significant contiguous voxels in the above analysis. The same VOIs were applied to $\lambda_1-\lambda_3$ images, and $\lambda_1-\lambda_3$ values were extracted. Axial (λ_1) and radial diffusivity ($[\lambda_2+\lambda_3]/2$) were compared.

2.5. Statistical analysis

Group differences in demographic characteristics between patients and healthy controls were examined by unpaired *t*-test and Pearson χ^2 test. To examine the group differences of FA values and axial/radial diffusivity in VOIs shown in the voxel-based analysis, we performed ANCOVA with age and gender as covariates. We included age and gender as covariates because they reportedly affect white matter integrity (Inano et al., 2011). Paired *t*-tests were performed to examine the changes in the mRS, MMSE, SDS, and HAM-D scores and the FA values of patients and controls during the 6-month period after their initial examinations. We computed Pearson's correlations to examine the relationship between FA values and depressive symptoms at the first assessment and at the 6-month follow-up assessment. Pearson's correlations were also used 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. To examine whether the ratio of the FA values was related to the change in depression scale scores (SDS and HAM-D scores at second minus initial examination), we performed a multiple regression analysis with the change in depression scale scores as the dependent variable and the ratio of the FA values as the independent variable, after adjustment for age and gender.

All statistical tests were 2-tailed and reported at $\alpha < 0.05$. 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).

3. Results

3.1. Demographic and clinical data

Table 1 summarizes the demographic and clinical characteristics of the participants. Patients differed significantly from healthy control subjects in MMSE, SDS and HAM-D scores. The MMSE score was lower, and SDS and HAM-D scores were higher, among the patients. Table 1 also shows the mRS score and the location and volume of the infarctions among the patients. The main locations of the infarctions were the basal ganglia (44.8%), the subcortical white matter in the frontal lobe (20.7%), and the thalamus (13.8%).

3.2. Between-group comparisons of FA values

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: $(x, y, z)=(-24, 16, 16)$, cluster voxel size=189, $T=6.41$; right anterior limb of internal capsule: $(x, y, z)=(16, 6, 10)$, cluster voxel size=756, $T=6.86$] (Fig. 1a). Table 2 shows the quantification of the differences in FA value and radial/axial diffusivity in these affected regions. These regions revealed decreased axial diffusivity but no change in radial diffusivity. When we added the MMSE, SDS, and HAM-D scores as covariates in the ANCOVA, the results did not change.

3.3. Change in FA values of patients over 6 months

There were no significant differences in demographic data between participants who were followed and those who were lost to follow-up, except the age of the healthy control groups (Table 3). Table 4 shows the changes in psychometric scores and FA values and axial/radial diffusivity over 6 months in the followed-up patients and controls. Healthy controls showed no significant change of FA values in the anterior limb of the internal capsule 6 months after the initial examination (Table 4). Patients showed significantly increased FA values in the anterior limb of the internal capsule 6 months after the infarction, although their FA values were still lower relative to those of healthy control subjects at this time point (Table 4, Fig. 1b). There were no significant changes in MMSE, SDS and HAM-D scores 6 months after the initial examination in the groups of patients and healthy controls (Table 4).

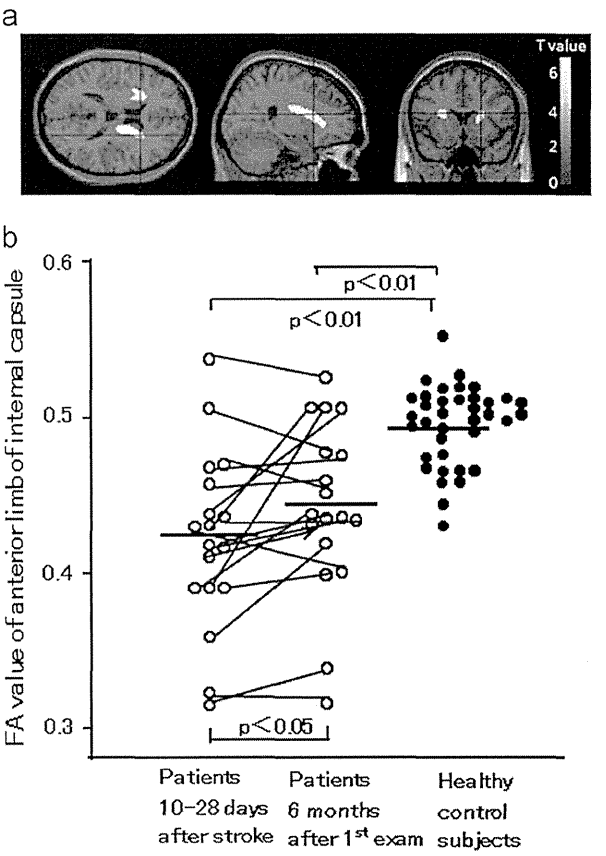

Fig. 1. White matter Fractional Anisotropy (FA) differences in voxel-based comparisons between stroke patients ($n=29$) and control subjects ($n=37$) (Fig. 1a), and scatter plots of FA values in the region of FA reduction among stroke patients ($n=18$) at 10–28 days after stroke/after 6-month follow-up and of control subjects ($n=37$) (Fig. 1b). (a) Images are presented in radiological orientation. Statistical parametric mapping projections were superimposed on a representative magnetic resonance image ($x=24, y=16, z=16$). 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 ($p<0.05$) after family-wise error correction for multiple comparisons with a minimum cluster size of 50 voxels. (b) Significant increase in FA of the patients was observed at 6-month follow-up ($p<0.05$), although the FA values of the patients were still lower than those of healthy subjects at both the initial and follow-up examinations ($p<0.01$).

There were no significant relationships between FA values and depressive symptoms at the first assessment and at the assessment performed 6 months later, but we found a significant negative correlation between the increased ratio of the FA values and the change in the depression scores on the SDS and HAM-D at 6-month follow-up ($r=-0.59$, $p=0.01$ for SDS; $r=-0.56$, $p=0.02$ for HAM-D) (Fig. 2). With Spearman's correlational analysis, the results were not changed ($r=-0.56$, $p=0.02$ for the SDS; $r=-0.73$, $p=0.001$ for the HAM-D). When we considered the influence of the volume of infarcts and lesion location [cortex ($n=2$), basal ganglia ($n=8$), thalamus ($n=1$), and subcortical white matter ($n=7$)] as a covariate in correlational analysis, the correlations were not changed ($r=-0.59$, $p=0.02$ for SDS; $r=-0.57$, $p=0.02$ for HAM-D). When we considered the influence of the degree of handicap by including the change in mRS scores as a covariate in the correlation analysis, which showed a significant decrease during follow-up (mRS score=2.1 ± 0.8 at first examination, 1.6 ± 0.6 at 6-month follow-up, $t=4.12$, $p<0.01$, paired

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

FA and axial/radial diffusivity	Patients ($n=29$)	Healthy controls ($n=37$)	Analysis of covariance [a]	
			$F(1, 62)$	P
Left anterior limb of internal capsule				
FA	0.44 ± 0.05	0.49 ± 0.05	16.7	<0.001**
Axial diffusivity ($\times 10^{-3}$)	4.16 ± 0.36	4.37 ± 0.35	5.46	0.02 *
Radial diffusivity ($\times 10^{-3}$)	3.94 ± 0.33	4.03 ± 0.33	1.38	0.25
Right anterior limb of internal capsule				
FA	0.45 ± 0.05	0.51 ± 0.05	21.8	<0.001**
Axial diffusivity ($\times 10^{-3}$)	4.15 ± 0.36	4.36 ± 0.36	5.19	0.03 *
Radial diffusivity ($\times 10^{-3}$)	3.91 ± 0.33	4.01 ± 0.33	1.38	0.24
Average of right and left anterior limbs of internal capsule				
FA	0.44 ± 0.05	0.50 ± 0.05	20.8	<0.001**
Axial diffusivity ($\times 10^{-3}$)	4.15 ± 0.36	4.36 ± 0.35	5.35	0.02 *
Radial diffusivity ($\times 10^{-3}$)	3.92 ± 0.33	4.02 ± 0.33	1.38	0.24

Data are mean ± S.D.

[a] Age and gender are entered as covariates.

* $p < 0.05$.

** $p < 0.01$.

Table 3
Demographic characteristics of patients and healthy control subjects who could be followed up and who were lost to follow-up.

	Follow-up	Lost to follow-up	t or χ^2	P
Patients	$n=18$	$n=11$		
Age (years)	69.2 ± 8.0	67.6 ± 9.0	0.58	0.57
Female sex (n, %)	5 (26.3)	1 (9.1)	1.45	0.24
mRS score	2.1 ± 0.8	2.4 ± 0.9	1.00	0.33
MMSE score	27.6 ± 3.5	27.5 ± 2.8	0.47	0.64
SDS score	26.9 ± 5.6	27.1 ± 7.3	0.42	0.68
HAM-D score	3.1 ± 2.7	2.3 ± 3.0	0.46	0.65
Controls	$n=19$	$n=18$		
Age (years)	69.1 ± 8.0	65.8 ± 8.1	5.22	<0.01
Female sex (n, %)	10 (55.5)	10 (55.5)	0.03	0.86
MMSE score	29.2 ± 1.2	29.2 ± 0.8	0.10	0.92
SDS score	22.1 ± 1.8	26.2 ± 1.8	1.64	0.11
HAM-D score	0.4 ± 0.6	1.8 ± 2.1	1.61	0.12

t-test), the correlational results were unchanged ($r=-0.61$, $p=0.01$ for the SDS; $r=-0.58$, $p=0.02$ for the HAM-D).

With multiple regression analysis evaluating whether the increased ratio of FA values was related to the change in the SDS and HAM-D depression scores at 6-month follow-up, the ratio of FA values was negatively related to both the changes in the SDS scores (beta$=-0.44$, $p=0.03$) and the HAM-D scores (beta$=-0.46$, $p=0.04$).

4. Discussion

Our findings showed that stroke patients had lower FA in the bilateral anterior limbs of the internal capsule relative to healthy control subjects. Six months after onset, a significant increase in FA was noted, and it was associated with a reduction in depression scale scores. The association of the increase in FA and the reduction in depression scale scores remained significant when we considered the influence of the volume of infarcts and lesion location and the severity of neurological symptoms as a covariate in the partial correlation analysis.

The reduced FA level of patients 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 (Song et al., 2005). Therefore, our finding was not a result of demyelination but rather of gross reduction in axonal number and/or size, possibly reflecting Wallerian degeneration secondary to neuronal loss due to stroke (Thomalla et al., 2004). From an anatomical perspective, the anterior limb of the internal capsule represents the intercept point in the course of the frontal-subcortical circuits (Axer and Keyserlingk, 2000), 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 spatially different sites of cortical and subcortical areas.

The frontal-striatal-thalamic-cortical circuits play an important role in behavioral regulation (Duran et al., 2009), and microstructural change in the anterior limb of the internal capsule was shown to be related to the severity of depressive symptoms in adults with Major Depressive Disorder (MDD) using MRI (Zou et al., 2008). Degeneration in this region may relate to a loss of white matter integrity of these neural circuits (Budde et al., 2007), and this abnormality might trigger the onset of negative mood change. Our finding of a significant negative correlation between the increased ratio of FA values and the change in the depression scale scores 6 months after the stroke might reflect the association between axonal damage of the internal capsule and the negative mood change in stroke patients, and it indicated that recovery from microstructural abnormalities decreased the vulnerability to post-stroke depression, as predicted by elevated depression scale scores.

Our study has some limitations. First, the sample size was not large enough to elucidate the moderate size of difference between groups, and further study with increased numbers of subjects is necessary to draw any definitive conclusions. Second, the stroke patients had predominantly suffered a modest ischemic insult. The extent to which our findings are related to the severity of the ischemic insult was uncertain. Third, patients with significant comprehension deficits were excluded because clinical verbal interviews could not be conducted. Finally, we were not able to control precisely for important variables such as social support, pre-morbid personality, and concurrent and past medication histories. Further analysis, inclusive of consideration of these points, is needed to confirm our present findings.

In conclusion, the present study suggests that FA reduction in the bilateral anterior limbs of the internal capsule is evident in stroke patients. This regional axonal damage should be related to abnormality of neuroanatomical pathways in frontal-subcortical circuits, recent studies of which have highlighted the specific relevance in the pathophysiology of depression due to stroke (Terroni et al., 2011; Paradiso et al., 2013), and it may increase

Table 4
Change in psychometric scores and FA values and axial/radial diffusivity over 6 months in patients ($n=18$) and controls ($n=19$).

	10–28 Days after stroke	6 Months after first exam	Paired *t*-test	*p*
Patients				
Age (years)	69.2 ± 8.0	–	–	–
Female sex (*n*, %)	5 (26.3)	–	–	–
mRS score	2.1 ± 0.8	1.6 ± 0.6	$t_{17}=4.12$	$<0.01^{**}$
MMSE score	27.6 ± 3.5	29.0 ± 2.1	$t_{17}=2.08$	0.05
SDS score	26.9 ± 5.6	29.2 ± 11.5	$t_{17}=0.93$	0.37
HAM-D score	3.1 ± 2.7	2.8 ± 4.9	$t_{17}=0.21$	0.84
Anterior limb of internal capsule				
FA	0.42 ± 0.06	0.44 ± 0.06	$t_{17}=2.16$	0.05^{*}
Axial diffusivity ($\times 10^{-3}$)	4.13 ± 0.37	4.29 ± 0.45	$t_{17}=1.32$	0.21
Radial diffusivity ($\times 10^{-3}$)	3.91 ± 0.34	4.01 ± 0.37	$t_{17}=1.38$	0.37
Controls				
Age (years)	69.1 ± 8.0			
Female sex (*n*, %)	10 (55.5)			
MMSE score	29.2 ± 1.2	29.7 ± 0.5	$t_{18}=0.95$	0.36
SDS score	22.1 ± 1.8	22.8 ± 2.6	$t_{18}=1.80$	0.10
HAM-D score	0.4 ± 0.6	0.3 ± 0.6	$t_{18}=1.00$	0.33
Anterior limb of internal capsule				
FA	0.49 ± 0.03	0.48 ± 0.03	$t_{18}=0.76$	0.46
Axial diffusivity ($\times 10^{-3}$)	4.18 ± 0.66	4.30 ± 0.55	$t_{18}=0.92$	0.37
Radial diffusivity ($\times 10^{-3}$)	3.86 ± 0.60	4.00 ± 0.46	$t_{18}=0.75$	0.46

mRS=Modified Rankin Scale. MMSE=Mini-Mental State Examination. SDS=Zung Self-Rating Depression Scale. HAM-D=Hamilton Rating Scale for Depression.
Data are mean ± S.D.
* $p<0.05$.
** $p<0.01$.

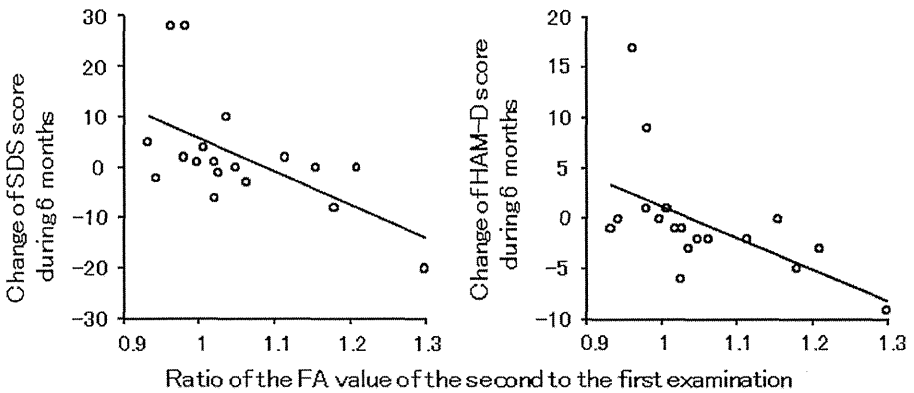

Fig. 2. Scatter plots showing the relationship between the ratio of the FA values of the second to the first examination and the change of depression scale scores among patients ($n=18$) Significant correlations were observed between the ratio of the FA values of the second examination to the first examination, and the changes in depression scale scores ($r=-0.59$, $p=0.01$ for the SDS; $r=-0.88$, $p=0.0001$ for the HAM-D).

the risk of post-stroke depression in conjunction with psychosocial factors following stroke. Further, a significant increase of FA in this region was noted 6 months after the stroke, and an association with a reduction in depression scale scores was revealed. Our findings suggested that the promotion of recovery from the axonal damage of stroke patients might prevent the onset of depressive symptoms. Further investigations are needed to clarify whether and how axonal damage of the internal capsule in stroke patients affects the clinical presentation, treatment response and outcome of depression or other psychiatric conditions in stroke survivors.

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References

Axer, H., Keyserlingk, D.G., 2000. Mapping of fiber orientation in human internal capsule by means of polarized light and confocal scanning laser microscopy. Journal of Neuroscience Methods 94, 165–175.
Brott, T., Adams, H.P.J., Olinger, C.P., Marler, J.R., Barsan, W.G., Biller, J., Spilker, J., Holleran, R., Eberle, R., Hertzberg, V., 1989. Measurements of acute cerebral infarction: a clinical examination scale. Stroke 20, 864–870.
Budde, M.D., Kim, J.H., Liang, H.F., Schmidt, R.E., Russell, J.H., Cross, A.H., Song, S.K., 2007. Toward accurate diagnosis of white matter pathology using diffusion tensor imaging. Magnetic Resonance in Medicine 57, 688–695.

Cohen, J., 1977. Statistical Power Analysis for the Behavioral Sciences. Academic Press, New York.
Drevets, W.C., Price, J.L., Furey, M.L., 2008. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. Brain Structure and Function 213, 93–118.
Duran, F.L., Hoexter, M.Q., Valente, A.A.J., Miguel, E.C., Busatto, G.F., 2009. Association between symptom severity and internal capsule volume in obsessive–compulsive disorder. Neuroscience Letters 452, 68–71.
Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. Journal of Psychiatric Research 12, 189–198.
Hamilton, M., 1960. A rating scale for depression. Journal of Neurology, Neurosurgery and Psychiatry 23, 56–62.
Hasler, G., Fromm, S., Carlson, P.J., Luckenbaugh, D.A., Waldeck, T., Geraci, M., Roiser, J.P., Neumeister, A., Meyers, N., Charney, D.S., Drevets, W.C., 2008. Neural response to catecholamine depletion in unmedicated subjects with major depressive disorder in remission and healthy subjects. Archives of General Psychiatry 65, 521–531.
Inano, S., Takao, H., Hayashi, N., Abe, O., Ohtomo, K., 2011. Effects of age and gender on white matter integrity. American Journal of Neuroradiology 32, 2103–2109.
Kim, D.H., Adalsteinsson, E., Glover, G.H., Spielman, D.M., 2002. Regularized higher-order in vivo shimming. Magnetic Resonance in Medicine 48, 715–722.
Paradiso, S., Ostedgaard, K., Vaidya, J., Ponto, L.B., Robinson, R., 2013. Emotional blunting following left basal ganglia stroke: the role of depression and fronto-limbic functional alterations. Psychiatry Research: Neuroimaging 211, 148–159.
Song, S.K., Yoshino, J., Le, T.Q., Lin, S.J., Sun, S.W., Cross, A.H., Armstrong, R.C., 2005. Demyelination increases radial diffusivity in corpus callosum of mouse brain. Neuroimage 26, 132–140.
Terroni, L., Amaro, E., Iosifescu, D.V., Tinone, G., Sato, J.R., Leite, C.C., Sobreiro, M.F., Lucia, M.C., Scaff, M., Fráguas, R., 2011. Stroke lesion in cortical neural circuits and post-stroke incidence of major depressive episode: a 4-month prospective study. The World Journal of Biological Psychiatry 12, 539–548.
Thomalla, G., Glauche, V., Koch, M.A., Beaulieu, C., Weiller, C., Rother, J., 2004. Diffusion tensor imaging detects early Wallerian degeneration of the pyramidal tract after ischemic stroke. Neuroimage 22, 1767–1774.
Vataja, R., Leppävuori, A., Pohjasvaara, T., Mäntylä, R., Aronen, H.J., Salonen, O., Kaste, M., Erkinjuntti, T., 2004. Poststroke depression and lesion location revisited. Journal of Neuropsychiatry and Clinical Neuroscience 16, 156–162.
Vataja, R., Pohjasvaara, T., Leppävuori, A., Mäntylä, R., Aronen, H.J., Salonen, O., Kaste, M., Erkinjuntti, T., 2001. Magnetic resonance imaging correlates of depression after ischemic stroke. Archives of General Psychiatry 58, 925–931.
Whyte, E.M., Mulsant, B.H., 2002. Post stroke depression: epidemiology, pathophysiology, and biological treatment. Biological Psychiatry 52, 253–264.
Zou, K., Huang, X., Li, T., Gong, Q., Li, Z., Ou-yang, L., Deng, W., Chen, Q., Li, C., Ding, Y., Sun, X., 2008. Alterations of white matter integrity in adults with major depressive disorder: a magnetic resonance imaging study. Journal of Psychiatry and Neuroscience 33, 525–530.
Zung, W.W., 1965. A self-rating depression scale. Archives of General Psychiatry 12, 63–70.