F. 研究発表

原著論文

1. Kitamura A, Fujita Y, Oishi N, Kalaria RN, Washida K, Maki T, Okamoto Y, Hase Y, Yamada M, Takahashi J, Ito H, Tomimoto H, Fukuyama H, Takahashi R, Ihara M. Selective white matter abnormalities in a novel rat model of vascular dementia. Neurobiol Aging 2012 ;33(5):1012.e25-35.

2. Hase Y, Okamoto Y, Fujita Y, Kitamura A, Ito H, Maki T, Washida K, Takahashi R, Ihara M. Cilostazol, a phosphodiesterase inhibitor, prevents no-reflow and hemorrhage in mice with focal cerebral ischemia. Exp Neurol 2012;233(1):523-533.

G. 知的財産権の出願・登録状況

1. 特許出願　　なし
2. 実用新案登録　なし
3. その他

Ⅲ 研究成果の刊行に関する一覧表

原著論文

発表者氏名	論文タイトル	発表誌名	巻号	ページ	出版年
Ihara M, Okamoto Y, Hase Y, Takahashi R.	Association of Physical Activity with the Visuospatial/Executive Functions of the Montreal Cognitive Assessment in Patients with Vascular Cognitive Impairment.	J Stroke Cerebrovasc Dis	22(7)	e146-151	2013
Kalaria RN, Ihara M.	Vascular and neurodegenerative pathways-will they meet?	Nat Rev Neurol	9(9)	487-88	2013
Ihara M, Taguchi A, Maki T, Washida K, Tomimoto H.	A mouse model of chronic cerebral hypoperfusion characterizing features of cognitive impairment.	Methods Mol Biol	1135	95-102	2014
Ihara M, Nishino M, Taguchi A, Yamamoto Y, Hattori Y, Saito S, Takahashi Y, Tsuji M, Kasahara Y, Takata Y, Okada M.	Cilostazol add-on therapy in patients with mild dementia receiving donepezil: a retrospective study.	PloS ONE	9(2)	e89516	2014
Tomimoto H, Wakita H.	Animal models of vascular dementia: translational potential at the present time and in 2050'	Future Neurology	9(2)	163-72	2014
Ota K, Oishi N, Ito K, Fukuyama H, Group S-JS (2014a)	A comparison of three brain atlases for MCI prediction.	J Neurosci Methods	221	139-50	2013

著書

発表者氏名	タイトル	発表誌名	巻号	ページ	出版年
猪原　匡史	認知症における血管病の重要性	脳循環代謝学会誌	24	83-88	2013

Ⅳ　研究成果の刊行物・別刷

Association of Physical Activity with the Visuospatial/Executive Functions of the Montreal Cognitive Assessment in Patients with Vascular Cognitive Impairment

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Background: The Montreal Cognitive Assessment (MoCA) is more suitable than the Mini-Mental State Examination (MMSE) for the detection of vascular cognitive impairment. In this study, we performed a correlation analysis of MoCA/MMSE scores with daily physical activity in patients with subcortical ischemic white matter changes. *Methods:* Ten patients (average 75.9 ± 9.1 years old) with extensive leukoaraiosis detected on magnetic resonance imaging underwent cognitive testing, including the MMSE and the Japanese version of the MoCA (MoCA-J). Physical activity was monitored with the Kenz Lifecorder EX device (Suzuken, Nagoya, Japan) to assess daily physical activity in terms of caloric expenditure, motor activity, number of steps, and walking distance for 6 months. Correlations of individual physical activity with total and subscale scores of MMSE/MoCA-J or 6-month interval change of MoCA-J scores were assessed. *Results:* The total or subscale scores of the MMSE did not correlate with any parameters of physical activity. However, the mean number of steps and walking distance significantly correlated with the total MoCA-J scores (r = .67 and .64, respectively) and its visuospatial/executive subscores (r = .66 and .66, respectively). The mean interval change of MoCA-J was + .6; those who improved number of steps (n = 4; 80.5 ± 3.0 years of age) had significantly preserved MoCA-J scores compared to those who did not (n = 6; 73.0 ± 11.6 years of age; +2.0 versus − .3; P = .016). *Conclusions:* These results suggest that MoCA is useful to detect a biologically determined specific relationship between physical activity and executive function. In addition, physical exercise, such as walking, may help enhance cognitive function in patients with vascular cognitive impairment of subcortical origin. **Key Words:** Leukoaraiosis—Montreal Cognitive Assessment—physical activity—stroke—vascular cognitive impairment.

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The widely used Mini-Mental State Examination (MMSE)[1] is inaccurate in screening vascular cognitive impairment (VCI) because it is insensitive to mild cognitive impairment (MCI) and complex cognitive deficits specifically.[2,3] By comparison, the Montreal Cognitive Assessment (MoCA) has been designed to be sensitive to mild deficits[4] and has been evaluated in amnestic MCI[4] and Parkinson disease.[5] Importantly, the MoCA has been reported to detect more cognitive abnormalities after ischemic stroke or transient ischemic attack compared to the MMSE, particularly in relation to frontal executive function.[6-9]

Executive function is positively associated with physical activity participation in a community-dwelling cohort

of older adults,[10] suggesting that the relationship between physical activity and executive function may be specifically biologically determined. This specific relationship may be explained by the increasing lines of evidence that physical exercise that targets cardiovascular fitness prevents ischemic changes in the frontal lobe, the primary target of the vascular cognitive impairment. However, such a relationship may not be determined with the MMSE, which lacks scope for screening visuospatial/executive performance.

The primary aim of the present study was to test the hypothesis that the MoCA is more sensitive than the MMSE in detecting the relationship between cognitive function and physical activity in patients with ischemic leukoaraiosis, namely subcortical VCI. Because no studies have investigated such relationships using cognitive screening tools, such as the MMSE and MoCA, this study may provide additional evidence that the assessment of physical activity with a continuous monitoring device may provide a practical predictor of domain-specific cognitive decline of ischemic origin, which may be detected with the MoCA in patients with VCI.

Methods

Subjects

Ten patients, whose fluid-attenuated inversion recovery– and T2-weighted magnetic resonance imaging scans revealed confluent hyperintensities in the subcortical white matter (Schmidt scale score of 3)[11] and several punctate high-intensity areas in the basal ganglia, were studied. Patients matching the criteria were enrolled in the study after admission to the Kyoto University Neurology Clinic between May 2008 and March 2011 with various neurologic signs and symptoms. Patients with mild to moderate leukoaraiosis (Schmidt scale score of 1 or 2) were not enrolled in this study in order to exclude the possibility of leukoaraiosis associated with neurodegenerative disorders, such as Alzheimer disease. Patients presenting with severe gait disturbances were also withdrawn, because their inclusion would prevent the assessment of spontaneous physical activity. Each subject was fully instructed on the experimental procedures, and all patients taking part in the study gave written, informed consent, as approved by the Committee of Medical Ethics within our faculty. None of the patients had apparent lesions in the cerebral cortex or hippocampus. Magnetic resonance angiography and duplex color-coded sonography did not reveal >50% stenosis in the major intracranial and extracranial vessels.

Assessment of Physical Activity

Physical activity intensity was monitored with the Kenz Lifecorder EX device (Suzuken, Nagoya, Japan)[12] to assess day-to-day physical activity in terms of caloric expenditure (kcal), motor activity (kcal), number of steps (no.), and walking distance (km) for 6 months. The data were retrieved with physical activity analysis software (Suzuken) in the outpatient clinic every 2 months to ensure adherence to the device and inform the patients of the results of the monitoring. To minimize individual gait variability, the above parameters were averaged over the observational period of 6 months and used for subsequent analysis. In addition, to avoid seasonal gait variability and to cover both warm and cool climates, 6 patients underwent gait assessment from winter to summer and 4 patients from summer to winter. Verbal instructions were given to the patients and their caregivers to encourage physical activity at the outpatient clinic. Those who walked more steps during the last 2 months, compared to the initial 2 months, were retrospectively labeled "improvers," while those who did not were labeled "nonimprovers."

Neuropsychological Evaluation

All subjects underwent a general physical and neurologic examination and neuropsychological assessment, including the MMSE and the Japanese version of the MoCA (MoCA-J).[13] The diagnosis of VCI was made according to the diagnostic criteria proposed by Gorelick et al.[14] To avoid habituation and interference effects, the MMSE was carried out at least 1 week after MoCA-J assessment. Two neurologists were involved in the neuropsychological assessment; if their assessments did not correlate, patients were reexamined for a final evaluation. Changes in MoCA-J over 6 months (ΔMoCA-J) were calculated and compared between the improvers and the nonimprovers.

Statistical Analysis

The statistical significance of intergroup differences was assessed with the Fisher exact test for categorical variables, and continuous variables of demographic data were assessed with the Mann–Whitney *U* test. Correlations of individual physical activity with total and subtest scores of MMSE/MoCA-J or ΔMoCA-J were assessed using Pearson correlation analysis.

Results

Patient Demographic Data

Patient clinical features and demographics are summarized in the Table 1. All patients had at least 1 risk factor for ischemic cerebrovascular disease, including hypertension and diabetes mellitus. All participants were right-handed and ambulant, though 7 patients (70%) had at least some slight postural instability. Most of the patients had lacunar infarctions or microbleeds in the basal ganglia or the thalamus as a manifestation of underlying small vessel pathology.

Table 1. *Patient clinical features and demographics*

Variable	
Mean age, y (range)	76.0 ± 9.1 (55-84)
Gender (M:F)	6:4
Dysarthria, no. (%)	3 (30)
Urinary incontinence, no. (%)	3 (30)
Hypertension, no. (%)	6 (60)
Diabetes mellitus, no. (%)	1 (10)
Cigarette smoking, no. (%)	2 (20)
Sleep apnea, no. (%)	1 (10)
Previous CVD, no. (%)	4 (40)
Use of antithrombotics, no. (%)	6 (60)
Use of statins, no. (%)	4 (40)
LDL cholesterol, mg/dL (±SD)	119 ± 40
Triglyceride, mg/dL (±SD)	118 ± 47
Hematocrit, % (±SD)	40.3 ± 2.8
Fibrinogen, mg/dL (±SD)	272 ± 46
No. of MBs in the BG/thalamus (±SD)	1.4 ± 2.0
No. of lacunas in the BG/thalamus (±SD)	2.6 ± 3.0

Abbreviations: BG, basal ganglia; CVD, cerebrovascular disease; F, female; LDL, low-density lipoprotein; M, male; MBs, microbleeds; SD, standard deviation.

Monitoring of Physical Activity

The monitoring of physical activity was successful at least in 60% of the observation period of 6 months in the 10 patients enrolled. The comparison in number of steps between the initial and the last 2 months found 4 "improvers" (mean age 80.5 ± 3.0 years; 1 man and 3 women) and 6 "nonimprovers" (mean age 73.0 ± 11.6 years; 5 men and 1 woman). Women tended to increase their steps in response to the instructions given in the outpatient clinic (Table 2). There were no significant correlations of age or educational years with the number of steps, walking distance, and motor activity. However, there was a significant inverse correlation of age with the total energy expenditure (r = − .743; P = .01).

Relationship Between Physical Activity and MMSE Scores

The total or subscale scores of the MMSE did not significantly correlate with any parameters assessed with the exercise-measuring device in terms of caloric expenditure, motor activity, number of steps, and walking distance (Fig 2).

Relationship Between Physical Activity and MoCA-J Scores

The total MoCA-J scores significantly correlated with mean number of steps per day (r = .67; P = .032; Fig 3A) and mean walking distance per day (r = .64; P = .045; Fig 3B). Among the subscores of the MoCA-J, only the visuospatial/executive subscores significantly correlated with mean number of footsteps per day (r = .66; P = .034; Fig 3C) and mean walking distance per day (r = .66; P = .037; Fig 3D).

Difference in ΔMoCA-J Between Physical Activity Improvers and Nonimprovers

The mean ΔMoCA-J over the 6 months of the 10 patients enrolled in this study was + .6. The mean ΔMoCA-J of the 4 improvers was +2.0, while that of 6 nonimprovers was − .3. There were significant differences in ΔMoCA-J between the 2 groups (P = .016). However, there were no statistical differences in ΔMoCA-J between the "physically active" group (n = 4, with >5000 steps per day; 79.0 ± 7.3 years of age) and "physically sedentary" group (n = 6, with <5000 steps per day; 74.0 ± 11.0 years of age; P = .38).

Discussion

The main finding of this study is that, in patients with VCI, physical activity (measured by number of steps and walking distance) significantly correlated with the total MoCA-J scores and the MoCA-J visuospatial/executive subscores. In addition, those who increased their physical activity after placement of the device had

Table 2. *Parameters obtained from the activity-monitoring device and the cognitive performance of the subjects included in this study*

Age and sex	83 F	83 F	79 M	77 F	82 M	68 M	67 M	82 F	55 M	84 M
Mean steps, no.	6959	5257	2364	2016	7179	6604	3802	3700	2468	676
% Increase of steps	29.0	52.4	33.6	14.2	−6.6	−33.1	−32.1	−52.8	−13.2	−51.7
Mean walking distance, km	4.36	3.12	1.53	1.44	4.54	4.56	2.48	2.48	1.64	.50
Mean calorie expenditure, kcal	1405	1377	1614	1279	1328	1848	1792	1434	1835	1655
Mean motor activity, kcal	116.7	104.8	65.5	30.6	134.2	177.6	103.0	77.0	61.2	19.9
Educational history, y	14	11	6	9	12	12	12	9	16	11
MMSE score	29	27	22	26	30	23	29	30	30	18
MoCA-J score	25	23	15	20	28	18	21	24	22	9
ΔMoCA-J score	1	1	2	4	−2	0	0	0	−1	1

Abbreviations: F, female; M, male; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; ΔMoCA-J, 6-month interval change of MoCA-J.

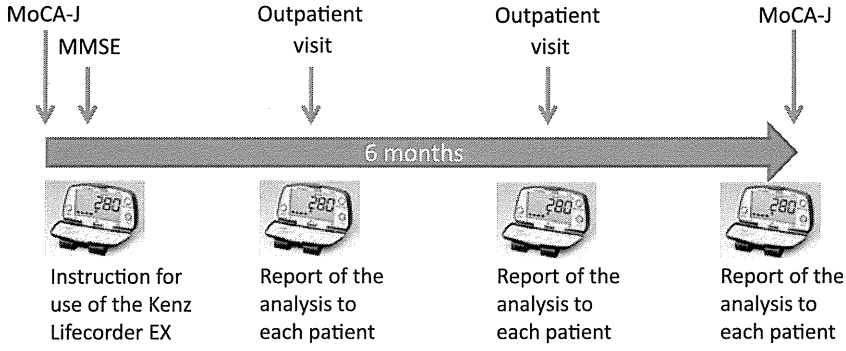

Figure 1. *Observational study protocol. Physical activity intensity was monitored with the Kenz Lifecorder EX device (Suzuken, Nagoya, Japan) to assess day-to-day physical activity in terms of caloric expenditure (kcal), motor activity (kcal), number of steps (no.), and walking distance (km) for 6 months. In the outpatient clinic, the data were retrieved every 2 months to ensure adherence to the device and to inform the patients of the results of the monitoring. All subjects underwent the Japanese version of the Montreal Cognitive Assessment (MoCA-J) and the Mini-Mental State Examination (MMSE) at the beginning of the study and the MoCA-J at the end of the study. To avoid habituation and interference effects, the MMSE was carried out at least 1 week after the MoCA-J assessment.*

significant preservation of the MoCA-J scores. However, the parameters of physical activity did not correlate with MMSE total scores or any subscores. The MoCA is reported to be more suitable in the detection of frontal executive function after ischemic stroke or transient ischemic attack when compared to the MMSE.[6-9] These results suggest that the MoCA is more appropriate in the detection of the biologically determined specific relationship between physical activity and executive function in patients with VCI, and that increased physical activity contributes to cognitive enhancement in such patients.

The Kenz Lifecorder EX physical activity measuring device (Suzuken) used in this study (Fig 1) is a community- and home-based monitoring system with a maximum of 200 days' memory for analyzing patient lifestyle.[15] Data from the device can be downloaded to a computer in an outpatient clinic and analyzed using physical activity analysis software. This allows continuous in-home monitoring, giving a more accurate assessment than, for example, a single gait test given at an annual physical examination, because walking speed taken at a single time point may overestimate walking abilities in the elderly, especially in a hospital setting. Because daily physical activity is thought to affect brain health, such a device could be valuable in monitoring sedentary behavior as a predictor of executive dysfunction in patients with VCI.

In this study, physical activity was monitored for 6 months, and the MoCA-J was assessed at the start and end of the study. Intriguingly, the mean MoCA-J scores increased by .6 points after the observation period of 6 months, while verbal instructions were given to patients to increase physical activity every 2 months. In addition, the patients were informed of the aim of this study at the beginning, which may have served as a motivating factor for physical activity and contributed to subsequent cognitive preservation in the patients, especially in the physical activity "improvers." Consistent with this notion, aerobic-based exercise may be of specific benefit in delaying the progression of cognitive decline among elderly individuals with VCI.[16] In addition, physical exercise appears to have a preventative effect on VCI in a group of elderly female patients.[17] Further study involving a longer observational period is required to clarify whether such exercise-monitoring devices are helpful in motivating VCI patients to continue exercise and thus potentially halt the progression of the cognitive impairment.

The main limitation of this study is the small number of patients enrolled, because only those with most severe white matter changes were eligible in order to minimize the heterogeneity of VCI and to maximally exclude those with white matter changes secondary to neurodegenerative changes.[18] To explore our findings, future studies should be performed in larger cohorts by recruiting not only patients with subcortical VCI but also those with

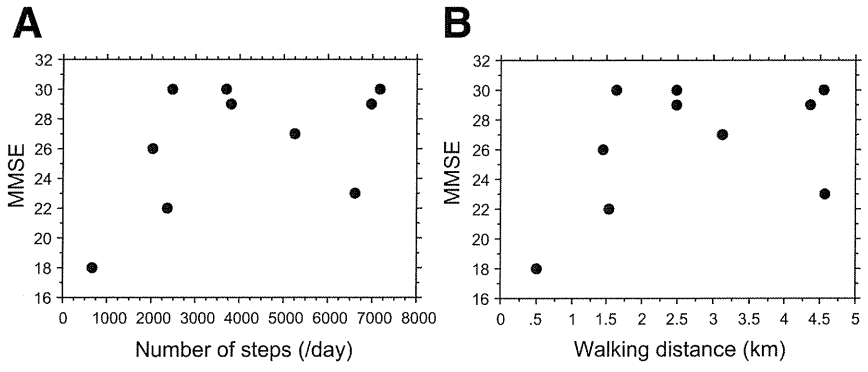

Figure 2. *There was no correlation of the Mini-Mental State Examination (MMSE) scores with physical activity. The total scores of the MMSE did not significantly correlate with the number of footsteps or walking distance assessed with the Kenz Lifecorder EX device (Suzuken, Nagoya, Japan).*

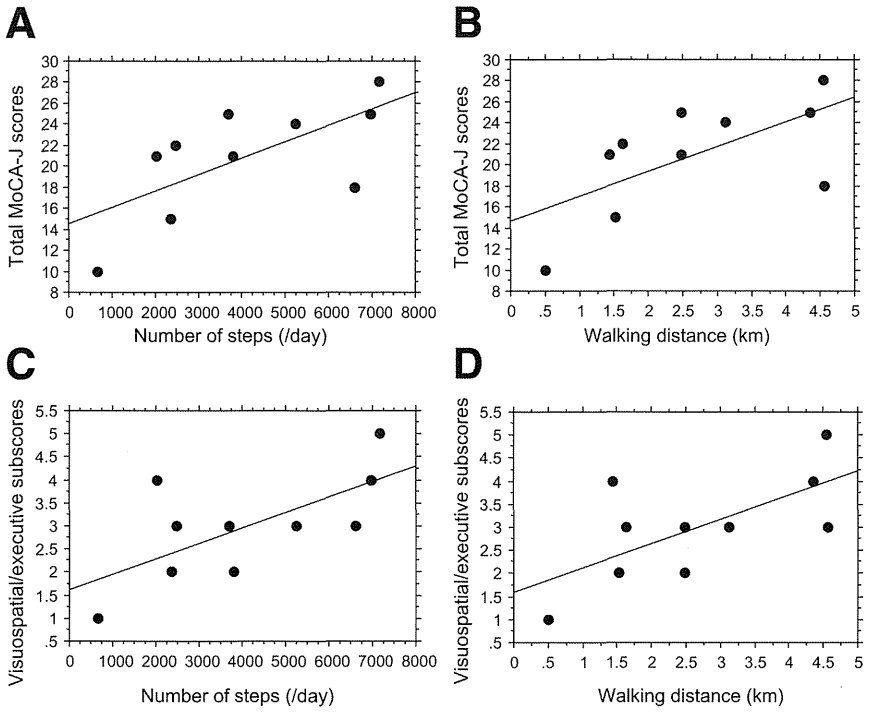

Figure 3. *There was significant correlation with the Japanese version of the Montreal Cognitive Assessment (MoCA-J) scores and physical activity. The total MoCA-J scores significantly correlated with the mean number of steps per day (r = .67; P = .032; A) and the mean walking distance per day (r = .64; P = .045; B). Among the subscores of the MoCA-J, only the visuospatial/executive subscores of the MoCA-J significantly correlated with the mean number of steps per day (r = .664; P = .034; C) and the mean walking distance per day (r = .658; P = .037; D).*

other VCI subtypes. Another limitation is that although the MoCA-J score was significantly preserved in the patients with improved physical activity, the 6-month follow-up period may be too short to influence the underlying mechanisms of VCI. Because of the above limitations, this preliminary study should be viewed as hypothesis-generating and should be followed by larger studies to confirm the results and to explore the lifestyle determinants of dementia. Nevertheless, our study has suggested that the assessment of physical activity with a continuous monitoring device may provide a practical predictor of domain-specific cognitive decline of ischemic origin and a rationale for intervention in patients with VCI.

In conclusion, the positive correlation of the executive and visuospatial performance of the MoCA-J with daily physical activity suggests potential benefits of exercise against domain-specific cognitive decline of subcortical ischemic origin.

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DEMENTIA

Vascular and neurodegenerative pathways—will they meet?

Raj N. Kalaria and Masafumi Ihara

Vascular and neurodegenerative pathologies are known to co-occur in Alzheimer disease, and were recently proposed to drive progression to dementia through independent pathways. A new study provides evidence in support of this hypothesis, by showing dissociation between the pathologies and regional brain metabolism. But might the two pathways converge?

Kalaria, R. N. & Ihara, M. *Nat. Rev. Neurol.* **9**, 487–488 (2013); published online 13 August 2013; doi:10.1038/nrneurol.2013.164

Loss of brain white matter (WM) is common in the elderly and is associated with cognitive decline and disability.[1,2] Given the heterogeneous nature of brain atrophy, however, these signs are not always apparent. MRI enables clinicians to observe the spectrum of changes in the brain as WM hyperintensities (WMHs)—measures that are widely accepted to represent underlying cerebrovascular disease (CVD) and are considered as a surrogate marker of small-vessel disease. Extensive WMHs in deep WM is thought to be a marker of subcortical ischaemic vascular disease, corresponding to chronic hypoperfusion. WMHs are common in neurodegenerative disorders such as Alzheimer disease (AD) and dementia with Lewy bodies, but to a lesser extent than in vascular dementia (VaD). Although marked microvascular changes are found in the majority of elderly people, it is now apparent that the combination of vascular pathology and neurodegenerative changes is additive, lowering the threshold of dementia risk.[3] WMHs in AD are often—although not always—linked to cerebrovascular risk factors such as hypertension, atherosclerosis and diabetes, and an interaction between vascular mechanisms and neurodegenerative processes that leads to late-onset dementia has been proposed. Support for this hypothesis has now been provided by data from a new study from Haight and colleagues for the Alzheimer's Disease Neuroimaging Initiative (ADNI).[4]

The pathological substrates of WMH and, therefore, the resultant degree and type of WM changes, are heterogeneous. The most common lesions are microvessel wall modifications, followed by perivascular space modifications, myelin loss and infarcts. Although such alterations are less prominent in AD than in VaD, they are more common in AD than in any other neurodegenerative disorder.[3] Disturbances in cerebrospinal fluid (CSF) production, disruption in blood–brain barrier permeability, and cerebral oedema have been cited as important factors that contribute to development of WM changes. In addition, age-related functional deficits in the branches of perforated arteries can also exist. However, WMHs might also be indicative of primary axonal degeneration that occurs as a result of neuronal damage in the grey matter. Thus, two disparate mechanisms of WM degeneration—vascular-associated and neurodegeneration-associated—could present as differential patterns of myelin loss in early stages of impairment and dementia.[5]

Temporal lobe WM exhibits more anomalies than WM in other brain regions in late-stage dementia of all types, but particularly in AD dementia, in which axonal degeneration with secondary demyelination is likely to be prominent. Frontal WM seems to be particularly vulnerable to age-related changes and vascular disease as compared with temporal WM.[6] Diffusion tensor imaging studies in the general population suggest that disintegration of WM microstructure occurs preferentially in fibre populations within the prefrontal region and internal capsule. Both the sclerotic changes in the medullary arteries and WM changes in subcortical VaD are most prominent in the frontal lobe—which may be more susceptible to a haemodynamic

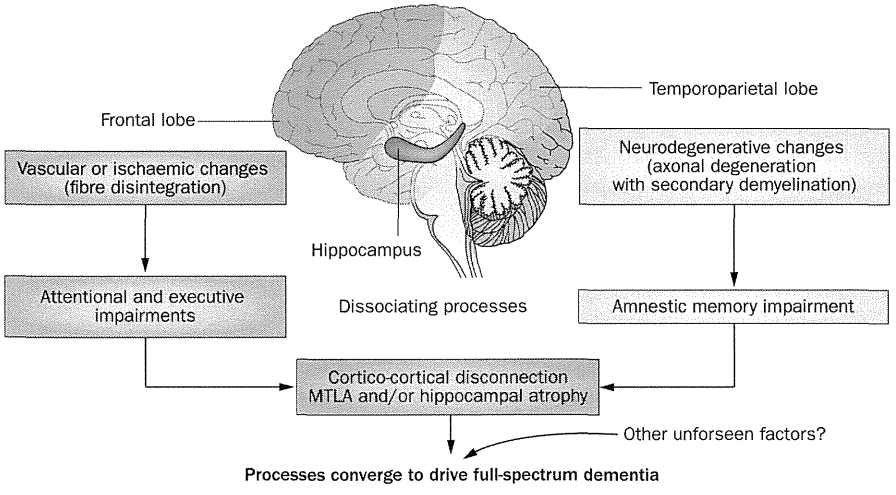

Figure 1 | Vascular and neurodegenerative pathways to dementia. The frontal white matter (WM) is preferentially vulnerable to vascular disease and ageing. By contrast, WM changes in the temporal lobe in Alzheimer disease (AD) may be preferentially caused by axonal degeneration consequent to neuronal damage in the grey matter. Frontal WM changes lead to attentional and executive impairments, whereas temporal alterations result in amnestic memory impairment in a mutually exclusive (dissociative) manner at earlier stages. At later stages of disease, the two pathologies converge to cause full-spectrum dementia, in which other as yet unknown factors might also play a part.

derangement than the temporal lobe—and lead to severe WM changes and reduced frontal lobe metabolism. Consistent with the 'frontal hypothesis', frontal lobe changes have implications for impairment in attentional and executive functions that are associated with fronto–subcortical circuits. By contrast, temporal lobe changes are closely linked to amnestic memory impairment, a feature that is characteristic of AD, although executive dysfunction has also been reported in AD.

As previously proposed,[7] it stands to reason that a vascular process is involved when frontal WM lesions are evident together with AD in patients who are in early disease stages or in those who convert to AD from mild cognitive impairment (MCI). The recent ADNI study[4] makes this long-accepted view clear, particularly given that the study excluded individuals with overt evidence of CVD: the study involved 203 patients with MCI with a Hachinski Ischaemic Score <4. Each participant was examined at baseline using MRI and fluorodeoxyglucose-PET, and re-evaluated over a 3-year period. CSF samples were collected from half of the cohort.

One profound finding of the ADNI study[4] was dissociation of the presumptive pathological substrates of dementia (namely, WMHs as a marker of vascular damage and amyloid-β [Aβ] as a marker of neurodegeneration) and regional glucose metabolism in patients who developed dementia. Whether standard voxel-based methods of PET analysis would have led to different conclusion is unknown, but the study found that greater burden of WMH (1% relative to total brain volume) was associated with decreased frontal metabolism, whereas low CSF Aβ was linked with temporoparietal hypometabolism and other AD-associated markers, such as hippocampal volume and possession of an apolipoprotein ε4 allele.

The inverse association between WMH burden and frontal metabolism is consistent with evidence showing that vascular-based insufficiency in WM can instigate cortical neuronal degeneration. The direct association between CSF Aβ load and temporoparietal metabolism is in agreement with proposed models of AD neurodegeneration in which the WMHs can appear subsequent to primary neurodegenerative changes in the cortex. Haight *et al.* found that these associations were similar in individuals who developed AD and in those who did not convert to AD,[4] suggesting that, regardless of dementia status, frontal hypometabolism is associated with higher WMH burden, and temporoparietal metabolism is increased in tandem with CSF Aβ concentrations. These observations emphasize that even if pathological processes that lead to AD dementia are accelerated in individuals with increased Aβ, such individuals still exhibit significant frontal WM vascular burden. Notably, a positive association between greater WMHs and greater temporoparietal metabolism was observed only in individuals with non-progessing MCI; in these patients with high WMH burden, elevated levels of temporoparietal metabolism might enable them to remain cognitively stable.

Medial temporal lobe atrophy (MTLA) is often associated with AD. Hypertension—the strongest vascular disease risk factor for AD—is strongly linked to frontal lobe symptoms, but is also associated with MTLA. Although postmortem verification is lacking in most studies, MTLA with hippocampal degeneration occurs in dementia and is caused by sporadic and familial small-vessel disease or post-stroke dementia that is unexplained by typical neurodegenerative pathology.[8] It is plausible that the results in the ADNI study[4] would have been different if vascular-disease risk factors were considered or if a community sample was evaluated in parallel, particularly as mixed pathologies (including CSF Aβ and tau) are common in the oldest old.[3] The observations of Haight *et al.*[4] provide evidence that the respective pathological conditions, when they co-occur, operate simultaneously through metabolic alterations in different brain regions, and that they potentially represent independent pathways to AD progression (Figure 1). The conclusions would be more robust, however, if they were supported by similar evidence of markers of tau pathology or of Aβ brain distribution.

Although the ADNI results[4] do not provide information on what factors might tip individuals over the dementia threshold, several other factors that could explain progression to dementia warrant consideration.[9] Prior observations of a strong direct association between Braak stage of neurofibrillary pathology and frontal lobe deep WMH burden in the oldest old suggests that, at least in this population, frontal lobe myelin or axonal abnormalities (presumably resulting from ischaemic lesions or vascular insufficiency) are linked to limbic neurodegeneration that is typical in AD. This observation further supports the linear relationship between hippocampal atrophy and WM lesions in the frontal lobe but, surprisingly, not in the temporal lobe in patients with AD.[10] To consider the frontal and temporoparietal lobes as entirely separate seems implausible. Whether these processes are linked through other factors and whether, through cortico-cortical disconnection, ischaemic and neurodegenerative processes could have additional and possibly synergistic effects on cognition, remain to be determined.

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Competing interests
The authors declare no competing interests.

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Chapter 8

A Mouse Model of Chronic Cerebral Hypoperfusion Characterizing Features of Vascular Cognitive Impairment

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Abstract

Vascular dementia or vascular cognitive impairment occurs as a result of persistently compromised blood flow to the brain and represents the second most common type of dementia after Alzheimer's disease. In order to investigate its underlying mechanisms, a mouse model of chronic cerebral hypoperfusion has been developed, which involves the narrowing of the bilateral common carotid arteries with newly designed microcoils. This mouse model provides a unique platform to investigate the mechanisms of angiogenesis following chronic cerebral hypoperfusion and to explore potential drugs or cell therapies designed to enhance angiogenesis as a preclinical step toward developing novel treatments for dementia of vascular origin.

Key words Chronic cerebral hypoperfusion, Vascular cognitive impairment, Dementia, Bilateral common carotid artery occlusion, Microcoil, Mouse, Cerebral blood flow, Laser speckle flowmetry

1 Introduction

Cerebral blood flow (CBF) is decreased diffusely, or at least focally, in elderly patients with vascular cognitive impairment [1, 2]. To mimic such persistent cerebral ischemia in humans, a chronic cerebral hypoperfusion model has been established in rats, gerbils, and mice [3–5]. The model can be generated by bilateral common carotid artery (CCA) occlusion in rats [3, 6], bilateral CCA stenosis in mice (BCAS) [4, 7–9] or in gerbils [5], and unilateral CCA occlusion in mice [10]. Although nonhuman primates appear to represent the best model for the study of vascular cognitive impairment, due to their similarities in cerebral vascular architectures with humans [11], most experiments studying chronic cerebral hypoperfusion have been performed in rodents because of the ease of handling and greater ethical acceptability.

Among rodent models of chronic cerebral hypoperfusion, the rat model is most widely used [12, 13], resulting in cognitive impairment and cholinergic deficits in the animal [3, 6, 14].

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The animals also develop white matter rarefaction [3, 9, 15], which appears very similar to that found in human cerebrovascular white matter lesions. However, the rat model does possess inherent drawbacks. For example, the visual pathway is invariably damaged by the occlusion of the ophthalmic arteries, thus potentially compromising behavioral assessment. Furthermore, genetic studies may be hampered because of limited accessibility to molecular technologies in the rat.

To circumvent such limitations, we have established a mouse model of chronic cerebral hypoperfusion, which is subjected to various degrees of CBF reduction by the narrowing of the bilateral CCAs with newly designed microcoils [4, 7–9]. The severity of ischemia/hypoperfusion can be easily controlled by internal diameter regulation of the microcoils [4]. The model demonstrates good reproducibility in terms of glial activation, blood–brain barrier disruption, white matter lesion appearance, and vascular cognitive impairment, which appear within a month after the surgery. In the longer-term model, significant hippocampal changes (atrophy and cell death) are documented 8 months after surgery, providing evidence linking chronic cerebral hypoperfusion with neurodegeneration [16]. The apparent advantage of using this surgical technique is that it provides a unique platform to investigate the mechanisms of angiogenesis and to explore potential drugs [17, 18] or cell therapies [19] designed to enhance angiogenesis as a preclinical step toward developing novel treatments for dementia of vascular origin. Another important advantage is its easy application to genetically engineered mice; for instance, β amyloid-overexpressing mice have been subjected to BCAS to clarify the wider question of whether and how chronic cerebral hypoperfusion has detrimental effects on Alzheimer's disease [20–22].

The aims of the current chapter are therefore to provide technical details that have accumulated since establishment of this mouse BCAS model, focusing particularly on a method of CBF monitoring using laser speckle flowmetry in order to consider the particular strengths and pitfalls of the method.

2 Materials

2.1 Animals and Surgical Appliances

1. Male C57BL/6J mice (weight, 24–29 g) (*see* **Note 1**).
2. Animal restraining device.
3. Heating pad.
4. Rectal thermometer.
5. Chemical depilatory.
6. Surgical scrub solutions: povidone-iodine scrub (Betadine scrub) or chlorhexidine scrub.

7. Anesthetics: halothane or isoflurane (*see* **Note 2**).
8. Surgical knife.
9. Silk suture.
10. Forceps: fine-tip and blunt-tip forceps.
11. Gauze.
12. Stainless steel wound clips.
13. Microcoils: manufactured in the Sawane Spring Co., Ltd. (Hamamatsu, Japan) (*see* **Note 3**).
14. Operating microscope.

2.2 CBF Monitoring Device

1. Laser speckle blood flow imager (Omegawave, Inc., Tokyo, Japan).
2. A calibration reference device (Calibrator S/N 080715-5, Omegawave, Inc.)
3. Gel (Aquasonic, Parker Laboratories, Inc., Fairfield, NJ).

3 Methods

Before performing surgery, one should be aware that there are legal and ethical requirements regarding the use of animals in research. The animal must be maintained in a surgical plane of anesthesia, and its vital signs monitored and regulated throughout the procedure.

3.1 Surgical Procedures

1. Anesthetize the mouse with gas anesthetics (*see* **Note 4**).
2. Place the anesthetized mouse in a dorsal recumbent position on the operating board with the tail toward the surgeon.
3. Shave the ventral neck area with a chemical depilatory and swab with surgical scrub.
4. Make a 1.0–1.5 cm midline skin incision from the base of the neck to the point below the lower jaw.
5. Remove the underlying fat and move the salivary glands laterally or upwards using forceps to maximize the operating field.
6. Expose and free both common carotid arteries (CCAs) from their sheaths under an operating microscope (*see* **Note 5**) (Fig. 1).
7. Place two 4–0 silk sutures around the distal and proximal parts of the CCA.
8. Gently lift the artery by the sutures and place between the loops of the microcoil just proximal to the carotid bifurcation (Fig. 1) (*see* **Note 6**).
9. Twine the microcoil by rotating it around the CCA (*see* **Note 7**).
10. 30 min later, twine another microcoil of the same size around the other CCA (Fig. 1) (*see* **Note 8**).

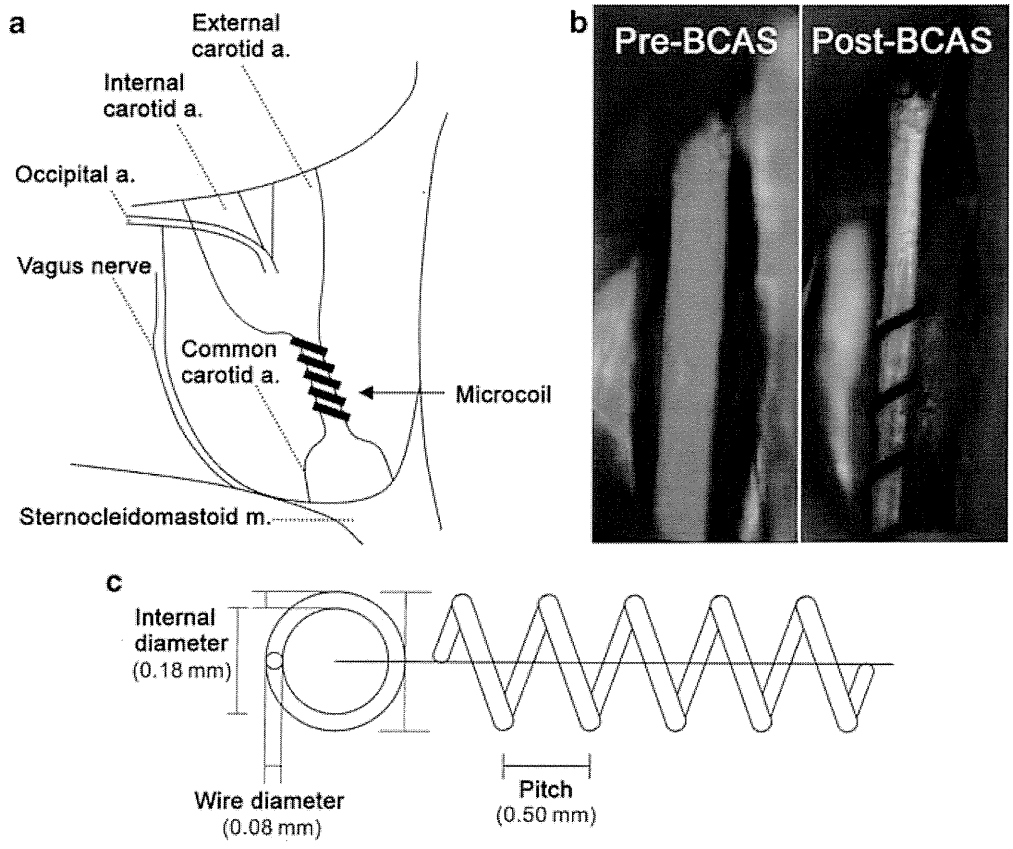

Fig. 1 The procedure for BCAS and microcoil placement. The microcoil is twined by rotating it around the CCA just proximal to the carotid bifurcation of a C57BL/6J mouse (**a**). Representative photographs of a FITC-perfused common carotid artery before (*left*) and after (*right*) placement of a microcoil (**b**). The most frequently used microcoil is made from piano wire (wire diameter of 0.08 mm) with an inner diameter of 0.18 mm, pitch 0.50 mm, and total length 2.5 mm (**c**)

11. Close the incision with stainless steel wound clips or fine sutures.
12. Return the mouse to the animal holding area after they appear normal (*see* **Note 9**).

3.2 Cerebral Blood Flow (CBF) Monitoring

1. Place the anesthetized mouse in a ventral recumbent position with the tail toward the surgeon (*see* **Note 4**).
2. Surgically remove the scalp to expose the skull.
3. Using fine-tip forceps, remove the periosteum, which adheres to the skull (*see* **Note 10**).
4. Wipe the skull surface with saline-soaked gauze and then cover with a thin layer of gel to prevent drying (*see* **Note 11**).
5. Perform calibration with a calibration reference device (*see* **Note 12**).
6. Perform CBF recordings through the skull using the laser speckle blood flow imager, (*see* **Note 13**) (Fig. 2). Define circular regions of interest on the image for quantitative measurement (*see* **Note 14**).

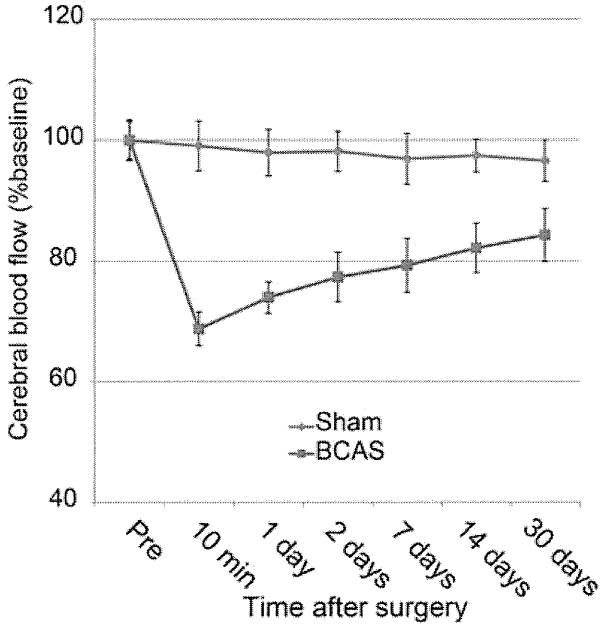

Fig. 2 Cerebral blood flow after BCAS. This figure shows temporal profiles of cerebral blood flow evaluated with laser speckle imager in mice at 2.5 months of age after the surgery using microcoils with diameter of 0.18 mm (*square*) and in sham-operated mice (*diamond*). The data represent mean values ± standard deviation expressed as a percentage of the preoperative value. The CBF values decreased significantly from the preoperative baseline after the surgery with the 0.18 mm diameter microcoils. Immediately after surgery (10 min), there was a significant reduction in CBF values to 68.8 ± 2.8 %, with gradual recovery to 84.3 ± 4.3 % at 1 month ($n=7$), probably due to compensatory mechanisms involving angiogenesis and arteriogenesis [4, 17]. Note that the CBF values, as a percentage of the preoperative value in the sham-operated mice ($n=7$), tended to decrease, although not significantly, probably as a result of minimal fibrous scar tissue build up and bone opacification regardless of appropriate treatment (*see* **Note 10**)

4 Notes

1. This model should be applied exclusively to the C57BL/6J strain, as CBF in other strains may have a greater variability after BCAS [4, 8, 23].
2. Although anesthetics are known to provide varying degrees of neuroprotection against ischemic injury, the selection of anesthesia did not appreciably affect the mortality rates, temporal profile of CBF, and ischemic white matter changes after BCAS.
3. Four types of microcoils are made from piano wire with varying inner diameters from 0.16 mm to 0.22 mm. Researchers in Japan may obtain the microcoils directly from the manufacturer (Sawane Spring Co., Ltd, Hamamatsu, Japan), but those outside Japan may purchase the microcoils from Invitrotech Co., Ltd. (Kyoto, Japan). The microcoils should be thoroughly disinfected with alcohol and air dried just before use.

4. Anesthesia is usually induced with 4 % isoflurane and maintained with 1.5 % isoflurane, via a face mask. Alternatively, halothane may be used with 4 % for induction and 1.5 % for maintenance.
5. Separate the carotid from the vagus nerve, which is a white, string-like object directly lateral to the carotid artery. Particular care should be taken to avoid damage to the vagus nerve.
6. Cessation of CBF for >1 min should be avoided.
7. For this manipulative procedure, one should avoid piercing the artery with either end of the microcoil. Instead of rotating the microcoil around the CCA, one may alternatively wind the artery along the groove of the coil.
8. In our original report, we used 30 min intervals between manipulations on the left and right CCAs to avoid early mortality [4]. However, no intervals may be required to generate this model. During the surgery, rectal temperature should be maintained between 36.5 °C and 37.5 °C.
9. The mortality rates range from 3 % to 5 % in mice with microcoils of 0.18 mm in diameter (unpublished data), although earlier studies suggested higher mortality rates, 13 % in mice with microcoils of 0.22 mm in diameter, 17 % in those of 0.20 mm, and 15–19 % in those of 0.18 mm [4, 24]. In contrast, 75 % (15/20) of mice with microcoils of 0.16 mm diameter administered died within 14 days after the surgery, most of whom were found to have cerebral infarctions [4]. In another study of a modified model with a 0.16 mm microcoil placed on the left CCA and the 0.18 mm microcoil on the right CCA, the mortality rate is reported to be 18.8 % [25].
10. It is important to remove the periosteum, which adheres tightly to the skull, with fine-tip forceps to minimize fibrous scar tissue buildup without significant changes in flow signals in sham-operated mice [19]. Swabbing the skull surface should be done gently. Rough or inappropriate treatment leads to scar tissue build up along the cranial sutures. It is difficult to reverse fibrous scar tissue buildup and bone opacification. If they do occur, another animal should be used for the experiment. Although most bleeding stops spontaneously, the wound should be checked postoperatively for blood stains on the skull, which, if present, must be removed gently with saline-soaked gauze.
11. For each recording, the skull surface should be wiped with saline-soaked gauze, covered with a thin layer of gel (Aquasonic, Parker Laboratories, Inc.), and immersed for 5–10 min. Care should be taken to ensure the surface is fully wet as indicated by a semitransparent appearance. A dry surface, as indicated by a white skull, or a partially wet surface results in a reduction in flow signal intensity.

12. Laser speckle imaging requires a baseline to anchor for repetitive measurement or comparison between different subjects. We use a calibration reference device (Calibrator S/N 080715-5, Omegawave, Inc.) and assign a value to this reference material (arbitrarily assigned value, 25.0). The value is attributed to the Brownian motion of red-colored particles (0.35 μm, 24 °C). Calibration with this device before each test provides standardized values for comparison.
13. CBF should be measured after CBF is stabilized after anesthesia. During the CBF recordings, the rectal temperature should be maintained between 36.5 °C and 37.5 °C. One can successfully image through the skull repeatedly up to 30 days after the operation [17, 19, 26]. Success is mostly dependent on the degree of removal of the periosteum.
14. It is ideal to measure the mean CBF in identically sized regions of interest (900 pixels) located 1 mm posterior and 2 mm lateral from the bregma.

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