光、山本雅、狩野方伸、武田雅俊、 p250GAP 遺伝子は統合失調症のリスク及び統合失調症型パーソナリティと関連する、第 22 回日本臨床精神神経薬理学会第 42 回日本神経精神薬理学会合同年会、栃木、10.18-20(19),2012. ポスター

27. 梅田 知美、橋本亮太、山森英長、岡田武也、安田由華、大井一高、福本素由己、伊藤彰、武田雅俊、統合失調症関連遺伝子 ZNF804A は TGF-β シグナリングに関与する遺伝子発現を制御する、第 22 回日本臨床精神神経薬理学会第 42 回日本神経精神薬理学会合同年会、栃木、10.18-20(19),2012. ポスター
28. 安田由華、橋本亮太、大井一高、福本素由己、山森英長、疇地道代、岩瀬真生、数井裕光、武田雅俊、自閉症スペクトラム障害におけるハノイの塔課題時の前頭葉の血流低下について：２チャンネル NIRS（Near Infra- Red Spectroscopy）による脳機能計測研究、第 22 回日本臨床精神神経薬理学会第 42 回日本神経精神薬理学会合同年会、栃木、10.18-20(19),2012. ポスター
29. 山森英長、橋本亮太、安田由華、福本素由己、大井一高、藤本美智子、武田雅俊、阪大病院における 23 症例の治療抵抗性統合失調症患者へのクロザピンの使用経験：副作用によって中止した症例のの転帰について、第 22 回日本臨床精神神経薬理学会第 42 回日本神経精神薬理学会合同年会、栃木、10.18-20(18),2012. ポスター
30. **Hashimoto R**, Ohi K, Yasuda Y, Fukumoto M, Yamamori H, Takahashi H, Iwase M, Okochi T, Kazui H, Saitoh O, Tatsumi M, Iwata N, OzakiN, Kamijima K, Kunugi H, Takeda M. Variants of the RELA gene are associated with schizophrenia and their startle responses. 第 34 回日本生物学的精神医学会、神戸、9.28-30(29), 2012. ポスター
31. 橋本亮太、安田由華、山森英長、梅田知美、深井綾子、大井一高、福本素由己、毛利育子、谷池雅子、松本直通、武田雅俊、広汎性発達障害の原因遺伝子とその探索ストラテジー- Research strategy for identifying ASD genes-、第 34 回日本生物学的精神医学会、第 11 回アジア太平洋神経化学会大会・第 55 回日本神経化学会大会合同シンポジウム、神戸、9.28-10.2(30), 2012. 口演
32. 大井一高、橋本亮太、安田由華、根本清貴、大西隆、福本素由己、山森英長、梅田知美、岩瀬真生、數井裕光、武田雅俊、統合失調症と NRGN 遺伝子：統合失調症の中間表現型への影響、The NRGN gene and schizophrenia: Impact of the gene on intermediate phenotypes for schizophrenia. 第 34 回日本生物学的精神医学会、神戸、9.28-30(30), 2012. 口演
33. 山森英長、橋本亮太、梅田知美、Cyndi Shannon Weickert、安田由華、大井一高 、福本素由己、藤本美智子、伊藤彰、武田雅俊、統合失調症患者死後脳における、GWAS で報告された統合失調症関連遺伝子の発現解析-Expression analysis of schizophrenia risk genes identified in GWAS using post mrtem brain-、第 34 回日本生物学的精神医学会、神戸、9.28-30(30), 2012. ポスター
34. 梅田 知美、橋本亮太、山森英長、岡田武也、安田由華、大井一高、福本素由己、伊藤彰、武田雅俊、統合失調症関連遺伝子 ZNF804A は TGF-β シグナリングに関与する遺伝子発現を制御する-The Regulation of gene expression involved in TGF-β signaling by ZNF804A, a risk gene for schizophrenia. 第 34 回日本生物学的精神医学会、神戸、9.28-30(29), 2012. ポスター
35. 沼田周助、木下誠、田嶋敦、大井一高、橋本亮太、下寺信次、井本逸勢、武田雅俊、

大森哲郎、メタアナリシス解析を用いたDISC 1機能多型と統合失調症の遺伝子関連研究- Meta-analysis of association studies between DISC1 missense variants and schizophrenia in Japanese population-、第34回日本生物学的精神医学会、神戸、9.28-30(30), 2012. ポスター

36. 安田由華、橋本亮太、大井一高、福本素由己、山森英長、畦地道代、岩瀬真生、数井裕光、武田雅俊、自閉症スペクトラム障害におけるハノイの塔課題時の前頭葉の血流低下：2チャンネルNIRSによる研究-Reduced prefrontal activation in Autism Spectrum Disorders:A two-channel NIRS study-第34回日本生物学的精神医学会、神戸、9.28-30(29), 2012. ポスター
37. 橋本亮太、大井一高、安田由華、山森英長、梅田知美、福本素由己、武田雅俊、「ゲノム研究による精神疾患診断は可能か？」、シンポジウム：バイオロジカルマーカー研究からDSM-Vに向けて、Biological marker research towards DSM-V、第34回日本生物学的精神医学会、神戸、9.28-30(30), 2012. 口演
38. 橋本亮太、大井一高、安田由華、山森英長、梅田知美、岩瀬真生、数井裕光、武田雅俊、シンポジウム：統合失調症の分子メカニズム研究の新しい潮流：中間表現型を用いた統合失調症の病態解明、第35回日本神経科学大会、愛知、9.18-21(19), 2012. シンポジウム、講演、座長
39. 中澤敬信、橋本亮太、武田雅俊、狩野方伸、シンポジウム：統合失調症の分子メカニズム研究の新しい潮流：p250GAP/TCGAPファミリーRhoGAP遺伝子と統合失調症との関連性の解析、第35回日本神経科学大会、愛知、9.18-21(19), 2012. シンポジウム、講演
40. 橋本亮太、「FMにおける精神疾患-治療構造の構築法と鑑別診断-」、日本線維筋痛症学会第4回学術集会、長崎、9.15-16(16).2012. 講演
41. Yamamori H, **Hashimoto R**, Umeda-Yano S, Yasuda Y, Ohi K, Ito A, Takeda M. Expression analysis of the genes identified by GWAS in postmortem brain tissues from BPD and SZ. The 2nd Meeting of East Asisan Bipolar Forum(2nd EABF). Fukuoka, 9.7-8(7),2012. 口演
42. 橋本亮太、うつ病：モノアミン仮説から神経栄養因子仮説へ—新しい治療とより早い診断を目指した神経科学の最前線—、第九回日本うつ病学会総会モーニングセミナー1、東京、7.27-28(28),2012.
43. 中澤敬信、橋本亮太、大井一高、安田由華、山森英長、山本雅、武田雅俊、狩野方伸、p250GAP/TCGAPファミリーRhoGAP遺伝子と統合失調症との関連性の解析、2012年度包括脳ネットワーク夏のワークショップ、仙台、7.24-27(26), 2012. ポスター
44. 橋本亮太、大井一高、安田由華、梅田知美、山森英長、武田雅俊、ヒトにおける脳表現型の分子機構の解明：ヒト脳表現型コンソーシアムについて、2012年度包括脳ネットワーク夏のワークショップ、仙台、7.24-27(26), 2012. ポスター
45. 橋本亮太、山森 英長、安田 由華、福本 素由己、大井 一高、梅田 知美、岡田 武也、武田 雅俊、阪大病院における20症例の治療抵抗性統合失調症患者へのクロザピンの使用経験、第108回日本精神神経学会学術総会、札幌、5.24-26(24), 2012. 口演
46. 橋本亮太、大井 一高、安田 由華、山森 英長、福本 素由己、梅田 知美、岡田 武也、武田 雅俊、統合失調症専門外来・入院プログラムによる地域医療と臨床研究の融合システムの構築、第108回日本精神神経学会学術総会、第108回日本精神神経学会学術

総会、札幌、5.24-26(25), 2012. 口演

47. 大井 一高、橋本亮太、安田 由華、根本 清貴、大西 隆 5、福本 素由己、山森 英長、岩瀬 真生、数井 裕光、武田 雅俊、統合失調症における全ゲノム関連解析による NRGN 遺伝子は前帯状回体積と関連する、第 108 回日本精神神経学会学術総会、札幌、5.24-26(25), 2012. ポスター
48. 橋本亮太、「統合失調症専門外来・入院プログラム」-地域医療機関と連携した臨床・教育・研究システム-、福井 DS フォーラム、福井、5.10, 2012. 講演
49. 橋本亮太、増井拓哉、伊藤圭人、小笠原一能、笹田和見、小出隆義、足立康則、福本素由己、久住一郎、尾崎紀夫、武田雅俊、小山司、加藤忠史、Alda スケール（双極性障害患者における長期治療効果の後方視的基準）の信頼性の検討、第 31 回リチウム研究会、東京、4.14,2012. 講演

研究分担者：玉浦明美

1. 研修会：玉浦明美, ネットワークを使った多施設共同研究でのコーディネーターの役割, 第 1 回筋ジストロフィー臨床試験ネットワーク研修会, ミッドタウンカンファレンス, 2012, 7. 29
2. シンポジウム：玉浦明美, 精神科領域の治験の推進を目指して —臨床研究コーディネーターの立場から—
3. 第 22 回日本臨床精神神経薬理学会・第 42 回日本神経精神薬理学会 合同年会 International Global Clinical Trials－課題と対応－ 宇都宮 10. 18. 2012
4. 研究会：玉浦明美：治験の質の向上と症例集積性を高めるために, 精神科治験実態調査, Roche/中外 NP25620 試験研究会, 東京 2012、11、3
5. 市民講座：玉浦明美, 治験って何だろう？-参加の流れと参加のメリット・デメリット-, 平成 23 年度治験市民講座, 2012, 11, 24
6. ワークショップ：玉浦明美, 臨床研究・治験に関わる臨床心理士のための基礎講座-臨床研究・治験の科学性・倫理性と CRC に役割-, 東京認知行動療法アカデミー, 早稲田大学国際会議場, 1. 6. 2013
7. Reiko Shimizu, Hirofumi Komaki, En Kimura, Katsuhisa Ogata, ○ Akemi Tamaura, Shin'ichi Takeda. Attempt to organize "Muscular Dystrophy Clinical Trial Network" 9th Japanese-French Symposium for 'muscular dystrophy' 2012/9/7-8 Tokyo

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The relationship between the prefrontal activation during a verbal fluency task and stress-coping style in major depressive disorder: A near-infrared spectroscopy study

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ABSTRACT

This study aimed to identify coping styles used by patients with major depressive disorder (MDD) in comparison with those used by healthy controls, and to explore their association with prefrontal hemodynamic response related to a cognitive task. Regional hemodynamic changes were monitored during a verbal fluency task (VFT) using a 52-channel near-infrared spectroscopy (NIRS) apparatus in 26 MDD patients in depressive state and 30 matched healthy controls, and their correlation with coping styles assessed by Coping Inventory for Stressful Situations (CISS) were examined. We found the Emotion-oriented coping style was significantly higher, whereas the Task-oriented coping and Avoidance-oriented coping style were lower in the MDD group compared with controls. Emotion-oriented coping style positively correlated with subjective assessment of depression severity. Regional hemodynamic changes were significantly smaller in the MDD group than in the control group in prefrontal and temporal regions, and positively correlated with Task-oriented coping (adaptive coping) in the bilateral ventrolateral and dorsolateral prefrontal cortex, and the midline fronto-polar and bilateral orbitofrontal cortex regions. These findings suggest coping styles may be considered an important source of knowledge for patients who struggle with the illness and for mental health professionals who work with MDD patients, and that hemodynamic response in the ventrolateral and dorsolateral prefrontal cortex, midline fronto-polar, and orbitofrontal cortex regions during a VFT may reflect the adaptive coping (Task-oriented coping) style in MDD patients in depressive state.

1. Introduction

Coping with stressful situations and adverse life events including mental disorders is both an important personality resource and a measure of one's adaptability. According to the cognitive-transactional theory of stress (Lazarus and Folkman, 1984), coping has been defined as one's cognitive and behavioral effort to manage the internal and external demands of a person–environment transaction that is considered taxing or exceeding one's resources. Endler and Parker (1990) categorized various coping strategies into three main styles (Task-oriented, Emotion-oriented, and Avoidance-oriented). Task-oriented coping is used to actively solve an underlying problem, cognitively reconceptualize it, and potentially minimize its adverse effects. Emotion-oriented coping strategies are person-oriented, and include emotional responses, e.g., self-preoccupation, self-blame, and fantasizing reactions. Avoidance-oriented coping involves both task and person orientation: one may avoid a stressful situation either by using social diversion, i.e., choosing to be with other people and seeking emotional support, or via self-distraction from stressful situation, e.g., "giving up", denial, or engaging in a substitute task.

Research has indicated that major depressive disorder (MDD) patients are inflexible in their use of coping strategies or styles (Gan et al., 2006), tend to use maladaptive or emotion-oriented coping styles (Bruder-Mattson and Hovanitz, 1990; Rohde et al., 1990; Tomczak-Witych, 2006), and the avoidance strategies are used the least often (Tomczak-Witych, 2006). Mentally ordered women, however, most commonly use problem (task)-focused strategies (Tomczak-Witych, 2006). Turner et al. (1992) found a significant

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relationship between coping strategies and both severity of symptoms and emotional distress. They found that Emotion-oriented coping being correlated positively with BDI scores and Task-oriented coping being correlated negatively with BDI scores. Another study found that less-adaptive coping strategies (i.e., Emotion-oriented coping) were associated with less-adaptive personality traits (i.e., Neuroticism) and with psychological distress (i.e., Depression), whereas the reverse was found regarding adaptive coping strategies (i.e., Task-orientated coping) (McWillams et al., 2003).

Coping style may be associated with a variety of clinical, personal, and biological factors including cognitive function (Van Den Bosch and Rombouts, 1997; Lysaker et al., 2001; Wilder-Willis et al., 2002) and personality traits (Uehara et al., 1999; McWilliams et al., 2003). Rabinowiz and Arnett (2009) suggested that the relationship between cognitive dysfunction and depression is dependent on coping style. Adaptive coping tend to protect individuals from experiencing depression related to their cognitive deficits; when individuals use maladaptive coping, cognitive dysfunction increases the risk for depression. Coping style as a mediational factor suggests that cognitive dysfunction leads to depression partially due to its effect on coping style. That is, cognitive deficits may impair individuals' ability to use adaptive coping strategies, leaving them more likely to use maladaptive strategies.

Coping strategies, the dependent variable of the study, can be evaluated with the Coping Inventory for Stressful Situations (CISS, Endler and Parker, 1990), a self-report measure of coping; the reliability and validity of its Japanese version have already been confirmed (Furukawa et al., 1993). It consists of 48 statements concerning ways in which people could react to various difficult, stressful, or upsetting situations. They comprise three 16-item orthogonal factors of Task-oriented coping, Emotion-oriented coping, and Avoidance-oriented coping. In the present study, the patients were asked to indicate how often they currently used each of the 48 coping strategies on a 5-point scale ranging from 1 ("not at all") to 5 ("very much"). The range of possible scores of each subscale is from 16 to 80, with higher scores indicating the greater use of a given coping style.

MDD is characterized by marked deterioration in affect as well as significant impairment in cognitive function (Veiel, 1997; Austin et al., 1999). Cognitive dysfunction has a severe impact on the patient's ability to cope with the demands of daily living. One of the regions of cognitive deficits reported in MDD (Hammar and Ardal, 2009), which is relevant to coping style, is executive function. Executive function is known to afford the ability to deviate from a stereotyped behavior locked to environmental stimuli, which ability is often necessary in coping adaptively with the stress caused by those stimuli. The neural basis of executive function appears to lie in the prefrontal cortex, a region also involved in other high-level cognitive functions. Considering the significance of coping style in MDD patients, elucidation of the relationship between the neural activity in prefrontal cortex underlying the executive function processes and coping style in depression is a worthwhile focus of study.

Multi-channel NIRS (ETG-4000, Hitachi Medical Co.), a recently developed functional neuroimaging technology, enables the non-invasive detection of spatiotemporal characteristics of brain function near the brain surface using near-infrared light (Strangman et al., 2002a; Boas et al., 2004). NIRS has enabled bedside measurement of the concentrations of oxygenated ([oxy-Hb]) and deoxygenated hemoglobin ([deoxy-Hb]) in micro-blood vessels. Assuming that hematocrit is constant, the changes in [oxy-Hb], [deoxy-Hb] and also [total-Hb] (summation of [oxy-Hb] and [deoxy-Hb]) are correlated with the changes in the regional cerebral blood volume (rCBV) as shown by simultaneous NIRS and positron emission tomography (PET) measurements (Hock et al., 1997; Villringer et al., 1997; Ohmae et al., 2006). In contrast to other neuroimaging methodologies, NIRS can be measured under a more restraint-free environment that is especially suitable for psychiatric patients. Indeed, NIRS has been used to assess brain functions in many psychiatric disorders (Matsuo et al., 2003; Suto et al., 2004; Kameyama et al., 2006; Pu et al., 2008).

Recently, NIRS measurement during VFT (verbal fluency task) has been approved by the Ministry of Health, Labor and Welfare in Japan as an advanced medical technology in the aid of differential diagnosis of psychiatric illnesses. It has thus been frequently applied in the clinical fields in Japan. In addition, there are a number of reports suggesting that the mean [oxy-Hb] changes activated by VFT in patients with depression are significantly decreased than those in normal controls (Suto et al., 2004; Ohta et al., 2008; Noda et al., 2012). We also showed reduced [oxy-Hb] activation of the prefrontal cortex during VFT in patients with MDD using NIRS, but the relationship between the hemodynamic response and severity of depressive symptoms has not been clarified (Pu et al., 2008). Moreover, we were especially interested in exploring the treatment strategy of depression, which needs to take into account the relationship among cognition, mood and coping styles. As we already noted, one of the regions of cognitive deficits reported in MDD, is executive functioning. As VFT is known to be one of the executive measures (Burgess, 2000; Alvarez and Emory, 2006), it may be possible that the hemodynamic response elicited by VFT may tap the neuronal activity in prefrontal cortex, which may be closely linked to stress coping styles.

The primary objective of the present study was to investigate more precisely the relationship between activity of the prefrontal cortex and stress coping styles, in patients with MDD, using a 52-channel NIRS machine (ETG-4000, Hitachi Medical Co.). We hypothesized that patients with MDD substantially differ from healthy individuals in coping styles and that activity in the prefrontal cortex associated with executive function is related to coping styles in patients with MDD.

2. Subjects and methods

2.1. Subjects

Twenty-six patients with MDD and 30 healthy controls participated in the study (Table 1). The patients were recruited from the outpatients at Tottori University Hospital, and were diagnosed using the criteria of Diagnostic and Statistical Manual of Mental Disorders, the fourth edition, text revision (DSM-IV-TR, American Psychiatric Association 2000).

To obtain detailed information on psychiatric symptoms, the participants were questioned using a structured interview, the Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998). None of the subjects had clinical evidence of other central nervous system disorders based on history and medical examination. Patients with previous head trauma, stroke, electroconvulsive therapy, and current or previous history of substance abuse were excluded from the study. Twenty-six individuals (11 male, 15 female) meeting these criteria participated in the investigation. All the patients with MDD were in a depressed mood state. Within the MDD sample, 13 patients were taking selective serotonin reuptake inhibitors (SSRIs), 9 taking serotonin noradrenaline reuptake inhibitors (SNRIs), and 4 taking tricyclic antidepressants.

Healthy individuals who were appropriate age and gender matches for the MDD patients participated as controls in the present study. Inclusion criteria for controls were similar to those for the patient sample, although controls were additionally required to have no previous or current psychiatric illnesses. Thirty individuals (12 male, 18 female) meeting these criteria were selected to participate in the study.

Table 1
Demographic characteristics of the subjects and scores of HAMD, BDI and CISS.

	Major depression disorder ($n = 26$)	Normal controls ($n = 30$)	Group difference P-value
Gender (f/m)	15 f/11 m	18 f/12 m	0.86[a]
Age (years)	47.9 ± 19.2	50.5 ± 19.7	0.61
Duration of illness (years)	3.0 ± 2.3	N/A	
Age of onset (years)	36.8 ± 10.8	N/A	
Hamilton Depression Rating Scale (HAMD)	17.6 ± 7.0	N/A	
Beck Depression Inventory (BDI)	22.9 ± 10.4	3.6 ± 3.1	<0.001
Coping Inventory for Stressful Situations (CISS)			
Task-oriented (T)	41.3 ± 10.0	56.7 ± 9.1	<0.001
Emotion-oriented (E)	49.1 ± 11.2	40.4 ± 9.8	<0.005
Avoidance-oriented (A)	37.4 ± 9.2	44.6 ± 11.2	<0.05
Antidepressants (imipramine equivalents) (mg/day)	62.0 ± 53.7	N/A	

[a] Chi-square test, otherwise Student's t-tests were used for between-group comparison.

All the participants were right-handed with criteria of more than 80% by the Edinburgh Inventory Index (Oldfield, 1971). All subjects gave their consent in a written form after receiving comprehensive information on the study protocol. The study was approved by the ethics committee of Tottori University Faculty of Medicine.

2.2. Clinical evaluation

Prior to NIRS measurement, all the subjects undertook self-assessments of depression severity and the coping styles: the Beck Depression Inventory (BDI, Beck et al., 1961) and CISS scale were used (Endler and Parker, 1990). In addition, only patients were assessed for depression severity using the Hamilton Rating Scale for Depression (HAMD, Hamilton, 1960) by two trained psychiatrists.

2.3. Activation task

The task procedure in the present study was similar to that of Takizawa et al. (2008). [Hb] changes were measured during VFT (letter version). Each subject sat on a comfortable chair and was instructed to minimize movement such as head movements, strong biting and eye blinking during the NIRS measurements, so as to avoid artifacts.

The cognitive activation task included a 30-s pre-task baseline, a 60-s VFT, and a 70-s post-task baseline. For the pre- and post-task baseline periods, the subjects were instructed to consecutively repeat the five Japanese vowels ("a", "i", "u", "e", "o") aloud. The subtraction method (task minus pre- and post-task baseline) minimized the vocalization effects during VFT. During the task period, they were instructed to generate as many Japanese words beginning with a designated syllable as possible. The three sets of initial syllables (A; /to/, /se/, /o/, B; /a/, /ki/, /ha/, C; /na/, /i/, /ta/) were presented in counterbalanced order among the subjects and each syllable changed every 20 s during the 60-s task. The total number of correct words generated during VFT was adopted as a measure of task performance.

2.4. NIRS measurements

The 52-channel NIRS machine (ETG-4000) measures relative changes of [oxy-Hb] and [deoxy-Hb] using two wavelengths (695 and 830 nm) of infrared light on the basis of the modified Beer–Lambert law (Yamashita et al., 1996). In this system, these [Hb] values include differential pathlength factor (DPF). The distance between pairs of source–detector probes was set at 3.0 cm and each measuring area between pairs of source–detector probes was defined as 'channel'. It is considered that the machine measures points at 2–3 cm depth from the scalp, that is, the surface of the cerebral cortex (Toronov et al., 2001; Okada and Delpy, 2003). The probes of the NIRS machine were placed on a subject's frontotemporal region with the midcolumn of the probe located over Fpz, and the lowest probes are located along the T3–Fp1–Fpz–Fp2–T4 line in accordance with the international 10/20 system used in electroencephalography. The arrangement of the probes enabled the measurement of [Hb] values from bilateral prefrontal and superior temporal cortical surface regions (Fig.1). The correspondence between the NIRS channels and the measurement points on the cerebral cortex was confirmed by a multi-subject study of anatomical craniocerebral correlation (Okamoto et al., 2004), and was presented on the basis of the results of the virtual registration method (Tsuzuki et al., 2007).

The rate of data sampling was 0.1 s. The obtained data were analyzed using the "integral mode"; the pre-task baseline was determined as the mean over a 10-s period just prior to the task period, and the post-task baseline was determined as the mean over the last 5 s of the post-task period; linear fitting was applied to the data between these two baselines. A moving average method using a window width of 5 s was applied to remove any short-term motion artifacts. However, a moving average method alone could not remove all the artifacts and, thus, we applied a semi-automatic method for removing those data with significant artifacts. First, we applied the algorithm developed by Takizawa et al. (2008) that enables a fully automatic rejection of data with artifacts separately for each channel using quantitative evaluation.

Although the criteria for quantifying artifacts have not been clarified, experience shows that there are three kinds of noise artifacts (high frequency noise, low frequency noise and no signal) and that body-movement artifacts show sharp signal changes compared with those of normal hemodynamics.

High frequency noise is caused by insufficient intensity of the detection light in the OT system and the digital gain and the analog gain are taken to the maximum value. Therefore, in the algorithm, the channels in this maximum value gain state are determined as artifact channels.

Low frequency noise has excessive FFT (Fast Fourier Transform) power in the 0.1–1 [Hz] spectrum of the oxy-Hb and the deoxy-Hb. In such cases we applied the FFT (Fast Fourier Transform) to the oxy-Hb($x_{oxy}(t)$) and the deoxy-Hb($x_{deoxy}(t)$), and the FFT power is calculated ($P_{oxy}(t)$ and $P_{deoxy}(t)$).

$$P_{oxy}(t) = \sqrt{\mathrm{real}\left(\sum_{j=1}^{N} x_{oxy}(t)e^{-j\omega t}\right)^2 + \mathrm{imag}\left(\sum_{j=1}^{N} x_{oxy}(t)e^{-j\omega t}\right)^2} \quad (1)$$

$$P_{deoxy}(t) = \sqrt{\mathrm{real}\left(\sum_{j=1}^{N} x_{deoxy}(t)e^{-j\omega t}\right)^2 + \mathrm{imag}\left(\sum_{j=1}^{N} x_{deoxy}(t)e^{-j\omega t}\right)^2} \quad (2)$$

We calculated the maximum value of 0.1–1 [Hz] spectrum from the $P_{oxy}(t)$ and $P_{deoxy}(t)$, the channels above this threshold are determined as artifact channels.

$$\max(P_{oxy}(N/100 : N/10)) > 15 \quad (3)$$

$$\max\left(P_{deoxy}(N/100 : N/10)\right) > 6 \quad (4)$$

where N is the number of OT measurement points

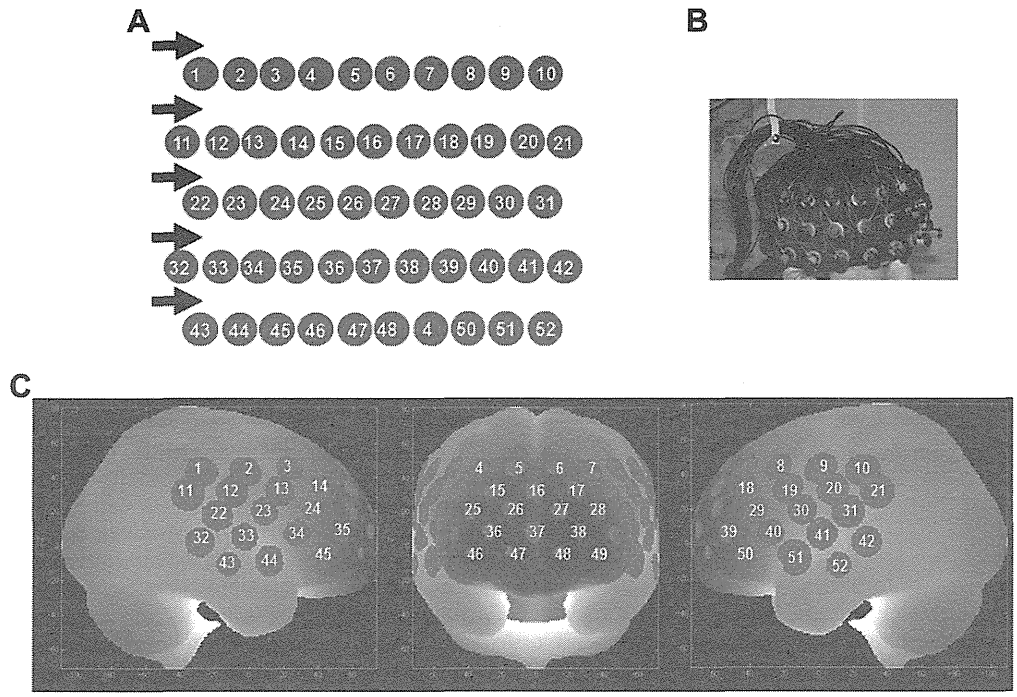

Fig. 1. Probe setting and measurement points for 52-channel near-infrared spectroscopy (NIRS). (A) The 52 measuring areas are labeled ch1-ch52 from the right posterior to the left anterior. (B–C) The probes with 3 × 11 thermoplastic shells were placed over a subject's bilateral prefrontal and superior temporal cortical surface regions. The channel numbers are indicated above the estimated cortical regions.

The "no signal" implies no change in the concentration of oxy-Hb and deoxy-Hb in all measurement time-points. Therefore, the channels in which the standard deviation value of all the measurement points is zero are determined as artifact channels.

The body-movement artifacts usually consist of sharp changes. Therefore, the channels that have body-movement artifacts with oxy-Hb and total-Hb changes over 0.15 [mMmm] in over 20 successive samples (during 2[s]) are determined as artifact channels.

Moreover, in some cases, the algorithm appeared to even reject data without artifacts. Therefore, in the next step, two researchers, who were both blind to the clinical background of the data, judged whether or not to save those data rejected by the algorithm through consultation. Consequently, the number of averaged data for each channel did not vary widely within and between the two diagnostic groups (MDD: $N = 21–26$ [mean = 25.2, SD = 1.2]; control: $N = 26–30$ [mean = 29.3, SD = 1.2]).

Next, for the analysis of the hemodynamic response data, [Hb] variables of each channel were averaged for the two time segments (pre-task baseline and task period). We focused on [oxy-Hb] concentrations during the 60-s task period, since [oxy-Hb] change (task period – pre-task baseline period) was assumed to more directly reflect cognitive activation than [deoxy-Hb] change as shown by a stronger correlation with blood-oxygenation level-dependent (BOLD) signal measured by fMRI (Strangman et al., 2002b).

2.5. Statistical analysis

First, the coping styles scores (Task-oriented, Emotion-oriented, and Avoidance-oriented coping), the task performance level of the VFT and the mean [oxy-Hb] changes for each channel were compared between groups using Student's *t*-test (MDD and control).

Secondly, Pearson's product moment correlation coefficients were calculated for a relationship between the mean [oxy-Hb] changes and the clinical characteristics such as HAMD, BDI in MDD patients and coping styles scores in both groups for each channel. If a significant relationship between the mean [oxy-Hb] changes and either coping style scores was observed, a step-wise multiple regression analysis was performed using the mean [oxy-Hb] changes in each channel, which showed a significant correlation with the coping style scores, as a dependent variable, and BDI and HAMD scores in addition to the coping style scores as independent variables, to test the influence of mood state on the relationship. We also investigated the relationship between [oxy-Hb] changes and task performance of VFT, age, age of onset and duration of illness in MDD patients using Spearman's rho because these variables did not show normal distribution. We adopted false discovery rate (FDR)-based procedure for the multiple testing correction in analyses using 52 channels data so that there are no more than 5% false positives on average (FDR-corrected) (Singh and Dan, 2006). Statistical analyses were performed using SPSS 17.0 software.

3. Results

3.1. Coping styles

The mean scores of the three CISS coping (Task-oriented, Emotion-oriented, and Avoidance-oriented) dimensions of controls and MDD patients were compared (Table 1). Compared with controls, the scores Emotion-oriented coping style was significantly higher, whereas the Task-oriented coping and Avoidance-oriented coping style were lower in the MDD group.

3.2. Task performance

The number of generated words on the VFT for MDD patients (mean, 12.2 ± 4.6) was not significantly different from that for the controls (mean, 13.9 ± 4.7; $t = -1.3$, n.s.) (Table 1)

3.3. Cognitive activation

MDD patients were associated with a significantly smaller increase in mean [oxy-Hb] changes than controls at 47 channels (ch3–4, ch6–10, ch12–22, ch24–52; FDR-corrected *P*: 0.001–

0.045), distributed predominantly in the prefrontal and temporal regions (Fig. 2).

3.4. Correlation analyses (Figs. 3 and 4)

In MDD patients, the mean [oxy-Hb] changes showed a significant positive correlation with Task-oriented coping scores in 14 channels (ch11–13, ch16, ch18–19, ch24–26, ch29, ch37, ch47–49; FDR-corrected *P*: 0.001–0.013; *R*: 0.49–0.64), with the highest correlations located approximately in the bilateral ventrolateral and dorsolateral prefrontal cortex, midline fronto-polar, and bilateral orbitofrontal cortex regions (Fig. 3). Meanwhile, there was no significant correlation between the mean [oxy-Hb] changes and either Emotion-oriented or Avoidance-oriented coping style scores in any channel (Fig. 4).

The mean [oxy-Hb] changes did not show any significant correlation with the task performance during VFT or other clinical variables, such as age, age of onset, duration of illness, BDI and HAMD in MDD patients.

Step-wise multiple regression analysis using the mean [oxy-Hb] changes in each channel, which showed a significant correlation with Task-oriented coping style scores, as a dependent variable and task-oriented coping style scores and BDI and HAMD scores as independent variables revealed that only Task-oriented coping style scores showed a significant effect on the mean [oxy-Hb] changes in all the channels.

As for the relationship between coping styles scores and HAMD, BDI scores, only Emotion-oriented coping style scores showed a significant positive correlation with BDI scores (*R*: 0.68, *P*: 0.0002).

4. Discussion

In the present study it was shown that the Emotion-oriented coping style was significantly higher, whereas the Task-oriented coping and Avoidance-oriented coping style were lower in the MDD group compared with controls. In addition, [oxy-Hb] activation during VFT was significantly smaller in MDD patients as compared with age- and gender-matched healthy controls, which was not explained by differences in task performance.

The activated area in the present study appeared to show a wide distribution compared to that observed in previous fMRI studies using VFT, which showed a relatively restricted area of activation such as left prefrontal cortex and anterior cingulate cortex (Schlosser et al., 1998; Okada et al., 2003). Meanwhile, the distribution is similar to other studies using NIRS during VFT including our own (Suto et al., 2004; Ohta et al., 2008; Pu et al., 2008; Noda et al., 2012). Although the reason for the discrepancy between NIRS and fMRI is not yet clear, one of the possibilities is the difference in spatial resolution; the poor resolution in NIRS may have resulted in wider distribution. Moreover, another difference between the two measures is that, while NIRS reflects the vasomotor activities of distensible capillary vessels, fMRI presents BOLD signal in the small veins, which may have caused the discrepancy in distribution. Finally, the difference in the task procedure should be noted. In the present study and also in other NIRS studies, the subjects were instructed to generate words beginning with a designated syllable which abruptly switched every 20 s for 3 sets. This procedure required the subjects to update their cognitive context, which may activate prefrontal cortex more potently than the conventional verbal fluency task.

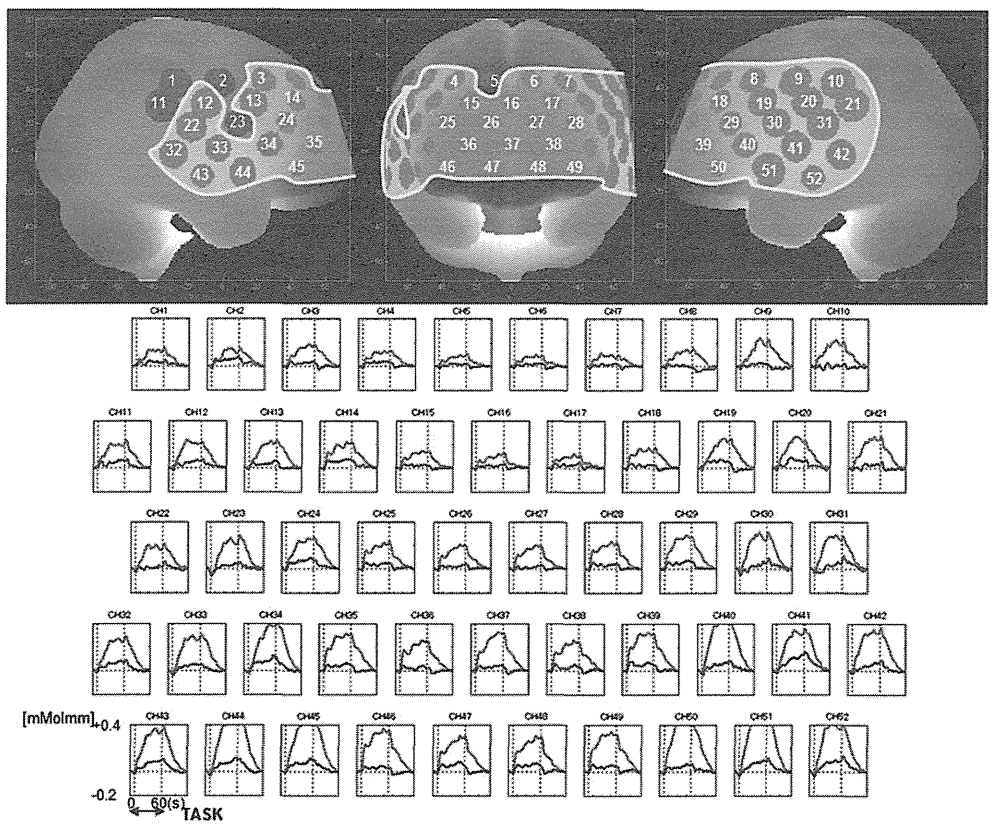

Fig. 2. Above: Brain area in yellow corresponds to the NIRS channels with significantly lower levels of activation in the MDD group than in the control group. The locations of NIRS channels were probabilistically estimated and anatomically labeled in the standard brain space in accordance with Tsuzuki et al. (2007). Below: Grand averaged waveforms of oxygenated hemoglobin ([oxy-Hb]) during VFT (between two dotted vertical lines in each graph) in 52 channels over frontal and temporal regions measured by NIRS. Red and blue lines represent MDD and control groups, respectively.

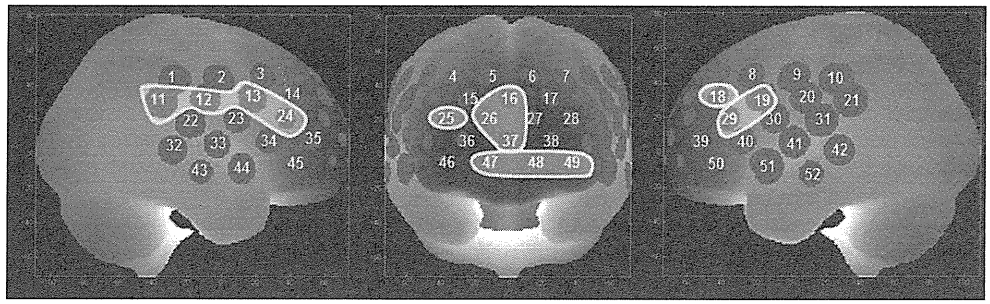

Fig. 3. Cortical distribution of the area where significant correlation between [oxy-Hb] changes and Task-oriented coping style scores was found. Brain area in yellow corresponds to the NIRS channels, [oxy-Hb] changes of which showed significant correlation (Pearson's product moment correlation; FDR-corrected $P < 0.05$) with Task-oriented coping style scores.

Furthermore, [oxy-Hb] activation during VFT positively correlated with Task-oriented coping style scores in the bilateral ventrolateral and dorsolateral prefrontal cortex, midline fronto-polar, and bilateral orbitofrontal cortex regions. These findings suggest that coping style may be considered an important source of knowledge for patients who struggle with the illness and for mental health professionals who work with MDD patients, and that hemodynamic response in the ventrolateral and dorsolateral prefrontal cortex, midline fronto-polar, and orbitofrontal cortex regions during VFT may reflect the adaptive coping (Task-oriented coping) style in MDD patients in depressive state. It may be assumed that Task-oriented coping style scores reflect cognitive aspect of stress coping while Emotion-oriented and Avoidance-oriented coping styles reflect emotional and behavioral aspects significantly. Considering that the mean [oxy-Hb] activation during an executive functioning task in ventrolateral and dorsolateral

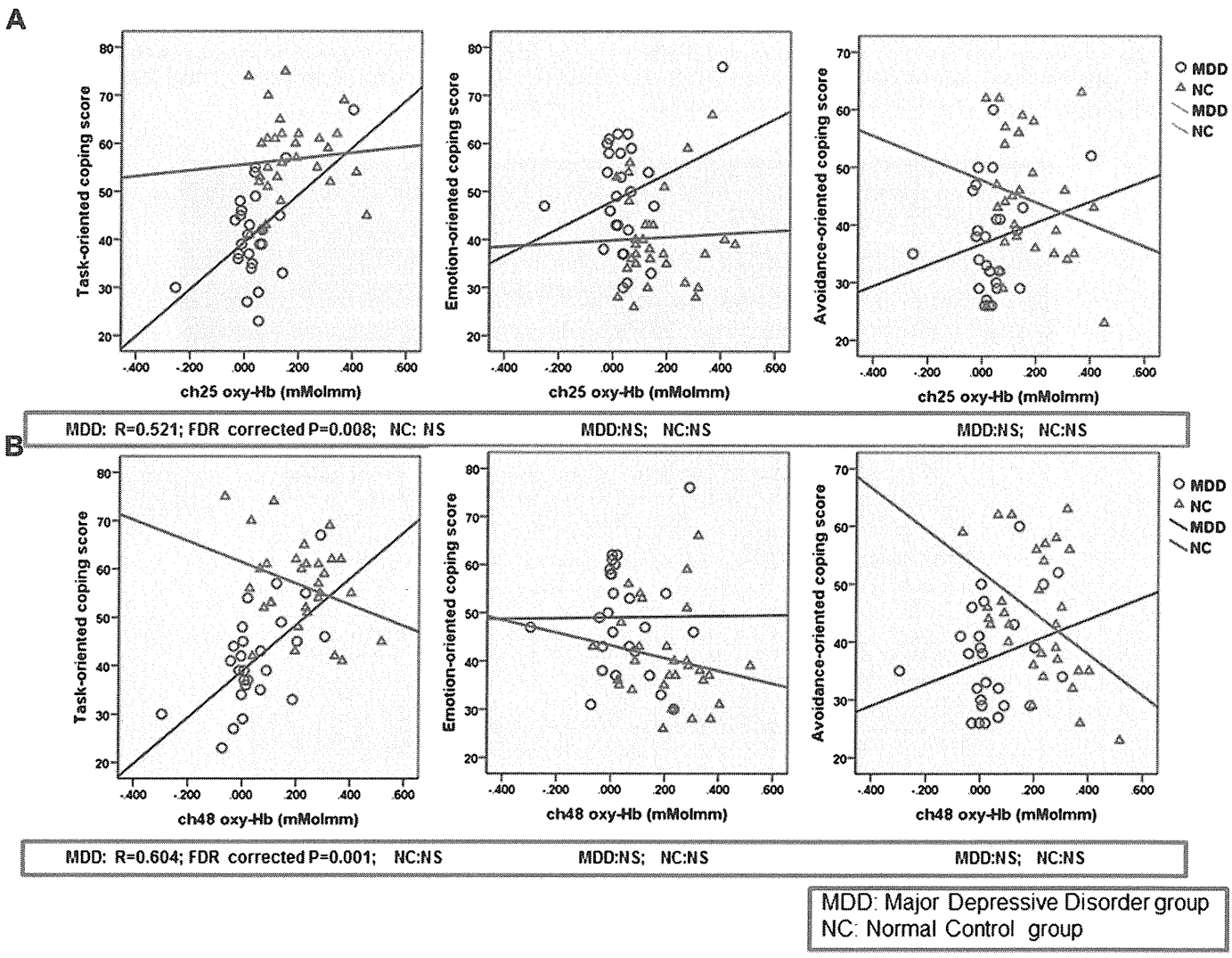

Fig. 4. Scatter diagrams showing the relationship between coping style scores and mean [oxy-Hb] changes in channels 25 (A) and 48 (B).

prefrontal cortex, and the midline fronto-polar and bilateral orbitofrontal cortex regions may be involved in high-order control processes of cognition, a closer link with Task-oriented coping style than Emotion-oriented and Avoidance-oriented coping styles may well be accepted.

Our results are in line with previous studies indicating that MDD patients tend to use Emotion-oriented coping styles (Bruder-Mattson and Hovanitz, 1990; Rohde et al., 1990; Tomczak-Witych, 2006), and that the avoidance strategies are used the least often (Tomczak-Witych, 2006). In addition, in our study, MDD patients scored markedly lower in Task-oriented coping style compared with controls, which is suggested to be socially adaptive, representing active attitude for solution of underlying problems. These results, together with the results of the present study, support the idea that coping strategies are maladaptive in MDD.

The neural pathways in the brain that are implicated in coping are increasingly coming to be understood. In particular, the amygdala and dorsal anterior cingulate cortex are implicated in threat detection, and regions of the prefrontal cortex are associated with adaptive responses to stress (Taylor and Stanton, 2007). The present study demonstrated an interesting relationship between neural activity in the prefrontal region and coping style. In line with the above study, MDD patients' Task-oriented coping (adaptive coping) style scores positively correlated with [oxy-Hb] activation during an executive functioning task in the prefrontal region, more specifically in the bilateral ventrolateral and dorsolateral prefrontal cortex, and the midline fronto-polar and bilateral orbitofrontal cortex regions. The prefrontal cortex governs the executive control of information processing and behavioral expression, including the ability to selectively attend to and maintain information, inhibit irrelevant stimuli and evaluate and select the appropriate response (Knight et al., 1995; Miller and Cohen, 2001). There is considerable evidence by now that the prefrontal cortex plays a major role in high-order control processes that exercise a top-down regulation of cognition and behavior (e.g. Petrides, 1996; Robbins, 1996; Shallice and Burgess, 1996; Duncan and Owen, 2000; Postle and D'Esposito, 2000). In addition, evidence indicates that the orbitofrontal cortex participates in the executive control of information processing and behavioral expression by inhibiting neural activity associated with irrelevant, unwanted, or uncomfortable (e.g. painful) information, sensations, or actions (Shimamura, 2000). Evidence across species points to the critical involvement of orbitofrontal cortex in the flexible control of behavior in changing environments (Rolls, 2004; Schoenbaum et al., 2009). These findings suggest that hemodynamic response in the prefrontal (in particular, ventrolateral and dorsolateral prefrontal cortex, midline fronto-polar, and orbitofrontal cortex) region during a VFT may reflect adaptive coping style in MDD patients in depressive state.

A relatively high correlation was obtained between Emotion-oriented coping style scores and BDI scores. The finding suggests that the high level of Emotion-oriented coping style is related to depression symptom severity, which is in line with previous studies (Turner et al., 1992; McWilliams et al., 2003). In the present study, we failed to find a straightforward relationship between Task-oriented coping style and mood symptomatology, presumably because cognitive dysfunction acted as a moderator variable. Although speculative, Task-oriented coping style may alleviate the mood symptoms for patients with relatively high cognitive function, whereas patients with severe cognitive dysfunction may experience strong psychological distress in using Task-oriented coping strategies, which is more challenging than the other two coping strategies. Considering the significant relationship between Task-oriented coping style and prefrontal neural activity underlying executive functioning, although cross-sectional as it is, cognitive enhancement treatment targeted on prefrontal neural activity may strengthen the patients' ability to make use of adaptive coping strategy and may lead to better social outcome.

On the other hand, we could not find any relationship between coping styles and [oxy-Hb] changes in normal controls. Although the reason is not clear, we could raise two possibilities. First, as MDD patients depend too much on Emotion-oriented coping strategy and less on Task-oriented or Avoidance-oriented coping strategy, greater use of Task-oriented coping strategy may be considered as adaptive, whereas normal controls already use Task-oriented coping strategy in an adaptive level and thus higher executive functioning do not necessarily relate to higher scores of Task-oriented coping style. Next, in MDD patients lower level of prefrontal activation may lead to impairment in executive functioning straight-forwardly, whereas in normal controls, who hold the capacity to highly activate the prefrontal neural systems, there might be some ceiling effect, which results in lack of correlation between prefrontal neural activity and Task-oriented coping style scores based on executive functioning.

There are some methodological considerations in this study. First, multichannel NIRS has limited spatial resolution compared with fMRI and PET. However, a recent MRI and NIRS combination study, which used a method for the probabilistic registration of NIRS data onto Montreal Neurological Institute (MNI) coordinate space, suggested the errors of spatial estimation, expressed as standard deviations, were approximately 10 mm (Okamoto and Dan, 2005; Tsuzuki et al., 2007). These suggest that multichannel NIRS could roughly detect sub-region-specific activation in the prefrontal cortex. Second, although we did not find any relationships between [oxy-Hb] and duration of illness and medication dosages in the patients with MDD, most of them were chronic and medicated. Thus, to fully rule out their effects, future studies are warranted using first-episode and/or drug-naïve patients with MDD. Moreover, considering the specific advantage that NIRS holds, such as its capacity for real-time monitoring in natural-settings, it may enable us to detect time-course of functional connectivity during an actual coping paradigm in real stressful situations in future studies.

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Contributors

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Drafting the article or revising it critically for important intellectual content (Shenghong Pu, Kazuyuki Nakagome, Takeshi Yamada, Katsutoshi Yokoyama, Hiroshi Matsumura, Izumi Nagata, Koichi Kaneko).

Final approval of the version to be published (Shenghong Pu, Kazuyuki Nakagome, Takeshi Yamada, Katsutoshi Yokoyama, Hiroshi Matsumura, Hideaki Mitani, Akiko Adachi, Izumi Nagata, Koichi Kaneko).

Conflict of interest

All the authors declare that they have no conflicts of interest with respect to this study or its publication.

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References

Austin MP, Mitchell P, Wilhelm K, Parker G, Hickie I, Brodaty H, et al. Cognitive function in depression: a distinct pattern of frontal impairment in melancholia. Psychological Medicine 1999;29:73–85.
Alvarez JA, Emory E. Executive function and the frontal lobes: a meta-analytic review. Neuropsychology Review 2006;16:17–42.
Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Archives of General Psychiatry 1961;4:53–63.
Boas DA, Dale AM, Franceschini MA. Diffuse optical imaging of brain activation: approaches to optimizing image sensitivity, resolution, and accuracy. Neuroimage 2004;23:275–88.
Bruder-Mattson SF, Hovanitz CA. Coping and attributional styles as predictors of depression. Journal of Clinical Psychology 1990;46:557–65.
Burgess PW. Strategy application disorder: the role of the frontal lobes in human multitasking. Psychological Research 2000;63:279–88.
Duncan J, Owen AM. Common regions of the human frontal lobe recruited by diverse cognitive demands. Trends in Neuroscience 2000;23:475–83.
Endler NS, Parker JDA. Coping Inventory for Stressful Situations (CISS) manual. Toronto: Multi-Health Systems, Inc; 1990.
Furukawa T, Suzuki-Moor A, Saito Y, Hamanaka T. Reliability and validity of the Japanese version of the coping inventory for stressful situations (CISS): a contribution to the cross-cultural studies of coping. Seishin Shinkeigaku Zasshi 1993;95:602–20 [in Japanese].
Gan Y, Zhang Y, Wang X, Wang S, Shen X. The coping flexibility of neurasthenia and depressive patients. Personality and Individual Differences 2006;40:859–71.
Hamilton M. A rating scale for depression. Journal of Neurology, Neurosurgery & Psychiatry 1960;23:56–62.
Hammar A, Ardal G. Cognitive functioning in major depression – a summary. Frontiers in Human Neuroscience 2009;3:26.
Hock C, Villringer K, Müller-Spahn F, Wenzel R, Heekeren H, Schuh-Hofer S, et al. Decrease in parietal cerebral hemoglobin oxygenation during performance of a verbal fluency task in patients with Alzheimer's disease monitored by means of near-infrared spectroscopy (NIRS) - correlation with simultaneous rCBF-PET measurements. Brain Research 1997;755:293–303.
Kameyama M, Fukuda M, Yamagishi Y, Sato T, Uehara T, Ito M, et al. Frontal lobe function in bipolar disorder: a multichannel near-infrared spectroscopy study. Neuroimage 2006;29:172–84.
Knight RT, Grabowecky MF, Scabini D. Role of human prefrontal cortex in attention control. Advances in Neurology 1995;66:21–34.
Lazarus RS, Folkman S. Stress, appraisal and coping. New York: Springer; 1984.
Lysaker PH, Clements CA, Wright DE, Evans J, Marks KA. Neurocognitive correlates of helplessness, hopelessness, and well-being in schizophrenia. Journal of Nervous and Mental Disease 2001;189:457–62.
Matsuo K, Taneichi K, Matumoto A, Ohtani T, Yamasue H, Sakano Y, et al. Hypoactivation of the prefrontal cortex during verbal fluency test in PTSD: a near-infrared spectroscopy study. Psychiatry Research: Neuroimaging 2003;124:1–10.
McWilliams LA, Cox BJ, Enns MW. Use of the Coping Inventory for Stressful Situations in a clinically depressed sample: factor structure, personality correlates, and prediction of distress. Journal of Clinical Psychology 2003;59:423–37.
Miller EK, Cohen JD. An integrative theory of prefrontal cortex function. Annual Review of Neuroscience 2001;24:167–202.
Noda T, Yohida S, Matsuda T, Okamoto N, Sakamoto K, Koseki S, et al. Frontal and right temporal activations correlate negatively with depression severity during verbal fluency task: a multi-channel near-infrared spectroscopy study. Journal of Psychiatric Research 2012;46(7):905–12.
Ohmae E, Ouchi Y, Oda M, Suzuki T, Nobesawa S, Kanno T, et al. Cerebral hemodynamics evaluation by near-infrared time-resolved spectroscopy: correlation with simultaneous positron emission tomography measurements. Neuroimage 2006;29:697–705.
Okada E, Delpy DT. Near-infrared light propagation in an adult head model. II. Effect of superficial tissue thickness on the sensitivity of the near-infrared spectroscopy signal. Applied Optics 2003;42:2915–22.
Okada G, Okamoto Y, Morinobu S, Yamawaki S, Yokota N. Attenuated left prefrontal activation during a verbal fluency task in patients with depression. Neuropsychobiology 2003;47(1):21–6.
Okamoto M, Dan H, Sakamoto K, Takeo K, Shimizu K, Kohno S, et al. Three-dimensional probabilistic anatomical cranio-cerebral correlation via the international 10–20 system oriented for transcranial functional brain mapping. Neuroimage 2004;21:99–111.
Okamoto M, Dan I. Automated cortical projection of head-surface locations for transcranial functional brain mapping. Neuroimage 2005;26:18–28.
Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971;9:97–113.
Ohta H, Yamagata B, Tomioka H, Takahashi T, Yano M, Nakagome K, et al. Hypofrontality in panic disorder and major depressive disorder assessed by multichannel near-infrared spectroscopy. Depression and Anxiety 2008;25(12):1053–9.
Petrides M. Specialized systems for the processing of mnemonic information within the primate frontal cortex. Philosophical Transactions of the Royal Society B 1996;351:1455–62.
Postle BR, D'Esposito M. Evaluating models of the topographical organization of working memory function in frontal cortex with event-related fMRI study. Psychobiology 2000;28:132–45.
Pu S, Matsumura H, Yamada T, Ikezawa S, Mitani H, Adachi A, et al. Reduced frontopolar activation during verbal fluency task associated with poor social functioning in late-onset major depression: a multi-channel near-infrared spectroscopy study. Psychiatry and Clinical Neurosciences 2008;62:728–37.
Rabinowitz AR, Arnett PA. A longitudinal analysis of cognitive dysfunction, coping, and depression in multiple sclerosis. Neuropsychology 2009;23:581–91.
Robbins TW. Dissociating executive functions of the prefrontal cortex. Philosophical Transactions of the Royal Society B 1996;351:1463–70.
Rohde P, Lewinsohn PM, Tilson M, Seeley JR. Dimensionality of coping and its relation to depression. Journal of Personality and Social Psychology 1990;58:499–511.
Rolls ET. Convergence of sensory systems in the orbitofrontal cortex in primates and brain design for emotion. The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology 2004;281:1212–25.
Schlösser R, Hutchinson M, Joseffer S, Rusinek H, Saarimaki A, Stevenson J, et al. Functional magnetic resonance imaging of human brain activity in a verbal fluency task. Journal of Neurology, Neurosurgery & Psychiatry 1998;64(4):492–8.
Schoenbaum G, Roesch MR, Stalnaker TA, Takahashi YK. A new perspective on the role of the orbitofrontal cortex in adaptive behaviour. Nature Reviews Neuroscience 2009;10:885–92.
Shallice T, Burgess DF. The domain of supervisory processes and temporal organization of behavior. Philosophical Transactions of the Royal Society B 1996;351:1405–11.
Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatic Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. Journal of Clinical Psychiatry 1998;59:22–33.
Shimamura AP. Toward a cognitive neuroscience of metacognition. Consciousness and Cognition 2000;9:313–23.
Singh AK, Dan I. Exploring the false discovery rate in multichannel NIRS. Neuroimage 2006;33:542–9.
Strangman G, Boas DA, Sutton JP. Non-invasive neuroimaging using near-infrared light. Biological Psychiatry 2002a;52:679–93.
Strangman S, Culver JP, Thompson JH, Boas DA. A quantitative comparison of simultaneous BOLD fMRI and NIRS recording during functional brain activation. Neuroimage 2002b;17:719–31.
Suto T, Fukuda M, Ito M, Uehara T, Mikuni M. Multichannel near-infrared spectroscopy in depression and schizophrenia: cognitive activation study. Biological Psychiatry 2004;55:501–11.
Takizawa R, Kasai K, Kawakubo Y, Marumo K, Kawasaki S, Yamasue H, et al. Reduced frontopolar activation during verbal fluency task in schizophrenia: a multichannel near-infrared spectroscopy study. Schizophrenia Research 2008;99:250–62.
Taylor SE, Stanton AL. Coping resources, coping processes, and mental health. Annual Review of Clinical Psychology 2007;3:377–401.
Tomczak-Witych A. Coping with stress strategies among female patients suffering from a depression. Psychiatria polska 2006;40:491–502.
Toronov V, Webb A, Choi JH, Wolf M, Michalos A, Gratton E, et al. Investigation of human brain hemodynamics by simultaneous near-infrared spectroscopy and functional magnetic resonance imaging. Medical Physics 2001;28:521–7.
Tsuzuki D, Jurcak V, Singh AK, Okamoto M, Watanabe E, Dan I. Virtual spatial registration of stand-alone fNIRS data to MNI space. Neuroimage 2007;34:1506–18.
Turner RA, King PR, Tremblay PF. Coping styles and depression among psychiatric outpatients. Personality and Individual Differences 1992;13:1145–7.
Uehara T, Sakado K, Sakado M, Sato T, Someya T. Relationship between stress coping and personality in patients with major depressive disorder. Psychotherapy and Psychosomatics 1999;68:26–30.
Van den Bosch RJ, Rombouts RP. Coping and cognition in schizophrenia and depression. Comprehensive Psychiatry 1997;38:341–4.
Veiel HO. A preliminary profile of neuropsychological deficits associated with major depression. Journal of Clinical and Experimental Neuropsychology 1997;19:587–603.
Villringer K, Minoshima S, Hock C, Obrig H, Ziegler S, Dirnagl U, et al. Assessment of local brain activation. A simultaneous PET and near-infrared spectroscopy study. Advances in Experimental Medicine and Biology 1997;413:149–53.
Wilder-Willis KE, Shear PK, Steffen JJ, Borkin J. The relationship between cognitive dysfunction and coping abilities in schizophrenia. Schizophrenia Research 2002;55:259–67.
Yamashita Y, Maki A, Ito Y, Watanabe E, Koizumi H. Noninvasive near-infrared topography of human brain activity using intensity modulation spectroscopy. Optical Engineering 1996;35:1046–9.

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The pilot study of a Neuropsychological Educational Approach to Cognitive Remediation for patients with schizophrenia in Japan

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ABSTRACT

The main aim of this study is to demonstrate the feasibility and efficacy of a Neuropsychological Educational Approach to Cognitive Remediation (NEAR) in Japan. This multi-site study used a quasi-experimental design. Fifty-one patients with schizophrenia or schizoaffective disorder participated. The NEAR program consisted of two 1-h computer sessions per week and an additional group meeting session lasting 30 to 60 min once a week. The subjects completed 6 months of NEAR sessions before being assessed. Moreover, taking into consideration the possible practice effect, we assessed 21 control patients twice with an interval of 6 months. We assessed cognitive function by using the Japanese version of the Brief Assessment of Cognition in Schizophrenia (BACS-J). Consequently, the NEAR group showed significant improvement in overall cognitive function, and in comparison with the control group, these findings were generally similar except for motor speed. Although the present study has its limitations, it demonstrates that the NEAR is feasible in Japan as well as it is in Western countries.

1. Introduction

It is widely accepted that cognitive dysfunction in schizophrenia plays a major role in determining social function (Green et al., 2000). Although there have been numerous reports that indicate the effectiveness of atypical antipsychotics (AAPs) on cognitive function, the size of the effect of AAPs is generally about 0.2–0.5 standard deviations (S.D.) (Woodward et al., 2005; Keefe et al., 2007), while the extent of cognitive dysfunction in schizophrenia is about 1–1.5 S.D. below the level of healthy populations (Bilder et al., 2000; Heinrichs, 2004). To bridge this gap, other treatment methods, such as cognitive remediation, have been considered in Western countries.

In Japan, the "Services and Supports for Persons with Disabilities Act" was established in 2006. Although disabled persons' employment, deinstitutionalization, and socialization were promoted by this law, there are actually many people with psychiatric illnesses, including patients with schizophrenia, who still suffer from social dysfunction. With the aim of alleviating the many difficulties that patients encounter in their lives, cognitive remediation therapy for patients with schizophrenia has gradually been launched in Japan (Nemoto et al., 2009).

We have become interested in one of the cognitive remediation therapies, namely, a Neuropsychological Educational Approach to Cognitive Remediation (NEAR) (Medalia and Freilich, 2008; Medalia et al., 2009), which is theoretically based on neuropsychology, educational psychology, learning theory, and cognitive psychology. After participating in 1-week clinician training for NEAR, we started implementing NEAR in Japan. NEAR is an evidence-based approach to cognitive remediation specifically developed for use with psychiatric patients. NEAR is a group-based treatment that provides a positive learning experience to each and every client, to promote independent learning, and to promote optimal cognitive function in everyday life. Sessions are structured in a way to enhance intrinsic motivation and learning. The main aim of this study is to demonstrate the feasibility and efficacy of NEAR in Japan by assessing its effectiveness on cognitive function using neuropsychological indices as a primary endpoint.

2. Methods

This multi-site study used a quasi-experimental design. All participants were recruited from five psychiatric hospitals in the western region of Japan called the 'San-in' district and exposed to NEAR in each hospital. All participants were recruited on the basis of consecutive referrals.

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Table 1
Baseline demographic variables.

	NEAR group	Control group
Number of patients		
Sch: Schizophrenia	Sch: 48	Sch: 21
SchAf: Schizoaffective disorder	SchAf: 3	SchAf: 1
Gender	Male: 31, Female: 20	Male: 14, Female: 8
Mean age	36.1 ± 10.6 y.o.	41.1 ± 12.4 y.o.
Years of education	13.5 ± 2.5 years	12.5 ± 2.6 years
Duration of illness	13.8 ± 9.8 years	16.1 ± 10.8 years
Age at onset of illness	22.3 ± 6.6 y.o.	22.6 ± 6.3 y.o.
Total number of hospitalizations	2.8 ± 3.1 times	4.6 ± 5.2 times
Total months of hospitalization	19.4 ± 29.4 Months	39.3 ± 65.8 months
Mean dosage of antipsychotics (Chlorpromazine equivalent dose)	634.5 ± 364.9 mg/day	699.2 ± 569.2 mg/day
Treatment settings (Outpatient or inpatient) *	Outpatients: 42 Inpatients: 9	Outpatients: 12 Inpatients: 10
NEAR attendance rate	0.90 ± 0.11	
BACS-J z score; Verbal memory**	−1.09 ± 0.92	−2.00 ± 1.05
BACS-J z score; Working memory	−0.95 ± 0.95	−1.30 ± 1.08
BACS-J z score; Speed	−1.60 ± 1.37	−2.25 ± 1.74
BACS-J z score; Verbal fluency	−0.47 ± 1.00	−0.71 ± 0.89
BACS-J z score; Attention and speed of information processing	−1.24 ± 0.88	−1.56 ± 0.77
BACS-J z score; Executive function	−0.57 ± 1.42	−1.56 ± 2.15
[EX]**	−0.79 ± 0.59	−1.10 ± 0.59
BACS-J composite score**	−1.65 ± 1.27	−2.61 ± 1.51

* $p<0.05$ Fisher's exact test.
** $p<0.05$ Student's t test.
[EX] = −log[2 − (Executive function BACS-J z score)].

2.1. Subjects (Table 1)

After a complete explanation of the study, informed consent was obtained from the participants. The protocol of this study was approved by the Ethics Committee of Tottori University. Inclusion criteria were outpatients or inpatients (a) with a diagnosis of schizophrenia or schizoaffective disorder made by two experienced psychiatrists according to DSM-IV-TR criteria, (b) between 13 and 65 years old, (c) able to sit for a 1-hour session, (d) willing to participate in the study, and (e) being recommended by their doctors. Exclusion criteria were patients (a) with active substance or alcohol abuse or having left a detoxification program within the last month, or (b) with traumatic head injury within the past 3 years.

Sixty-two patients were referred to the program, and 11 dropped out at the midway point (the dropout rate was 17.4%). Among these 11 patients, five patients dropped out owing to a lack of motivation and five patients dropped out because of relapse of psychotic symptoms. One patient found a job and left the program. Six of the patients who withdrew left the program within the first half of the 6-month trial. Finally, 51 patients with schizophrenia or schizoaffective disorder completed the NEAR program. The NEAR program consisted of two 1-h computer sessions per week and an additional group meeting session lasting 30 to 60 min once a week. The subjects completed approximately 6 months before the program's efficacy was assessed.

Moreover, we assessed 22 control patients twice with an interval of 6 months, taking into consideration a possible practice effect, which may have affected the scores of neuropsychological tests. They did not receive any cognitive training program including NEAR. As for the clinical backgrounds, the treatment settings were significantly different between the two groups, with more inpatients being included in the control group than in the NEAR participant group.

In each computer session, patients engaged with some educational computer software that was related to various domains of cognitive function, including attention, memory, and executive function, taking into account the profiles of the patients' cognitive impairments. The software available in Japan is not identical to that in Western countries; however, it appeared to cover the relevant cognitive domains (Table 2).

The main aim of the group meeting sessions was to contextualize the computer training into the patients' everyday activities. The process should lead to enhancing motivation and generalization of cognitive skills to real-life activities.

One of our co-authors is certified as a supervisor of NEAR and she supervised NEAR sessions periodically. In order to use consistent methods across sites, all clinicians participated in 1-week clinician training, and they attended trimonthly meetings.

Although the medications were changed throughout the whole period as little as possible, there were 16 patients whose medications needed to be changed because of clinical decisions. However, the change in the medication status of these 16 patients was only related to daily dosage levels.

2.2. Assessments

We assessed cognitive function using the Japanese version of the Brief Assessment of Cognition in Schizophrenia (BACS-J) (Keefe et al., 2004; Kaneda et al., 2007). Z scores were calculated for each subcomponent score using means and standard deviations based on the dataset of 340 healthy control Japanese populations; however, it must be noted that age, sex, and socio-economic status of the healthy controls were not necessarily matched to those of the patients in the present study. Composite scores were calculated by averaging all z scores of the six subcomponents (verbal memory, working memory, motor speed, verbal fluency, attention and speed of information processing, and executive functions), and then re-normed based upon the standard deviations (SD) of the average of those scores in the normative sample (SD = 0.6).

2.3. Statistical analysis

Two-tailed paired t-tests were performed for the assessment of change between the two measurements of BACS-J data, which were administered before (baseline) and after (post-treatment) the NEAR sessions. Each subcomponent score was normally distributed except for the executive function score. Through a logarithmic transformation of the executive function score, the curve was modified to a normal distribution, described by [EX] = −log[2 − (Executive function BACS-J z score)]. Therefore, we used [EX] instead of "executive function BACS-J z score" for analysis.

Except for the treatment settings, baseline verbal memory, baseline [EX], and baseline composite scores, neither socio-demographic nor clinical variables differed significantly between the two groups (Table 1). Therefore, repeated measures analyses

Table 2
Sample educational computer software used in the computer sessions.

Task	Software	Activity	Target cognitive domain
The mail room	Monsters Inc.: Scream Team Training	Sort all the mail into the proper mailboxes before the clock hits 9 a.m.	Attention, speed
Lunch room	Monsters Inc.: Scream Team Training	Select food items and daily specials to serve to each monster in accordance with the figure presented on the lunch-order ticket.	Attention, speed
Moonfish	Finding Nemo: Nemo's Underwater World of Fun	Repeat the shape patterns made by the moonfish.	Working memory
Spark! Mejikara	Let's refresh your brain	Memorize the illustrations that appear one after another on the screen, and recollect them in order.	Working memory
Hustle memory	Let's refresh your brain	Memorize the character's clothes that are put on within 10 s.	Visual learning and memory
Frippletration	Thinkin' Things 2	Visual and auditory memory matching game.	Visual/auditory learning and memory
Stocktopus	Thinkin' Things 3	Repeat trading items to get the items you need for your portfolio.	Working memory, executive function,
Build it	Factory Deluxe	Build up the presented goal product by selecting and using appropriate tools.	Executive function
The puzzles	Logical Journey Of The Zoombinis	Solve puzzles with various rules using as clues physical features of hair, eyes, nose, and feet of little creatures called Zoombinis.	Executive function

"Thinkin' Things 2", "Thinkin' Things 3", and "Factory Deluxe" were English versions; however, English ability was not necessary to accomplish the tasks. Other software programs were Japanese versions.

Table 3
The result of paired t test on BACS-J data with NEAR participants.

	Baseline	Post treatment	t	p	Cohen's d
Verbal memory	−1.09 ± 0.92	−0.13 ± 0.99	8.80	<0.0001	1.01
Working memory	−0.95 ± 0.95	−0.54 ± 1.17	4.11	<0.0005	0.39
Motor speed	−1.60 ± 1.37	−1.04 ± 1.42	3.28	<0.005	0.41
Verbal fluency	−0.47 ± 1.00	−0.14 ± 1.10	3.41	<0.005	0.32
Attention and speed of information processing	−1.24 ± 0.88	−0.99 ± 0.96	3.19	<0.005	0.28
[EX]	−0.79 ± 0.59	−0.55 ± 0.55	3.02	<0.005	0.44
Composite score	−1.65 ± 1.27	−0.79 ± 1.33	8.96	<0.0001	0.67

[EX] = −log[2 − (Executive function BACS-J z score)].

of variance were performed on BACS-J data using 'group' (NEAR group, control group) and 'treatment settings' (inpatient, outpatient) as inter-individual factors, while 'time' (baseline, post-treatment) was used as an intra-individual factor. Moreover, in the analyses of verbal memory, [EX], and composite scores, baseline data were used as covariates.

3. Results (Tables 3, 4, Fig. 1)

3.1. The within-NEAR treatment change of BACS-J data

There were significant improvements in the scores of all subcomponents in the BACS-J (Table 3).

3.2. In comparison with control patients

There were significant interactions between 'group' and 'time' in verbal memory, working memory, verbal fluency, attention and speed of information processing, [EX], and composite scores (Table 4). The improvement of these areas was significantly greater in the NEAR group than in the control group. There was no difference between groups in terms of the change in motor speed.

4. Discussion

In the present study, we found significant improvement for all cognitive domains related to the BACS-J. According to the meta-analysis of the effectiveness of cognitive remediation in schizophrenia, neurocognitive benefit varied from small (Cohen's $d = 0.2$) to very large ($d = 1.2$) effect size (Medalia and Choi, 2009). Medalia et al. (2009) also suggested that heterogeneity of response to cognitive remediation might depend on instructional techniques, intellectual ability, and intrinsic motivation. In NEAR, instructional techniques are devised to enhance intrinsic motivation. It has already been shown that the use of NEAR educational software without an instructional approach did not achieve clinically meaningful change in neurocognitive capacity (Bellack et al., 2005; Dickinson et al., 2010). In our study, we complied with the principle of NEAR by attaching great importance to instructional approach and could find small to very large effect sizes in broad domains ($d = 0.28$–1.01). In comparison with the control group, the positive findings remained significant except for the motor speed. NEAR proved to be a feasible psychosocial therapy, even in Japan with its different cultural background and with the use of software programs that differ from those in Western countries.

In BACS-J, motor speed was assessed by the "Token Motor Task". The task requires the participants to put 100 plastic tokens into a container bimanually as quickly as possible within 60 s, and the outcome measure is the total number of tokens put in the container (Keefe et al., 2004). In the NEAR session, participants were engaged in the computerized learning tasks selected to address specific domains of cognitive function (Medalia et al., 2009); however, we may have failed to include those tasks that required considerable motor speed to perform in the session. This may explain why the NEAR participants were not able to achieve greater improvement in motor speed than the controls.

In this study, the two groups were heterogeneous in many points, and although several subcomponent scores of the BACS-J were significantly lower in the control group than in the NEAR group, correlations between baseline BACS-J data and the improvement in

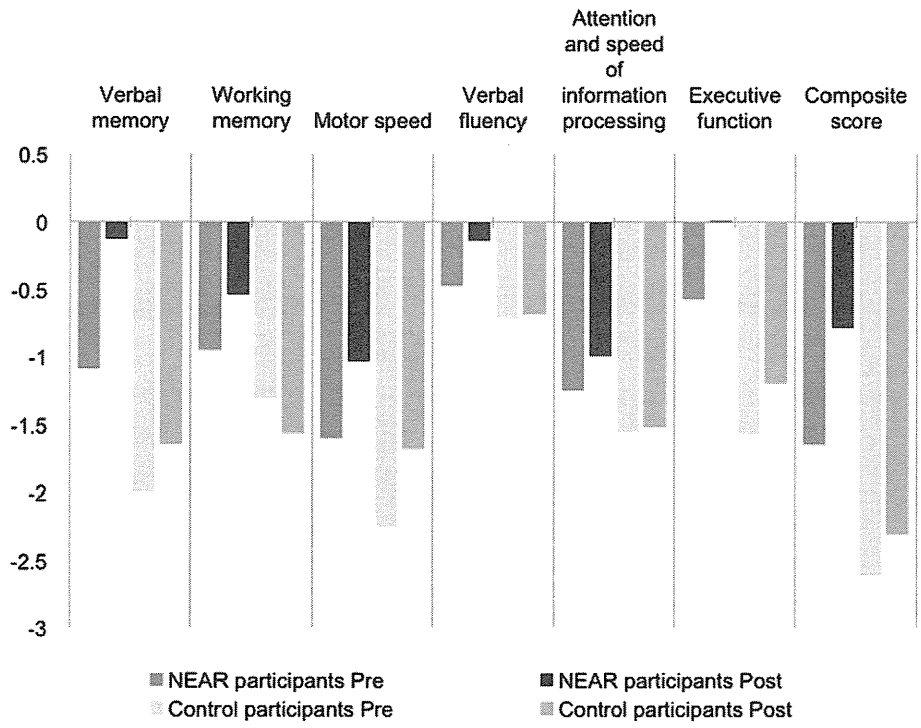

Fig. 1. Changes in cognitive function over a 6-month period.

Table 4
"Time × group" interaction effect on ANOVA with BACS-J data in comparison with control group.

	d.f.	*F*	*p*
Verbal memory#	1,69	16.1	<0.0005
Working memory	1,70	16.9	<0.0005
Motor speed	1,70	1.53	n.s.
Verbal fluency	1,70	4.39	<0.05
Attention and speed of information processing	1,70	5.79	<0.05
[EX]#	1,69	4.69	<0.05
Composite score#	1,69	19.1	<0.0001

# baseline data were used as covariates.
[EX] = − log[2 − (Executive function BACS-J *z* score)].

BACS-J data were negative ($r = -0.57$ to -0.06) in the NEAR group. This implies that the NEAR program is more effective when baseline neurocognitive ability is weaker. Although it is possible that there was recruitment bias to include higher functioning subjects in the NEAR group at baseline, it may be assumed that taking into account the difference in neurocognition would not negate the effect of NEAR.

There are several limitations of the present study. First, although only the difference in treatment settings between the NEAR participants and the controls appeared significant, clinical and demographic variables were not well matched between the two groups. Second, subjects were not randomly assigned to either of the groups. Third, some clinicians who managed the NEAR session also had to take a role as a tester in the BACS-J. To resolve these issues, randomized control studies of the NEAR program with testers being blinded to the treatment assignment are warranted. Moreover, while we focused on the neurocognitive effect of NEAR in Japan in the present report, we should also take into consideration its effectiveness on social function and/or quality of life in patients with schizophrenia.

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References

Bellack, A.S., Dickinson, D., Morris, S.E., Tenhula, W.N., 2005. The development of a computer-assisted cognitive remediation program for patients with schizophrenia. Israel Journal of Psychiatry & Related Sciences 42, 5–14.
Bilder, R.M., Goldman, R.S., Robinson, D., Reiter, G., Bell, L., Bates, J.A., Pappadopulos, E., Willson, D.F., Alvir, J.M., Woerner, M.G., Geisler, S., Kane, J.M., Lieberman, J.A., 2000. Neuropsychology of first-episode schizophrenia: initial characterization and clinical correlates. The American Journal of Psychiatry 157, 549–559.
Dickinson, D., Tenhula, W., Morris, S., Brown, C., Peer, J., Spencer, K., Li, L., Gold, J.M., Bellack, A.S., 2010. A randomized, controlled trial of computer-assisted cognitive remediation for schizophrenia. The American Journal of Psychiatry 167, 170–180.
Green, M.F., Kern, R.S., Braff, D.L., Mintz, J., 2000. Neurocognitive deficits and functional outcome in schizophrenia: are we measuring the "right stuff"?. Schizophrenia Bulletin 26, 119–136.
Heinrichs, R.W., 2004. Meta-analysis and the science of schizophrenia: variant evidence or evidence of variants? Neuroscience and Biobehavioral Reviews 28, 379–394.
Kaneda, Y., Sumiyoshi, T., Keefe, R., Ishimoto, Y., Numata, S., Ohmori, T., 2007. Brief assessment of cognition in schizophrenia: validation of the Japanese version. Psychiatry and Clinical Neurosciences 61, 602–609.
Keefe, R.S., Goldberg, T.E., Harvey, P.D., Gold, J.M., Poe, M.P., Coughenour, L., 2004. The Brief Assessment of Cognition in Schizophrenia: reliability, sensitivity, and comparison with a standard neurocognitive battery. Schizophrenia Research 68, 283–297.
Keefe, R.S., Sweeney, J.A., Gu, H., Hamer, R.M., Perkins, D.O., McEvoy, J.P., Lieberman, J.A., 2007. Effects of olanzapine, quetiapine, and risperidone on neurocognitive function in early psychosis: a randomized, double-blind 52-week comparison. The American Journal of Psychiatry 164, 1061–1071.
Medalia, A., Choi, J., 2009. Cognitive remediation in schizophrenia. Neuropsychology Review 19, 353–364.
Medalia, A., Freilich, B., 2008. The neuropsychological educational approach to cognitive remediation (NEAR) model: practice principles and outcome studies. American Journal of Psychiatric Rehabilitation 11, 123–143.
Medalia, A., Revheim, N., Herlands, T., 2009. Cognitive Remediation for Psychological Disorders: Therapist Guide. Oxford, New York.
Nemoto, T., Yamazawa, R., Kobayashi, H., Fujita, N., Chino, B., Fujii, C., Kashima, H., Rassovsky, Y., Green, M.F., Mizuno, M., 2009. Cognitive training for divergent thinking in schizophrenia: a pilot study. Progress in Neuro-Psychopharmacology & Biological Psychiatry 33, 1533–1536.
Woodward, N.D., Purdon, S.E., Meltzer, H.Y., Zald, D.H., 2005. A meta-analysis of neuropsychological change to clozapine, olanzapine, quetiapine, and risperidone in schizophrenia. The International Journal of Neuropsychopharmacology 8, 457–472.

Protein-anchoring Strategy for Delivering Acetylcholinesterase to the Neuromuscular Junction

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Acetylcholinesterase (AChE) at the neuromuscular junction (NMJ) is anchored to the synaptic basal lamina *via* a triple helical collagen Q (ColQ). Congenital defects of ColQ cause endplate AChE deficiency and myasthenic syndrome. A single intravenous administration of adeno-associated virus serotype 8 (AAV8)-*COLQ* to *Colq*$^{-/-}$ mice recovered motor functions, synaptic transmission, as well as the morphology of the NMJ. ColQ-tailed AChE was specifically anchored to NMJ and its amount was restored to 89% of the wild type. We next characterized the molecular basis of this efficient recovery. We first confirmed that ColQ-tailed AChE can be specifically targeted to NMJ by an *in vitro* overlay assay in *Colq*$^{-/-}$ mice muscle sections. We then injected AAV1-*COLQ-IRES-EGFP* into the left tibialis anterior and detected AChE in noninjected limbs. Furthermore, the *in vivo* injection of recombinant ColQ-tailed AChE protein complex into the gluteus maximus muscle of *Colq*$^{-/-}$ mice led to accumulation of AChE in noninjected forelimbs. We demonstrated for the first time *in vivo* that the ColQ protein contains a tissue-targeting signal that is sufficient for anchoring itself to the NMJ. We propose that the protein-anchoring strategy is potentially applicable to a broad spectrum of diseases affecting extracellular matrix molecules.

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INTRODUCTION

Acetylcholine (ACh) released from the nerve terminal is rapidly hydrolyzed by acetylcholinesterase (AChE) at the vertebrate neuromuscular junction (NMJ) to terminate cholinergic transmission. Three tetramers of catalytic AChE subunits are linked by a triple helical collagen Q (ColQ) to constitute a ColQ-tailed AChE.[1] The ColQ-tailed AChE is assembled in the endoplasmic reticulum and the Golgi apparatus.[2,3] ColQ carries three domains: (i) an N-terminal proline-rich attachment domain that organizes the catalytic AChE subunits into a tetramer, (ii) a collagenic domain that forms a triple helix, and (iii) a C-terminal domain enriched in charged residues and cysteines. ColQ-tailed AChE is organized in a secretory pathway, excreted, and anchored into the synaptic basal lamina using two domains of ColQ (**Figure 1**). First, the collagen domain harbors two heparan sulfate proteoglycan (HSPG)-binding domains[4] that bind to heparan sulfate proteoglycan such as perlecan in the synaptic basal lamina.[5] Second, the C-terminal domain of ColQ binds to MuSK, a muscle-specific receptor tyrosine kinase, on the postsynaptic membrane.[6] Human congenital defects of ColQ cause endplate AChE deficiency, in which the neuromuscular transmission is compromised.[7–9] Endplate AChE deficiency is an autosomal recessive disorder, which manifests as generalized muscle weakness, fatigue, amyotrophy, scoliosis, and minor facial abnormalities. Thirty-nine mutations of *COLQ* are currently registered in the Human Gene Mutation Database at http://www.hgmd.cf.ac.uk/. Ephedrine is effective for myasthenic symptoms to some extent,[10,11] though the underlying mechanisms of ephedrine efficacy remain elusive. We have developed a mouse model deficient in ColQ by deletion of the PRAD domain.[12] This strain recapitulates the phenotype of congenital myasthenic syndromes with AChE deficiency.

Gene therapy of endplate AChE deficiency is a complex issue both in humans and mice because ColQ is encoded by alternative promoters with a specific expression in subsynaptic nuclei of slow- and fast-twitch muscles.[13] The levels of AChE at the NMJ are supposed to be precisely controlled by the expression of ColQ and AChE,[14] as well as by a post-translational mechanism.[3] To treat endplate AChE deficiency in *Colq*-deficient mice, we delivered *COLQ* using adeno-associated virus (AAV) serotype 8, which has a tropism for muscles.[15] We used human *COLQ* instead of mouse *Colq* to foresee if the recombinant human *COLQ* is applicable to clinical practice in the future. Efficient rescue of AChE at the NMJ of AAV8-*COLQ*-injected mice prompted us to search for the molecular basis of these unexpected effects. We found that ColQ carries tissue-targeting signals that are necessary and sufficient to cluster AChE at the NMJ. This is the first report of a long-distance delivery of a large extracellular matrix complex over 50 nm in length and weighing over one million kDa in skeletal muscle. The findings of

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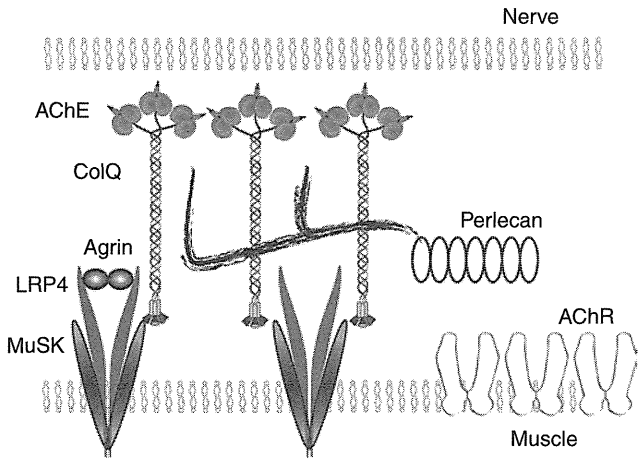

Figure 1 Schematic of anchoring of collagen Q (ColQ) to neuromuscular junction (NMJ). Twelve catalytic subunits of acetylcholinesterase (AChE) are attached to ColQ to form ColQ-tailed AChE. Two heparan sulfate proteoglycan-binding domains of ColQ are bound to perlecan. C-terminal domain of ColQ is bound to muscle-specific kinase (MuSK). Nerve-derived agrin binds to an LRP4–MuSK complex and induces rapsyn-mediated clustering of acetylcholine receptors (AChR) by phosphorylating AChR.

the present study open a new therapeutic avenue for treating many inherited defects of extracellular matrix proteins.

RESULTS

Intravenous administration of AAV8-*COLQ* normalizes motor functions of *Colq*$^{-/-}$ mice

We explored the recovery of the muscular phenotype of *Colq*$^{-/-}$ mice by viral delivery of a functional ColQ molecule. Therefore, we constructed a recombinant AAV serotype 8 carrying human *COLQ* cDNA. AAV serotype 8 (AAV8) is efficiently delivered to skeletal muscle after systemic injection.[16] We intravenously administered 1×10^{11}–2×10^{12} viral genome (vg) copies of AAV8-*COLQ* into 4-week-old *Colq*$^{-/-}$ mice. These mice exhibit muscle weakness, myasthenia, tremor, kyphosis, involuntary vocalization, and a slower growth rate than their wild-type littermates.[12] However, a single injection of 2×10^{12} vg, gradually improved their motor function to reach the level of that of wild type (**Figure 2a**). Furthermore, there were no signs of fatigue 6 weeks after the therapeutical injection (**Figure 2b**). Voluntary exercise in the treated mice also increased gradually but did not reach the level of wild type even at 5 weeks after injection (**Figure 2c**). The improved motor activities of treated mice are also demonstrated in **Supplementary Video 1**. Pairs of treated mice gave birth to *Colq*$^{-/-}$ pups and reared them to maturity. In longitudinal studies of three treated mice, all survived 18–20 months. Motor functions of the treated mice were declined at 48 weeks after injection but to the similar levels as those of wild type (**Figure 2a,c**). These observations clearly indicate the long-term therapeutic potential of a single viral injection of AAV.

AAV8-*COLQ* normalizes the neuromuscular synaptic transmission

To estimate recovery of neuromuscular transmission, we performed electrophysiological studies (**Table 1**). Treatment with

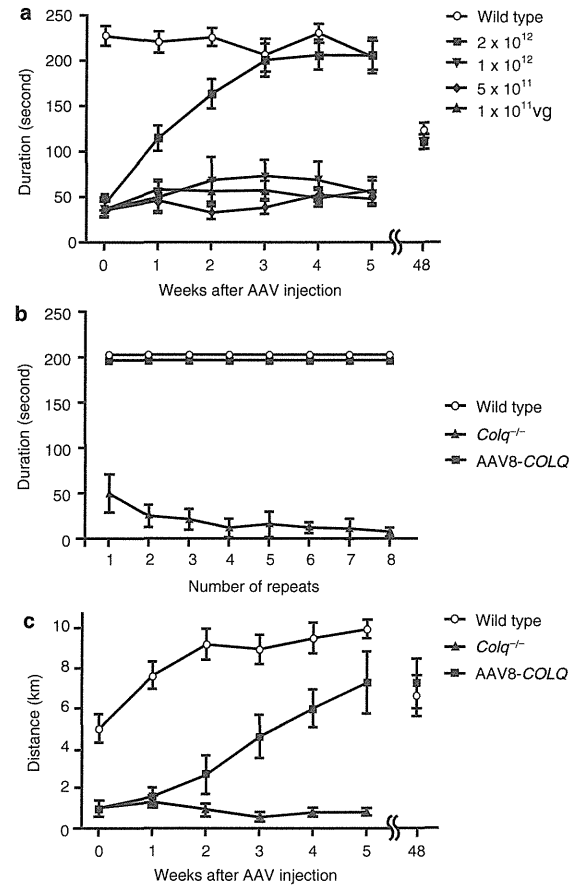

Figure 2 Exploration of motor function after intravenous injection of AAV8-*ColQ* to the tail vein of *Colq*$^{-/-}$ mice. (**a**) Motor function on the rotarod. The rotation was linearly accelerated from 0 to 40 r.p.m. in 240 seconds. Five groups of six mice were studied. Each group consisted of 4-week old mice and was either injected or not (control group) with increasing numbers of viral particles. Three weeks after their AAV8-*COLQ* injection, only the group of mice treated with 2×10^{12} vg remained on the rod as long as the wild-type littermates. Importantly, there was a progressive motor function recovery during the first 3 weeks after injection of *Colq*$^{-/-}$ mice. Symbols indicate mean and SE of six mice for each experiment. Mean and SE of the durations on the rotarod of two treated mice at 48 weeks after treatment is indicated along with that of the four age-matched wild-type mice. (**b**) Fatigue test using the rotarod was performed on three groups of a total of 18 mice. The rotation speed was fixed at 10 r.p.m. and the mice were immediately placed back on the rod each time they fell. Mice injected with 2×10^{12} vg exhibited no fatigue at 6 weeks after injection, whereas untreated *Colq*$^{-/-}$ mice fell increasingly more rapidly off the rod. (**c**) Voluntary movements were quantified by a counter-equipped running wheel. Plots show mean and SE of the number of rotations over 24 hours in each group of six mice (wild type, *Colq*$^{-/-}$, and AAV8-*COLQ*). Only the group of mice treated with 2×10^{12} vg increased the number of rotations every week but they did not reach the level of wild-type mice at 5 weeks after injection. Mean and SE of the number of rotations of two treated mice at 48 weeks after treatment is indicated along with that of the four age-matched wild-type mice. AAV8, adeno-associated virus serotype 8; ColQ, collagen Q.

AAV8-*COLQ* reduced decrements of the compound muscle action potentials in response to repetitive nerve stimulation at 2 Hz, reduced the amplitudes of miniature endplate potentials (MEPPs), shortened the miniature endplate potential decay time constants (**Figure 3**), and acquired responses to neostigmine. Endplate potential quantal content, which was decreased in

Table 1 Repetitive nerve stimulation and microelectrode studies

	Wild type	Wild type with neostigmine	*Colq*$^{-/-}$	*Colq*$^{-/-}$ with neostigmine	Treated *Colq*$^{-/-}$	Treated *Colq*$^{-/-}$ with neostigmine
Repetitive nerve stimulation[a]	0.92 ± 0.01* (2)	n.a.	0.58 ± 0.05 (3)	n.a.	0.76 ± 0.02* (3)	n.a.
EPP quantal content[b]	39.8 ± 2.3** (18)	n.a.	28.2 ± 1.8 (19)	n.a.	24.1 ± 1.6 (18)	n.a.
MEPP amplitude (mV)[c]	0.77 ± 0.04** (31)	1.52 ± 0.12 (18)	1.52 ± 0.11 (19)	1.52 ± 0.07 (10)	0.68 ± 0.02** (25)	0.98 ± 0.05** (24)
EPP amplitude (mV)[d]	30.6	n.a.	42.9	n.a.	16.4	n.a.
MEPP decay time (ms)[c]	1.77 ± 0.06** (31)	2.27 ± 0.08** (18)	3.07 ± 0.12 (19)	2.99 ± 0.09 (10)	2.45 ± 0.08** (25)	3.66 ± 0.09** (24)

Abbreviations: AChR, acetylcholine receptors; ColQ, collagen Q; EPP, endplate potential; MEPP, miniature endplate potential; n.a., not applicable.
Values represent mean ± SE. T = 29 ± 0.5 °C for EPP and MEPP recordings. Numbers in parenthesis indicate the number of recordings for repetitive nerve stimulation and the number of EPs from one or two mice for the other assays.
[a]Repetitive nerve stimulations were performed at 2 Hz, and the relative areas of compound muscle action potential (CMAP) of the fourth to the first stimulations are indicated. [b]Quantal content of EPP at 0.5 Hz stimulation corrected for resting membrane potential of –80 mV, nonlinear summation, and non-Poisson release. As the quantal contents of EPP are higher than 10, corrected values are indicated according to Cull-Candy *et al.*[45c] Normalized for resting membrane potential of –80 mV and a mean muscle fiber diameter of 55 µm. The actual fiber diameters were 45 ± 3.6 µm (mean ± SD, n = 31) for wild-type mice, 43 ± 3.0 µm (n = 19) for *Colq*$^{-/-}$ mice, and 46 ± 4.2 µm (n = 25) for the treated *Colq*$^{-/-}$ mice. [d]Estimated EPP amplitude is the product of the EPP quantal content and the MEPP amplitude. As AChR was partly blocked with curare for EPP recordings and not for MEPP recordings, we could not directly measure EPP amplitudes. Predicted low EPP amplitudes in treated mice suggest that the improvement of motor function was likely due to amelioration of depolarization block and/or of endplate myopathy.
*P < 0.05 and **P < 0.001 compared to *Colq*$^{-/-}$ mice by Student's t-test.

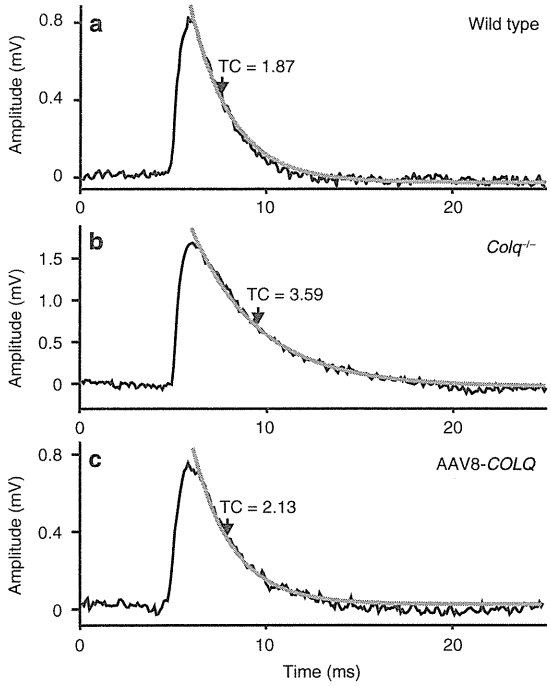

Figure 3 Representative miniature endplate potential (MEPP) recordings of diaphragm muscles of (a) wild type, (b) *Colq*$^{-/-}$, and (c) AAV8-*COLQ*-treated mice. (**b**) *Colq*$^{-/-}$ mice have higher MEPP amplitude and a longer decay time constant (TC) than (**a**) wild-type mice. AAV8-*COLQ* treatment shortened the decay TC and lowered the MEPP amplitude. Gray lines represent fitted exponential decay curves. AAV8, adeno-associated virus serotype 8; ColQ, collagen Q.

Colq$^{-/-}$ mice, was further decreased by the treatment, in contrast to our expectation.

Human ColQ-tailed AChE is anchored to the mouse NMJ *in vivo*

To further evaluate that the rescue was due to restitution of AChE at the NMJ, we used histological methods to visualize ColQ and AChE on muscle sections (**Figure 4a–c**). ColQ and AChE were colocalized to acetylcholine receptors (AChR) at the NMJ, confirming that ColQ-tailed AChE was specifically clustered to the target tissue. Although, we failed to observe improvement of motor functions with 1 × 10^{12} vg or less (**Figure 2a**), we still detected ColQ and AChE at NMJs with smaller amounts (data not shown). This suggests that a certain amount of viral genomes is required to exhibit improvement of motor deficits.

The ultrastructural morphology of treated mice also improved compared with age-matched *Colq*$^{-/-}$ mice (**Figure 4d–f**). The NMJ ultrastructures were variable from one to another in wild type, *Colq*$^{-/-}$, and treated mice, and we quantified the electron micrograph pictures (**Supplementary Table S1**). Quantitative analysis of presynaptic ultrastructures demonstrated that, in soleus slow-twitch muscle, Schwann cell invagination was mitigated, which increased the nerve terminal length, but the nerve terminal area remained essentially the same. Postsynaptic area and postsynaptic membrane length were also increased in soleus muscle of treated mice. In the extensor digitorum longus fast-twitch muscle, however, significant improvement was observed only in the ratio of enwrapped nerve terminal. Thus, the morphological improvements were more prominent in the soleus rather than in extensor digitorum longus muscles.

AAV8-*COLQ* restores the amount of ColQ-tailed AChE in the muscle to 89.3% of wild type

To estimate the efficiency of intravenous administration of AAV8-*COLQ*, we quantified the amount of the transduced *COLQ* mRNA, as well as ColQ-tailed AChE, in the muscle. We estimated the amount of *COLQ* mRNA in hindlimbs by a TaqMan probe, and found that the treated mice expressed the transduced *COLQ* at 92.5 ± 47.8% (mean ± SE, n = 4) of wild type. ColQ-tailed AChE from hindlimbs of the treated mice was fractionated by sucrose density-gradient ultracentrifugation. Sedimentation analysis revealed that AAV8-*COLQ* muscles have similar peaks of ColQ-tailed AChE species as those of wild type (**Figure 5a–c**). We also quantified the amount of globular AChE and ColQ-tailed AChE in gastrocnemius muscles of treated mice (**Figure 5d**). As previously reported, the amount of globular AChE was slightly lower in *Colq*$^{-/-}$ mice,[12] and this was normalized by treatment

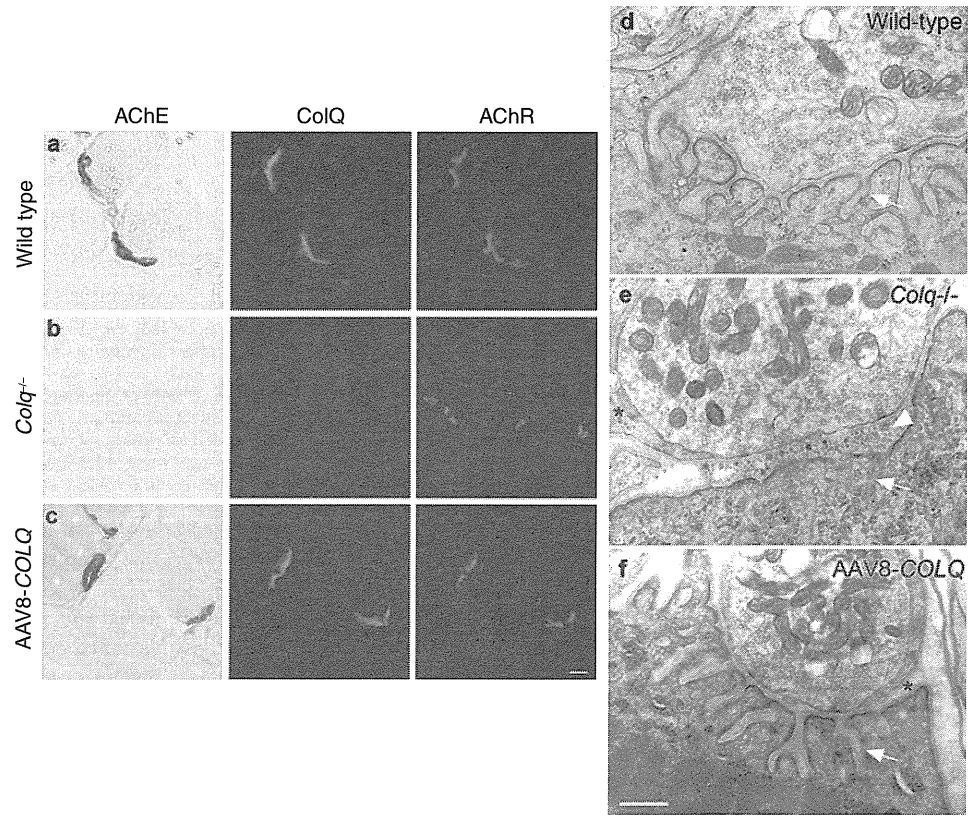

Figure 4 Histologies and ultrastructures of the neuromuscular junctions (NMJs). Localization of acetylcholinesterase (AChE) activity, collagen Q (ColQ), and acetylcholine receptors (AChR) in quadriceps muscles of (**a**) wild type, (**b**) *Colq*$^{-/-}$, and (**c**) AAV8-*COLQ* mice. Mice treated with 2 × 10^{12} vg of intravenous AAV8-*COLQ* express ColQ-tailed AChE at NMJ. AChE is stained for its activity. ColQ and AChR are detected by the polyclonal anti-ColQ antibody and α-bungarotoxin, respectively. Bar = 10 μm (**a–c**). Representative stainings of six mice in each group are indicated. Ultrastructures of soleus muscle NMJ (**d–f**). (**e**) *Colq*$^{-/-}$ mice show simplified synaptic clefts (arrow) and widening of the synaptic space (arrow head), whereas the NMJ ultrastructure of AAV8-*COLQ* mice (**f**) is indistinguishable from that of wild type (**d**). AAV8-*COLQ* mice still have small nerve terminals and invaginated Schwann cells (*). Bar = 1 μm (**d–f**). Representative ultrastructures of 27–41 electron micrograph (EM) pictures (see **Supplementary Table S1**) are indicated. AAV8, adeno-associated virus serotype 8.

with AAV8-*COLQ*. Treatment with AAV8-*COLQ* also restored ColQ-tailed AChE to 89.3 ± 9.6% (mean ± SD, n = 4) of wild type at 6 weeks after treatment. We also quantified ColQ-tailed AChE at 48 weeks after treatment and found that the amount was still 81.8 ± 21.6% (mean ± SD, n = 2) of the age-matched wild-type mice (n = 3). Although soleus slow-twitch muscle exhibited prominent improvement with the ultrastructural analysis, the available amount of soleus muscle was too small for the biochemical assay.

We also examined whether ColQ-tailed AChE was produced in the liver because AAV8 efficiently transduces hepatocytes.[15] AAV8-*COLQ* increased the *COLQ* mRNA level in the liver from 3.4 ± 0.34% (mean ± SE of five wild-type mice) to 61.3 ± 12.6% (mean ± SE of five treated mice) compared to those in the muscle of wild-type mice (n = 5). The *Ache* mRNA levels in the liver of wild type, *Colq*$^{-/-}$, and treated mice, however, were estimated to be <0.5% of that in wild-type muscle. The *Ache* mRNA levels in the liver were too low to be accurately quantified by real-time reverse transcription-PCR. Sedimentation profiles revealed no peaks of ColQ-tailed AChE in the liver of either wild type, *Colq*$^{-/-}$, or treated mice (**Supplementary Figure S1a–c**). This was probably due to lack of *Ache* expression. Globular AChE species observed in the sedimentation analysis was likely to represent AChE on the erythrocyte cell membrane.[17] These data suggest that AAV8-*COLQ* did not induce expression of ColQ-tailed AChE in the liver.

Local intramuscular injection of AAV8-*COLQ* expresses ColQ-tailed AChE at NMJs of noninjected limbs

Prominent improvements that we observed in AAV8-*COLQ*-treated mice raised a possibility that ColQ-tailed AChE moved from the transduced muscle cells to other muscle cells. We thus tested this possibility in the following experiments.

First, we have previously reported that the human recombinant ColQ-tailed AChE can be anchored to the synaptic basal lamina of the frog NMJ.[18] We tested this anchoring using mouse NMJs. We purified ColQ-tailed AChE expressed in HEK293 cells and incubated this with a section of skeletal muscle from *Colq*$^{-/-}$ mice. As expected, ColQ and AChE were detected at the mouse NMJ (**Supplementary Figure S2**), which supports the notion that ColQ-tailed AChE can be moved and anchored to the target *in vitro*.

Next, we tested whether ColQ-tailed AChE moved from the transduced muscles to the nontransduced muscles. We injected AAV8-*COLQ* to the left anterior tibial muscle. As expected, AChE and ColQ were rescued at the NMJs of the injected muscle. In addition, AChE and ColQ were also detected at all the examined