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DOCTORAL DISSERTATION

IDENTIFICATION OF EARLY NEUROPHYSIOLOGICAL MARKERS RELATED TO COGNITIVE-BEHAVIOURAL DISORDERS AND PROGRESSION OF VASCULAR COGNITIVE IMPAIRMENT: A TRANSCRANIAL MAGNETIC STIMULATION STUDY

Tutor: Prof. Giovanni Pennisi

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ABSTRACT

Transcranial magnetic stimulation (TMS) highlighted functional changes in dementia, whereas there are few data in patients with Vascular Cognitive Impairment–No Dementia (VCI-ND) at risk for cognitive worsening. Similarly, little is known about the neurophysiological impact of Vascular Depression (VD) on deterioration of cognitive functions. We performed a longitudinal TMS study to test whether the presence of depression might affect not only cognition but also the functioning of specific cortical circuits in patients with subcortical vascular damage. In this study, 16 VCI-ND and 11 VD patients, age-matched with 15 healthy controls, underwent a baseline evaluation including clinical-cognitive, neuroimaging and TMS assessment. After approximately two years of follow-up, all participants were prospectively re-evaluated. At baseline, a significant more pronounced intracortical facilitation (ICF) at paired-pulse TMS was found in VCI-ND patients only. Re-evaluation revealed an increase of the global excitability at single-pulse TMS in both VCI-ND and VD. At follow-up, the ICF of VCI-ND become similar to the other groups. Only VD patients showed cognitive deterioration. In conclusion, in VCI-ND specific measures of cortical excitability, namely the high level of ICF found at baseline, suggests an enhanced glutamatergic neurotransmission that might contribute to the preservation of cognitive functioning; conversely, a lack of this hyperfacilitation in VD might be associated with clinical progression. The hyperexcitability to single-pulse TMS observed at follow-up in both group of patients also suggests functional changes in glutamatergic neurotransmission. This suggests that the mechanisms enhancing the risk of dementia in VD might be related either to subcortical changes produced by vascular lesions or to the lack of compensatory functional cortical changes.

Key words: cognitive decline; cerebrovascular disease; late-life depression; non-invasive barin stimulation; cortical excitability; synaptic plasticity.
La Stimolazione Magnetica Transcranica (TMS) ha messo in luce specifiche alterazioni funzionali in pazienti con demenza. Tuttavia, i dati in soggetti con deterioramento cognitivo vascolare a rischio per demenza (VCI-ND) sono limitati, così come poco è noto sull’impatto neurofisiologico della depressione vascolare (VD) nel deterioramento delle funzioni cognitive nell’anziano. In questo studio, abbiamo valutato prospetticamente le modificazioni elettrocorticali alla TMS in soggetti con VCI-ND con e senza depressione al fine di verificare se la presenza di sintomatologia depressiva nel contesto della cerebrovascolopatia cronica sottocorticale possa influire negativamente non solo sulle capacità cognitive ma anche sul funzionamento di specifici circuiti cortico-sottocorticali. Sedici soggetti con VCI-ND ed 11 con VD, paragonati a 15 anziani sani di pari età, sono stati sottoposti ad una valutazione di base comprendente l’esame clinico-cognitivo, neuroradiologico e TMS. All’ingresso, i pazienti con VCI-ND esibivano un aumento della facilitazione intracorticale (ICF) alla TMS a doppio stimolo rispetto agli altri 2 gruppi. Dopo circa 2 anni, tutti i pazienti (con e senza depressione) mostravano un aumento globale dell’eccitabilità corticale alla TMS a singolo stimolo; inoltre, l’ICF dei VCI-ND risultava ora sovrapponibile agli altri due gruppi. Solo i pazienti depressi si erano deteriorati cognitivamente. In conclusione, specifiche misure di eccitabilità corticale, quale l’iperfacilitazione di base dei pazienti con VCI-ND, suggeriscono un’aumentata neurotrasmissione glutammatergica che potrebbe contribuire a preservare lo status cognitivo di coloro senza depressione; al contrario, la carenza di facilitazione dei soggetti depressi potrebbe accompagnarsi alla loro progressione clinica. L’ipereccitabilità osservata al follow-up potrebbe anch’essa essere espressione di modificazioni funzionali dell’attività glutammatergica cerebrale, sebbene senza correlazione con la presenza di depressione. I meccanismi neurofisiologici che espongono maggiormente al rischio di demenza i soggetti depressi potrebbero dunque risiedere sia in alterazioni sottocorticali dovuti alla malattia cerebrovascolare sia al deficit di risposte plasticо-compensatorie a livello corticale.
**List of abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>A ratio</td>
<td>CMAP/MEP amplitude ratio</td>
</tr>
<tr>
<td>ACC</td>
<td>anterior cingulate cortex</td>
</tr>
<tr>
<td>ACE</td>
<td>angiotensin converting enzyme</td>
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<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
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<tr>
<td>ADL</td>
<td>Activity of Daily Living</td>
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<tr>
<td>AGTR</td>
<td>angiotensin receptor</td>
</tr>
<tr>
<td>ApoE</td>
<td>Apolipoprotein E</td>
</tr>
<tr>
<td>AS</td>
<td>Apathy Scale</td>
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<tr>
<td>BDNF</td>
<td>Brain-derived Neurotrophic Factor</td>
</tr>
<tr>
<td>CBF</td>
<td>cerebral blood flow</td>
</tr>
<tr>
<td>CMCT</td>
<td>central motor conduction time</td>
</tr>
<tr>
<td>CMCT-F</td>
<td>CMCT estimated by using the F wave</td>
</tr>
<tr>
<td>CoG</td>
<td>center of gravity</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebro-spinal fluid</td>
</tr>
<tr>
<td>CSP</td>
<td>contralateral cortical silent period</td>
</tr>
<tr>
<td>DLPFC</td>
<td>dorsolateral prefrontal cortex</td>
</tr>
<tr>
<td>DSM-IV-TR</td>
<td>Diagnostic and Statistical Manual for Mental Disorders-Forth Edition-Text Revised</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
</tr>
<tr>
<td>EEG</td>
<td>electroencephalography</td>
</tr>
<tr>
<td>EMG</td>
<td>electromyographic</td>
</tr>
<tr>
<td>FA</td>
<td>fractional anisotropy</td>
</tr>
<tr>
<td>FAB</td>
<td>Frontal Assessment Battery</td>
</tr>
<tr>
<td>FDI</td>
<td>first dorsal interosseous</td>
</tr>
<tr>
<td>FLAIR</td>
<td>Fluid-attenuated Inversion Recovery</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma-aminobutyric acid</td>
</tr>
<tr>
<td>HDRS</td>
<td>17-item Hamilton Depression Rating Scale</td>
</tr>
<tr>
<td>IADL</td>
<td>Instrumental Activity of Daily Living</td>
</tr>
<tr>
<td>ICF</td>
<td>intracortical facilitation</td>
</tr>
<tr>
<td>IFCN</td>
<td>International Federation of Clinical Neurophysiology</td>
</tr>
<tr>
<td>ISI</td>
<td>interstimulus interval</td>
</tr>
<tr>
<td>LAI</td>
<td>long-latency afferent inhibition</td>
</tr>
<tr>
<td>LLD</td>
<td>late-life depression</td>
</tr>
<tr>
<td>LTD</td>
<td>long-term depression</td>
</tr>
<tr>
<td>LTP</td>
<td>long-term potentiation</td>
</tr>
<tr>
<td>M1</td>
<td>primary motor cortex</td>
</tr>
<tr>
<td>MCI</td>
<td>Mild Cognitive Impairment</td>
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<tr>
<td>MEP</td>
<td>motor evoked potential</td>
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<tr>
<td>MID</td>
<td>multi-infarct dementia</td>
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<tr>
<td>MMP</td>
<td>Matrix metalloproteinases</td>
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<tr>
<td>MMSE</td>
<td>Mini Mental State Examination</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>PAS</td>
<td>paired-associative stimulation</td>
</tr>
<tr>
<td>PSD</td>
<td>post-stroke dementia</td>
</tr>
<tr>
<td>RAS</td>
<td>renin-angiotensin system</td>
</tr>
<tr>
<td>rMT</td>
<td>resting motor threshold</td>
</tr>
<tr>
<td>rTMS</td>
<td>repetitive Transcranial Magnetic Stimulation</td>
</tr>
<tr>
<td>SAI</td>
<td>short-latency afferent inhibition</td>
</tr>
<tr>
<td>SICI</td>
<td>short-latency intracortical inhibition</td>
</tr>
<tr>
<td>SIVD</td>
<td>subcortical ischemic vascular disease</td>
</tr>
<tr>
<td>SNRI</td>
<td>Serotonin Noradrenaline Reuptake Inhibitors</td>
</tr>
<tr>
<td>SSRI</td>
<td>Selective Serotonin Re-uptake Inhibitors</td>
</tr>
<tr>
<td>Stroop E</td>
<td>Stroop Color-Word test – number of errors</td>
</tr>
<tr>
<td>Stroop T</td>
<td>Stroop Color-Word test – total time</td>
</tr>
<tr>
<td>TMS</td>
<td>Transcranial Magnetic Stimulation</td>
</tr>
<tr>
<td>VaD</td>
<td>Vascular Dementia</td>
</tr>
<tr>
<td>VCI</td>
<td>Vascular Cognitive Impairment</td>
</tr>
<tr>
<td>VCI-ND</td>
<td>Vascular cognitive impairment – No Dementia</td>
</tr>
<tr>
<td>VD</td>
<td>Vascular Depression</td>
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<tr>
<td>WML</td>
<td>white matter lesion</td>
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CHAPTER 1
FROM VASCULAR COGNITIVE IMPAIRMENT TO VASCULAR DEMENTIA:
A PREDICTABLE CONTINUUM?

1.1 Definition and terminology

Vascular cognitive impairment (VCI) is a heterogeneous group of cognitive disorders that share a presumed vascular origin. VCI is of interest to both clinicians and researchers because it is a common condition, costly, and possibly preventable. As known, about a third of cases of dementia show substantial vascular pathology on autopsy, and depending on our understanding of cerebrovascular mechanisms, VCI can be deemed to be the most common form of cognitive impairment, affecting about 5% of people over the age of 65. However, unlike neurodegenerative cognitive disorders, such as Alzheimer’s disease (AD), VCI can be potentially prevented and the course of cognitive decline significantly improved.

Historically, the idea of VCI grew from dissatisfaction with the term “multi-infarct dementia” (MID), which replaced the term “senile dementia” due to hardening of the arteries. MID is reported to account for 15% of dementias (figure 1), and even today, it is sometimes incorrectly used interchangeably with the broader concept of Vascular Dementia (VaD). As shown in Figure 1, VCI should be considered as an “umbrella term”, that includes VCI–No Dementia (VCI-ND), VaD, and cognitive impairment of mixed origin (usually AD + VaD). Within the VCI construct, the parallel condition to Mild Cognitive Impairment (MCI) is VCI–ND, and indeed the term “vascular MCI” is sometimes used for VCI–ND. The MCI concept distinguishes amnestic from non-amnestic MCI, as well as single-domain from multi-domain impairment. Non-amnestic MCI can, in some cases, be vascular in origin, and other subtypes of MCI can progress to VCI.
Figure 1 Links between the main entities associated with Vascular Cognitive Impairment and Alzheimer’s disease. 

CI = cognitive impairment; MCI = mild cognitive impairment; AD = Alzheimer’s disease. 

1.2 Subtypes

Because it is a large and heterogeneous group of disorders, VCI has been subgrouped for clinical and research uses,\(^1\) being the subtypes characterised by different risk factors, mechanisms, pathology, clinical features, neuroimaging findings, and response to treatment.\(^{16}\)

- The VaD subtype includes disorders that are in the original VaD construct, such as post-stroke dementia (PSD), MID, subcortical ischemic VaD, and hemorrhagic dementia.\(^2\)
- VCI–ND describes those individuals whose symptoms are not associated with substantial functional impairment, including a high proportion with subcortical ischemia with cognitive impairment of presumed vascular cause. Patients with VCI–ND have a high risk of progression into dementia, mixed primary neurodegenerative dementia with VaD, or VaD per se,\(^{17,18}\) particularly if they have recurrent strokes.\(^{19}\)
- Mixed dementia describes the presentation of individuals with clinical, and commonly neuropathological features of AD and VaD. The way in which AD relates to VCI is incompletely understood but their coexistence is well recognised\(^{20}\) (Figure 2).
1.3 Pathogenesis and pathophysiology

The pathogenesis and pathophysiology of VCI continue to be investigated. A mechanistic approach separates VCI due to large vessel disease from that associated with small vessel disease, including subcortical ischaemic vascular disease, and non-infarct ischemic changes² (Figure 3).
1.3.1 Large vessel disease

The clinical archetype of large vessel disease is PSD, defined as a substantial cognitive impairment that follows stroke (usually within 3 months).\(^2\) The prevalence of dementia after stroke ranges from 14% to 32%, and incident PSD from 20% at 3 months to 33% at 5 years.\(^{21,22}\) Risk factors for PSD include age and low education; of note, the number of vascular risk factors might be more important for predicting cognitive impairment than any individual factor.\(^{23}\) PSD is more common in people who have had strokes and pre-existing neurodegenerative dementia.\(^{24,25}\)

PSD can follow a single strategic infarct in the thalamus, angular gyrus, caudate, globus pallidus, basal forebrain, or hippocampus. Such strokes commonly have characteristic cognitive features; for instance, case reports indicate that angular gyrus infarction is associated with an acute onset of fluent dysphasia, visuospatial disorientation, agraphia, and memory loss that can be mistaken for AD.\(^{26}\) Dementia can also result from the cumulative effects of several cortical infarcts of varying size and number, which is the basis of MID, described by Hachinski.\(^7\) MID can also result from thromboembolic disease or, less commonly, cerebral vasculitis.\(^2\)

PSD is seldom found in isolation. In older patients, small vessel disease (overt or covert) is ubiquitous and can accelerate the clinical progression of AD.\(^{27}\) Small vessel disease includes leukoaraiosis, subcortical infarcts, and incomplete infarction associated with cognitive impairment, and might be the most common cause of VCI.\(^{28,29}\) When both large and small vessel disease are present, vascular pathology can act in association with neurodegenerative changes.\(^2\)

1.3.2 Small vessel disease

Small vessel disease is commonly discussed in relation to the white matter changes seen with neuroimaging, and the term “leukoaraiosis” is often used to describe them. The increased sensitivity of Magnetic Resonance Imaging (MRI) has led to reduced specificity and predictive validity of leukoaraiosis, which can now be detected in more than 90% of older patients.\(^{30}\) White matter lesions (WMLs) are not specific to infarcts; frank infarction might be rare in leukoaraiosis.
compared with deep WMLs, and the causal pathway between leukoaraiosis and vascular changes is not well understood.\textsuperscript{31} The cognitive domains affected by leukoaraiosis are not clearly established, but the association with slow AD-like cognitive and functional decline is robust\textsuperscript{31,32} (Figure 4). In general, patients with confluent lesions have a worse prognosis than those with punctuate lesions, but decline in cognition and function are more consistently related to measures of atrophy.\textsuperscript{23}

In contrast to cortical infarcts, subcortical vascular injury due to small vessel infarct or ischemia occurs within the cerebral white matter, basal ganglia, and brainstem. Lacunae are typically seen in the corona radiata, internal capsule, centrum semiovale, thalamus, basal ganglia, and pons.\textsuperscript{29} Infarcts less than 3 mm in diameter are up to 20 times more prevalent than overt infarcts and occur in 20\% of patients older than 65 years.\textsuperscript{33}

Lesions in the prefrontal subcortical circuit are associated with impairments in verbal fluency, executive function (the ability to sequence, plan, organise, initiate, and shift between tasks),\textsuperscript{34} increased risk of stroke and dementia, and more rapid cognitive decline, even when controlling for other vascular risk factors.\textsuperscript{2}

O’Brien and colleagues\textsuperscript{1} proposed executive dysfunction as part of a specific cognitive profile that might distinguish VCI from AD. Although impaired executive function, and specifically abstract reasoning, might differentially predict AD and VCI,\textsuperscript{35} not all studies agree on the importance of executive dysfunction in VCI.\textsuperscript{36-40} Indeed, executive dysfunction might not be unique to VCI at any stage.\textsuperscript{41-43} Support for this contention comes from a recent prospective neuropsychological and autopsy-based study in which the cognitive effects of small vessel cerebrovascular disease were variable and not particularly distinct, which raises questions about the use of executive impairment as a diagnostic marker for VaD.\textsuperscript{44} Nevertheless, although executive dysfunction is not specific to VCI, it remains a prominent feature of subcortical ischaemic vascular disease,\textsuperscript{45} including its common manifestation as subcortical ischemic VaD.\textsuperscript{46}
1.3.3 Non-ischemic changes and atrophy

Not all the neuropathology in VCI involves overt infarction but is more probably a continuum of processes related to ischemia. Nowadays, non-infarct ischemia is accepted as an integral part of the disease process that affects both clinical presentation and future outcomes; for example, the robust association between age and PSD can indicate both previous infarcts and non-infarct ischemia. Diffusion tensor imaging (DTI) at MRI can be used to detect abnormalities that extend beyond the visible borders of leukoaraiosis (the so called “normal-appearing white matter”), and these abnormalities show a more robust association with cognition than leukoaraiosis alone. Ischemia can also contribute to mixed dementia by promoting the neuropathological changes of AD. In animals, ischemic changes in the vascular endothelium increase cleavage of amyloid precursor protein, promote tau phosphorylation, and inhibit clearance of extracellular amyloid.

Moreover, hypoperfusive hypoxic changes are associated with concurrent AD neuropathology, and might explain the poor outcomes in people with VCI who have no apparent lesions at neuroimaging.
1.4 Diagnosis

The current criteria for VaD, most commonly the NINDS-AIREN [National Institute for Neurological Disorders and Stroke-Association Internationale pour la Recherché et l’Enseignement en Neurosciences] criteria do not include VCI–ND, although up to half of people with VCI do not meet the criteria for dementia. The current diagnostic criteria for VaD are not concordant, which makes comparisons difficult. The more recent harmonisation standards from NINDS and the Canadian Stroke Network represent substantial progress towards diagnostic criteria (Table 1).

<table>
<thead>
<tr>
<th>Essential diagnostic criterion</th>
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<tr>
<td><strong>A. Presence of cognitive impairment plus cerebrovascular lesion</strong></td>
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<tr>
<td>Dementia or mild cognitive impairment (Table 1)</td>
</tr>
<tr>
<td>Cerebrovascular lesion: presence may be suggested by clinical information (history of stroke, focal neurological signs with or without a history suggesting a lesion). Presence of lesions should always be demonstrated by cranial MRI/CT and/or anatomical pathology study (Table 2).</td>
</tr>
<tr>
<td><strong>B. Onset of cognitive impairment occurs immediately after stroke, plus any one of the following:</strong></td>
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<tr>
<td>Cognitive impairment does not Intensify or improve over time</td>
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<tr>
<td>Cognitive impairment with stopped progression</td>
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<td>Younger patient unlikely to show associated AD (especially early onset familial Alzheimer disease)</td>
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<tr>
<td><strong>C. MRI or anatomical pathology study revealing a cerebrovascular lesion affecting a strategic area or network for cognitive functions</strong></td>
</tr>
<tr>
<td>Localisation in border zone (in dominant hemisphere or bilaterally): superior frontal (watershed between the ACA and the MCA), parieto-occipital (watershed between the MCA and PCA), and internal (between the ACA, MCA, PCA, and the area supplied by the recurrent artery of Heubner, lentictulostrate, and anterior choroid arteries)</td>
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<tr>
<td>Angular gyrus (of dominant hemisphere or bilaterally)</td>
</tr>
<tr>
<td>Orbitofrontal and cingulate (bilateral ACA)</td>
</tr>
<tr>
<td>Anterior thalamus, dorsal-paramedian or dorsomedial (unilateral or bilateral thalamic perforating artery)</td>
</tr>
<tr>
<td>Medial temporal, hippocampus (bilateral PCA)</td>
</tr>
<tr>
<td>Caudate nucleus (lentictulostrate branch of the MCA in the dominant hemisphere)</td>
</tr>
<tr>
<td>Other key areas of the grey or white matter in the dominant hemisphere or bilaterally: anterior part of the putamen, anterior limb of internal capsule, genu of the internal capsule</td>
</tr>
<tr>
<td><strong>D. Identification of a genetic biomarker for cerebrovascular disease that causes dementia</strong></td>
</tr>
<tr>
<td>Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: mutation in the NOTCH3 gene located on chromosome 19</td>
</tr>
<tr>
<td>Hereditary cerebral haemorrhage with amyloidosis: mutation in the gene for amyloid precursor proteins</td>
</tr>
<tr>
<td><strong>Exclusion criteria</strong></td>
</tr>
<tr>
<td>Absence of cerebrovascular lesion in multimodal MRI study</td>
</tr>
<tr>
<td>Evidence of a disorder severe enough to cause cognitive impairment: major depression, toxic-metabolic disorder (requires specific studies), intracranial neoplasia, subdural haematoma, chronic hydrocephalus, intracranial infection</td>
</tr>
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</table>

Table 1. Useful criteria for diagnosing Vascular Cognitive Impairment.
1.4.1 Clinical evaluation

VCI remains a clinical diagnosis (Figure 5). The harmonisation standards recommend a detailed account of the complaints by the patient and informant on different cognitive domains, such as memory, speed of thinking or acting, mood, and functional status. Information about vascular risk factors, such as hypertension, hyperlipidaemia, diabetes, alcohol or tobacco use, and physical activity, should be sought. History taking should also include checks for atrial fibrillation, coronary artery bypass surgery, angioplasty and stenting, angina, congestive heart failure, peripheral vascular disease, transient ischaemic attacks or strokes, and endarterectomy. Other elements, including hypercoagulable states, migraine, and depression, might also be helpful. Finally, the history should also include details of the acuity of onset, progression, and occurrence of urinary incontinence, gait disturbance, and motor deficit.

Physical examination should focus on blood pressure, pulse, body mass index, waist circumference, and examination of the cardiovascular system for evidence of arrhythmias or peripheral vascular disease. Neurological examination should note focal neurological signs and assess gait initiation and speed.

Figure 5. Relevant medical history in the clinical assessment of vascular cognitive impairment.
1.4.2 Cognitive assessment

The pattern of cognitive deficits in patients with VCI varies considerably. Single strategic infarcts can lead to specific psychopathological profiles, whereas subcortical lesions are often associated with abnormalities of information processing speed, executive function, and emotional lability. This cluster of features (the “subcortical syndrome”) can also result from cortical lesions.60

Standard cognitive assessments, such as the Mini Mental State Examination (MMSE), are usually insensitive to these abnormalities,61 even in patients followed up for one year after a confirmed stroke.62 Cognitive tests can usefully differentiate between VaD and AD, but the inclusion of mixed dementia and VCI–ND within the VCI construct affects the specificity of the diagnosis. In patients who have had a stroke, impairment in global cognition and attention is more closely associated with worse functional outcomes than are executive dysfunction and isolated memory deficits.63 Three subsets of the Montreal Cognitive Assessment,64 a five-word immediate and delayed recall test, a six-item orientation task, and a phonemic fluency test, were selected for the 5 minute cognitive assessment, with the recommendation that the entire assessment, the trail-making test,65 or a semantic fluency test were also administered (Table 2).

![Table 2. Neuropsychological findings of vascular-related cognitive impairment.66](image)

*AD = Alzheimer’s disease; MMSE = Mini Mental State Examination; MoCA = Montreal Cognitive Assessment.*
1.4.3 Neuroimaging

Neuroimaging studies require a clinical correlation in the assessment of VCI patients. Indeed, VCI shows no pathognomonic neuroimaging features. Infarct location often does not correlate with the cognitive profile, and neuroimaging cannot reliably confirm the chronology of lesions or disentangle the contribution of neurodegenerative and ischemic processes to the clinical presentation. For a diagnosis of probable VaD, the NINDS–AIREN and the California criteria require evidence of cerebrovascular disease at neuroimaging and that infarcts and leukoaraiosis fit specific criteria with regard to their location and the amount of white matter affected. These criteria have proved to be insensitive in practice, however, which makes their use problematic for the routine clinical diagnosis of VCI. Increasing recognition of the importance of incomplete infarction and hypoperfusion inform the understanding that VCI might be present in the absence of neuroimaging abnormalities (Figure 6).

Although Computed Tomography is widely available and in many parts of the world is a pragmatic choice for patients, it is less sensitive than MRI. Newer MRI-based neuroimaging techniques continue to advance our knowledge of the pathophysiology of VCI. The results of DTI-MRI studies have enhanced our understanding of lesion location in relation to clinical presentation, and suggest that such white matter changes are not necessarily ischaemic. In general, WMLs are associated with loss of neuronal integrity, which leads to higher mean diffusivity and lower fractional anisotropy. DTI-MRI technique might eventually enable measurement of the number of fibres per tract and the functional areas connected by white matter.

The importance of atrophy in VCI is increasingly recognised and might show a stronger association with disease progression and depressive symptoms than WMLs. Medial temporal atrophy is emerging as an important correlate of cognitive dysfunction even in VCI. Likewise, even small amounts of white matter abnormalities are associated with significant memory and language impairments.
Figure 6. Vascular imaging changes on MRI.\textsuperscript{72}
(A) Arrows indicate extensive (>25\%) white matter lesions (Fluid-attenuated Inversion Recovery – FLAIR – image);
(B) Arrow indicates large cortical infarction (FLAIR image);
(C) Arrow indicates microbleed (T2*-weighted image);
(D) Arrows indicate multiple lacunar infarcts (T1-weighted image).
1.4.4 Neuropathology

Many researchers believe that neuropathology is the gold standard for the diagnosis of VCI, despite a considerable body of evidence that undermines it as a test of 100% sensitivity and specificity.\textsuperscript{3,4,27,69} Other researchers have accepted that neuropathology is not ideal, and propose neuroimaging as an alternative gold standard. Anyway, there is currently no single standard for neuropathology in relation to VCI, and neuropathological criteria need to be understood in relation to clinical presentation. Moreover, there is currently no neuropathological threshold of cerebrovascular disease reliably distinguishes between no cognitive impairment and dementia.\textsuperscript{3}

The types of neuropathological lesions associated with VCI include large and small vessel infarcts, white matter changes, haemorrhage, gliosis, and, in mixed dementia, the neuropathological changes of AD\textsuperscript{75} (Figure 7). In a population-based neuropathological study, in which 13\% of participants had pure VaD without major evidence of AD, the requirement that dementia follows a known stroke resulted in high specificity but low sensitivity in autopsy-verified cases.\textsuperscript{76}

Figure 7. Some of the major pathological changes underlying vascular dementia.\textsuperscript{66} A) Ischemic infarct. Note the wedge-shape border of the infarct (arrows). Both white and gray matter are involved. Nissl staining. B) Lacunar infarct characterized by an irregular cavity and a central blood vessel surrounded by a rim of gliotic, rarefied brain tissue (arrow). Hematoxylin and Eosin stain (H&E). C) Histopathological counterpart of a white matter lesion detected by MRI in a 62 years-old male. Note the regions of myelin pallor (**) and an enlarged perivascular space (arrow). Kluever-Barrera stain. D) Cerebral amyloid angiopathy (CAA). The Amyloid β-deposition in the wall of a cortical artery is colored in brown (arrow). In this case, there is a microbleed around this artery (note the anuclear red blood cells). Immunostain with an antibody against Ab17-24 (4G8; Covance). E) Small vessel disease. Two white matter arteries exhibit fibrosis and hyalinization of wall (arrows). These lesions are also referred to as arteriolosclerosis, arteriohyalinosis or lipohyalinosis (arrow). H&E. The calibration bars correspond to 100 μm.
1.4.5 Biomarkers

Biomarkers are indicators that might or might not be in the causal pathway of the disease but correlate with the disease process and its progress. Candidate biomarkers should aid early detection, discriminate the neuropathology, estimate the prognosis, and monitor disease progression or treatment response. The search for biomarkers in VCI is hampered by the clinical and pathological heterogeneity and the presence of pathology, such as white matter hyperintensities, even in healthy individuals. Furthermore, the high prevalence of mixed dementia within VCI poses challenges for the discriminative abilities of biomarkers. Indeed, to date the results of studies that compare the phenotypes associated with the presence or absence of biomarkers are inconsistent.

Markers in the cerebro-spinal fluid (CSF) have shown more discriminative ability in patients with VCI than have serological biomarkers. The CSF-albumin index is a measure of blood-brain barrier integrity, which is compromised in many types of dementia, particularly in subcortical vessel disease. Matrix metalloproteinases (MMP) attack tight junctions in the cerebral vessels, thereby opening the blood–brain barrier and contributing to demyelination. Of 26 known metalloproteinases, MMP-2 and MMP-9 are the most studied: MMP-2 is constitutively expressed, whereas MMP-9 is associated with inflammation and has variable specificity in VCI compared with AD. The light neurofilament subunit of normal myelin has been found in higher concentrations in the CSF of people with subcortical vessel disease compared with those with AD, and might be a marker of demyelination. CSF tau and phospho-tau concentrations are elevated in non-vascular dementias and therefore might be useful as negative biomarkers.

Nevertheless, biomarkers cannot take the place of clinical diagnosis although they can inform on the relationship between risk factors and disease progression. However, their contribution to our understanding requires considerable empirical data and standardisation of collection, storage, and measurement techniques.
1.5 Disease progression and management

There is over-reliance on objective measures considered as useful and sensitive indexes for the evaluation and prediction of progression of VCI.\textsuperscript{44,45} Notwithstanding, the unifying empirical theme is that the subtype VCI–ND is an at-risk state for future dementia and should be the target of prevention models. Moreover, the progression of dementia in patients with VCI is neither linear nor unidirectional. There is still much plasticity in VCI–ND, and careful evaluation of patients with this condition who do not progress to dementia or who revert to no cognitive impairment might enhance our understanding of how exposures interact with neuropathology.

Although some cross-sectional evidences suggest that VCI lies on a spectrum between normal cognition and VaD,\textsuperscript{39,84} little is known about the progression of VCI–ND. It is true that VCI-ND does not always progress to dementia, and data from epidemiological and clinical series indicate that improvement is even possible.\textsuperscript{85} Moreover, in clinic-based longitudinal studies, VCI–ND showed less progression compared with other VCI subtypes,\textsuperscript{52} which might also be the case for post-stroke VCI–ND.\textsuperscript{86}

In a population-based study, progression from VCI–ND to incident dementia was not associated with a particular neuropsychological profile but occurred at the highest rate in those patients with both memory and functional impairment at baseline.\textsuperscript{37} There was no difference in the rates of progression between those patients with predominant functional impairment and those patients with predominant memory impairment, and a half of the patients with VCI–ND were later diagnosed with AD. However, in the same study, 52% of people with VCI–ND died, and 42% developed dementia within 5 years.\textsuperscript{17} VCI–ND also carries a high risk of PSD.\textsuperscript{18} Figure 8 illustrates the course of patients with cerebrovascular disease, from the “brain at risk” stage to an overt dementia or different origin (PSD, VaD, AD, mixed).

Similar to VCI–ND, considerable variability is seen in the progression of PSD; up to a third of people change diagnostic category (no cognitive impairment; cognitive impairment without
dementia; overt dementia) within one year after a stroke. Age, previous cognitive impairment, polypharmacy, hypotension during acute stroke, and depression are risk factors for progression and functional decline. In general, infarct location is less important in predicting cognitive decline than medial temporal atrophy, and medial temporal atrophy is more important than are WMLs to predict dementia after stroke.

Figure 8. Course of cerebrovascular disease, from the “brain at risk” stage to a picture of overt dementia. AD = Alzheimer’s disease; CVD = cerebral vascular disease; MCI = Mild Cognitive Impairment; PSD = post-stroke dementia; VaD = Vascular Dementia; VCI = Vascular Cognitive Impairment.

The available data on vascular risk factors for the progression of VCI show surprisingly equivocal support for primary and secondary prevention. Pre-stroke hypertension is associated with post-stroke dementia, treatment of hypertension with perindopril + indapamide reduced the dementia and cognitive decline associated with recurrent stroke. However, a recent Cochrane review found no convincing evidence that lowering of blood pressure prevents the development of dementia or cognitive impairment in patients with hypertension without prior cerebrovascular
disease, although methodological difficulties cast doubt on the true effects.\textsuperscript{92} Diabetes has been associated with risk of stroke, cognitive decline, and dementia in several population studies.\textsuperscript{93-95} Hypercholesterolemia is a well recognised risk factor for stroke, and the recent Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) study found a reduction in recurrent stroke in those patients with a history of stroke or transient ischemic attack who were treated with high doses of statins.\textsuperscript{96}

To date, however, apart from controlling vascular risk factors, the overall effect of treatment on patients with VaD is modest. There is variable but generally limited enthusiasm for the drugs used to treat AD; the results of a meta-analysis concluded that only small benefits of uncertain clinical meaningfulness were available from cholinesterase inhibitors or memantine.\textsuperscript{97} Even so, some patients (who had probably a mixed dementia) experience clinical benefit, although the responders cannot be easily identified. Despite reasons to believe that cholinesterase inhibitors might benefit people without concomitant AD,\textsuperscript{98} the most persuasive studies, which assembled patients with VaD into groups,\textsuperscript{90} had small effect sizes, no dose-response effect, limited evidence for convergence of treatment effects within and across trials, and no clear translation into responses that physicians might look for in routine clinical care.\textsuperscript{99}

Neuroimaging, particularly the DTI-MRI technique, gives some insight into disease mechanisms, although their relationship with clinical effects is not always clear. In a secondary analysis of the results of the Study on Cognition and Prognosis in the Elderly, patients treated with the angiotensin receptor blocker candesartan showed reduced risk of WMLs.\textsuperscript{100} Similarly, physical activity was not associated with a slower progression of WMLs seen on MRI,\textsuperscript{101} and a relationship with whole brain volume is not clear from the published literature. Perhaps physical activity prevents VCI through enhancement of cognitive reserve,\textsuperscript{101} which refers to the ability to maintain cognitive function despite increasing neuropathological burden.
CHAPTER 2

“Vascular Depression”, the hypothesis linking vascular disease and late-life depression

2.1 Definition

The “Vascular Depression” (VD) hypothesis proposed that cerebrovascular disease may predispose, precipitate, or perpetuate late-life depression (LLD). First, Alexopoulos and colleagues proposed a working definition based on the presence of vascular risk factors. The clinical presentation of VD was characterized by cognitive deficits, psychomotor retardation, lack of insight, and disability disproportional to the depression severity. Investigators subsequently focused on the cognitive dysfunction occurring in LLD and its relationship to response to antidepressants. Second, Krishnan and colleagues proposed a MRI-based definition requiring evidence of vascular changes on neuroimaging, referred to as MRI hyperintensities (WMLs). Both definitions are relevant as subsequent work demonstrated the validity of a VD diagnostic subtype characterized by peculiar MRI findings and involvement of executive functions.

2.2 The role of white matter lesions: “MRI-defined Vascular Depression”

The hallmark of MRI-defined VD is the presence of WMLs identified as white matter hyperintensities on T2-weighted or fluid attenuated inversion recovery (FLAIR) MRI. WMLs are associated with advanced age and cerebrovascular risk factors, including diabetes, cardiac disease, and hypertension. Vascular dysregulation contributes to WML development as white matter is sensitive to transient ischemia and many larger WMLs are ischemic in origin.
Hypertension and blood pressure variability are associated with LLD\textsuperscript{117-119} and also contribute to WML development,\textsuperscript{120,121} particularly when accompanied by impaired cerebral vasomotor reactivity and altered autoregulatory processes.\textsuperscript{122,123} Such deficits reduce cerebral blood flow (CBF) and may lead to WMLs.\textsuperscript{124,125} LLD is consistently associated with greater WML severity\textsuperscript{126-129} and volumes.\textsuperscript{111,130-132} Compared with early-onset depression, individuals with a later onset (e.g., after 50 year old) exhibit greater WML severity\textsuperscript{105,119,132,133-137} and cognitive impairment.\textsuperscript{138-139}

However, VD as a potential diagnostic entity may not be limited to late-onset patients. Individuals with an earlier onset are at increased vascular risk as precedent depression is associated with increased risk of vascular disease and stroke.\textsuperscript{140-144} Depression in early and mid-life may promote inflammation\textsuperscript{145-147} or epigenetic modifications of genes related to vascular homeostasis.\textsuperscript{148} Thus some individuals with early onset depression may be prone to VD later in life.

WMLs location is also important. In non-depressed samples, periventricular WMLs are more closely associated with cognitive impairment than are deep WMLs.\textsuperscript{32,149-150} It is possible this is due to anatomical differences, as the periventricular region has a high density of long associating fibers with cortical-subcortical connections, while subcortical deep white matter has a high density of shorter U-fibers connecting adjacent cortical regions.\textsuperscript{150,151} Others have localized WMLs associated with depression to the frontal\textsuperscript{152-155} and temporal lobe.\textsuperscript{156} More recently, several groups reported that LLD is associated with greater WML severity in specific white matter fiber tracts including the cingulum bundle, uncinate fasciculus, and superior longitudinal fasciculus.\textsuperscript{157-159}

### 2.3 Neuropathological evidences

Studies using both neuroimaging and pathological techniques demonstrate that WMLs represent a wide range of pathological processes, including perivascular demyelination,
arteriosclerosis, ischemia, gliosis, or partial loss of myelin and axons.\textsuperscript{116,160,161} Generally, confluent deep WML but not periventricular appear to be related to ischemic processes.\textsuperscript{162}

There are also regional differences in WML etiology. Deep WML and punctuate lesions of depressed older adults are most likely to have ischemic origins. In LLD ischemic lesions are also more likely to occur in the dorsolateral prefrontal cortex (DLPFC), instead of the anterior cingulate cortex (ACC) or occipital lobe.\textsuperscript{115} Similarly, depressed elders exhibit increased expression of cellular adhesion molecules (CAMs) in the DLPFC, but not the ACC or occipital lobe.\textsuperscript{163-165} CAMs are inflammatory markers whose expression is increased by ischemia, supporting a role for ischemia in LLD and highlighting the relationship between vascular and inflammatory processes. Although ischemic pathology is thus consistently localized to the DLPFC, other frontal regions may also be involved. Depressed elders exhibit decreased density of pyramidal neurons in the orbitofrontal cortex, which may be the result of vascular processes\textsuperscript{166} in arterioles and medium arteries\textsuperscript{167} or alterations in astrocyte-associated immune function.\textsuperscript{168}

Neuropathological studies also demonstrate that LLD can develop in the absence of significant vascular abnormalities. In a study of late-onset depressed elders, depression was not related to either lacunes or microvascular lesions.\textsuperscript{169} Similarly, in a population-based study where depression was ascertained in a pre-mortem diagnostic interview, depression was associated neither with cerebrovascular nor AD pathology.\textsuperscript{170} However, depression was associated with the Lewy bodies,\textsuperscript{170,171} highlighting the heterogeneity of neuropathologies that present as depression.

2.4 “Depression-executive dysfunction syndrome” and other cognitive deficits

A “depression-executive dysfunction syndrome” was conceptualized as the clinical expression of frontal network impairment caused by vascular and other aging related factors.\textsuperscript{104}
Accordingly, this syndrome describes depressed patients with vascular disease and evidence of impairment in networks related to mood and executive function.\textsuperscript{172}

Executive function is a frontally mediated domain that encompasses cognitive processes including selective attention, response inhibition, and performance monitoring.\textsuperscript{173-175} It is clinically expressed as difficulty with planning, sequencing, organizing, and abstracting. These deficits are common in depression,\textsuperscript{176-179} particularly in LLD.\textsuperscript{139,178,180} When compared with depressed elders without executive dysfunction, patients with depression-executive dysfunction exhibit reduced fluency, impaired visual naming, suspiciousness, anhedonia, psychomotor retardation, and significant disability.\textsuperscript{181-183} Importantly, studies across eight different samples identified executive dysfunction as a predictor of poor antidepressant response.\textsuperscript{184-191}

Individuals with LLD exhibit also deficit across other cognitive domains, including episodic memory, working memory, visuo-spatial ability, and processing speed.\textsuperscript{176,179,192-197} Processing speed deficits may influence other cognitive deficits\textsuperscript{195-197} and mediate in part executive task performance.\textsuperscript{198} Although cognitive performance improves with successful antidepressant treatment, such deficits can persist.\textsuperscript{192,199-201}

Greater WML burden is associated with executive dysfunction, perseveration, and slowed processing speed\textsuperscript{113,158,202-205} but also episodic and visuo-spatial memory deficits.\textsuperscript{118,150,194,206} These cross-sectional observations are supported by longitudinal studies demonstrating that progression of WML severity parallels cognitive decline.\textsuperscript{207-209}

2.5 Course of Vascular Depression

2.5.1 Cognitive dysfunction and antidepressant outcomes

Multiple studies demonstrate that executive dysfunction predicts poor acute response of LLD to antidepressants.\textsuperscript{191,210} Executive dysfunction is also associated with high relapse and
recurrence rates, although not all studies found this association. Much of this work used the Mattis Dementia Rating Scale Initiation-Perseveration (I/P) subtest, which yields a composite score of several executive skills. Notably, a recent meta-analysis examined the relationship between antidepressant response and performance across multiple executive tests, including the I/P subtest, the Wisconsin Card Sort Test, the Stroop Color-Word test, and others. The authors concluded that the I/P subtest was the only test providing reliable discrimination between antidepressant responders and non-responders. Further analysis of I/P subtest components showed that semantic strategy while performing the I/P verbal fluency task explained most of the variance in predicting remission. Effective semantic strategy appears to be associated with treatment response regardless of the probing task; during verbal learning task, it was also associated with high LLD remission rate. These observations suggest that preserved semantic organization, rather than fluency or verbal learning, is critical for remission during antidepressant treatment. However, impaired response inhibition is another aspect of executive dysfunction that influences antidepressant response.

Other cognitive deficits can influence outcomes. Poorer performance on tests of episodic memory, language processing, and processing speed are associated with poorer acute antidepressant response and may predict poorer long-term course of depression.

2.5.2 White matter lesions as a predictor of antidepressant outcomes

Most studies used cross-sectional WML measures to predict outcome. However, WML is a progressive rather than a static process, and the rate of change may be a more important predictor. The few studies in LLD cohorts examining this question found that greater increases in WML volume over two- and four-year intervals are associated with non-remission or relapse. Some have examined both WML severity and cognitive dysfunction as predictors of outcome. In these studies, cognitive measures are generally a better predictor of antidepressant response than are WML measures. The largest published study examining a 12-week response to sertraline found that
poorer response was independently associated with greater WML severity and poorer performance in cognitive domains of episodic memory, language processing, processing speed and executive function. However, in models controlling for baseline depression severity, WML severity was no longer associated with response. Smaller studies have found similar patterns, wherein initial associations between WML severity and response were not statistically significant in models incorporating cognitive measures.

Other measures of white matter integrity are associated with antidepressant response. In voxel-wide analysis, microstructural white matter abnormalities (low fractional anisotropy – FA) in multiple frontolimbic brain areas, including the rostral and dorsal anterior cingulate, DLPFC, genu of the corpus callosum, hippocampus, and posterior cingulated, were associated with non-remission. In region of interest analyses, low FA in the corpus callosum, left superior corona radiata, and right inferior longitudinal fasciculum was associated with lower remission rates. In contrast, others found that higher prefrontal white matter FA was associated with a failure to remit to antidepressants, a finding that parallels reports in depression of increased functional connectivity to a dorsal nexus. Moreover, depressed elderly carriers of 5-HTTLPR polymorphism short allele had lower FA than long allele homozygotes in frontolimbic areas, including the anterior cingulate, posterior cingulate, dorsolateral and medial prefrontal regions. Notably, such differences in FA can be caused by several pathologies, not all of which are vascular in nature.

2.6 Vascular risk genes in late-life depression

There is a wide literature examining genetic influences in LLD, including reports examining polymorphisms in Brain-derived Neurotrophic Factor (BDNF), Apolipoprotein E (ApoE), and serotonin transporter genes, amongst others. Within this field, there are an increasing number of reports associating LLD with genetic polymorphisms increasing vascular risk.
One example of this literature includes studies examining genetic variation of the renin-angiotensin system (RAS). Beyond influencing blood pressure, RAS polymorphisms may also increase the risk of depression via modulation of monoamines,\(^2^{29}\) or contributing to hypothalamic-pituitary-adrenal axis dysregulation.\(^2^{30}\) RAS variation is further related to differences in brain structure and function. Several studies in older adults have now associated variants in angiotensin converting enzyme (ACE) and angiotensin receptor (AGTR) blocker with altered cerebral fronto-temporal structure\(^2^{31-234}\) and abnormal default mode network activity.\(^2^{35}\) AGTR-1 variants are associated with greater WMH progression, particularly in men,\(^2^{36}\) and this effect may be localized to specific tracts.\(^1^{57}\) RAS polymorphisms may also influence antidepressant outcomes. In a general adult population, the ACE-D variant and the AGTR-1 \(C1166\) allele are associated with better acute antidepressant response.\(^2^{37,238}\) However, this contrasts with a study of LLD, wherein AGTR-1 \(C1166\) allele homozygous individuals exhibited a poorer antidepressant response over a longer period.\(^2^{39}\) Such age differences could be important, reflecting stress reactivity in earlier life and allostatic effects of an overly active RAS later in life.\(^1^{07}\)

### 2.7 Mechanistic hypotheses

According to a model by Alexopoulos,\(^1^{02}\) the clinical expression of LLD is mediated by altered brain activity in cognitive and affective circuits, characterized as hypometabolism of dorsal cortical regions and hypermetabolism of ventral limbic structures. The impact of these neurobiological contributors can be increased by vulnerability factors originating from pre-existing differences in mood circuitry. Such etiological factors may lead to depression only after crossing a certain threshold above which they acquire a dose effect relationship. In this conceptualization, single or multiple potential etiological factors may reach an initial severity threshold and contribute to LLD vulnerability. When etiological factors further increase in severity and cross a second
threshold, they may have a greater and direct effect on mood circuit function, leading to affective and cognitive symptomatology (Figure 9). Of course, instead of threshold effects, such relationships could be cumulative following linear or curvilinear patterns. This threshold model can account for multifactorial contributions to LLD.107

Figure 9. Proposed mechanisms underlying vascular depression.
MID = multi-infarct dementia; SIVD = subcortical ischemic vascular disease.
2.7.1 The “Disconnection hypothesis”

Building on the concept of “Disconnection syndromes,” ischemia and WMLs may contribute to depression by disrupting neural connections among regions regulating mood-affect and cognition. In this model, global cerebral WML severity is less determinant to LLD than is focal damage to specific fiber tracts and neural circuits. Such focal damage would adversely affect the tract structural and functional connectivity. In turn, this state adversely affects the function of connected regions at rest and during cognitive tasks, thus contributing to neural circuitry alterations that mediate clinical symptoms and influence antidepressant response.

This view is supported by studies examining WML location. As stated, LLD is associated with greater WML severity in the cingulum bundle, uncinate fasciculus, and superior longitudinal fasciculus. Additionally, greater WML severity in the uncinate and superior longitudinal fasciculi is associated with executive dysfunction and greater depression severity. These macrostructural MRI findings are supported by DTI studies examining white matter microstructure. DTI changes reflect various pathologies leading to decreased myelin integrity, including demyelination secondary to cerebrovascular and inflammatory changes. WMLs occurring in fiber tracts are also associated with DTI changes.

2.7.2 The “Inflammation hypothesis”

Aging- and disease-related processes promote pro-inflammatory states in older individuals. Further, immune system activation can be a characteristic of depression and precipitate depressive symptoms. Alexopoulos and Morimoto recently proposed that immune dysregulation may promote the development of affective and cognitive symptoms in LLD.

Pro-inflammatory cytokines affect monoamine neurotransmitter pathways, including indoleamine 2,3-dioxygenase up-regulation and kynurenine pathway activation. This results in decreased tryptophan and serotonin and increased synthesis of detrimental tryptophan catabolites that promote hippocampal damage and apoptosis. Cytokines, including interleukine (IL)-1β,
also reduce extracellular serotonin levels by activating the serotonin transporter. Additionally, proinflammatory cytokines disrupt glucocorticoid receptor function and reduce neurotrophic support (Figure 10).
2.7.3 The “Hypoperfusion hypothesis”

Vascular dysregulation is common in LLD, and CBF reduction can impair regional brain function, contributing to affective and cognitive symptoms. Blood flow to the brain is influenced by systemic hemodynamic and cerebrovascular autoregulation, with cerebral arteries contracting or dilating as arterial pressure changes. However, these processes are impaired in the context of vascular disease: hypertension, diabetes, and atherosclerosis lead to vascular wall hypertrophy, reduced arterial lumen diameter, reduced arterial distensibility, and endothelial cell dysfunction. Such vascular changes, including increased intima-media thickness, increased arterial stiffness, and endothelial dysfunction, are pronounced in LLD populations, and endothelial function may be particularly poor in antidepressant non-responders.

Perfusion deficits do not need to cause ischemia in order to influence brain function. Reduced CBF impairs protein synthesis crucial for cognitive processing and for maintaining the integrity of cortical functional maps. Thus mild CBF reduction may impair cognitive and affective processes, while greater CBF reduction in the context of autoregulatory deficits may cause ischemic injury. The subcortical white matter is particularly sensitive to these changes because it is supplied by terminal arterioles with limited collateral flow and so susceptible to infarction due to impaired autoregulation. Greater WML severity may be a marker of broader deficits in perfusion and autoregulation as individuals with greater WML severity exhibit reduced CBF in both white and gray matter regions. Depressed elders with greater WMLs severity also exhibit decreased perfusion in the cingulate gyrus, a region involved in cognitive and affective processing.

Advanced age is itself associated with decreased fronto-temporal CBF, an effect mediated by vascular risk factors. In LLD, perfusion deficits are more severe, particularly in the medial and lateral prefrontal cortex and temporal structures. Others report no change of CBF in antidepressant non-responders, suggesting that persistently reduced regional CBF may be a biomarker of non-response.
CHAPTER 3
Dementia through the looking glass of Transcranial Magnetic Stimulation

3.1 Introduction

Clinically introduced approximately 30 years ago as a diagnostic tool to study the central motor pathways, today Transcranial Magnetic Stimulation (TMS) goes well beyond the simple assessment of the cortical-spinal tract, providing novel insights into the pathophysiology of the neural circuitry that underlies several neurological and psychiatric diseases. TMS may give information about the excitability of the human brain cortex, the conduction along cortical-spinal tract, the functional integrity of intracortical neuronal structures and callosal fibers. TMS has also a strong talent to unveil motor system impairments in their pre-clinical phase. Moreover, integrated approaches using electrophysiological techniques together with structural and functional neuroimaging have allowed the study of connectivity across motor and non-motor areas. By evaluating the effects of agonists or antagonists for specific neurotransmitters, it has been shown that TMS can selectively and non-invasively explore the function of glutamatergic, gamma-aminobutyric acid (GABA)-ergic and cholinergic cortical circuits. Although the physiological abnormalities revealed by TMS are not disease-specific, there may be specific neurophysiological changes that co-segregate in each dementing illness, consistent with the involvement of distinct neurobiological substrates in the pathogenesis of each disease.

In the last years, there has been a significant growth in the number of publications exploiting TMS techniques to aid the diagnostic approach in patients with primary dementia. Although not always clinically evident, the involvement of motor areas in dementia has been shown by clinical, neuropathological and neuroimaging studies. Changes in motor areas may be secondary to the direct
structural alterations caused by the disease process but, more often, they are the consequence of indirect remodeling mechanisms.\textsuperscript{307} In this context, increasing evidences support the hypothesis that phenomena of brain plasticity are involved in different kind of dementia, related to functional and structural components, each entailing a number of cellular mechanisms operating at different time scales, synaptic loci, and developmental phases within an extremely complex framework.\textsuperscript{308}

However, the exact relationship between brain plasticity and excitability of cortical areas and their connections is still unclear.

### 3.2 Cortical excitability and plasticity in Vascular Cognitive Impairment

It is well known that motor cortex hyperexcitability is a relatively stable electrophysiological feature of both AD and VaD.\textsuperscript{305} This has been considered part of a plastic compensatory response to neuronal loss, supporting the concept of dementia as a dynamic condition and changes of specific TMS parameters as indexes of neural plasticity.\textsuperscript{309} The enhanced cortical plasticity might counteract cognitive decline and shed light on the reasons underlying decline or preservation of cognitive functions. This hypothesis has been demonstrated by means of TMS mapping technique in AD patients who showed a frontal and medial shift of the motor cortical output’s center of gravity (CoG), suggesting functional reorganization compensating for disease progression, at least in the early stages.\textsuperscript{310} Similarly, a clear pattern of global hyperexcitability has been observed in subcortical ischemic VaD,\textsuperscript{311} which is a homogeneous subtype of VaD of particular interest because of the relatively slow progression often making difficult the differentiation from AD.\textsuperscript{5}

However, although a cortical reorganization similar to that occurring in AD was hypothesized, it has not been demonstrated yet. Based on this theoretical background, Guerra et al. proved this hypothesis with a TMS mapping study exploring the relationship between excitability
and plasticity in subcortical ischemic VaD. Although obtained from small sample size, they found that motor cortex had enhanced excitability in both AD and subcortical ischemic VaD patients with respect to controls, and it was plastically rearranged although with a slightly lesser CoG frontal shift of subcortical ischemic VaD compared to AD. Moreover, a significant direct correlation between parameters associated to cortical excitability and those associated to synaptic plasticity was evident, suggesting the existence of mechanisms that partially overlap and probably act in the same neurophysiological way although they are, at least in principle, different both in localization (subcortical vs cortical) and in origin (vascular vs degenerative). The authors conclude that AD and VaD can share a common neurophysiological platform, related to the progressive neuronal loss within motor areas and to the ischemic disconnection, respectively. This alteration finally could promote a functional rearrangement that could allow the preservation of motor programming and execution despite disease progression. Nevertheless, as stated before, vascular lesions, even in the absence of any motor deficit, give a significant contribution to the development and progression of degenerative dementia, so that it cannot be excluded that some of those patients had rather a mixed form of dementia. With this respect, it is worth to highlight that TMS is not currently able to clearly distinguish VaD from AD based only on their neurophysiological profiles or to disentangle the vascular and degenerative burden.

A crucial issue is whether it is possible to identify non-demented patients at risk for progression. A recent study on non-demented elderly patients with subcortical vascular disease has suggested that the ischemic lesion of prefrontal subcortical loops implicated in cognition and mood-affection regulation might result in functional changes of intracortical excitatory neuronal circuits, specifically in an enhanced intracortical facilitation. After a 2-year follow-up, the same patients showed an increase of global cortical excitability along with a significant worsening of frontal lobe abilities, but without substantial functional impairment. The question here is whether these changes might represent a marker of “brain at risk” for dementia. It was hypothesized that the
critical point at which the excitability becomes abnormal might discriminate patients advancing to VaD from those with no conversion.\(^{316}\)

Although it is not possible at the moment to determine whether these findings are reflected in changes of decision-making in the care of patients, an integrated approach utilizing modern neurophysiological techniques, including high-density electroencephalography (EEG), event-related potentials and TMS, together with biological markers and advanced Neuroimaging, is promising for a large-scale, affordable and non-invasive intercept of at-risk population. This approach might also guarantee the possibility of studying drug-induced changes in the electrical properties of the human cortex, probing models of brain connectivity and testing neuromodulatory techniques as therapeutic tools for cognitive rehabilitation.\(^{314}\)

### 3.3 TMS: basic principles and derived parameters

#### 3.3.1 Single-pulse TMS measures

A single TMS pulse applied over the primary motor cortex (M1) elicits a motor evoked potential (MEP) in the contralateral target muscles\(^{317}\) and may provide a functional assessment of the cortical-spinal conduction. In particular, the latency of MEP and the central motor conduction time (CMCT), defined as the latency difference between the MEPs induced by motor cortex stimulation and those evoked by motor root stimulation, are measures of the integrity of the cortical-spinal pathways, while the amplitude of the MEP is an aggregate measure of the excitation state of output cells in the motor cortex\(^{304}\) (Figure 11).

Resting motor threshold (rMT) is defined as the minimum stimulus intensity which is required to produce a MEP amplitude >50 \(\mu\)V in at least 5 of 10 consecutive trials at rest, and it is believed to provide information about a central core of neurons in the muscle representation of the M1.\(^{318}\) Resting MT is increased by drugs that block voltage-gated sodium channels,\(^{319}\) whereas it is
not affected by drugs with effects on GABA,\textsuperscript{320} and is lowered by drugs increasing glutamatergic transmission not mediated by N-methyl-D-aspartate (NMDA),\textsuperscript{321} suggesting that rMT reflects both neuronal membrane excitability and non-NMDA receptor glutamatergic neurotransmission. Resting MT is typically increased if a significant portion of the cortical-spinal tract is damaged, while it decreases in situations of hyperexcitable cortical-spinal system.\textsuperscript{304}

Figure 11. Basic principle and measures of single-pulse Transcranial Magnetic Stimulation (TMS). Left side (top): example of motor evoked potential (blue arrow: latency; red arrow: amplitude; green arrow: duration). Left side (bottom): example of cortical silent period. Right side: schematic registration of cortical motor response and peripheral component; method to calculate the central motor conduction time is illustrated.\textsuperscript{304}

When the single magnetic pulse is delivered during a voluntary contraction of the contralateral target muscle, the MEP is followed by a suppression of the electromyographic (EMG) activity (Figure 11). This phenomenon, called contralateral cortical silent period (CSP), is indeed a measure of the suppression of the pyramidal output at a cortical level, probably due to the activation, after an early spinal phase (its first 50-75 ms), of inhibitory cortical interneurons mainly mediated by GABA-B transmission.\textsuperscript{322,323}
3.3.2 Paired-pulse TMS measures

TMS may also be used to investigate the intracortical inhibitory and facilitatory mechanisms within the M1 and other non-motor areas. Some of these TMS techniques involve paired stimulation, in which a conditioning subthreshold stimulus precedes a suprathreshold test stimulus by a programmable interstimulus interval (ISI). By this way, paired-stimuli TMS has revealed the existence of a complex of inhibitory and excitatory intracortical interactions within the human brain, mainly depending on the ISI employed. At short ISIs (1-5 ms), the conditioning stimulus determines a short-latency intracortical inhibition (SICI) of motor responses with respect to the test stimulus, whereas at longer ISI (7-20 ms), the effect is an intracortical facilitation (ICF).

SICI and ICF are considered to arise from different neural circuits: SICI is thought to reflect mostly the excitability of inhibitory GABAergic intracortical circuits; ICF is considered to depend on the activity of glutamatergic excitatory circuits, since dextromethorphan, an NMDA receptor antagonist, reduces the ICF. However, neurochemical networks underlying ICF seem to be more complex, supporting the hypothesis of a complex cortical-subcortical neurophysiological phenomenon, mainly mediated by the glutamatergic system.

![Figure 12. Schematic representation of paired-pulse Transcranial Magnetic Stimulation (TMS) and obtained curve of intracortical excitability at the different interstimulus intervals.](image-url)
3.3.3 TMS measures of sensory-motor modulation

Using a different TMS paradigm, it is possible to investigate the sensory-motor interaction within the cerebral cortex as well as the cortical phenomena of the short-latency afferent inhibition (SAI) and long-latency afferent inhibition (LAI). SAI refers to the suppression of the amplitude of the MEP produced by a conditioning afferent electrical stimulus applied to the median nerve at wrist approximately 20 ms prior to TMS of the hand area of the contralateral M1. SAI is thought to reflect the integrity of central cholinergic neural circuits, as it has been shown to be reduced or abolished by the muscarinic antagonist scopolamine in healthy subjects, whereas it is positively modulated by acetylcholine. On the other hand, it has been suggested that SAI may depend on the integrity of circuits linking sensory input and motor output, although other neurotransmitters (dopamine, in particular) are supposed to play a modulatory role on the cholinergic transmission.

LAI is probably related to cortical-cortical connections involving the motor cortex and both primary and secondary somatosensory cortical areas.

3.3.4 Plasticity-related measures and repetitive TMS

TMS allows also to probe the synaptic plasticity at different levels. In healthy subjects, TMS applied after a brief period of exercise reveals the “post-exercise facilitation” and the “delayed facilitation” phenomena, providing valuable information on cortical excitability and synaptic reorganization underlying motor learning.

Single TMS pulses delivered in trains are the principle of repetitive TMS (rTMS), an approach that can transiently influence the function of stimulated and connected brain areas, mainly depending on the frequency of stimulation. Repetitive TMS might have therapeutic and rehabilitative applications since the effects of repeated sessions may persist in time. Generally low-frequencies of stimulation (stimulus rates of <1 Hz) induces inhibitory effects on motor cortical excitability, allowing creation of a reversible “virtual lesion”, while high-frequencies (5-20 Hz) usually promotes an increase of cortical excitability. This modulation can last for several
minutes, depending on the duration of the train itself, thus providing an index of plasticity. The mechanisms of these changes are not clear, but seem to be related to phenomena of long-term potentiation (LTP) and long-term depression (LTD) within the Central Nervous System.305,341

Similarly it is possible to induce LTP-like changes in the sensory-motor system at the level of the M1, by means of an experimental intervention known as paired-associative stimulation (PAS).342 PAS protocol involves a stimulus to a peripheral nerve (usually the median nerve) followed by a single TMS pulse applied over the hand area of the M1. PAS induces a lasting increase of cortical-spinal excitability, which can be considered as a marker of motor cortex plasticity, with long-term plasticity-like mechanisms thought to play a major role.342

Figure 13 shows common magnetic stimulators used for both clinical and research purposes.

CHAPTER 4

TMS study of depression in the course of Vascular Cognitive Impairment-no Dementia

4.1 Aim and hypothesis

At present, little is known about the functional changes that take place in patients with VCI-ND at risk for future VaD and the impact of LLD on any plastic change that could contribute to the preservation of cognitive functions. To better characterize the possible role of depression in cognitive decline of patients with vascular damage, we investigated the relationship between the progression of the neurophysiological changes and cognitive impairment in patients with VCI-ND with those obtained in a group of patients with VD, both compared with healthy age-matched controls. Our hypothesis was that the presence of late-onset depression might affect not only cognition but also the functioning of specific cortical circuits that can be explored by TMS.

4.2 Materials and methods

4.2.1 Participants

Sixteen VD (68.1 ± 8.6 years) and 11 VCI-ND patients (70.0 ± 7.0 years) were consecutively recruited from the Cerebrovascular Disease Center of the University of Catania (Italy), and compared with 15 age-matched controls (63.8 ± 7.2 years). Participants were included as VCI-ND when they met the imaging criteria for subcortical vascular disease with predominant WMLs.46 They also did not satisfy the criteria for dementia according to the Diagnostic and Statistical Manual for Mental Disorders-Forth Edition-Text Revised (DSM-IV-TR), although they
were required to show deterioration in at least one cognitive domain but normal functional status in their activities of daily living.\textsuperscript{5}

Exclusion criteria were: other neurological disorders (i.e. dementia, stroke, Parkinson’s disease, Multiple Sclerosis, head trauma, epilepsy); major psychiatric illness, including depression or dythimic disorder; acute medical illness or organ failure (such as heart failure, liver cirrhosis, kidney failure, respiratory failure, severe metabolic imbalance) or diffuse neoplasm; alcohol or drug abuse; score at MMSE\textsuperscript{343} <24; exposure to drugs able to affect cortical excitability, such as benzodiazepines, zolpidem, antipsychotics, mood stabilizers, and antiepileptic drugs; any condition precluding MRI or TMS execution. None of the VCI-ND participants was on antidepressant, other psychotrophic drugs or cholinesterase inhibitors medications.

VD participants were required to fulfill the DSM-IV-TR diagnostic criteria for unipolar major depressive disorder and MRI criteria for subcortical vascular disease with predominant WMLs.\textsuperscript{46} Before the enrolment, 3 VD patients were on tricyclic antidepressant, whereas 6 and 7 of them were treated with SSRI and Serotonin Noradrenaline Reuptake Inhibitors (SNRI), respectively. A pharmacological wash out was performed two weeks before any TMS procedure, as recommended.\textsuperscript{344} Patients with a history of major psychiatric illness (except for personality disorders and anxiety, if secondary to depression), major neurological disorders (see above), history of epilepsy, acute medical illness, organ failure or diffuse neoplasm, mood or cognitive disorder due to endocrinopathies, alcohol or drug abuse, intake of drugs causing depressive symptoms (i.e. steroids, beta-blockers, clonidine) or modulating cortical excitability (see above), MMSE <24, and contraindication to MRI or TMS were excluded.

Conventional EEG was performed prior to the enrolment to rule out predisposition to seizure. The study was approved by the local Ethics Committee based at the “Policlinico-Vittorio Emanuele” University Hospital of Catania (Italy), and written informed consent was obtained from all participants prior to the participation, in accordance with the Declaration of Helsinki. All assessments were performed in a controlled laboratory environment.
4.2.2 Assessment

All subjects underwent clinical assessment, including: age, gender, education, handedness, presence of cardiovascular risk factors (hypertension, diabetes, hypercholesterolemia, coronaryopathy, atrial fibrillation, and smoking habit), and both general and neurological examinations. Patients and controls were treated for their vascular risk factors with anti-platelet or anti-coagulant medications (aspirin, clopidogrel, warfarin), anti-hypertensive drugs (ACE inhibitors, AGTR blockers, diuretics, calcium channel blockers), cholesterol lowering medications (statins), and anti-diabetic medications (oral drugs or insulin).

None of the patients had focal neurological deficit. The three groups were similar in terms of educational level and vascular risk factors profile; VD participants exhibited a more frequent family and personal history of depression. The neuropsychological battery of tests assessed overall cognitive impairment (MMSE),\(^{343}\) frontal lobe abilities (Frontal Assessment Battery – FAB),\(^{345}\) and the interference task Stroop Color-Word test (total time – Stroop T – number of errors – Stroop E).\(^{346}\) The presence of depressive symptoms and apathy was quantified by means of the 17-item Hamilton Depression Rating Scale (HDRS)\(^{347}\) and the Apathy Scale (AS),\(^{348}\) respectively. Functional status was defined by using the scores of the Activity of Daily Living (ADL) and the Instrumental Activity of Daily Living (IADL).

Brain MRI was acquired from all participants with a 1.5 Tesla General Electric\(^{©}\) system. The imaging protocol consisted of T1-, T2-, proton density-weighted, and Fluid-attenuated Inversion Recovery (FLAIR) scans; slice thickness was 5 mm, with 0.5 mm slice gap. In all subjects, the severity of WMLs was graded according to the Fazekas visual scale\(^{349}\) (Figure 14): 0 = absence; 1 = punctuate foci; 2 = beginning confluence of foci; 3 = large confluent areas. Accordingly, WML severity was graded as mild in 8 VD patients (grade 1), moderate in 4 (grade 2), and severe in 4 (grade 3); 6 VCI-ND patients were rated as grade 1, 4 as grade 2 and 1 as grade 3; brain MRI was normal in all controls (grade 0).
4.2.3 Transcranial magnetic stimulation protocol

TMS was performed using a High-power Magstim 200 mono pulse magnetic stimulator© (Magstim Co., Whitland, Dyfed, UK). A 70 mm figure-of-eight coil was held over the motor cortex at the optimum scalp position to elicit MEPs in the contralateral first dorsal interosseous (FDI) muscle of the dominant hand, according to the Edinburgh Handedness Inventory. The flat surface of the coil was positioned tangentially on the scalp over the M1. EMG activity was recorded from a silver/silver chloride surface active electrode placed over the motor point of the FDI muscle, with
the reference electrode placed distally at the metacarpal-phalangeal joint of the index finger. Motor responses were amplified and filtered (bandwidth 3-3.000 Hz) using a 2-channel Medelec Synergy® (Oxford Instruments Medical, Inc., UK) system with an amplification factor of the screen of 100 μV per division unit for the measurement of the rMT and 1 mV per division unit during the MEP recording. The temporal resolution of the screen was 5 ms per division unit, in such a way that the TMS artefact, the beginning and the end of the MEP were all always visible.  

For the motor nerve conduction study (M and F waves from the FDI muscle), a bipolar nerve stimulation electrode with 6-mm diameter felt pads and an inter-electrode separation of 25 mm was used. M and F waves were elicited by giving supramaximal electrical stimulation (constant current square wave pulse of 0.2 ms) to the ulnar nerve at wrist. Three reproducible artefact-free M responses and ten F waves were recorded for each subject. While FDI was relaxed, the peak-to-peak amplitude of M and F waves were determined. We identified the F waves according to the criteria reported by the International Federation of Clinical Neurophysiology (IFCN) as responses that are variable in their latency, amplitude, and configuration but that occur grouped with a consistent range of latency. The F wave with the shortest latency, providing a measure of conduction in the fastest motor axons, was considered.

Measures of motor cortex excitability included rMT, CSP, MEPs, and CMCT from both hemisphere. Resting MT was defined, according to the IFCN Committee recommendation, as the lowest stimulus intensity able to elicit MEPs of an amplitude >50 μV in at least 5 of 10 trials, with the muscle at rest. It is a global measure of cortical excitability reflecting the excitability of cortical-spinal neurons and interneurons projecting into these neurons in the motor cortex, as well as of spinal motor neurons, neuromuscular junctions and muscle. The CSP was determined with an approximately 50% of maximum tonic voluntary contraction of the FDI muscles, induced by contralateral TMS pulses delivered at 130% of the rMT. During the CSP recordings, the subjects maintained the isometric tonic contraction by abducting the index finger against a strain gauge. The mean CSP duration based on trial-by-trial measurements of 10 rectified traces was calculated.
Following the IFCN guidelines,\textsuperscript{351,352} in a single trial the CSP was measured as the time elapsing from the onset of the MEP until the recurrence of voluntary tonic EMG activity. If voluntary EMG activity did not recover abruptly but gradually, making the identification of the end of the CSP difficult, the following criteria on a single trial basis were used: when the EMG activity reached or exceeded the pre-TMS baseline level and lasts for at least 50 ms, reoccurring EMG activity marked the end of the CSP. As known, the CSP is mainly mediated by the activity of GABAergic intracortical neurons.\textsuperscript{351,352}

CMCT reflects the integrity of the cortical-spinal tract, from the upper to the lower motor neurons. It was calculated by subtracting the conduction time in peripheral nerves from MEP latency obtained during moderate active muscle contraction (10-20\% of maximum background force), at a stimulus intensity set at 130\% of the rMT.\textsuperscript{352} By using the F wave latency, CMCT was estimated as: \( T - (F + M - 1)/2 \) \( [T = \text{onset latency of MEP elicited by TMS}; F = \text{onset latency of F wave by electrical ulnar nerve stimulation}; M = \text{onset latency of M wave by electrical ulnar nerve stimulation}] \).\textsuperscript{352} Moreover, in order to assess spinal motor excitability, the mean amplitude of the F wave was measured in the target muscle.\textsuperscript{353,354}

Intracortical circuits were studied bilaterally using the conditioning-test paradigm described by Kujirai and co-workers\textsuperscript{325} through a BiStim \textsuperscript{©} module (Magstim Co., Whitland, Dyfed, UK) connected to a Cambridge Electronic Design Micro 1401 \textsuperscript{©} interface (Cambridge, UK). The procedure consisted of applying two magnetic stimuli in rapid succession through two magnetic stimulators connected to each other. The conditioning stimulus was applied at 80\% of the subject’s rMT, and the intensity of the test stimulus was set at 130\% of the rMT. The ISIs tested were 1, 3, 5, 7, 10, and 15 ms; ten trials for each ISI were recorded randomly. The responses were expressed as the ratio of the MEP amplitude produced by paired stimulation to that produced by test stimulation alone. SICI was obtained at short ISIs in which the conditioning stimulus determines an inhibition with respect to the test stimulus; it is attributed to an activation of inhibitory neuronal system transmission.\textsuperscript{306,325} ICF was studied at longer ISIs in which the conditioning stimulus determines an
enhanced response with respect to the test stimulus; it is modulated by multiple neurotransmission pathways, although mainly through excitatory glutamatergic neuron.\textsuperscript{328,329}

All TMS measurements were conducted while subjects were seated on a comfortable chair with continuous EMG monitoring to ensure either a constant level of muscular activity during tonic contraction or complete relaxation at rest. Data were collected on a computer and stored for off-line analysis. Hardware setting, data collection and off-line processing were performed by using an \textit{ad hoc} tool which is detailed in the article by Giordano and co-workers.\textsuperscript{355}

\textbf{4.2.4 Follow-up}

All participants were re-evaluated after a median period of approximately two years (VD 24.1 ± 2.1 months; VCI 23.9 ± 1.8 months; controls 23.2 ± 1.7 months; \(p = 0.15\)), with the same assessment performed at the entry of the study, including clinical-demographic evaluation, neuropsychological tests, as well as single- and paired-pulse TMS. Brain MRI was repeated in all patients, showing a progression of the vascular burden from grade 1 to grade 2 in one VCI-ND, and in two VD patients. Of the original cohort, one VCI-ND subject and one with VD were no more eligible to TMS due to a permanent pacemaker implantation and the poor medical condition, respectively; nevertheless, these patients were re-assessed for their cognitive profile.

\textbf{4.2.5 Statistical analysis}

The non-parametric Kruskal-Wallis ANOVA test was used for the comparison of clinical, neuropsychological and electrophysiological parameters of patients and controls at baseline (time \(t_0\)), at follow-up (time \(t_1\)), and their differences (assessed as \(t_1-t_0\)). The Mann-Whitney test was employed as a \textit{post hoc} analysis for the pairwise comparison. The Wilcoxon test for paired data sets was used for the comparison of clinical, neuropsychological and electrophysiological variables at time \(t_1\) and \(t_0\) for each group patient. Non-parametric statistics analysis was required given the categorical nature of the neuropsychological testing results and the non-uniform distribution of the
results of the TMS studies. Correlations between neuropsychological and TMS variables were evaluated by means of the Spearman’s correlation coefficient.

A $p$ value lower than 0.05 was considered as statistically significant. To account for multiple comparison, Bonferroni correction as well as Benjamini-Hochberg procedure were employed.

### 4.3 Results

#### 4.3.1 Baseline

Neuropsychological characteristics of all participants at the entry of the study are summarized in Table 3. As shown in Table 4, no statistically significant difference between patients and controls was found at baseline for any single-pulse TMS parameter.

The mean time course of intracortical excitability of all subjects at time $t_0$ is shown in Figure 15. As shown, at baseline there was a significantly more pronounced ICF in VCI-ND than in controls and in VD. In detail, conditioned MEP amplitudes from both hemispheres at ISI of 10 ms ($left$ hemisphere: VD 1.5 ± 0.9; VCI-ND 3.0 ± 2.7; controls 1.4 ± 0.6 – $p= 0.0009$; $right$ hemisphere: VD 1.6 ± 0.6; VCI-ND 2.5 ± 2.4; controls 1.3 ± 0.3 – $p= 0.0092$), and at ISI of 15 ms ($left$ hemisphere: VD 1.7 ± 1.1; VCI-ND 2.5 ± 1.2; controls 1.3 ± 0.7 – $p= 0.0021$; $right$ hemisphere: VD 1.8 ± 0.8; VCI-ND 2.7 ± 1.4; controls 1.4 ± 0.6 – $p= 0.0033$) were significantly larger in the VCI-ND patients than in the other two groups, suggesting an increase of the ICF.
Table 3. Neuropsychological characteristics of the three groups at baseline.

<table>
<thead>
<tr>
<th></th>
<th>VD</th>
<th>VCI-ND</th>
<th>Controls</th>
<th>Kruskal Wallis ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>H (2.42)</td>
<td>p</td>
</tr>
<tr>
<td>Age (years)</td>
<td>68.1 ± 8.6</td>
<td>70.0 ± 7.0</td>
<td>63.8 ± 7.2</td>
<td>4.04</td>
</tr>
<tr>
<td>Education (years)</td>
<td>7.5 ± 5.1</td>
<td>6.8 ± 4.0</td>
<td>9.7 ± 4.4</td>
<td>5.11</td>
</tr>
<tr>
<td>MMSE</td>
<td>26.6 ± 1.8</td>
<td>27.2 ± 2.1</td>
<td>28.5 ± 1.6</td>
<td>8.88</td>
</tr>
<tr>
<td>ADL</td>
<td>5.8 ± 0.4</td>
<td>5.9 ± 0.3</td>
<td>6.0 ± 0.0</td>
<td>3.08</td>
</tr>
<tr>
<td>IADL</td>
<td>7.4 ± 1.4</td>
<td>7.8 ± 0.4</td>
<td>7.8 ± 0.4</td>
<td>0.39</td>
</tr>
<tr>
<td>HDRS</td>
<td>14.8 ± 6.1</td>
<td>4.2 ± 2.0</td>
<td>4.1 ± 2.3</td>
<td>29.28</td>
</tr>
<tr>
<td>AS</td>
<td>1.3 ± 0.5</td>
<td>0.4 ± 0.3</td>
<td>0.3 ± 0.4</td>
<td>23.68</td>
</tr>
<tr>
<td>Stroop T</td>
<td>42.3 ± 15.5</td>
<td>43.9 ± 17.8</td>
<td>24.6 ± 12.5</td>
<td>12.80</td>
</tr>
<tr>
<td>Stroop E</td>
<td>2.4 ± 2.5</td>
<td>3.8 ± 3.5</td>
<td>0.5 ± 0.6</td>
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<tr>
<td>FAB</td>
<td>14.8 ± 2.2</td>
<td>14.3 ± 2.4</td>
<td>17.1 ± 1.5</td>
<td>15.35</td>
</tr>
</tbody>
</table>

Post hoc analysis

<table>
<thead>
<tr>
<th></th>
<th>VD vs VCI-ND</th>
<th>VD vs Controls</th>
<th>VCI-ND vs Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z</td>
<td>p</td>
<td>Z</td>
</tr>
<tr>
<td>MMSE</td>
<td>0.91</td>
<td>1.000</td>
<td>2.93</td>
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<tr>
<td>HDRS</td>
<td>4.39</td>
<td>0.00003</td>
<td>4.74</td>
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<tr>
<td>AS</td>
<td>3.52</td>
<td>0.0013</td>
<td>4.55</td>
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<tr>
<td>Stroop T</td>
<td>0.24</td>
<td>1.000</td>
<td>3.10</td>
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<tr>
<td>Stroop E</td>
<td>0.86</td>
<td>1.000</td>
<td>2.67</td>
</tr>
<tr>
<td>FAB</td>
<td>0.45</td>
<td>1.000</td>
<td>3.28</td>
</tr>
</tbody>
</table>

Table 4. Single-pulse TMS parameters obtained from patients and controls at baseline.

<table>
<thead>
<tr>
<th></th>
<th>VD</th>
<th>VCI</th>
<th>Controls</th>
<th>Kruskal Wallis ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>H (2.42)</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left hemisphere</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rMT (%)</td>
<td>47.2 ± 9.8</td>
<td>47.4 ± 9.0</td>
<td>43.0 ± 5.5</td>
<td>2.86</td>
</tr>
<tr>
<td>CSP (ms)</td>
<td>86.4 ± 38.3</td>
<td>93.2 ± 37.0</td>
<td>71.0 ± 16.6</td>
<td>3.22</td>
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<td>MEP latency (ms)</td>
<td>19.6 ± 1.8</td>
<td>20.4 ± 1.3</td>
<td>20.3 ± 1.6</td>
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<td>CMCT (ms)</td>
<td>5.7 ± 1.0</td>
<td>5.3 ± 0.4</td>
<td>6.0 ± 1.0</td>
<td>4.81</td>
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<tr>
<td>CMCT-F (ms)</td>
<td>5.3 ± 0.9</td>
<td>5.4 ± 0.8</td>
<td>5.9 ± 0.8</td>
<td>4.20</td>
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<tr>
<td>A ratio</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>1.91</td>
</tr>
<tr>
<td>F amplitude (μV)</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.1</td>
<td>1.96</td>
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<tr>
<td>Right hemisphere</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>rMT (%)</td>
<td>46.1 ± 9.6</td>
<td>44.1 ± 6.2</td>
<td>42.9 ± 4.6</td>
<td>1.54</td>
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<tr>
<td>CSP (ms)</td>
<td>97.1 ± 48.3</td>
<td>91.5 ± 36.8</td>
<td>67.7 ± 22.8</td>
<td>3.52</td>
</tr>
<tr>
<td>MEP latency (ms)</td>
<td>19.7 ± 1.7</td>
<td>20.1 ± 1.6</td>
<td>19.9 ± 1.6</td>
<td>0.62</td>
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<tr>
<td>CMCT (ms)</td>
<td>5.7 ± 0.8</td>
<td>5.5 ± 0.4</td>
<td>5.7 ± 1.0</td>
<td>0.13</td>
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<tr>
<td>CMCT-F (ms)</td>
<td>5.3 ± 0.7</td>
<td>5.5 ± 1.0</td>
<td>5.6 ± 1.1</td>
<td>0.51</td>
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<tr>
<td>A ratio</td>
<td>0.5 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>1.24</td>
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<tr>
<td>F amplitude (μV)</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>3.98</td>
</tr>
</tbody>
</table>

Table 3. Neuropsychological characteristics of the three groups at baseline.

VD = patients with vascular depression; VCI-ND = patients with vascular cognitive impairment-no dementia; MMSE = Mini Mental State Examination; ADL = Activity of Daily Living; IADL = Instrumental Activity of Daily Living; HDRS = 17-item Hamilton Depression Rating Scale; AS = Apathy Scale; Stroop T = Stroop Color–Word test interference – time (sec); Stroop E = Stroop Color–Word test interference – number of errors; FAB = Frontal Assessment Battery; numbers in bold and italic = statistically significant p values.

Table 4. Single-pulse TMS parameters obtained from patients and controls at baseline.

VD = patients with vascular depression; VCI = patients with vascular cognitive impairment-no dementia; rMT = resting motor threshold; CSP = cortical silent period; MEP = motor evoked potential; CMCT = central motor conduction time; central motor conduction time estimated by using the F wave latency; A ratio = CMAP/MEP amplitude ratio.
Figure 15. The mean time course of intracortical excitability in the patients and controls at baseline. MEP = motor evoked potential; ISI = interstimulus interval; VD = patients with vascular depression; VCI = patients with vascular cognitive impairment-no dementia.
4.3.2 Follow-up

The comparison of neuropsychological and TMS characteristics of the three groups between baseline and follow-up is summarized in Table 5 and Table 6, respectively. Unlike VCI-ND, depressed patients showed a significant decline of their functional status (IADL), together with a worsening of the mean FAB score (Table 5). The TMS evaluation showed that the median rMT decreased significantly in both VCI-ND and VD patients (without significant difference between the two groups) compared to controls, whereas the CSP lengthened its duration from both hemispheres in controls but not in patients (Table 6). The Figure 16 shows the comparison over time of the paired-pulse TMS curves between patients and controls; in particular, the ICF of VCI-ND group become now similar to that found in the other two groups.

<table>
<thead>
<tr>
<th></th>
<th>VD Mean SD</th>
<th>VCI-ND Mean SD</th>
<th>Controls Mean SD</th>
<th>Kruskal Wallis ANOVA H (2,40)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMSE</td>
<td>-2.86 3.54</td>
<td>-1.00 1.73</td>
<td>-1.42 1.70</td>
<td>1.59</td>
<td>0.451</td>
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<tr>
<td>ADL</td>
<td>-0.67 1.35</td>
<td>-0.10 0.57</td>
<td>0.00 0.00</td>
<td>3.11</td>
<td>0.211</td>
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<td>IADL</td>
<td>-1.20 1.52</td>
<td>-0.70 0.82</td>
<td>0.07 0.26</td>
<td>11.81</td>
<td><strong>0.0027</strong></td>
</tr>
<tr>
<td>HDRS</td>
<td>1.80 6.79</td>
<td>0.30 4.40</td>
<td>0.53 2.59</td>
<td>0.95</td>
<td>0.622</td>
</tr>
<tr>
<td>AS</td>
<td>-0.30 0.64</td>
<td>-0.08 0.34</td>
<td>-0.04 0.47</td>
<td>2.67</td>
<td>0.263</td>
</tr>
<tr>
<td>Stroop T</td>
<td>10.47 25.18</td>
<td>16.03 31.14</td>
<td>5.43 14.18</td>
<td>1.03</td>
<td>0.597</td>
</tr>
<tr>
<td>Stroop E</td>
<td>1.47 3.45</td>
<td>-0.24 2.68</td>
<td>0.37 0.85</td>
<td>0.73</td>
<td>0.695</td>
</tr>
<tr>
<td>FAB</td>
<td>0.48 3.49</td>
<td>0.39 1.88</td>
<td>-1.14 1.35</td>
<td>8.45</td>
<td><strong>0.0146</strong></td>
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</table>

Table 5. Differences (computed as t1 – t0) of neuropsychological features of patients and controls.

<table>
<thead>
<tr>
<th>Post hoc analysis</th>
<th>VD vs VCI-ND</th>
<th>VD vs Controls</th>
<th>VCI-ND vs Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z  p</td>
<td>Z  p</td>
<td>Z  p</td>
</tr>
<tr>
<td>IADL</td>
<td>0.39 1.000</td>
<td>2.76</td>
<td><strong>0.0171</strong></td>
</tr>
<tr>
<td>FAB</td>
<td>0.80 1.000</td>
<td>2.85</td>
<td><strong>0.0127</strong></td>
</tr>
</tbody>
</table>

VD = patients with vascular depression; VCI-ND = patients with vascular cognitive impairment-no dementia; SD = standard deviation; MMSE = Mini Mental State Examination; ADL = Activity of Daily Living; IADL = Instrumental Activity of Daily Living; HDRS = 17-item Hamilton Depression Rating Scale; AS = Apathy Scale; Stroop T = Stroop Color-Word test interference – time (sec); Stroop E = Stroop Color-Word test interference – number of errors; FAB = Frontal Assessment Battery; numbers in bold and italic = statistically significant p values.
### Table 6.
Comparison of clinical and neuropsychological features of the three groups between baseline ($t_0$) and follow-up ($t_1$); differences are computed as $t_1 - t_0$.

*VD* = patients with vascular depression; *VCI-ND* = patients with vascular cognitive impairment-no dementia; *SD* = standard deviation; *rMT* = resting motor threshold; *CSP* = cortical silent period; *MEP* = motor evoked potential; *CMCT* = central motor conduction time; *CMCT-F* = central motor conduction time estimated by using the F wave latency; *A ratio* = CMAP/MEP amplitude ratio; numbers in bold and italic = statistically significant p values.

<table>
<thead>
<tr>
<th></th>
<th>VD</th>
<th>VCI-ND</th>
<th>Controls</th>
<th>Kruskal Wallis ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td><em>Left hemisphere</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rMT (%)</td>
<td>-5.27</td>
<td>6.53</td>
<td>-5.70</td>
<td>3.83</td>
</tr>
<tr>
<td>CSP (ms)</td>
<td>1.10</td>
<td>2.59</td>
<td>3.20</td>
<td>24.26</td>
</tr>
<tr>
<td>MEP latency (ms)</td>
<td>0.32</td>
<td>1.32</td>
<td>0.09</td>
<td>0.54</td>
</tr>
<tr>
<td>CMCT (ms)</td>
<td>0.12</td>
<td>1.42</td>
<td>0.32</td>
<td>0.61</td>
</tr>
<tr>
<td>CMCT-F (ms)</td>
<td>-0.30</td>
<td>1.03</td>
<td>0.27</td>
<td>0.67</td>
</tr>
<tr>
<td>A ratio</td>
<td>0.03</td>
<td>0.19</td>
<td>0.07</td>
<td>0.20</td>
</tr>
<tr>
<td>F amplitude ($\mu$V)</td>
<td>0.02</td>
<td>0.08</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td><em>Right hemisphere</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rMT (%)</td>
<td>-3.93</td>
<td>5.83</td>
<td>-3.10</td>
<td>2.92</td>
</tr>
<tr>
<td>CSP (ms)</td>
<td>15.83</td>
<td>41.03</td>
<td>4.20</td>
<td>15.18</td>
</tr>
<tr>
<td>MEP latency (ms)</td>
<td>0.11</td>
<td>0.81</td>
<td>0.15</td>
<td>1.17</td>
</tr>
<tr>
<td>CMCT (ms)</td>
<td>-0.17</td>
<td>1.31</td>
<td>-0.05</td>
<td>0.79</td>
</tr>
<tr>
<td>CMCT-F (ms)</td>
<td>-0.19</td>
<td>1.14</td>
<td>-0.46</td>
<td>2.20</td>
</tr>
<tr>
<td>A ratio</td>
<td>-0.02</td>
<td>0.27</td>
<td>-0.05</td>
<td>0.10</td>
</tr>
<tr>
<td>F amplitude ($\mu$V)</td>
<td>0.01</td>
<td>0.07</td>
<td>0.10</td>
<td>0.14</td>
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</table>

### Post-hoc analysis

<table>
<thead>
<tr>
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<th>VD vs VCI-ND</th>
<th>VD vs Controls</th>
<th>VCI-ND vs Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z</td>
<td>p</td>
<td>Z</td>
</tr>
<tr>
<td>rMT (left)</td>
<td>0.75</td>
<td>1.000</td>
<td>2.41</td>
</tr>
<tr>
<td>CSP (left)</td>
<td>0.09</td>
<td>1.000</td>
<td>2.34</td>
</tr>
<tr>
<td>rMT (right)</td>
<td>0.37</td>
<td>1.000</td>
<td>2.15</td>
</tr>
<tr>
<td>F amplitude (right)</td>
<td>2.30</td>
<td>0.062</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Finally, the correlation between psychopathological and TMS variables revealed a positive correlation in VCI-ND group between ISI of 15 ms from left hemisphere at baseline and MMSE score at follow-up ($\rho = 0.604961$, $p < 0.0025$ – $p$ value lowered according to the Bonferroni correction). The correlation resulted significant even after controlling for the false discovery rate with Benjamini-Hochberg procedure (critical value set using 0.20 as false discovery rate).
Figure 16. Comparison of the time course of intracortical excitability of patients and controls between baseline ($t_0$) and follow-up ($t_1$). Normalized MEP difference is computed as $(t_1-t_0)/t_0$. The comparison over time of the paired-pulse TMS curves between patients and controls did not show significant differences in term of intracortical inhibition and intracortical facilitation between the three groups. Y-axis shows the normalized MEP difference at baseline ($t_0$) and at follow-up ($t_1$) (computed as the value at time $t_1$ minus the one at time $t_0$ divided by $t_0$).

$MEP = motor \text{ evoked potential}; \ ISI = \text{intestimulus interval}; \ VD = \text{patients with vascular depression}; \ VCI = \text{patients with vascular cognitive impairment-no dementia}; \ CON = \text{healthy controls}$.
4.4 Discussion

This is the first longitudinal study assessing the neurophysiology of late-onset depression as a potential risk factor for future VaD in patients with subcortical vascular disease. The main finding is that the high level of intracortical facilitation observed at baseline in non-depressed patients only might be protective from cognitive decline, possibly through an enhancement of glutamate-related neuroplasticity. Moreover, the hyperexcitability at single pulse TMS observed at follow-up in both group of patients also points out an involvement of glutamatergic neurotransmission, although without a specific neurophysiological change that parallels cognitive decline in depressed patients. This suggests that the mechanisms that contribute to cognitive deterioration in VD might be related either to subcortical changes produced by vascular lesions or to the lack of compensatory functional cortical adaptation.\textsuperscript{356}

These results are in line with previous TMS studies in subjects with subcortical vascular disease and clinical-cognitive features of VCI-ND, showing an enhanced ICF with respect to age-matched healthy controls.\textsuperscript{315} An increase of cortical excitability, together with a significant worsening of frontal lobe abilities but without the development of dementia, was found after two years of follow-up.\textsuperscript{316} Interestingly, a slight enhancement of ICF was also observed in patients with VD,\textsuperscript{357} but not in those with early-onset major depression disorder.\textsuperscript{358}

In the present study monitoring vascular depressed and non-depressed individuals, the cortical excitability at follow-up increased significantly in both groups, although VD only showed clinical progression. This different behaviour may lie on the fact that the burden of subcortical vascular lesions constitute a neuropathological platform for both cognitive decline and affective disorder in old age.\textsuperscript{191,359} In this context, this neurophysiological contribution might shed lights on the mechanisms underlying progression or preservation of cognitive functions in depression. In particular, an increased ICF, probably trough plastic compensatory phenomena involving the excitatory glutamatergic interneurons within the motor cortex, might act preserving cognition in
Conversely, a lack of this hyperfacilitation in VD might contribute to their cognitive and functional deterioration, suggesting an impaired level of neural plasticity. This hypothesis is in agreement with a growing body of evidences in the literature indicating that the glutamate neurotransmission, which is known to play a major role in synaptic plasticity, is disrupted in depressive disorder, and that drugs targeting the NMDA receptor have shown antidepressant properties.

It is noteworthy that the increased cortical excitability observed at follow-up might be also related to a parallel degeneration of inhibitory GABAergic terminals. Indeed, in both groups of patients we did not find the same prolongation of the CSP duration observed in the control group. Given that the CSP is a well-known measure of motor cortex inhibition largely mediated by GABA-B receptors, this finding confirm the physiological role of the GABAergic transmission in controls with normal brain aging; conversely, it highlights an altered inhibitory control in elderly VCI patients, with or without depression. However, it should be kept in mind that discrepant results on CSP modification with aging, probably related to technical and experimental differences, have emerged even in healthy adults.

Finally, in the last decade, research has been focused on the intriguing role of the neurotrophin release in mood disorders. The “neurogenic and neurotrophic hypothesis” assumes that development of depression would be, at least partially, related to the reduced neurogenesis and/or depletion of neurotrophic factors, which can eventually lead to functional impairment of brain network implicated in mood-affect regulation. In particular, serum level of BDNF was found to be lower in late-onset depressed subjects than in age-matched controls. Low concentrations of both BNDF and Vascular Endothelial Growth Factor (VEGF) can contribute to the progression of depression as well. Other investigations have also addressed the relevance of the BDNF val66met polymorphism in depression, being the met allele associated with the incidence of PSD or with greater WMLs load in the elderly.
The results of the present investigation should be considered taking into account some limitations related to both samples studied and methodology used, as detailed below.

The main limitation, as usual in TMS research, is the relatively small number of patients, although they were very homogeneous in terms of age, gender, neuroradiological lesion load and with a well-defined vascular risk profile. Moreover, they were age-matched with healthy controls without evidence of cerebrovascular disease at neuroimaging (that is strikingly prevalent among elderly), and any cognitive impairment at neuropsychological evaluation.

Second, although some TMS parameters change consistently with the involvement of different pathophysiological substrates even in the earliest stages of the disease, there is not pathognomonic measure, and therefore it cannot be excluded that, at this stage, TMS is not entirely able to quantify the risk of progression in patients with or without depression. Therefore, the hypothesis to identify a characteristic signature in patients with subcortical vascular disease at risk for developing VaD or mixed dementia could be risky given the paucity of previous data and the difficulty of similar approaches in other dementing conditions, such as non-AD dementias. Consequently, the identification of a pattern of cortical excitability rather than single marker of the disease process and progress would allow a more reliable and reproducible prediction.

Finally, it cannot be excluded that some patients enrolled in the studies here reviewed had a mixed form of cognitive decline rather than a pure VCI. With this respect, as stated before, TMS is not currently capable to clearly distinguish VaD from AD based only on their neurophysiological profile, or to clearly elucidate the vascular or degenerative burden.
CHAPTER 5
Conclusions and future outlooks

Studying cortical excitability by means of TMS provides a potentially new window into the neurophysiological mechanisms behind neurogeriatric depression and its reciprocal relationship with vascular-related cognitive disorders. Moreover, these data seem to support the “Vascular depression hypothesis” as a different syndrome with respect to non-vascular major depression also at the TMS level, suggesting that depressive syndrome is probably not the primary disorder but rather one of the clinical manifestations in the wide spectrum of cerebrovascular disease. Further independent investigations with larger group size are needed to confirm the present findings and to understand their modifications and clinical correlates over time.

Despite the fact that patients cohorts and methodologies are not always homogeneous between studies, much of the literature agrees on the utility of TMS in dementia. Although a single measure is not sufficient to define a diagnosis, all together the abnormalities detected in the TMS parameters of interest are footprints of specific pathophysiological processes that affects motor and non-motor areas in the various form of dementia. Hence, not the single alterations, but the whole frame on how all the pieces of the puzzle fit together can help to build a comprehensive knowledge on the diseases, predict their progression and identify a “brain at risk”.

This is of utmost importance in front of the raising of the average age and the life expectancy of the population, which, in turn, dramatically increases the prevalence of dementia and the growing need of expenses rationalization of the public health systems. TMS, coupled with imaging, biology and genetics, could furnish the tools necessary for an early, diagnosis that finds the process of involution in a preclinical phase, when it is still attackable. The early and accurate diagnosis will also be of pivotal importance when designing trials of disease modifying drugs or innovative non-pharmacological approaches based on non-invasive brain stimulation.
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