Depression is the most common and serious emotional symptom following a stroke and is associated with excess disability, cognitive impairment and mortality (Whyte and Mulsant, 2002). Although there is no consensus about the relationship between lesion location and post-stroke depressive symptoms, Magnetic Resonance Imaging (MRI) studies have found a high prevalence of depressive symptoms in patients with lesions that affect structures of the prefronto-subcortical circuit (Vataja et al., 2001, 2004). Recent studies have highlighted the specific relevance of the Limbic-Cortical-Striatal-Pallidal-Thalamic (LCSPT) circuit in the pathophysiology of major depressive disorder (Drevets et al., 2008; Hasler et al., 2008) and of depression due to stroke (Terroni et al., 2011; Paradiso et al., 2013).

Diffusion Tensor Imaging (DTI) combines a conventional MRI sequence with additional magnetic field gradients to quantify water diffusion, namely, Fractional Anisotropy (FA), the degree to which diffusion is directionally hindered, which reflects the microstructural integrity of the white matter tracts. Microstructural damage to white matter tracts may confer a biological vulnerability to the onset of depressive symptoms in stroke patients. To our knowledge, however, no studies have investigated the existence of microstructural abnormalities of white matter in stroke patients and examined whether the diminution of microstructural abnormalities decreased the vulnerability to post-stroke depression, as measured by increases in depression scale scores in stroke patients that are noted before the onset of severe depression.

Thus, the primary aim of the present study was to investigate the existence of microstructural abnormalities in white matter tracts in stroke patients, as well as the relationship between the recovery from these microstructure abnormalities and the change in depression scale scores 6 months after a stroke. DTI was performed and whole brain voxel-based analysis was used to compare FA between groups of acute ischemic stroke patients

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and healthy control subjects. Six-month follow-up examinations were conducted. On the day of the MRI scan, depressive symptoms were evaluated with the Hamilton Rating Scale for Depression and the Zung Self-rating Depression Scale.

2. Methods

2.1. Participants

After complete description of the study to the subjects, written informed consent was obtained. The study was approved by the medical ethics committee of the National Cerebral and Cardiovascular Center in Japan. The patients were of Japanese ethnicity and were recruited from the neurology unit of the National Cerebral and Cardiovascular Center hospital. These patients had initially been hospitalized for treatment of acute ischemic stroke.

Stroke was diagnosed by neurologists according to World Health Organization (WHO) criteria. After the assessment, a group of psychiatrists and neurologists reviewed the data and reached a consensus regarding the presence or absence of psychiatric disease, including dementia, according to DSM-IV criteria. Patients were included if they met the following criteria: (1) a focal lesion of either the right or left hemisphere on MRI; (2) absence of other neurological, neurotoxic, or metabolic conditions; (3) modest ischemic insult (modified Rankin scale ≤ 4) with absence of a significant verbal comprehension deficit; and (4) occurrence of stroke 10–28 days before the examinations. Exclusion criteria were as follows: (1) transient ischemic attack, cerebral hemorrhage, subdural hematoma or subarachnoid hemorrhage; (2) history of a Central Nervous System (CNS) disease such as tumor, trauma, hydrocephalus, and Parkinson's disease; and (3) pre-stroke history of depression. Thirty-eight patients who volunteered to participate in the study were screened for eligibility. We excluded 5 subjects who did not meet the study criteria. In addition, four patients had not completed the MRI scan due to fatigue. A final group of 29 patients met the criteria and participated in this study.

Thirty seven healthy volunteers were recruited from the local area by poster advertisement. Exclusion criteria for the volunteers were a history or present diagnosis of any DSM-IV axis I or any neurological illness. Major characteristics of this cohort are summarized in Table 1. To reliably elucidate differences in white matter integrity between groups, the target total sample size was set at above 52, which was expected to yield power ≥ 0.8, based on $\alpha \leq 0.05$ and assuming a large effect size ($f=0.4$) with the analysis of covariance (ANCOVA) used in this study (Cohen, 1977), and the sample size of this study met the power requirement.

All patients and volunteers were assessed with a series of standardized, quantitative measurements of depressive symptoms [Hamilton Rating Scale for Depression (HAM-D) (Hamilton, 1960), Zung Self-rating Depression Scale (SDS) (Zung, 1965)] and cognitive function [Mini-Mental State examination (MMSE) (Folstein et al., 1975)] on the day of the MRI scan. A neurological examination [modified Rankin scale: mRS (Brott et al., 1989)] was also carried out in the patients. MRIs were conducted for all of the subjects.

Six-month follow-up MRI examinations were also conducted for 18 of 29 patients and 19 of 37 healthy subjects. The other patients and controls were lost to follow-up because we were unable to contact them at 6 months after the first study or they declined to further participate in this study due to health problems, business, feeling of rejection, and so on. On the day of the follow-up MRI scan, the participants underwent the same battery of depressive, cognitive function and (for the patients) neurological measurements that had been performed at the time of the initial MRI. There were no changes in medication between baseline and follow-up. No patients and healthy subjects were diagnosed as meeting DSM-IV criteria for major depression on the day of the initial MRI. Two patients were diagnosed as meeting criteria for major depression for the first time on the day of the follow-up MRI, and they were prescribed medication after the examinations. No patients were on antidepressant treatment during the examinations.

2.2. MRI acquisition

All MRI examinations were performed using a 3-T whole-body scanner (Signa Excite HD V12M4; GE Healthcare, Milwaukee, WI, USA) with an eight-channel phased-array brain coil. DT images were acquired with a locally modified single-shot Echo-Planar Imaging (EPI) sequence by using parallel acquisition at a reduction (ASSET) factor of 2, in the axial plane. Imaging parameters were as follows: repetition time (TR)=17 s; echo time (TE)=72 ms; b=0, 1000 s/mm^2; acquisition matrix, 128 × 128; field of view (FOV), 256 mm; section thickness, 2.0 mm; no intersection gap; 74 sections. The reconstruction matrix was the same as the acquisition matrix, and 2 mm × 2 mm × 2 mm isotropic voxel data were obtained. Motion Probing Gradient (MPG) was applied in 55 directions, the number of images was 4144, and the acquisition time was 15 min, 52 s.

To reduce blurring and signal loss arising from field inhomogeneity, an automated high-order shimming method based on spiral acquisitions (Kim et al., 2002) was used before acquiring DTI scans. To correct for motion and distortion from eddy current and B0 inhomogeneity, FMRIB software (FMRIB Center, Department of Clinical Neurology, University of Oxford, Oxford, England; http://www.fmrib.ox.ac.uk/fsl/) was used. B0 field mapping data were also acquired with the echo time shift (of 2.237 ms) method based on two gradient echo sequences.

High-resolution three-dimensional T1-weighted images were acquired using a spoiled gradient-recalled sequence (TR=12.8 ms, TE=2.6 ms, flip angle=8°, FOV, 256 mm; 188 sections in the sagittal plane; acquisition matrix, 256 × 256; acquired resolution, 1 × 1 × 1 mm). T2-weighted images were obtained using a fast-spin echo (TR=4800 ms; TE=101 ms; echo train length (ETL)=8; FOV=256 mm; 74 slices in the transverse plane; acquisition matrix, 160 × 160, acquired resolution, 1 × 1 × 2 mm).

Table 1
Demographic characteristics of patients and healthy control subjects.

Characteristic	Stroke patients (n=29)	Healthy controls (n=37)	t_{64} or X^2	P
Age (years)	68.7 ± 8.2	67.5 ± 5.2	t=0.77	0.45
Female sex (n, %)	6 (20.7)	15 (40.5)	χ^2=2.95	0.10
MMSE score	27.8 ± 3.0	29.2 ± 1.0	t=2.45	0.02*
SDS score	26.5 ± 5.6	24.1 ± 3.6	t=2.03	0.05*
HAM-D score	2.6 ± 2.5	1.1 ± 1.8	t=2.64	0.01*
mRS score	2.2 ± 0.8	–		
Number of acute infarcts	1.2 ± 0.6	–		
Volume of acute infarcts (ml)	2.0 ± 2.3	–		
Acute infarcts (n, %) in				
Frontal cortex	1 (3.4)	–		
Occipital cortex	1 (3.4)	–		
Basal ganglia	13 (44.8)	–		
Thalamus	4 (13.8)	–		
Subcortical white matter infarcts in				
Frontal lobe	6 (20.7)	–		
Parietal lobe	1 (3.4)	–		
Temporal lobe	1 (3.4)	–		
Occipital lobe	1 (3.4)	–		
Genu of internal capsule	1 (3.4)	–		
Total	10 (34.5)	–		
Laterality of acute hemisphere infarcts				
Left hemisphere (n, %)	17 (58.6)	–		

MMSE=Mini-Mental State Examination. SDS=Zung Self-Rating Depression Scale. HAM-D=Hamilton Rating Scale for Depression. DWMH= deep white matter hyperintensity. PVH=Periventricular hyperintensity. mRS=Modified Rankin Scale. Data are mean ± S.D. * $p < 0.05$.

2.3. Image processing

FMRIB software was used to generate FA maps and three eigenvalues ($\lambda 1$, $\lambda 2$, and $\lambda 3$) from each individual. First, brain tissue was extracted using the Brain Extraction Tool in FSL software. Brain maps for each of the 55 directions were eddy-corrected, subsequent to which FA values were calculated at each voxel using the FSL FMRIB Diffusion Toolbox.

Image preprocessing and statistical analysis were carried out using SPM8 software (Wellcome Department of Imaging Neuroscience, London, England). Each subject's echo planar image was spatially normalized to the Montreal Neurological Institute echo planar image template using parameters determined from the normalization of the image with a b value of 0 s/mm^2 and the echo planar image template in SPM8. Images were resampled with a final voxel size of 2 × 2 × 2 mm^3. Normalized maps were spatially smoothed using an isotropic Gaussian filter (8-mm full-width at half-maximum).

2.4. Voxel-based analysis

Voxel-based analysis was performed using SPM8 software. FA maps were compared between patients and healthy subjects by ANCOVA with age and gender as covariates of no interest. We included age and gender as covariates because it has been reported that they affect the white matter integrity (Inano et al., 2011). Statistical inference was done with a voxel-level threshold of $p < 0.05$, after family-wise error correction for multiple comparisons, with a minimum cluster size of 50 voxels. The regional FA value was calculated by averaging the FA values for all voxels within the voxel of interest (VOI) corresponding to the cluster composed of