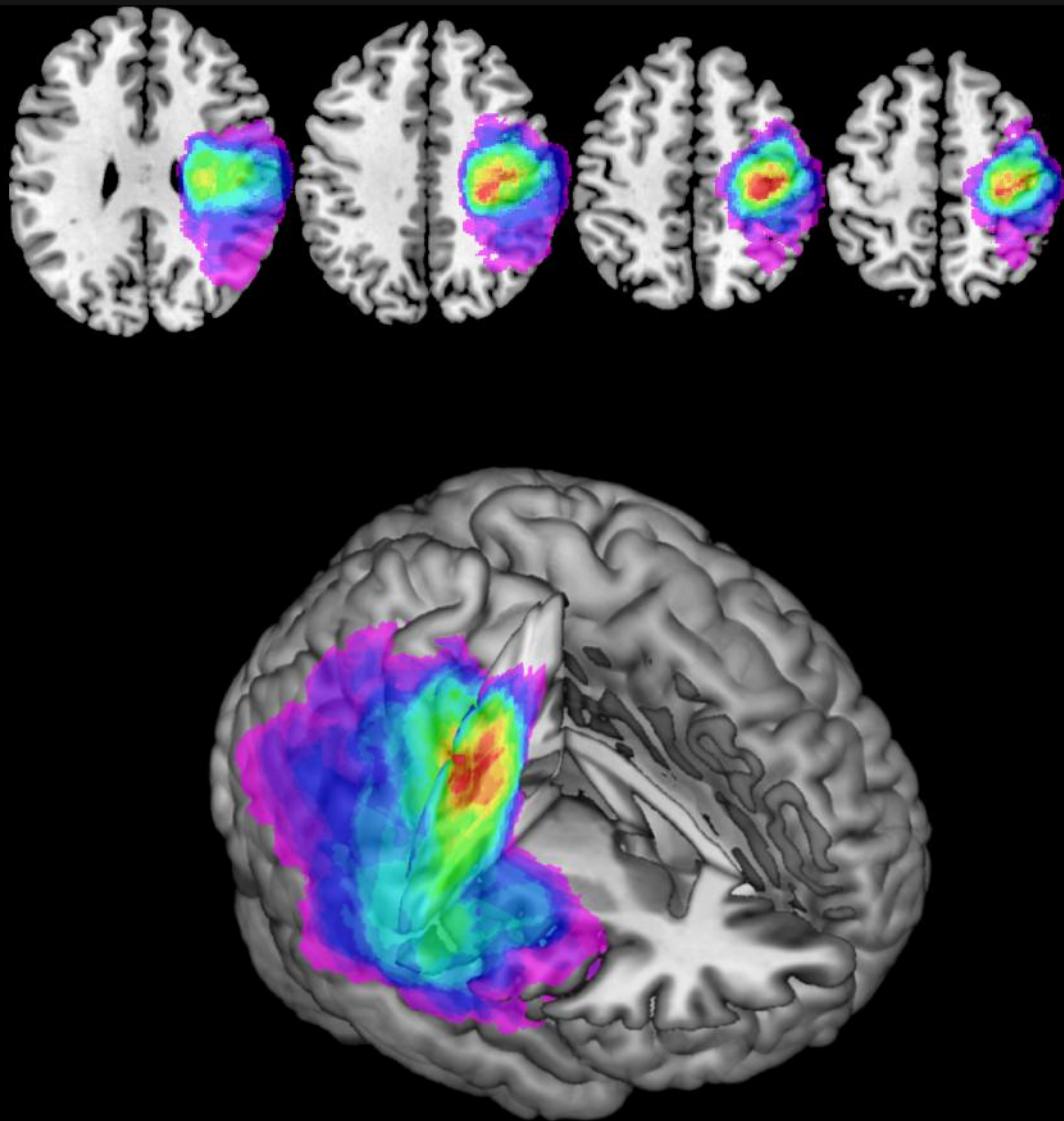
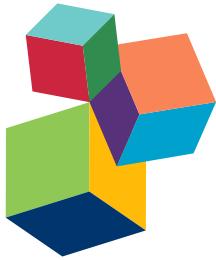


PRINCIPLES UNDERLYING POST-STROKE RECOVERY OF UPPER EXTREMITY SENSORIMOTOR FUNCTION – A NEUROIMAGING PERSPECTIVE

EDITED BY: Bruno J. Weder, Roland Wiest and Rüdiger J. Seitz

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PRINCIPLES UNDERLYING POST-STROKE RECOVERY OF UPPER EXTREMITY SENSORIMOTOR FUNCTION – A NEUROIMAGING PERSPECTIVE

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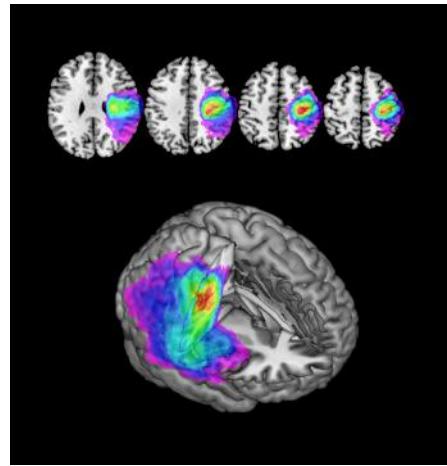
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Neuroimaging post-stroke has the potential to uncover underlying principles of disturbed hand function and recovery characterizing defined patient groups, including their long term course as well as individual variations. The methods comprise functional magnetic resonance imaging (MRI) measuring task related activation as well as resting state. Functional MRI may be complemented by arterial spin labeling (ASL) MRI to investigate slowly varying blood flow and associated changes in brain function. For structural MRI robust and accurate computational anatomical methods like voxel-based morphometry and surface based techniques are available. The investigation of the connectivity among brain regions and disruption after stroke is facilitated by diffusion tensor imaging (DTI). Intra- and interhemispheric coherence may be studied by electromagnetic techniques such as electroencephalography and transcranial magnetic stimulation.

Consecutive phases of stroke recovery (acute, subacute, early chronic and late chronic stages) are each distinguished by intrinsic processes. The site and size of lesions entail partially different functional implications. New strategies to establish functional specificity of a lesion site include calculating contrast images between patients exhibiting a specific disorder and control subjects without the disorder. Large-size lesions often imply poor cerebral blood flow which impedes recovery significantly and possibly interferes with BOLD response of functional MRI. Thus, depending on the site and size of the infarct lesion the patterns of recovery will vary. These include recovery sensu stricto in the perilesional area, intrinsic compensatory mechanisms using alternative cortical and subcortical pathways, or behavioral compensatory strategies e.g. by using the non-affected limb. In this context, behavioral and neuroimaging measures should be developed and employed to delineate aspects of learning during recovery. Of special interest in recovery of hand paresis is the interplay between sensory and motor areas in the



This image shows lesion overlap maps for patients in the acute phase after a cortical sensorimotor (MI/SI) stroke, confirmed on diffusion-weighted MRI, exhibiting contralateral hand paresis or plegia as common denominator (upper half). The corresponding three-dimensional rendering with a vertical cut through the maximum overlap of the complete cohort is added in the lower half. The image relates to the paper of Abela E, Missimer J, Wiest R, Federspiel A, Hess C, Sturzenegger M, Weder B (2012) (Lesions to Primary Sensory and Posterior Parietal Cortices Impair Recovery from Hand Paresis after Stroke. *PLoS ONE* 7(2): e31275. doi:10.1371/journal.pone.0031275).

posterior parietal cortex involved during reaching and fine motor skills as well as the interaction with the contralesional hemisphere. The dominant disability should be characterized, from the level of elementary to hierarchically higher processes such as neglect, apraxia and motor planning.

In summary, this Research Topic covers new trends in state of the art neuroimaging of stroke during recovery from upper limb paresis. Integration of behavioral and neuroimaging findings in probabilistic brain atlases will further advance knowledge about stroke recovery.

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Editorial: Principles Underlying Post-Stroke Recovery of Upper Extremity Sensorimotor Function – A Neuroimaging Perspective

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Keywords: stroke recovery, multimodal neuroimaging, computational biophysical modeling, motor control, motor imagery, somatosensory disorders, perilesional plasticity, network reorganization

The Editorial on the Research Topic

Principles Underlying Post-Stroke Recovery of Upper Extremity Sensorimotor Function – A Neuroimaging Perspective

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A substantial proportion of stroke survivors suffer from long-term sensorimotor deficits of the contralateral arm and hand (1). Neuroimaging, using a diversity of methods, has the potential to uncover underlying principles of functional disabilities and recovery characterizing patient groups as well as individual variability (2–6). The present issue aims at (i) revealing the physiological mechanisms and the long-term course of stroke recovery with respect to site and size of lesions, (ii) correlating behavioral deficits and electrophysiological parameters with imaging patterns, (iii) delineating neural networks involved, and (iv) identifying sites where interventions enhance the recovery process.

Seitz and Donnan give an overview of mechanisms and disease-related limitations in post-stroke recovery. They address two informative subsections delineating time courses of the recovery process and state-of-the-art of neurorehabilitative training to improve the stroke-induced neurological deficit.

Auriat et al. complete this clinical perspective with an overview on the use of transcranial magnetic stimulation and multimodal neuroimaging to estimate functional resources post-stroke. They provide a review of data from studies utilizing DTI, MRS, fMRI, EEG, and brain stimulation techniques, focusing on TMS and its combination with uni- and multimodal neuroimaging methods with respect to their benefits and limitations.

Falcon et al. used “The Virtual Brain (TVB),” an open source platform based on local biophysical models. Using this platform, they simulated individuals’ brain activity linking structural data directly to a TVB model. Correlating TVB parameters with graph analysis metrics, they obtained evidence for a shift of global to local dynamics in chronic stroke patients.

Buetefisch reviews the role of an intact contralateral motor cortex (M1) in post-stroke recovery of upper extremity motor function. The impact of the contralateral M1, on the lesioned motor cortex, seems to be promoting activity in the acute and inhibiting it in the chronic stage. Supportive evidence comes from animal studies, including changes in neurotransmitter systems, dendritic growth, and synapse formation. Thus, the contralateral M1 may represent a treatment target during rehabilitation.

Sharma and Baron report an fMRI study of a finger-thumb opposition sequence in chronic, well-recovered subcortical stroke patients. Using independent component analysis, they could show that recovery of motor function involved pre-existing cortical networks contributing to recovery in a differentiated manner.

The study of Abela et al. complements these investigations of functional networks associated with recovery in the case of cortical sensorimotor stroke. The structural covariance network in patients recovering from hand paresis encompassed (i) a cortico-striato-thalamic loop involved in motor execution and (ii) higher order sensorimotor cortices affected by the stroke lesions. The network emerged in the early chronic stage post-stroke was related to gray matter volume increases in the ipsilesional mediodorsal thalamus, and its expression depend on an interaction of recovered hand function and the lesion size.

Bannister et al. report about neuroimaging evidence for the significance of the contralesional hemisphere in the recovery process after hemispheric supratentorial ischemic stroke, thus supplementing the review of Buetefisch. They followed the time course of touch sensation in the upper extremity using resting state - fMRI to explore functional connectivity. Improvement of touch sensation was related to changes in the contralesional hemisphere and cerebellum: (1) an increase in connectivity strength between the secondary somatosensory area seed and both inferior parietal cortex and middle temporal gyrus as well as the thalamus seed and cerebellum and (2) a decrease in connectivity strength between SI seed and the cerebellum.

Primaßin et al. deal with four exemplary cases in which motor and language domains were affected differently. They focused on dissociative outcomes after 7 weeks of rehabilitative treatment following the predominant failure at baseline. Primarily, precise location of the lesions in the corticospinal tract and/or fasciculus arcuatus, respectively, turned out to be critical for recovery. Motor and language improvement seemed to occur together, rather than to compete for recovery resources.

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Ben-Shabat et al. investigated changes in human proprioception, its specific brain activation, laterality, and changes following stroke. Brain activation involved the supramarginal gyrus (SMG) and dorsal premotor cortex (PMd) with a prominent lateralization in the former. Lateralization was diminished in three patients exhibiting proprioceptive deficits post-stroke and a common lesion within the thalamus. The findings underline the role of SMG and dPM in spatial processing and motor control.

Brugge et al. investigated the intriguing role of supplementary motor complex (SMC) and disturbed motor control, a retrospective clinical and lesion analysis of 10 patients presenting anterior cerebral artery stroke. In the very acute phase, alien hand syndrome (AHS) dominated accompanied by failed conscious awareness of motor intention and a missing sense of agency while performing externally triggered movements. In the follow-up, motor signs specifically related to AHS, i.e., disturbed self-initiated movements, grasping, and intermanual conflict, were mainly related to lesions of the pre-supplementary motor area and medial cingulate cortex.

Camilleri et al. studied the neural substrate underlying the performance of the trail making test (TMT) that is often used in the follow-up of stroke. In healthy volunteers, they found that performance in terms of motor speed to be related to the local brain volume of a region in the lower bank of the left inferior sulcus. Conjunction analysis of four connectivity approaches has shown this area to represent a constituent of the so-called multiple demand network, highlighting the TMT as related rather to executive than primary motor function.

In summary, the neurological deficits, recovery mechanisms, and the prognosis for recovery after stroke are hot spots of clinical neurology and systems neuroscience research. Multimodal imaging, applied neurophysiology, and careful neurobehavioral *in vivo* correlations have opened new vistas on the pathophysiological mechanisms underlying post-stroke recovery of upper extremity sensorimotor deficits paving new avenues for future research.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Recovery Potential After Acute Stroke

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In acute stroke, the major factor for recovery is the early use of thrombolysis aimed at arterial recanalization and reperfusion of ischemic brain tissue. Subsequently, neurorehabilitative training critically improves clinical recovery due to augmentation of postlesional plasticity. Neuroimaging and electrophysiology studies have revealed that the location and volume of the stroke lesion, the affection of nerve fiber tracts, as well as functional and structural changes in the perilesional tissue and in large-scale bihemispheric networks are relevant biomarkers of post-stroke recovery. However, associated disorders, such as mood disorders, epilepsy, and neurodegenerative diseases, may induce secondary cerebral changes or aggravate the functional deficits and, thereby, compromise the potential for recovery.

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INTRODUCTION

Stroke is one of the leading causes of persistent disability in Western countries (1). It induces acute deficits of motion, sensation, cognition, and emotion. In the majority of patients, stroke results from an interruption of cerebral blood supply and subsequent ischemic brain damage, while >25% of patients suffer from intracranial hemorrhage (2, 3). Recovery from stroke is a multifaceted process depending on different mechanisms that become operational at different phases after the acute insult ranging from hours to many months (4). Importantly, intravenous and intra-arterial thrombolyses have opened new avenues to substantially reverse the amount of brain damage and the neurological deficit after stroke (5–8). Furthermore, neuroscience-based strategies in neurorehabilitation have improved the fate of stroke patients. Specifically, training approaches including very early mobilization, antigravity support for walking, basic arm training, and arm ability training can be tailored to the neurological deficits to optimally engage the residual capacities of the patients (9–11). From a technical point of view, neuroimaging and neurophysiological methods have offered means to investigate the recovery potential of stroke patients already in the acute stage of stroke (12–14). In particular, these non-invasive neuroscientific measures substantiate clinical observations and have opened new insights into the neuroscientific basis of recovery mechanisms from stroke. More recently, the recovery potential after stroke has been studied by using multivariate analyses in which epidemiological factors have also been taken into account (15). We address here the mechanisms of post-stroke recovery including postlesional plasticity and disease-related limitations of the recovery potential in acute ischemic stroke.

MECHANISMS OF POST-STROKE RECOVERY

Dynamics of Cerebral Ischemia

A sudden interruption of arterial blood supply leads to disturbances of neural function and the clinical appearance of neurological or neuropsychological deficits. In the most severe cases, ischemia is so severe that structural brain damage and the formation of ischemic brain infarction occur (**Figure 1**). The cessation of cerebral blood circulation induces an immediate suppression of cerebral electrical activity with peri-infarct depolarization leading to repeated episodes of metabolic stress (16, 17). There is good evidence from animal experiments that ischemic damage of neurons and brain tissue occurs in proportion to the reduction of regional cerebral blood flow (rCBF) (16). Thus, the acute occlusion of a cerebral artery, the thereby caused local depression of rCBF, and its subsequent electrical, metabolic, and ionic changes are critical factors determining the extent of a cerebral ischemic infarct (18). Imaging and neurophysiological studies in humans have shown that, similar to animal experiments, spreading depression occurs in severe ischemic stroke leading to progressive infarct expansion (19, 20).

After occlusion of a cerebral artery, an area of impaired perfusion surrounds an area with a complete cessation of perfusion whose extent is determined by the compensatory recruitment of arterial collaterals. In the area of misery perfusion, the so-called penumbra, the extraction of oxygen from blood into brain tissue is enhanced as was shown in stroke patients by multiparametric imaging with positron emission tomography (21, 22). The advent of magnetic resonance imaging (MRI) has allowed a spatial dimension to be introduced. It has been shown that the area of impaired perfusion typically exceeds the area of reduced extracellular water diffusion, thus signifying virtually reversible brain tissue damage due to ischemia (23–25). In fact, there is a good correspondence between the area with enhanced oxygen extraction and the perfusion–diffusion mismatch area in acute stroke (26, 27).

The area of reduced brain perfusion undergoes a dynamic lesion transformation within the first 24 h after onset of ischemia

(28–30). In a persisting arterial occlusion, the infarct lesion expands up to 24 h (31, 32). Beyond the acute time window of about 24 h, secondary changes including an early phase with vasogenic edema and a later phase with inflammatory infiltration evolve (33–35). Lymphocytes and macrophages have been shown to accumulate in the perivascular vicinity ~6 days after a cerebral infarction and are heterogeneously distributed within the infarct area (36). Due to their immunological competence, these cells are suited to augment the infarct lesion raising the interesting notion that immunosuppression may have a beneficial effect in acute stroke (37).

Reversal of Cerebral Ischemia

In acute ischemic stroke, intravenous thrombolysis is targeted toward the rescue of brain tissue by early recanalization of the occluded cerebral artery. It has been shown to be effective up to 4.5 h with maximal efficacy within the first 90 min after symptom onset (5, 6, 38). The beneficial role of early recanalization was demonstrated by functional brain imaging (39–42) and monitoring with transcranial Doppler sonography (43, 44). More recently, neuroradiological interventions with intra-arterial thrombolysis and/or thrombectomy have been shown to be at least as effective as intravenous thrombolysis even in distal carotid or proximal middle cerebral artery (MCA) occlusion (8). By multiparametric MRI, it became evident that brain tissue at the risk of ischemic damage can be salvaged by tissue reperfusion (**Figure 1**). Important factors determining the extent of a brain infarct are the severity and duration of ischemia, the dimension and composition of the causal arterial emboli, the anatomy and the vascular changes of the cerebral arteries, and the presence of diabetic hyperglycemia (29, 41, 45–47). In failed reperfusion, severe edema formation will develop that can hardly be limited pharmacologically. Thus, to rescue patients from malignant brain swelling after stroke craniectomy has been advocated as a symptomatic therapy which is a life-saving action but does not reduce the neurological deficit in patients older than 60 years (48).

Brain infarcts may result from cardiac or artery to artery embolism, from thrombotic occlusion of the small penetrating arteries complicating vessel hyalinosis or microatheroma (49, 50). While infarcts in the territory of the posterior cerebral artery (PCA) are typically embolic in origin affecting the entire supply area of the PCA (51), infarcts in the anterior cerebral artery (ACA) territory are usually of atherosclerotic origin and more variable in lesion pattern and neurological deficit (52). The situation is most complex in the MCA territory because of the arborization of the MCA, the large territory supplied by the artery, and the widespread anastomoses of the leptomeningeal arterial branches fed from the ACA or PCA. The poorer these collaterals are due to arterosclerotic changes in the intracranial arteries, the more severe is the initial ischemic event and the resulting stroke lesion (41, 53, 54).

The location and the volume of the cerebral infarct determine the neurological deficit in an individual patient as shown for sensorimotor as well as cognitive and emotional functions (55–61). Large brain infarcts involving subcortical white matter may affect multiple brain systems which may result in complex neurological syndromes, such as apraxia, neglect,

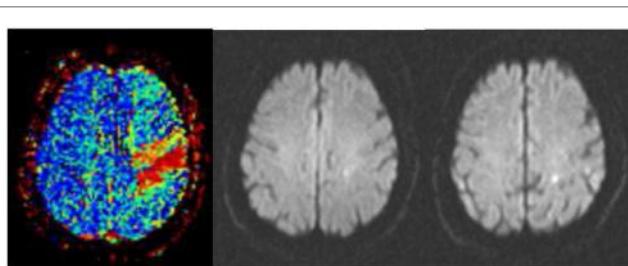


FIGURE 1 | Successful thrombolysis. (Left) Severe perfusion deficit in the precentral gyrus (red) as assessed in a time-to-peak map before thrombolysis. (Middle) Point-like abnormality in diffusion-weighted imaging at the same time signifying the perfusion–diffusion mismatch. (Right) Two small lesions in diffusion-weighted imaging 24 h after intravenous thrombolysis accompanied by complete recovery from hemiparesis.

and Gerstman's syndrome (62–64). In such patients, measures of fiber tract damage or cortical activations have been found to predict the degree of recovery (55, 65–68). Similar observations have also been made for language, somatosensory and visual functions (69–72).

Residual Brain Infarct Lesions After Thrombolysis

The successful recanalizing therapy is of fundamental importance for the topography and volume of the resulting ischemic infarct lesion (73, 74). This was taken into consideration in developing a refined classification of ischemic brain infarcts (75). It should be stated, however, that the functional prognosis of ischemic stroke is worse than that in cerebral hemorrhage in stroke survivors (76). This most likely reflects the structural damage of brain tissue in ischemic stroke, while in cerebral hemorrhage recovery can occur largely upon absorption of the hematoma. Accordingly, territorial Type I infarcts depend on the size of the emboli and the location of the arterial occlusion (Table 1). Distal arterial branch occlusion gives rise to small infarcts entirely limited to the cerebral cortex, while proximal arterial branch occlusions result in larger infarcts involving the cerebral cortex and the underlying

white matter (77, 78). In MCA stroke, these territorial infarcts do not destroy the entire motor and somatosensory representation areas, nor the complete descending motor cortical output or afferent sensory input tracts (55, 79, 80). This allows sufficient recovery potential associated with perilesional reorganization in the adjacent cerebral tissue in response to various neurorehabilitative approaches.

Ischemic lesions of large parts of or the entire striatocapsular region typically result from an embolic occlusion of the MCA stem (81) (Table 1). If reperfusion is achieved early, only the deep perforating arteries and the arteries that supply the insular cortex may remain obstructed causing infarcts of the lentiform nucleus and insula (82). However, when collaterals are insufficient due to arteriosclerotic changes in multiple cerebral arteries (41, 53, 54), the infarct lesions become larger involving to a larger extent also the hemispheric white matter. This causes hemispatial neglect and conduction aphasia due to cortico-cortical and cortico-subcortical disconnections (62, 83, 84).

Small-sized, lacunar-type, infarcts (Type III infarcts) result from an occlusion of the small penetrating cerebral arteries or even arterioles. They typically occur in the anterior choroidal artery, the deep perforating lenticular MCA branches, the thalamic branches of the PCA, or in brainstem structures and the pons (85, 86). In spite of their small spatial dimension, but due to their strategic location, they cause well-defined neurological syndromes, such as pure motor and pure sensory stroke (Table 1). These infarcts have a limited recovery potential as predicted by a loss of motor-evoked potentials and asymmetry of water diffusivity on MR imaging (55, 87, 88). The crucial role of the white matter for functional outcome becomes apparent from the observation that small infarcts in the precentral gyrus allow for profound motor recovery, whereas infarcts of similar volume in the periventricular white matter or the internal capsule may induce a severe and persistent hemiparesis (89, 90). Interestingly, white matter damage in stroke was found in a large genome-wide association study to be related to a mutation in chromosome 17 (91).

Patients with a chronic occlusion of extracranial cerebral arteries resulting from dissection or long-standing cerebrovascular disease constitute Type IV infarcts (Table 1). These patients may become symptomatic with transient ischemic attacks due to small embolic or hemodynamically induced watershed infarcts in cerebral white matter (92, 93). In these patients, blood flow depression induces a reactive vasodilatation of the intracranial blood vessels resulting in a severe delay in cerebral brain perfusion in the presence of an enhanced cerebral blood volume (94, 95).

Perilesional Plasticity

Ischemia and reperfusion evoke a large number of biochemical, metabolic, and immunological processes that evolve sequentially as identified in animal experiments (96). In addition, there are rapid changes in the expression of genes, neurotransmitters, such as glutamate and GABA, as well as neurotrophic mediators implicated as molecular substrates related to perilesional reorganization (21, 97–101). These biochemical changes are accompanied on the microscopical level by the growing of axons

TABLE 1 | Classification of ischemic brain infarcts.

Type	Infarct location	Pathogenesis	Response to thrombolysis
I	Territorial	Occlusion of cerebral artery branch	
I.1	Cortical	Distal branch	Early
I.2	Cortico-subcortical	Proximal branch	Limited
II	Striatocapsular	Occlusion of MCA stem	
II.1	± Insula	Infarct core	Early
II.2	+ Periventricular white matter	Large lesion	Limited
III		Lacunar hyalinosis of arterioles	Limited
III.1	Fiber tracts		
III.2	Internal capsule (anterior choroidal artery)		
III.3	Basal ganglia, lateral thalamus		
III.4	Medial and anterior thalamus (perforating branches of posterior cerebral artery)		
IV		Chronic hemodynamic deficit + downstream emboli	
IV.1	Cortico-subcortical	Extracranial artery occlusion ± intracranial large artery occlusion ± accompanied by reactive vasodilation	Limited
IV.2	Arterial borderzone	Extracranial artery occlusion	

Adapted from Seitz and Donnan (75).

and formation of new synapses in the perilesional vicinity and in remote locations in functionally related areas in the affected and contralesional “non-affected” hemisphere (102, 103). In particular, they occur when animals recover in an enriched environment or are subjected to dedicated training (104, 105).

Non-invasive brain stimulation techniques have provided means to explore changes of cortical excitability following stroke in humans. There are different technical approaches that allow to enhance or to suppress brain activity (106). By these methods, diagnostic and therapeutic goals were aimed for as summarized in **Table 2**. For example, using paired-pulse TMS, it was found that within the first 7 days after a brain infarct, there is an enhanced cortical excitability in the cortex adjacent to the brain lesion (107–109). In fact, the sites of residual motor representation move into the region of maximal cortical disinhibition (110). Also, fMRI activation areas related to finger movements were found to remap to spared more dorsal locations of the motor cortex (111, 112). Notably, an enhanced excitability was propagated to the contralesional hemisphere (14, 107–109, 113). It decreased in the patients who showed a good recovery within the 90 days, while it persisted in those patients with poor recovery (114). In keeping with these observations, functional MRI performed ~2 days after stroke revealed an area in the ipsilesional postcentral gyrus and posterior cingulate gyrus that correlated with motor recovery ~3 months after stroke (115). Conversely, recovery of hand function was associated with progressively lateralized activation of the affected sensorimotor cortex (116–118).

Non-invasive electrical anodal stimulation of the affected motor cortex was found to augment motor skill acquisition due to improved consolidation but not due to long-term retention of the task (120). In contrast, application of 1-Hz repetitive TMS (rTMS) that downregulates the contralesional motor cortex improved the kinematics of finger and grasp movements in the affected hand (121). This was accompanied by an overactivity in the contralesional motor and premotor cortical areas predicting improvement in movement kinematics. One may wonder if long-term retention of the induced effects can be achieved by longer lasting stimulation or by the combination of voluntary action and direct brain stimulation preferentially in the acute phase after stroke. The combination of electrical stimulation of finger extensor muscles and training over 2–3 weeks did not result in a greater improvement of dexterity of the affected hand as assessed with the Jebsen test than each intervention alone (122). Subjects with an intact motor cortex showed a greater improvement than

those who had damage of the motor cortex. Similarly, in chronic stroke-induced aphasia rTMS over the left inferior frontal gyrus resulted in an increase of reaction time or error rate in a semantic task suggesting restoration of a perilesional tissue in the left hemisphere after stroke (123, 124). Given the human postlesional changes of cortical excitability it may be intriguing to rebalance the interhemispheric rivalry by direct cortical stimulation or peripheral stimulation (125–128). An even greater effect was observed when bihemispheric direct cortical stimulation was used to activate the affected motor cortex and to inhibit the contralesional motor cortex (129). Cortical stimulation in association with motor training also improved motor performance (128, 130–132). Along the same line, combining peripheral nerve stimulation to the affected hand with anodal direct current stimulation of the affected motor cortex in chronic stroke facilitates motor performance beyond levels reached with either intervention alone (133).

Infarct Induced Damage to Cortico-Cortical and Cortico-Subcortical Connections

Corticospinal fibers are key factors for the recovery of motor function after stroke as demonstrated with different imaging modalities as well as electrophysiological measures (55, 87, 134–136). In non-human primates, the cortico-reticulo-spinal and cortico-rubro-spinal tracts are known to mediate motor functions in case of corticospinal tract lesions (137, 138), since these tracts have been described as functionally redundant in healthy animals (139). In humans, however the corticospinal tract is of key relevance for motor recovery (**Figure 2**). In fact, the integrity of the corticospinal tract determines the movement related motor cortex activation (65, 87). When there are no motor evoked potentials and there is poor recovery in chronic patients, the fractional anisotropy of the posterior part of the internal capsule as assessed by diffusion tensor imaging was altered in the affected hemisphere (68, 87). Notably, these patients had bilateral fMRI activations in relation to finger movements, while in the patients with a lower asymmetry, there was an activation lateralized to the affected hemisphere.

There are not only changes in the efferent motor fiber tracts but also in the cortico-cortical and probably also cortico-subcortical fiber tract systems during recovery. In fact, the intracortical excitability as assessed with TMS was increased in motor cortex of

TABLE 2 | Techniques, actions, and effects of non-invasive stimulation of the human brain.

Transcranial magnetic stimulation (TMS)				Transcranial electrical stimulation		
				Neuromodulatory effects		
Single pulse TMS	Paired-pulse TMS	Repetitive TMS	Patterned rTMS	Direct current stimulation tDCS	Alternating current stimulation	Random noise stimulation
	Intracortical (single coil)	1 Hz TMS (inhibitory)	Continuous theta-burst stimulation (inhibitory)	Cathodal tDCS		
	Cortico-cortical (two coils)	>4 Hz TMS (excitatory)	Intermittent theta-burst stimulation (excitatory)	Anodal tDCS		

After Liew et al. (119).

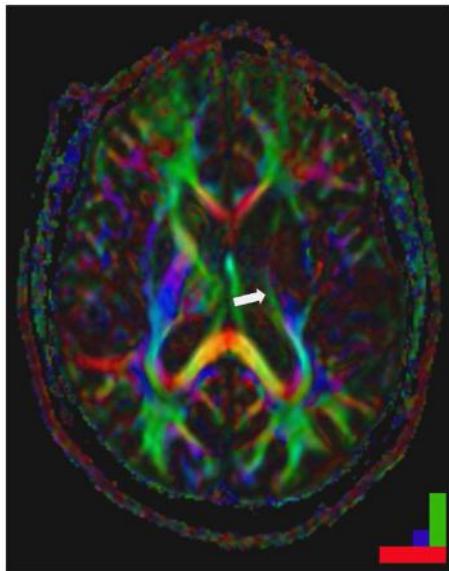


FIGURE 2 | Striatocapsular stroke (Type II.1) in a patient with persistent hemiplegia. Note the small but complete destruction of the posterior limb of the internal capsule (arrow). Color bar: green fronto-occipital diffusion, red right-left diffusion, blue dorso-ventral diffusion. By permission of Oxford University Press (URL www.oup.com), Free permission Author reusing own material, p. 82 fig: 6.4 (left part) from "Stroke Rehabilitation" edited by Carey and Leeanne (140).

both hemispheres both in subcortical and cortical infarcts (108, 114, 141, 142). Conversely, ipsilesional MEPs were more easily elicited from proximal muscles in stroke patients than in healthy subjects (143–145). Moreover, motor cortical connectivity was shown by diffusion tensor imaging to be enhanced after stroke (146). Additionally, orientation uncertainty and greater white matter complexity correlated with functional outcome and were possibly triggered by functional demands (146, 147). In addition, it was found recently that the pyramidal tract splits up in the pons forming a ventral and a dorsal tract. When both tracts are affected, patients have a poor recovery, while continuity of the projections in the dorsal portion was characterized by good recovery (136). In addition, in chronic stroke patients, DTI-derived measures of transcallosal motor fibers as well as ipsilesional corticospinal tracts pyramidal tract and alternate fiber tract determine the therapeutic response to rehabilitation. The more the diffusivity profiles resembled those observed in healthy subjects, the greater a patient's potential for functional recovery (88). These findings accord with the evidence from functional imaging suggesting that the concerted action of both cerebral hemispheres is required for recovery. This corresponds well to the observation that even patients with an excellent recovery may show a bilateral activation pattern (148, 149). This abnormal activity involved premotor cortical areas and was largely reminiscent of activity patterns in learning but are essentially transient in nature (84, 115, 149). Notably, tiny activation areas in contralateral motor cortex were related to mirror movements that frequently occur initially after stroke (150).

Network types of neuroimaging data analysis have revealed that there is a pathological interhemispheric interaction between the ipsi- and contralateral motor cortex as well as between the ipsilesional supplementary motor area (SMA) and contralateral motor cortex in patients with a single infarct lesion (151, 152). In unilateral movements of the affected hand, there was an inhibitory influence from the contralateral to the ipsilesional motor cortex which correlated with the degree of motor impairment (152). In bimanual movements, the interaction of the ipsilesional SMA and the contralateral motor cortex was reduced, and this correlated with impaired bimanual performance. This can be related to the observation that there was less activation in contralateral motor cortex when the motor task did not require working memory demands and no change when the task required online visual feedback monitoring (153). Furthermore, connectivity strength of the prefrontal cortex to the premotor cortex was enhanced in relation to motor imagery highlighting its role for higher order planning of movement (154).

DISEASE-RELATED LIMITATIONS OF THE RECOVERY POTENTIAL

Associated Diseases

It has been known for 30 years that patients with acute stroke may develop cognitive impairment and mood disorders which may aggravate their clinical conditions (155, 156). However, only recently it was shown in a large database of stroke patients subjected to systemic thrombolysis that the pre-existing functional impairment may reduce the patients' response to thrombolysis and the survival rate (157). In a prospective, open label study of 192 patients (68 ± 13 years, 50% males) subjected to intravenous thrombolysis the patients was found to improve ($P < 0.0001$), while 18% deceased within 100 days (158). This was predicted by older age (76 ± 10 years, $P < 0.05$) and more severe affection on admission ($P < 0.0001$). Also, these patients more frequently had atrial fibrillation ($P < 0.03$) than the surviving patients. Furthermore, it was found that stroke patients with a severe pre-stroke disability have a virtually 50% risk of deceasing. It seems that women are particularly liable of depression after stroke and that this is related to a greater stroke severity (159). Of note are patients with migraine that to a large proportion suffer from small vessel disease (160) or hemorrhagic stroke (161). This is of great functional relevance since white matter disease due to small vessel disease enhances the risk of depression, physical disability, and a reduction of quality of life (162). Furthermore, there is evidence from a huge meta-analysis that ischemic stroke is associated with the presence and subsequent development of dementia, particularly in recurring ischemic stroke (163). In addition, dementia was found to be associated with increased lethality (164). Interestingly, small vessel disease is the most frequent vascular abnormality in patients with Parkinson's disease (165, 166). These vascular changes seem to predispose patients with Parkinson's disease to cerebrovascular accidents (167). Arteriosclerosis was found to be of particular relevance for Parkinsonian gait, while macroscopic infarcts seem to result in rigidity (168). Moreover, infarcts induce epileptic seizures (169), which may mimic stroke as in

Todd's paresis and impair recovery due to reduced consciousness. Beyond that stroke may induce changes of affect including alexithymia (58) or depression (170). The latter was found to be most severe in chronic obstructive pulmonary disease, smoking, and in patients with poor socioeconomic status. Also the increasing lesion load with recurrent strokes in the elderly may predispose to depression (171) and death (172). Thus, there is an intimate interaction of stroke and comorbidities the latter of which impair the recovery potential of stroke patients. Deeper insight into the pathophysiology of these interactions is required to counteract these detrimental effects and to enhance the recovery potential of the multimorbid stroke patients.

Functional Deficits in Brain Infarcts

The neurological deficit has two expressions. There is the impairment to perform actions on command which is usually assessed in clinical examinations. And there is the decrease in spontaneous motor activity which may be functionally relevant (Figure 3). In a prospective study of 25 patients (63 ± 10 years) with acute MCA stroke and seven control patients without neurological disease (61 ± 14 years), movement activity was measured continuously

for 4 days in both arms using Actiwatches (Cambridge Research Instruments, UK). Stroke patients with an initial decline in arm movement activity showed no increase in movement activity in either arm over 4 days after stroke, while other patients improved steadily after admission. The impairment continued to be different among the two groups 3 months after stroke (173). Stroke severity, location and treatment, as well as arterial blood pressure and body temperature were not different among the groups. But, in the non-recovering patients, the C-reactive protein was elevated and related to a low number of waking hours. These results support the notion that in the acute stage after MCA stroke, there are patients with a secondary decline in general motor activity and an enhanced sleep demand which was related to systemic inflammation.

Moreover, recordings with the electroencephalogram (EEG) revealed that stroke patients may exhibit focal slow wave activity (SWA) as well as focal epileptic changes in the affected hemisphere (175–177). Focal SWA (1–4 Hz) has been reported to predict poor recovery from stroke (178–180) but can last even for years (181). Notably, EEG recordings have revealed that, in addition to their neurological deficit, stroke patients also have

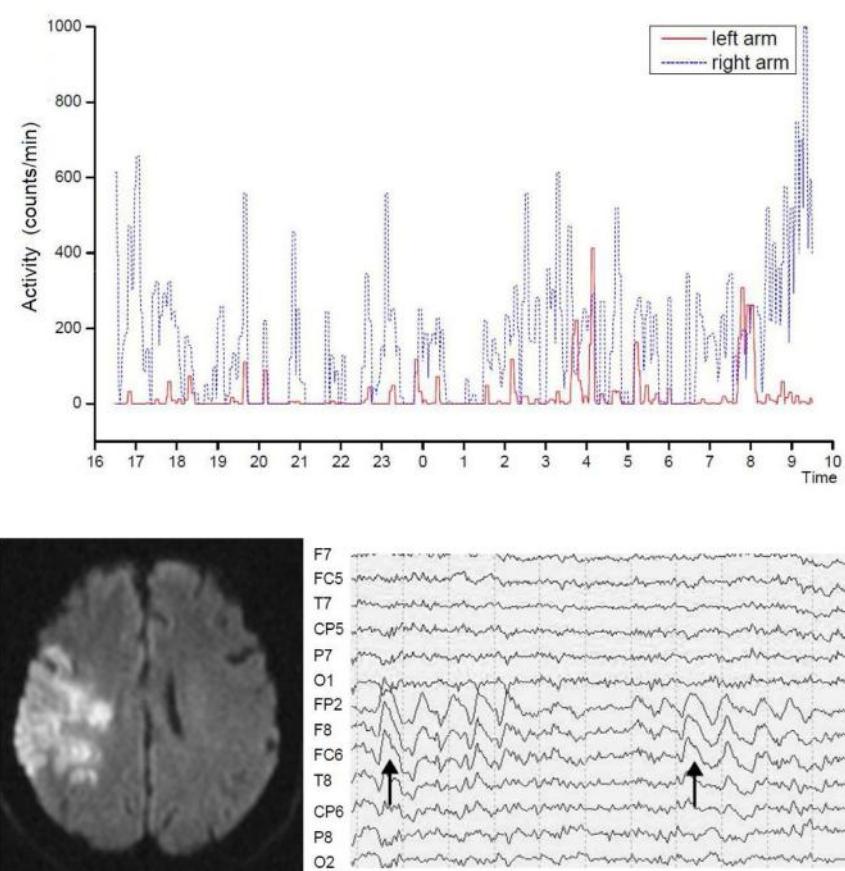


FIGURE 3 | Severely reduced spontaneous movement activity in the affected left arm in right hemispheric brain infarct. Shown is the recording time between 4 p.m. until 10 a.m. the following day. The intermittent slow wave activity in electroencephalographic recordings predicted poor motor recovery. Dotted lines indicate seconds. From Ruan and Seitz (174).

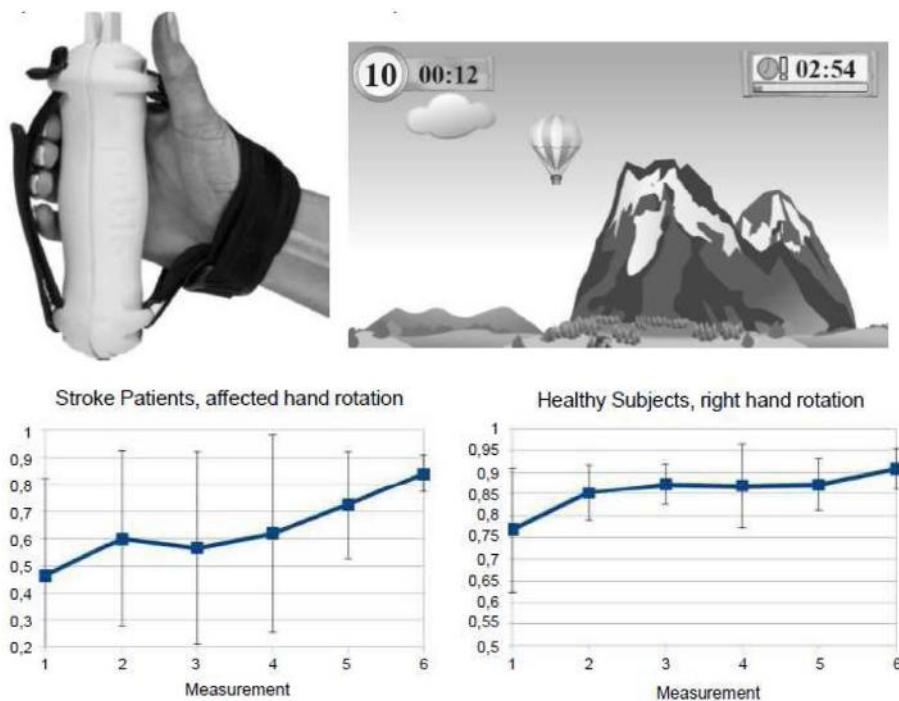


FIGURE 4 | Gaming-based training scenario using the commercially available hand hold PABLO^R-device. Hand movements are measured by acceleration and force sensors and thereby steer objects in virtual reality games. Training on consecutive days enlarged the angle of hand rotations and decreased the heterogeneity of movement execution both in healthy subjects and stroke patients. From Seitz et al. (213).

an abnormal sleep architecture (182, 183). It is unclear, however, what the functional impact of SWA is on spontaneous movement activity of the affected side after stroke. In fact, stroke patients with similar infarcts concerning lesion location and volume may show recovery patterns of the formal neurological assessment that are not reflected by the spontaneous movement activity of the affected limbs (184, 185). In acute stroke patients (68 ± 8 years) and age-matched controls (68 ± 12 years), movement activity was measured continuously and synchronously with the EEG for 24 h in both arms using actiwatches (174). The stroke patients had lower total sleep time ($P = 0.031$), sleep efficiency ($P = 0.019$), percent non-rapid eyement movement sleep ($P = 0.034$), and percent sleep stage N2 ($P = 0.003$) and showed reduced spontaneous movement activity in the affected arm during wakefulness. Stroke patients with abnormal focal SWA showed less spontaneous arm movement activity than those without SWA, while there were no differences in the sleep parameters (Figure 3). These findings accord with earlier observations by Bassetti and Aldrich (175) supporting the notion that sleep architecture is impaired in stroke patients leading to sleep fragmentation, increased wakefulness, and increased REM latency (186). Furthermore, the stroke patients with SWAs enjoyed a limited recovery as assessed with the NIHSS. Thus, focal SWA is a marker of profound brain pathology.

Times-Lines for Post-Stroke Recovery

The neurological deficits can regress substantially in the early period after ischemic stroke following acute stroke treatment

with arterial recanalization and effective reperfusion. The relatively early recovery in patients with small cortical lesions steadily evolves over weeks and levels out over the subsequent months (112, 187, 188). In contrast, the processes of cerebral reorganization are slow and may need many months to complete. In the acute phase of stroke, it is difficult to predict the degree of ultimate recovery, since there is a large heterogeneity of recovery over the first 3 months after stroke (12). Prediction becomes progressively better the more specific and differentiated the physiological assessment measures are and the longer the time since stroke (70, 189, 190). For example, the neurological state by day 4 predicts the long-term neurological outcome (188, 191). The recovery of activities of daily living usually develop within 26 weeks after the stroke insult and is often accompanied by compensatory hand use (192, 193).

Neurorehabilitative Training

There are numerous reports about rehabilitative approaches to improve the neurological deficit following stroke (4, 13). Notably, patients older than 65 years benefit as much as younger patients from intensive rehabilitation (190, 194), while younger patients typically improve more on mobility, balance, walking, and grip strength (195). The intensity of the training rather than the type of training appears to determine long-term improvement of motor function (113, 196–198). While passive training of wrist movements was reported to be clinically effective and associated with change in cortical activation (199), volitional control of finger and thumb extensions was found to play an important role

for successful hand shaping and grasping of objects (147, 214). Importantly, repetitive training of the affected arm resulted in an increase of activation in the sensorimotor cortex related to hand movements which initially persisted for weeks after training completion and then decreased in magnitude in relation to the functional gain (200, 201). In contrast, mirror therapy was found to improve the neurological status immediately after the intervention and to be effective even at long-term follow-up (202, 203).

Training of the affected limb as well as training targeting the non-affected limb has been proposed to be effective. For example, use of bilateral synergies has been reported to improve the motor capacity of the paretic arm (204). It was described that active–passive bilateral arm therapy can produce sustained improvements in upper limb motor function in chronic stroke patients. This was paralleled by an enhanced ipsilesional motor cortex excitability and an increased transcallosal inhibition from ipsilesional to contralesional motor cortex (205). Conversely, the concept of “learned non-use” was implemented in new approaches of rehabilitative strategies in chronic patients with brain infarction (206, 207). This therapy has been shown to be successful even when applied in the chronic state to moderately affected patients (65, 208, 209). This beneficial effect of constraint-induced movement therapy is likely to be composed of focusing the patient’s attention to the affected side and imposing repetitive training. It was shown

to result in improved motor function and enhanced activation in the partially damaged sensorimotor cortex and other gray matter areas including the hippocampus (210).

Recently, computer-based training approaches employing virtual reality scenarios have been developed for neurorehabilitative training purposes, since it was assumed that they engage the patients emotionally and thereby enhance their inclination to embrace rehabilitation training activities. For example, the rehabilitation gaming system (RGS) is a flexible, virtual reality-based device for rehabilitation of neurological patients (211). In fact, it was shown to effectively improve arm function in acute and chronic stroke patients. Furthermore, it was shown by fMRI that the RGS engages human mirror neuron mechanisms that underly visuomotor coordination (212). Similarly, the handheld multifunctional PABLO^R-device was applied for the training of visuomotor-tracking paradigms. It was observed that training of the right dominant hand improved visuomotor coordination of hand rotation movements in both hands in healthy subjects. Notably, it was successful only in the trained hand in stroke patients (**Figure 4**). Since these gaming applications capitalize on the positive affect of the patients and engage brain structures known to be related to emotional processing (212), these approaches point into new avenues of post-stroke rehabilitation opening new frames for the recovery potential after stroke.

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A review of transcranial magnetic stimulation and multimodal neuroimaging to characterize post-stroke neuroplasticity

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Following stroke, the brain undergoes various stages of recovery where the central nervous system can reorganize neural circuitry (neuroplasticity) both spontaneously and with the aid of behavioral rehabilitation and non-invasive brain stimulation. Multiple neuroimaging techniques can characterize common structural and functional stroke-related deficits, and importantly, help predict recovery of function. Diffusion tensor imaging (DTI) typically reveals increased overall diffusivity throughout the brain following stroke, and is capable of indexing the extent of white matter damage. Magnetic resonance spectroscopy (MRS) provides an index of metabolic changes in surviving neural tissue after stroke, serving as a marker of brain function. The neural correlates of altered brain activity after stroke have been demonstrated by abnormal activation of sensorimotor cortices during task performance, and at rest, using functional magnetic resonance imaging (fMRI). Electroencephalography (EEG) has been used to characterize motor dysfunction in terms of increased cortical amplitude in the sensorimotor regions when performing upper limb movement, indicating abnormally increased cognitive effort and planning in individuals with stroke. Transcranial magnetic stimulation (TMS) work reveals changes in ipsilesional and contralesional cortical excitability in the sensorimotor cortices. The severity of motor deficits indexed using TMS has been linked to the magnitude of activity imbalance between the sensorimotor cortices. In this paper, we will provide a narrative review of data from studies utilizing DTI, MRS, fMRI, EEG, and brain stimulation techniques focusing on TMS and its combination with uni- and multimodal neuroimaging methods to assess recovery after stroke. Approaches that delineate the best measures with which to predict or positively alter outcomes will be highlighted.

Keywords: multimodal neuroimaging, stroke, sensorimotor recovery, diffusion tensor imaging, magnetic resonance spectroscopy, functional MRI, electroencephalography, transcranial magnetic stimulation

INTRODUCTION

Recent advances in stroke treatment have stressed early intervention, greatly reducing the risk of mortality after stroke (1). Yet, development of treatments aimed at improving function after stroke has failed to keep pace, in part because rehabilitation specialists do not yet understand how to best help the brain recover from stroke. The importance of this issue is underscored by work from the Boyd Lab showing a *clinically meaningful decline* in population-based quality of life for Canadians with stroke from 1998 to 2005 (2). In this work, declines in health-related quality of life in the Canadian population were associated with increases in the proportion of individuals with impaired motor function post-stroke. Together the high incidence, increased survival rates, and decreased quality of life following stroke demonstrate a critical need for improved understanding of brain recovery after stroke.

Many have attempted to define the neural mechanisms of post-stroke impairment and recovery in the hope that understanding these processes will improve rehabilitation interventions and enhance function (**Figure 1**, Part I). Since the development of neuroimaging techniques, such as magnetic resonance imaging (MRI) and functional MRI (fMRI), it is possible to identify both structural and functional brain changes, termed neuroplasticity, as individuals with stroke re-learn motor skills. In addition, the use of transcranial magnetic stimulation (TMS) allows cortical excitability to be temporarily enhanced or reduced, which enables researchers to experimentally test the influence of specific brain regions on motor learning and recovery from stroke. To date, numerous studies show neuroplastic change after stroke by documenting recovery of function that is independent of spontaneous change associated with acute recovery (3, 4). Our work (3, 5–9) and that of others (10–12) clearly shows that motor learning and capacity for neuroplastic change (13, 14) are preserved, even during the chronic stage after stroke. Experience-dependent neuroplasticity likely explains a portion of the change associated with motor learning after stroke in this work (15), yet despite these advances in knowledge, no clear pattern of motor-related brain activation has emerged that fully explains how the brain compensates for stroke-related damage during motor learning.

In part, our failure to grasp how the damaged brain learns stems from an incomplete understanding of the relationships between behavior and brain function. Key to improving functional recovery after stroke is more fully understanding and mapping experience-dependent neuroplasticity (17), which demonstrates that the functional organization of the motor system can be modified by use. Technological advances have enabled detailed structural assessment of the brain with volumetric analysis of white and gray matter, the indexing of white matter connectivity using diffusion imaging, quantifying metabolic changes with magnetic resonance spectroscopy (MRS), mapping of brain activity with fMRI and electroencephalography (EEG), and assessing experience-dependent neuroplasticity through the manipulation of cortical excitability using repetitive TMS (rTMS). In this review, we highlight the use of these neuroimaging techniques to map the neuroplasticity of motor learning and sensorimotor recovery, as well as the advances in knowledge that have been stimulated

from their use. In combination, the knowledge gained from these approaches is contributing significantly to the genesis of novel, evidence-based interventions designed to promote functional recovery after stroke.

NEUROIMAGING

Structural Imaging

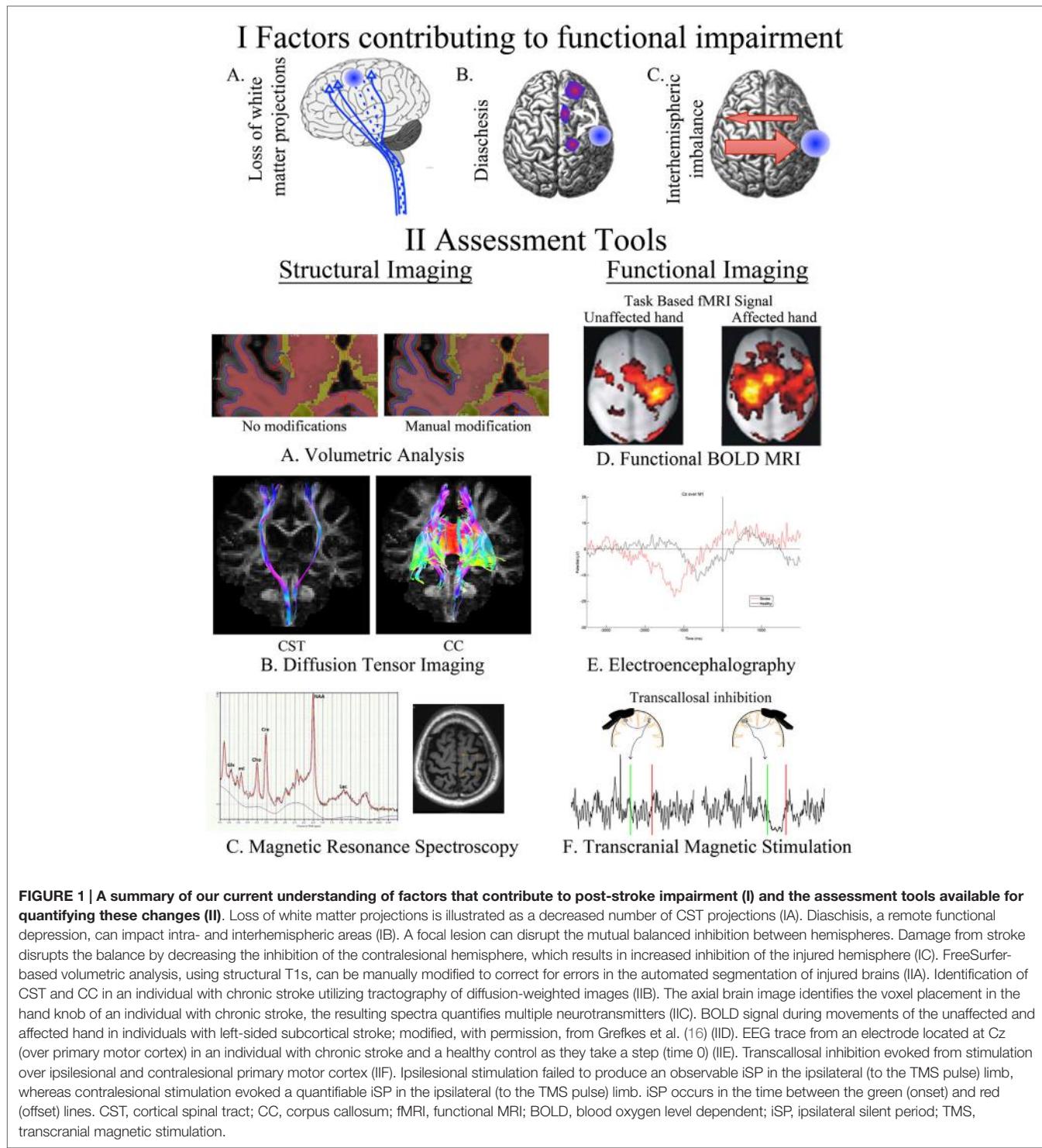
Volumetric Analysis

It has long been recognized that lesion location rather than size explains the bulk of neurological deficits after stroke (18). For instance, the degree damage to the cortical spinal tract (CST) rather than lesion volume correlates with motor ability after stroke (19). However, stroke-related damage also has effects on regions remote from the site of injury (20). The time point of assessment is important because of delayed atrophy in areas remote from the stroke (21). Advances in volumetric analysis of MRI have allowed for the automated quantification of brain volumes after segmentation into gray and white matter (22, 23) often using only an anatomical T1 scan (**Figure 1**, Section IIA). The quality of the scan influences the precision of segmentation and having additional scans, such as fluid-attenuated inversion recovery (FLAIR), T2, or proton density (PD), can improve accuracy and identification of subtle lesions (23). Unfortunately, difficulties arise when using these methods to quantify brains with a neurological pathology (24, 25). Caution must be taken to ensure programs designed to use anatomical landmarks to segment and quantify brain volumes are functioning as expected with analysis of chronic post-stroke brains, where landmarks may shift or be non-existent due to direct damage or atrophy. Recently, our group has utilized the FreeSurfer-based image analysis package (26, 27) for volumetric segmentation in chronic stroke and found that segmentation was unaffected by small subcortical lesions (24). However, participants with more extensive damage had to be excluded from the analysis due to segmentation errors. Alternative segmentation programs or using more extensive manual edits will allow for the inclusion of participants with larger lesions.

Volumetric analysis may be a valuable predictor of responders to post-stroke interventions (23, 28). Future use of volumetric analysis in rehabilitation studies will likely provide more useful information on the influence of structural integrity on post-stroke recovery. However, extreme caution and manual review/intervention of computerized assessments must be used to ensure accurate quantification of post-stroke brains (24, 25).

Diffusion-Weighted Imaging

Diffusion-weighted magnetic resonance imaging (DW-MRI) non-invasively provides information on white matter pathways in the human brain. Based on its ability to determine water diffusion characteristics, DW-MRI has been extensively used to identify the orientation and integrity of white matter after stroke, and to relate these measures to motor function [see Ref. (28) for review]. Brain regions, such as the corpus callosum (CC) (29, 30) and the corticospinal tract (CST) (29, 31–33), have been repeatedly studied and related to both motor function and functional potential (30, 31, 33). DW-MRI has been touted as a



promising tool for rehabilitation planning and prognosis after stroke (31), and may predict neural changes after motor learning. Importantly, preliminary studies have demonstrated that the integrity of CST (24, 34) and CC (35) influences the efficacy of rTMS, suggesting that DW-MRI can provide valuable information when selecting rTMS protocols and predicting the efficacy of an intervention.

The motion of water molecules is restricted based on its location and in white matter movement of water is restricted across the tracts, with a relatively greater freedom of movement parallel to the white matter fibers. It is this basic principle, which allows for DW-MRI to identify the diffusion characteristics of white matter and predict specific white matter pathways. Several diffusion-based measures have been related to post-stroke outcome,

primarily, fractional anisotropy (FA), apparent diffusion coefficient (ADC), axial diffusivity (AD), radial diffusivity (RD), number of tracts, and tract volume. FA is the most commonly reported DW-MRI measure, and indicates the degree of directionality within the tissue microstructure, which is determined by tissue features, such as axons, myelin, and microtubules. FA ranges from 0 (completely isotropic) to 1 (completely anisotropic); therefore, higher FA indicates greater directionality (36, 37). ADC, AD, and RD are all based on the eigenvalues of the apparent diffusion tensor [λ_1 , λ_2 , and λ_3 (38)]. AD is an indicator of water diffusion along the parallel, principal, direction of axonal water diffusion [$AD = \lambda_1$ (38)]. RD is an index of water diffusion perpendicular to the principal direction of water [$RD = \lambda_2 + \lambda_3/2$ (38)]. ADC is the mean value of eigenvalues of the apparent diffusion tensor [$ADC = \lambda_1 + \lambda_2 + \lambda_3/3$ (38)]. Tractography methods allow for the visualization of fiber architecture and also allow for the identification of fiber number and volume in pathways of interest to stroke recovery (**Figure 1**, Section IIB).

Although the reproducibility of tractography has been established in a stroke population (39, 40), different analysis methods can affect the interpretation of results (41). At present, no “gold standard” method for fiber tractography exists for *in vivo* application (42–44). For example, our group has recently found that diffusion tensor imaging (DTI) and constrained spherical deconvolution (CSD) methods produce significantly different results when applied to individuals with chronic stroke (41). Although DTI is the most commonly applied method of tractography analysis in stroke research, CSD analysis provided a stronger relationship between CST and CC white matter characteristics, and post-stroke outcome. Additionally, DTI-based tractography often fails to reconstruct fibers projecting to the lateral aspect of the cortex (41, 42). Lateral projections of the CST play a significant role in motor recovery after stroke (45), specifically fine motor control of the hand (46). The failure of DTI to detect these lateral projections likely hinders correlations between CST and CC diffusion measures and motor function. If DW-MRI tractography is to become a feasible tool for assessing prognosis, functional potential, or rehabilitation strategies, it is important that this technique be as sensitive and specific to actual white matter fiber architecture as possible. Inability to detect an intact CST or an under-estimation of the projection of fiber populations may undermine patients’ expected potential for recovery resulting in minimized rehabilitation efforts. Additional studies are needed to identify optimized tractography strategies for identifying the fiber projections important for stroke recovery.

In addition to tractography, several strategies are utilized to interpret the microstructural white matter information provided from DW-MRI. Many studies use a FA map to place a region of interest (ROI) over a section of white matter (29), or use tract-based spatial statistics (TBSS) to isolate specific regions of change (47). Each of these methods has been able to correlate FA and/or diffusion measures of the CST with sensorimotor function and impairment following stroke (29, 32, 48). Lindenberg et al. found a correlation between fiber number asymmetry (ipsilesional – contralateral/ipsilesional + contralateral) and motor outcome in chronic stroke (32). Cho et al. used DTI

tractography to classify CST integrity after corona radiata infarct (49) and intra-cerebral hemorrhage (50), and found a relationship between tract involvement and functional outcome. ADC of the CST appears to be elevated in the chronic stage of stroke (51, 52), and has been related to functional outcomes (28, 52). AD and RD have been less frequently reported after stroke. Nonetheless, studies in individuals with acute stroke found AD of the CST to be related to motor outcomes (53, 54). One study found increased RD in several regions, including the posterior CC, in acute stroke patients compared to controls; however, increased AD occurred only in the corona radiata (55). These results are consistent with the work by Lindenberg et al., who assessed individuals with chronic stroke in comparison to controls (30). Recent work has shown that ADC, AD, and RD are elevated in the ipsilesional CST and are related to motor outcome in individuals with chronic stroke (41).

Several studies have assessed the relationship between DW-MRI-based diffusion measures of the CC and post-stroke outcome. Recently, Takenobu et al. used a combination of voxel-based statistical tractography and a deterministic ROI-based approach to determine callosal FA in acute ischemic stroke patients (47). A significant positive correlation between FA values within a ROI placed in the callosal midbody and motor impairment was reported. Lindenberg and colleagues employed a probabilistic tractography method, identifying white matter tracts passing through contralateral primary motor cortex, and found that several DTI-based outcomes were related to baseline motor function and improvements in motor function after a 5-day intervention combining non-invasive brain stimulation and motor practice (30). Specifically, transcallosal FA was negatively correlated with baseline motor function, and both AD and RD were positively correlated with change in function between pre- and post-intervention assessments.

Together these findings indicate multiple measures of white matter microstructure of the CST and CC correlate with stroke outcome. It remains to be seen which diffusion measure(s) and method(s) will provide the most reliable indication of CST and CC function. Populations with stroke tend to have heterogeneous characteristics, such as, varied time since stroke onset, wide range of functional and cognitive impairments, and differences in lesion size and location. The contribution of these factors to white matter microstructure have not been comprehensively explored, and should be evaluated in future work to enhance the use of DW-MRI to predict stroke outcome and the response to interventions.

Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy allows for the non-invasive measurement of metabolites *in vivo*, within a defined region of tissue. H¹MRS uses resonance signals from hydrogen protons to quantify cerebral metabolites, which have different identifiable resonance signals (or peaks) in a static magnetic field, measured in parts per million (ppm). Thus, the magnitude of the peak resonance at the chemical shift point for each metabolite can be measured and a spectral map computed, providing information on the presence and concentration of metabolites within the target tissue [see Ref. (56) for review] (**Figure 1**, Section IIC).

The process of acquiring MRS data (shimming, water suppression, and phasing curve fitting) has now been automated and is available in programs, such as linear combination (LC) model (57) or magnetic resonance user interface (MRUI) (58).

The number of metabolites that can be differentiated in the MRS spectrum depends on the field strength of the MRI scanner (59, 60). With a 3T MRI, it is normally possible to obtain reliable peaks for six different metabolites: *N*-acetylaspartate (NAA), myo-inositol (mI), choline, creatine, glutamate, and lactate. Signals from the different peaks overlap making detection of less-abundant metabolites, such as gamma-aminobutyric acid (GABA), difficult without specific optimization of the MRS procedure that involves editing the spectra to obtain the GABA peak at the cost of losing information from other observable peaks (61). The physiological roles of the five identifiable metabolites are still under examination. The physiological role of NAA in the CNS is unclear; however, it is considered a marker of viable neurons. Lowered levels of NAA may indicate neural loss or death (62). mI is a cerebral osmolyte and occurs in astrocytes. It is considered a marker of glial cells and elevated mI is often considered a sign of gliosis or cytotoxic edema (63). mI is elevated in spared neural tissue in chronic stroke (64, 65). Choline represents the sum of four choline-containing compounds in the CNS, all of which are contained in cellular membranes; choline is considered a marker of cell membrane integrity. Elevated choline levels may indicate increased cell membrane turnover or demyelination (62, 66). Creatine also represents the sum of creatine-containing compounds, creatine and phosphocreatine, both of which are cellular energy reserves and are markers of energy metabolism in the brain (62). Creatine and choline levels are commonly believed to be stable across the brain and are often used to normalize levels of other cerebral metabolites; however, this may not be an appropriate approach in neuropathological conditions, such as stroke, as choline and creatine levels may be unstable after cerebral infarct (67). Glutamate represents the sum of glutamate and its precursor glutamine; it is not possible to differentiate these two compounds at 3T field strength (59). Glutamate is the principle excitatory neurotransmitter in the CNS and levels of glutamate may be of particular interest in indexing changes related to *N*-methyl-D-aspartate receptor (NMDAR)-mediated neuroplasticity, or glutamate excitotoxicity post-stroke.

Magnetic resonance spectroscopy has significant potential to act as an index of metabolic changes in surviving neural tissue after stroke (68). Thus, MRS has primarily been useful as a marker of neuronal loss or to indicate altered metabolic processes in penumbral tissue following infarction. NAA levels are reduced in areas of cerebral infarct (69), consistent with neural death, and appear to reduce further from acute to chronic stroke, perhaps indicating neuronal loss by diaschisis (70). Combining lactate peaks with NAA data provides useful predictive information about the viability of peri-infarct tissue in acute stroke (71–73). MRS has been less utilized in evaluating sensorimotor outcomes in chronic stroke, though analyses of spared ipsilesional tissue have provided interesting insights into neural adaptations in motor networks following distal infarct. In individuals with subcortical stroke, there is lower NAA and higher mI in spared ipsilesional primary motor cortex (M1) (65, 74); this is consistent

with neuronal stress or atrophy as result of an infarct to the motor network. Lower NAA and higher mI have also been reported in the ipsilesional supplementary motor area (SMA) and premotor cortex, respectively (64). NAA levels in M1 and non-primary motor areas positively correlate to motor function in several reports (64, 74–76) suggesting motor outcomes after stroke rely in part on the integrity of surviving neural tissue. There have been fewer reports on neurotransmitter levels and functional outcomes after stroke. Cirstea et al. report levels of glutamate in ipsilesional M1 correlate with motor impairment, with higher levels of glutamate relating to better motor function, though glutamate was not significantly reduced in ipsilesional M1 compared to contralateral M1 (65). A recent study from Blicher et al., using optimized MRS protocols for detection of GABA, reveals GABA is reduced in ipsilesional M1 after stroke (77). Further, Blicher et al. report improvements in motor function in response to constraint-induced therapy (CIT) related to individual differences in GABA levels, with higher baseline GABA in ipsilesional M1 relating to greater improvements in motor function after CIT (77). Future studies linking MRS measures to functional outcomes are needed, particularly in relation to glutamate's potential role in motor adaptation after stroke. MRS provides significant potential benefit as a modality to link observations of changes in neural activity post-stroke from fMRI or TMS imaging with changes in metabolic function.

Magnetic resonance spectroscopy could also be a valuable tool to advance our understanding of the neurochemical effects of rTMS, and may be used as a predictive measure to identify responders from non-responders. It is thought that rTMS does not change NAA levels, instead it shifts neuronal metabolism and neurotransmitter levels (78). Studies examining the effects of rTMS on MRS measures have, thus far, largely been conducted on high-frequency rTMS over the dorsolateral prefrontal cortex (DLPFC) in the treatment of depression. These studies have begun to examine individual variability in pre-stimulation metabolite levels and how these relate to treatment response. Participants who responded to high-frequency rTMS over DLPFC for treatment of depression showed lower baseline levels of glutamate prior to rTMS stimulation, and greater increases in cortical glutamate in response to rTMS (79, 80), while non-responders showed a decrease in glutamate levels in response to stimulation (80). Therefore, response to rTMS appears to rely in part on baseline levels of glutamate in target brain regions. These studies were conducted on rTMS for depression, with different stimulation targets and network effects than rTMS for sensorimotor recovery. However, they highlight the potential value of MRS as an index of treatment response to stimulation for stroke patients.

There is scant research to date on MRS response to rTMS over sensorimotor regions as it relates to stroke recovery. To our knowledge, only one such study has been conducted, by Stagg et al., using GABA-optimized MRS in examination of the effects of continuous theta burst stimulation (cTBS) to M1 (81). The authors report cTBS, which has inhibitory effects on cortical circuitry, increases GABA levels without affecting glutamate levels in M1 (81). Individual baselines in GABA levels relate to improvement gains on upper limb motor function (77), and a study in healthy adults demonstrated that individuals with greater reductions in GABA levels after transcranial direct-current stimulation (tDCS)

showed improved motor learning and greater M1 activation in fMRI (82). It remains to be seen whether individuals with differing levels of baseline GABA following stroke show differing responses to rTMS protocols, this is an avenue that should be examined in future research. Not only would future MRS work expand our understanding of the neurobiological actions of rTMS, but it also could allow for improved understanding of baseline neurochemical characteristics that predict response to rTMS protocols, and thus more targeted individualized treatment approaches in stroke rehabilitation.

Functional Imaging

Functional MRI

Functional MRI measures changes in blood movement in the brain over time. This signal is the blood oxygen level dependent (BOLD) signal. The BOLD signal is an indirect measure of neural activity and reflects the amount of deoxyhemoglobin in a tissue. The amount of deoxyhemoglobin depends on the local rate of metabolism of oxygen, the volume of blood in the region, and the amount of blood flow in a region (83). As neural activity increases in a brain region, local oxygen metabolism, blood volume, and blood flow all increase together (83). When MRI measures the BOLD signal, there is a time delay between the neural event and the signal measurement. The “fast response” occurs 2–3 s after an event with the main BOLD signal recorded ~5 s later. In the literature, the BOLD signal is sometimes described as a “hemodynamic response.”

Measurement of BOLD signal can occur as the study participant is performing a task (**Figure 1**, Section IID), or while “resting” – the participant is typically asked to think of nothing in particular but to remain awake (84). After collecting the study data, it can be processed and analyzed in very similar ways. The difference is that some analysis techniques are designed for use with certain experiment types (i.e., for resting state). Resting-state fMRI is defined as the spontaneous low-frequency (<0.1 Hz) BOLD fluctuations with spatio-temporal correlations in networks (85). What the BOLD signal fluctuations mean is not yet clear, but increasing evidence suggests it does have a neural basis (85).

In the past, stroke rehabilitative research using neuroimaging focused on the analysis of local lesion-specific activity and subsequent impairments (86, 87). Limitations in computation and mathematical modeling restricted study to isolated brain regions, though clinically the effects of an isolated stroke can demonstrate large sensorimotor and cognitive effects in remote areas (88). Recent advancements in technological and scientific knowledge have allowed for broader study of brain activity upstream and downstream from the stroke lesion, namely network analysis. Network analysis allows for the study of potential widespread changes in neural activity after a focal lesion. Analyzing patterns of network activity can inform researchers and clinicians of the effect a lesion has on the output of brain activity and may indicate whether certain “compensatory” network patterns are better than others for producing functional motor performance. A recent review of network analysis demonstrated altered activity both adjacent to and distant from a stroke lesion, affecting both hemispheres, and a pattern of change in network activity linked

with motor impairments and recovery (89). Reorganization in the lesioned hemisphere includes interactions between the fronto-parietal regions and the primary motor cortex, which may suggest greater cortical control is needed for motor performance of the paretic upper extremity (89). These studies underline the ability of network analysis to determine connectivity patterns after a stroke, and its potential for determining the effectiveness of current rehabilitative therapies. If network analysis can link certain patterns of early post-stroke activity with better prognosis, it may have a role in informing the direction of future therapies.

Brain network activity after a stroke is commonly studied with task-based fMRI. The challenges with using fMRI in individuals after a stroke, is that the post-stroke motor impairments can make motor performance difficult often resulting in movement synergies (90), mirror movements (91), and head motion during an fMRI scan (92). If during an fMRI study, participants produce head movement beyond a few millimeters, move in synergies or produce mirror movements, the scan may be rendered useless. People who have sustained a severe stroke with resulting severe motor impairments are often not studied with task-based fMRI, as motor performance of even simple tasks are frequently not possible without assistance, though some studies attempt to overcome this limitation by studying passive movements (93, 94). Even those who have sustained a mild or moderate stroke may have difficulty performing common functional tasks, such as individuated finger movements, so researchers are limited to studying basic and simple motor tasks, limiting generalizability to other motor tasks. Imaging the brain during rest allows for the study of individuals with a wide range of post-stroke motor impairments, and permits the examination of network activity without the need for task performance. For these reasons, resting-state imaging is an attractive method for studying stroke network activity.

Resting-State fMRI

Resting-state fMRI can characterize functional deficits after a stroke and provide important predictive evidence that links brain behavior with functional sensorimotor recovery of the upper limb. After a cortical stroke, participants demonstrate increased network activity in the ipsilesional fronto-parietal cortex, bilateral thalamus and cerebellum, while contralesional M1 and occipital cortical activity are decreased compared with healthy controls (95). Furthermore, the functional connectivity of the ipsilesional M1 with the contralesional thalamus, SMA, and middle frontal gyrus during the acute stroke phase positively correlate with motor recovery after 6 months (95), suggesting that changes in upper extremity motor impairment can be predicted by alterations in resting-state activity. Recently, participants with impaired upper extremity function received 12 weeks of training with shoulder and elbow robotic rehabilitation (96). Resting-state fMRI and upper extremity motor impairment was assessed before and after training. Decreased impairment could be predicted from functional connectivity changes measured by resting-state fMRI. Resting-state fMRI can reveal disrupted functional connections within hours of stroke as well as during recovery. Individuals with ischemic stroke were scanned within 24 h, 1 week, and 3 months post-stroke (97). Within hours after

stroke, lower connectivity was found in individuals with motor deficits. Interestingly, connectivity was restored 1 week later in those with recovered hand function. However, residual decreased subcortical connectivity remained 3 months later, even in those individuals without remaining hand motor impairment. These findings indicate that though motor function improves for some individuals after stroke, resting-state fMRI may remain altered. Resting-state fMRI also allows for the analysis of multiple networks simultaneously. Recent work has proposed that disrupted whole brain connectivity in both the sensorimotor and dorsal attention network is closely linked with functional impairment more than the intra-hemispheric connectivity (98).

Task-Based fMRI

Task-based fMRI can inform the capacity of individuals to recover after stroke, specifically with regard to motor function and learning. fMRI studies have found that paretic hand movement early after stroke is linked to widespread bilateral activity within the motor system, with greater bilateral activity found in individuals with greater motor impairment (99). Research directed at understanding the function of this bilateral pattern of activity suggests that the surviving brain regions influence distant regions during movement (99). It is now known that brain regions that survive the initial stroke influence one another during movement, and that multiple brain regions and pathways participate in reorganization and functional recovery, such as the CST, brainstem pathways, interhemispheric connections (100). The contralesional hemisphere also provides support for paretic hand movements (100). Task-specific practice in individuals with chronic stroke facilitated motor learning and reduced the volume of contralesional cortical activity while using the paretic arm (101). Performing the learned task altered cortical activation by producing a more normalized contralateral pattern of brain activation, which suggests task-specific motor learning may be an important stimulant for neuroplastic change and can remediate maladaptive patterns of brain activity after stroke. Our group has found motor learning and overall improvements in motor control are associated with increased response in the prefrontal-based attentional network in individuals with chronic stroke (14). Additionally, evidence of plasticity is also noted for movement of the non-paretic arm; this activity is related to alterations in neural activation in areas anatomically and functionally connected to the lesion, implying an extensive bilateral network is involved (102).

Electroencephalography

Electroencephalography uses surface electrodes placed on the scalp to detect fluctuating electrical voltages, which result from the small electrical currents generated by active neurons (103). EEG recordings are mainly generated by pyramidal neurons in cortical layers III, V, and VI, with summation of cortical activity producing a voltage field that can be recorded on the scalp (103). EEG is used for diagnosis, prognosis, treatment monitoring, and clinical management in acute ischemic stroke (104). Additionally, in chronic stroke, the EEG signal can identify subtle changes in the brain that cannot be detected by clinical measures; further, quantification of the EEG signal before and after rehabilitation

interventions can assess neuroplasticity both locally surrounding the lesion and within whole brain networks (105).

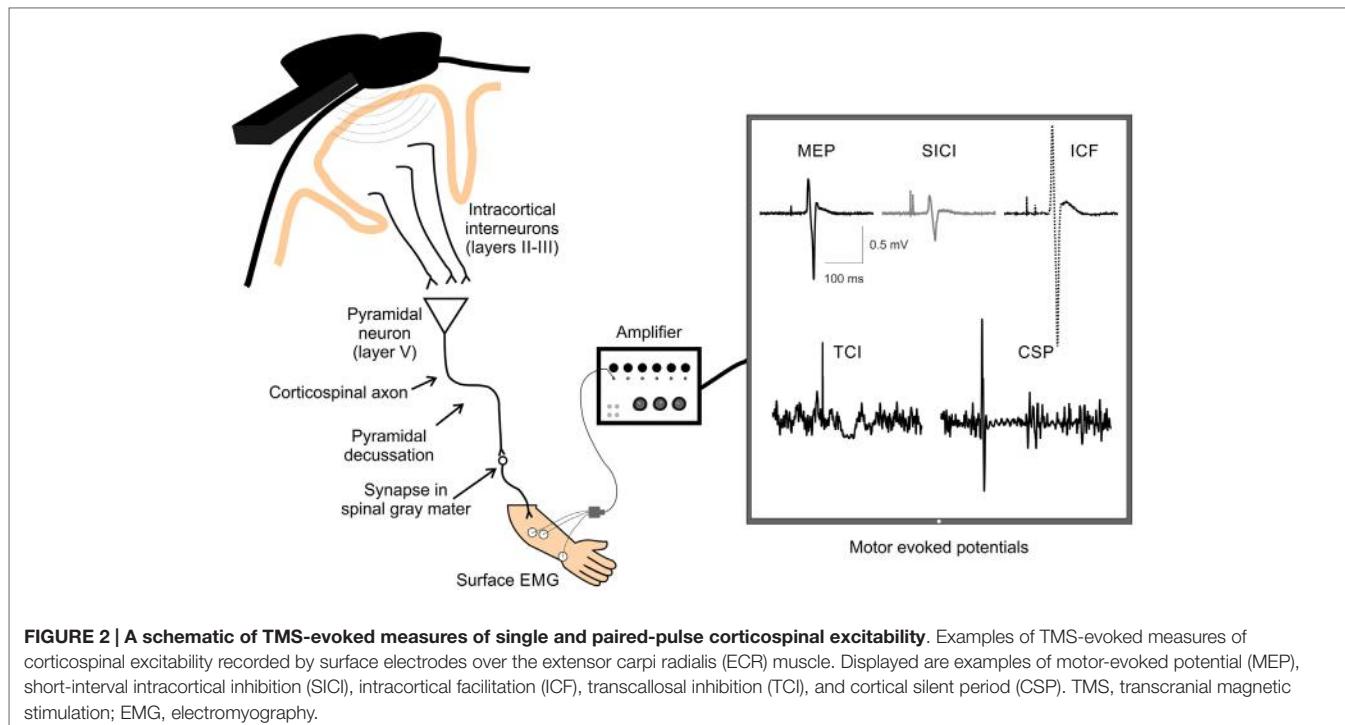
For EEG, resting-state activity can provide valuable predictive information regarding network activity after a stroke, but has limitations with regard to spatially localizing the sources, or regions of interest, within the network. Stroke can affect the synchrony of electrical oscillations in neural networks and these changes in network coherence can be associated with neurological deficits. In individuals with sub-acute stroke, functional connectivity of resting-state EEG correlated with motor performance. Individuals with stroke presented with disrupted alpha band connectivity where the spatial distribution of alpha activity reflected the pattern of motor and cognitive deficits of the individual participant (106). Even 1 month after stroke, measures of delta and alpha power were correlated with stroke severity scores (107). Focal brain lesions affect functional brain networks. In individuals 3 months after ischemic stroke, the synchrony of alpha band oscillations decreased between affected brain regions with the rest of the brain and this decrease was related to cognitive and motor deficits (108). Resting-state EEG can measure the synchronization of neuronal firing, and this can occur in the form of phase coupling or amplitude correlation. Behavioral performance after a stroke can be predicted by two distinct resting-state EEG coupling patterns: (1) amplitude of beta activity between homologous regions and (2) the lagged phase synchronization in EEG alpha activity from one brain region to rest of the cortex (109). A disruption of these coupling patterns is found to be associated with neurological deficits in individuals with stroke (109). Robot-aided rehabilitation programs are a relatively new and promising therapy, promoting brain plasticity and supporting improvements in upper extremity motor control. In a pilot study of seven individuals with stroke, 12 weeks of robotic rehabilitation decreased upper limb impairment and changed brain connectivity as indicated by altered coherence in the high beta band (24–33 Hz) (110). These studies demonstrate the ability of EEG to provide information about the patterns of impairment and recovery after stroke.

Transcranial Magnetic Stimulation

Transcranial magnetic stimulation is a useful way to non-invasively measure and modulate cortical excitability. TMS activates neurons in the cortex under the coil, which at high enough intensities transsynaptically depolarizes corticospinal output neurons. The corticospinal volleys activated by TMS reach the target muscle and can be recorded by surface electromyography (EMG) (111). Multiple single and paired-pulse techniques can be used to index neuroplasticity, providing useful information about how stroke and subsequent interventions modify brain function (see **Figure 2** for overview).

Single Pulse

Motor Thresholds In order to account for individual responses to TMS across individuals, a standardized motor threshold value is determined. Resting motor threshold is most commonly defined as the lowest percent of stimulator output that is required to produce a motor-evoked potential (MEP) with a peak-to-peak amplitude of 50 μ V on five out of 10 trials while the individual is at rest (112). Similarly, active motor threshold is defined as the



lowest percent of stimulator output that is required to produce an MEP with a peak-to-peak amplitude of 200 μ V on five out of 10 trials while the individual maintains a light background contraction (113). Threshold values are often used to determine the stimulation intensity to use in the assessment and modulation of cortical excitability with TMS techniques.

MEP Input–Output Curves Motor-evoked potential input–output (IO) curves utilize single-pulse TMS over a range of intensities to measure the increase in excitability within the corticospinal system in response to increased stimulus intensity, as indexed by MEP amplitude (114, 115). The linear slope of the curve (115) or area under the curve (116) produced by increasing stimulator intensity is quantified as a representation of the ability of the excitability of the M1 representation to be up-regulated, and the strength of the corticospinal connections. MEP IO curves can be measured while the participant is at rest, or during a sustained contraction. Resting MEP IO curves activate lower threshold neurons, while active MEP IO curves utilize the voluntary contraction to activate higher threshold neurons, thus stimulating unique neuronal pools, which may have different functional significance (117).

M1 Cortical Mapping Single-pulse TMS can also be utilized to probe the excitability of M1 in terms of quantifying the distribution and amplitudes of MEPs in the target muscle(s). TMS mapping of M1 follows the principles of motor homunculus (118) where stimulation of different motor regions produces systematic responses in the corresponding peripheral musculature. The amplitudes and distribution of MEPs when different scalp sites are systematically stimulated can be analyzed and displayed as

topographical maps showing the greatest activity produced from a corresponding scalp location over M1. Mapping the M1 representation of particular muscles is used to understand the healthy and pathological cortex, as well as to map change in neuronal representation of muscle groups over time or following an intervention (119–129).

Silent Period When single-pulse TMS is applied while holding a slight contraction in the contralateral limb, a cortical silent period (CSP) is produced, which presents a prolonged reduction in EMG activity following the MEP (130–132). The CSP originates largely from activation of inhibitory cortical and spinal interneurons, and there is evidence that the latter half of the CSP is associated with GABA_B-like activity at the cortical level (133). Therefore, single-pulse TMS can be indicative not only of motor cortical excitability, or increases in corticospinal tract excitability in response to increasing stimulator output, but also inhibitory circuit activity within the corticospinal system.

Transcallosal inhibition (TCI), important in interhemispheric communication, can be quantified via an ipsilateral silent period (iSP) derived from single-pulse TMS (134, 135). Specifically, during a sustained unilateral muscle contraction, a single TMS pulse over the ipsilateral M1 is delivered to evoke a reduction in the background EMG activity in the ipsilateral muscle, known as the iSP. Since the iSP is diminished or absent in patients with lesions of the corpus callosum (135, 136), it is likely a result of inhibition via transcallosal projections.

Paired Pulse

Intracortical Inhibition and Facilitation The excitation of M1 pyramidal neurons that ultimately translates into corticospinal

output to target muscles is also influenced by intracortical circuitry within the motor cortex. Inhibitory intracortical circuitry within M1 influences corticospinal output, and can be quantified using TMS. Specifically, short-interval intracortical inhibition (SICI) and long-interval intracortical inhibition (LICI), quantify inhibitory circuitry. SICI is produced when two TMS pulses (a subthreshold conditioning stimulus followed by a suprathreshold test stimulus) are administered over M1 with an interstimulus interval (ISI) of 1–6 ms and results in a decreased MEP amplitude than that elicited by a single TMS pulse alone (137). Intracortical facilitation (ICF), from 10 to 15 ms after the stimulation, measures the facilitatory circuits in M1. The protocol for measuring ICF is identical to that with SICI (subthreshold conditioning stimulus and suprathreshold test stimulus), with only the ISI differing. At longer ISIs of 50–200 ms, there is again a period where inhibition is produced due to paired-pulse TMS called LICI (138, 139). Unlike SICI, LICI is evoked with two identical suprathreshold pulses. SICI is likely mediated by GABA-A (140) and LICI by GABA-B (133, 141–143) receptor-mediated circuitry, due to the differences in the time course of activation of the respective circuitry (**Figure 1**, Section II F). ICF appears to be mediated by different neural circuitry than SICI (144), and glutamate may play a role in mediating ICF (145). Assessing these inhibitory and facilitatory circuits is an important component of understanding how neuroplastic change may be mediated and underlies associated behavioral changes, functional improvement, and assessment of neurological injury (i.e., stroke).

Short-Afferent Inhibition and Long-Afferent Inhibition Measures of short (SAI) and long-afferent inhibition (LAI) use single-pulse TMS in conjunction with peripheral nerve stimulation to examine the integration of sensory information into the motor output system. Specifically, an electrical stimulation is delivered at the contralateral median nerve prior to a TMS pulse delivered over M1 while the participant is at rest, which results in a reduced MEP relative to a single pulse alone. SAI applies this technique with an ISI of 20 ms and LAI utilizes an ISI of 200 ms (130, 146–150). SAI provides only enough time for activation of the primary somatosensory cortex and secondary somatosensory cortex, whereas LAI is long enough to ensure activation of primary somatosensory cortex, bilateral secondary somatosensory cortex, and contralateral posterior parietal cortex (130). While the mechanisms underlying both SAI and LAI have not been described, they provide information on the impact of peripheral nerve stimulation on M1 excitability, which is an important component to consider when studying sensorimotor integration in regards to neuroplasticity and neurological injury.

TMS Assessment of Cortical Excitability and Connectivity in Stroke Several methods of TMS assessment have shown that there is altered brain excitability and connectivity during all phases post-stroke (acute, sub-acute, and chronic). In approximately the first week after stroke, the ability to elicit MEPs in the paretic limb after single-pulse stimulation over the ipsilesional hemisphere predicts good recovery (151–156). A lack of elicited MEPs in the paretic limb along with increased MEP amplitudes in the non-paretic limb after contralateral stimulation predicts

poor motor recovery (31, 157), although this is not always the case (151, 158, 159). The appearance of MEPs where there were none before and improvement of TMS measures of corticospinal integrity during the first few months of recovery (160–162), both correlate with better functional outcome. An imbalance of motor cortex excitability (decrease lesioned cortex excitability and overly increased excitability of contralesional cortex) occurs following severe stroke and a restoration of balance is associated with functional recovery (151, 157, 162, 163). Several studies utilizing motor cortical mapping have shown that there are a decreased number of excitable scalp sites over the ipsilesional compared to contralesional cortex (160, 164–168), which has been suggested to indicate a hemispheric imbalance between the cortices that accompanies motor impairment of the more affected limb.

After stroke, measures of intracortical inhibition and excitation within the ipsilesional hemisphere are altered. There is increased inhibition as measured by a prolonged CSP after subcortical stroke (169). Conversely, SICI and LICI are suppressed (158, 170, 171), and ICF remains within normal ranges (172–174). Recent reports have shown that SAI is reduced in the acute phase of stroke, where increased suppression of SAI has been correlated with better motor function 6 months after stroke (175). In the contralesional hemisphere, motor thresholds and MEP amplitudes remain generally normal (151, 162, 173, 176–181), but SICI is suppressed in some (158, 172, 173, 177).

The connectivity between hemispheres is also altered following stroke, showing asymmetric transcallosal interactions. Several studies show that ipsilesional M1 generates less TCI than usual (177, 182), and contralesional M1 continues to demonstrate normal, or even increased, levels of interhemispheric inhibition (IHI) (183, 184). The net result is increased inhibition acting on ipsilesional M1 (183) that can depress ipsilesional M1 excitability. These changes may interfere with neuroplasticity in ipsilesional cortex (4, 185, 186), as increased IHI from contralesional M1 onto ipsilesional M1 reduces excitability in neurons that survived the stroke (177, 187) and is associated with more severe functional deficits (183, 184). Additionally, work from our group with chronic stroke participants has found increased TCI from the ipsilesional to contralesional M1 while maintaining a contraction, suggesting greater inhibitory signals sent from the ipsilesional to contralesional M1 (188). Further, we have recently shown that contralesional TCI was negatively correlated with hemiparetic arm function and impairment, demonstrating decreased inhibition from the contralesional to ipsilesional hemisphere is associated with greater impairment (189). Therefore, bilateral alterations in cortical excitability and circuitry are associated with the degree of motor impairment and post-stroke recovery.

Modulation of Cortical Excitability with Repetitive TMS

Repetitive TMS can be applied in specific patterns to uniquely modulate cortical excitability; the effects of rTMS may last for periods of time exceeding that of stimulus application, from minutes to an hour beyond stimulation (190–192). Therefore, rTMS can be used to index neuroplasticity or enhance cortical excitability before a behavioral intervention, such as skilled motor practice (193, 194).

Repetitive TMS, when applied in specific patterns, can excite or inhibit a local cortical region for a short duration. rTMS can be applied at low frequencies of under 1 Hz that suppresses excitability in the targeted area, or at high frequencies over 1 Hz, which transiently excites the targeted area for ~15 min (195). Similarly, theta burst stimulation (TBS) uses a 5-Hz stimulation pattern, with triplets of 20 Hz stimulation, to inhibit or facilitate cortical excitability if the TBS is applied continuously (inhibitory cTBS), or intermittently (facilitatory iTBS), respectively (190). The effects of cTBS and iTBS can last up to 60 min post-stimulation (190, 191). Importantly, the specific effects of cTBS and iTBS show substantial inter-individual variability, which likely depends upon which interneuron populations are activated by the TMS pulse (196). rTMS protocols, like TBS, have been shown to modulate cortical excitability, and at times behavior, when applied over motor-related areas, such as M1 (190), contralateral M1 (197, 198), the SMA (199), the dorsal premotor cortex (PMd) (200), the primary somatosensory cortex (S1) (194), area 5 (201), as well as non-motor areas, such as the cerebellum (202) and the DLPFC (203). Not only does rTMS modulate cortical activity directly below the magnetic coil, but activity in remote cortical and subcortical regions can be modified by application of rTMS over a single cortical target (204). Specifically, changes in MRI activity can be detected in M1/S1, SMA, PMd, cingulate motor area, the putamen, and thalamus after rTMS over left hemisphere M1 or S1 (204). These methods for modulating cortical excitability are thought to mimic early stages of long-term potentiation (LTP) or long-term depression (LTD)-like mechanisms, and are proposed to be dependent upon NMDA receptors (205). Due to the ability to modulate cortical excitability in motor and non-motor-related cortical areas beyond the time of stimulation itself (206), rTMS has been utilized by researchers to developed protocols to test whether the application of stimulation alone, or in conjunction with other behavior and therapy can further rehabilitation from neurological impairment, such as stroke.

Repetitive Brain Stimulation as an Intervention After Stroke
Since rTMS is known to modulate cortical excitability in local and remote regions to the areas stimulated, it has been suggested to be a viable therapeutic approach to aid in the recovery of motor function after stroke (207), yet there is accumulating evidence that the response to rTMS is inconsistent and variable (34, 193, 194). When targeting stimulation over M1, rTMS has been delivered in isolation (34, 208–210) and in combination with rehabilitation training (193, 194, 211, 212) in individuals with stroke. Since the effects of rTMS can outlast the period of stimulation itself (190, 206), the prevailing thought is that the aftereffects may be capitalized on by pairing it with skilled motor practice and/or rehabilitation training to promote neuroplastic change (193, 194, 213).

Theoretically, rTMS can be used to increase cortical excitability in the ipsilesional cortex by directly applying excitatory rTMS over the ipsilesional hemisphere (**Figure 3**) or by applying inhibitory rTMS over the contralateral to potentially decrease abnormally increased inhibition to the lesioned M1 (**Figure 4**). This manipulation of cortical excitability is supported by observations of imbalanced IHI after stroke (214). Impaired motor

performance following stroke is often attributed to a disruption in IHI where an overactive contralesional area suppresses the activity of the lesioned hemisphere.

Repetitive Brain Stimulation as an Intervention After Stroke: Ipsilesional Stimulation

Studies have shown promising preliminary findings using high-frequency excitatory (>1 Hz) rTMS applied over the ipsilesional hemisphere. One study showed that 3-Hz rTMS over the ipsilesional hemisphere for 10 days combined with passive limb manipulation, which gradually increased to active manipulation of the paretic limb, resulted in improvements in function and recovery of MEPs in certain individuals, with no relationships between improvements in function and MEP increases (215). Another study demonstrated increases in MEPs and improvements in a sequential finger motor task when 10-Hz rTMS was applied over the ipsilesional M1, and that cortical excitability was associated with improvements in motor learning (216). Similarly, improvements in motor skill learning have been shown when 5-Hz rTMS is applied over ipsilesional S1 (193) and this improvement is dependent on the white matter volume in the somatosensory cortex in the lesioned hemisphere (24). Although variable depending on stroke location, individuals with subcortical stroke only showed improved movement kinematics after 10-Hz rTMS over ipsilesional M1, whereas hand dexterity actually deteriorated in the majority of those with cortical stroke (217). This study also found that rTMS reduced activation of contralesional cortex for those with subcortical stroke, and caused bilateral activation of primary motor and sensory areas in those with cortical stroke (217). The authors concluded that it is likely that the extent and location of stroke may determine the beneficial response to ipsilesional excitatory rTMS. Studies have also reported little effects of applying excitatory rTMS over the ipsilesional cortex. Talelli and colleagues used iTBS over ipsilesional M1 followed by intensive physiotherapy of the paretic upper limb for 10 days that did not show any significant improvements (212). Another study combined 20-Hz rTMS over ipsilesional M1 with CIMT in chronic stroke for 2 weeks, finding that no additional improvements beyond that of CIMT alone were observed except slightly lower motor thresholds (218). However, it could be that the pairing of excitatory rTMS over the index finger muscle representation in M1 followed by reaching, grasping and other gross arm movements contributed the lack of positive effects in the above two studies. A recent study suggested that 10-Hz rTMS applied over ipsilesional M1 delivered 5 days per week for 2 weeks enhanced motor function of the paretic limb only in those with subcortical stroke and those who presented with MEPs immediately after the intervention and at a 2-week follow-up (219).

Repetitive Brain Stimulation as an Intervention After Stroke: Contralateral Stimulation

An alternative to directly enhance ipsilesional M1 excitability by applying excitatory rTMS over the lesioned hemisphere is to deliver inhibitory rTMS over contralateral M1. This approach potentially releases contralesional IHI and indirectly enhances ipsilesional M1 excitability. Some studies

Ipsilesional excitatory rTMS

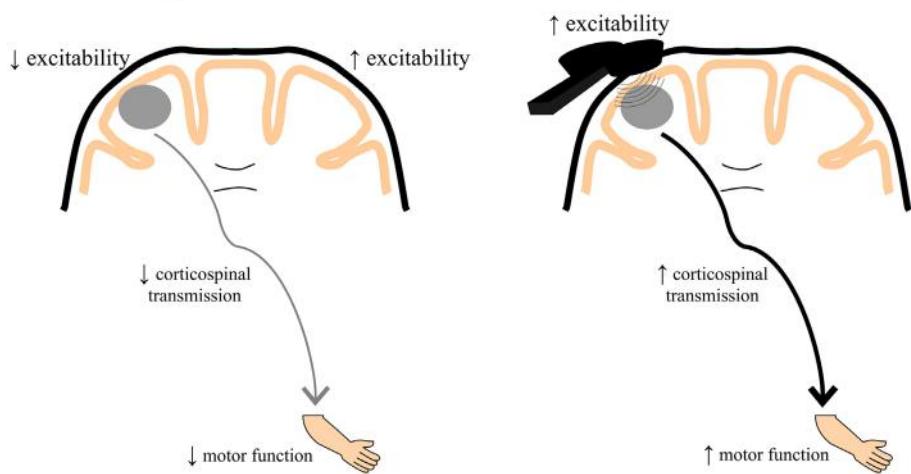


FIGURE 3 | A schematic of the theoretical effects of excitatory rTMS over the ipsilesional cortex. Decreased ipsilesional cortical excitability may contribute to decreased corticospinal transmission resulting in diminished motor function of the paretic upper limb. Ipsilesional excitatory rTMS may increase the excitability of the damaged cortex, thereby contributing to enhanced corticospinal transmission potentially leading to better motor function of the paretic upper limb. rTMS, repetitive transcranial magnetic stimulation.

Contralesional inhibitory rTMS

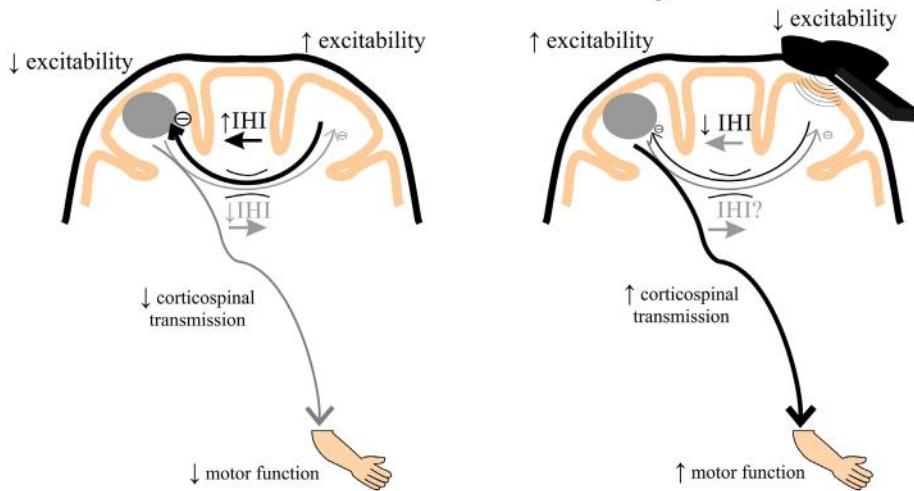


FIGURE 4 | A schematic of the theoretical effects of inhibitory rTMS over the contralesional cortex. Increased interhemispheric inhibition (IHI) from the contralesional to ipsilesional cortex via the corpus callosum may contribute to decreased ipsilesional corticospinal excitability and diminished motor function of the paretic upper limb. Contralesional inhibitory rTMS may suppress contralesional to ipsilesional IHI and assist in improving ipsilesional corticospinal transmission, potentially leading to better motor function of the paretic upper limb. rTMS, repetitive transcranial magnetic stimulation.

demonstrate that low-frequency inhibitory (<1 Hz) rTMS or cTBS applied over the contralesional hemisphere improves hand function (209, 210), reach-to-grasp movements (220), motor learning (194), and brief improvements in hand dexterity, which was associated with a reduction in TCI to the ipsilesional M1 (221).

Other studies have investigated functional brain activation changes following rTMS in stroke. One study showed a significant increase in the peri-infarct fMRI-related activity in the ipsilesional M1 after 6-Hz low-frequency rTMS over the contralesional M1 (208). Another study demonstrated improved motor performance of the paretic hand following 1-Hz rTMS

over the contralesional M1 that was associated with a decrease in over-activation of contralesional M1 activation during paretic hand movements (222). Additionally, connectivity between SMA and M1 within the ipsilesional hemisphere was enhanced after inhibitory rTMS over contralesional M1 (222). Recently, it was shown that 10 sessions of 1-Hz rTMS over contralesional M1 showed a change in contralesional plasticity (35), and that mild improvements in motor ability was associated with more normal transcallosal white matter. These data suggest that the condition of callosal white matter may influence the impact of contralesional rTMS on recovery of motor function after stroke (35).

Variability in Response and Application of rTMS in Stroke

Overall, the reported effects of rTMS in individuals with stroke are moderate (223) but inconsistent (24, 34, 193, 194, 224). Varied effects are noted regardless of what type of rTMS is employed (34, 193, 194) and irrespective of the targeted brain region (34, 193, 194, 216, 225). Further, research to date has suggested that varied responses to rTMS are not explained by simple demographic factors, such as age, sex, or stroke severity (24, 34). There are several potential reasons for this variability, such as stroke location and extent (217, 226), post-stroke duration (219, 225), presence of MEPs (31, 219, 227), hemispheric dominance pre-stroke (228), callosal (33, 189, 229) and corticospinal structural integrity (24, 34, 189), cortical target location for rTMS (193, 219, 224), brain-derived neurotrophic factor genotype (230), different interneuron populations activated by TMS (196), and combination with a well-controlled motor learning task or individualized physical therapy. Despite its broad use, a comprehensive understanding of the physiologic effects of rTMS on the brain is lacking. Further, there is no consensus on which brain region to stimulate, whether it is somatosensory (193) or motor execution (194, 216) or preparation (231) areas, and if stimulation should be applied over the ipsilesional or contralesional hemisphere (194).

Although rTMS demonstrates great potential to enhance post-stroke recovery future work is needed to address the issue of response variability. With a greater understanding of the factors driving response variability, we will be better able to target rehabilitation to the individual.

MULTIMODAL ASSESSMENTS

Multimodal Neuroimaging: Combined TMS, MRI, and EEG Assessment After Stroke

Few studies have utilized multiple methods of neuroimaging in order to predict motor function and impairment due to stroke (188, 189, 229, 232, 233). Studies have shown that those with decreased MEP amplitudes also have a weaker paretic hand, with greater activation in the ipsilesional M1 as recorded by task-based fMRI (234). A study using TMS to assess corticospinal integrity via single-pulse assessment of MEPs and fMRI during isometric hand gripping determined that in patients with less corticospinal excitability, there was an associated increase in activation of contralesional premotor and cerebellar areas (232). The suggestion from these combined methods is that other

non-primary motor cortical areas may be playing a functionally relevant role in controlling force production in more severely affected individuals with stroke. A combined paired-pulse dual coil TMS and fMRI study showed that both TMS and fMRI neurophysiological function in the contralesional PMd was associated with the degree of impairment (233). Specifically, a lack of inhibition from contralesional PMd to ipsilesional M1 measured by paired-pulse TMS and greater activation of PMd during hand-grip was correlated with the level of clinical impairment. The authors suggested that contralesional PMd may support recovery in ipsilesional M1 (233).

Stinear and colleagues have demonstrated through DTI and TMS that weak or absent MEPs evoked ipsilesionally and greater asymmetry in FA of the posterior limb of the internal capsule are predictive of poor motor recovery (227). Recent work from our lab has demonstrated the utility of combined TMS and MRI measures to predict motor function (188). Specifically, bilateral hand dexterity was found to correlate with resting motor threshold and precentral gyral thickness. Those with higher resting motor thresholds and decreased precentral gyral thickness presented with decreased bilateral hand dexterity. Furthermore, increased levels of TCI were associated with greater midcallosal white matter volume (188). In another study, we demonstrated that altered microstructure of transcallosal fiber tracts in anterior sub-regions were associated with TCI and upper extremity impairment in chronic stroke (189). Specifically, anterior transcallosal tract FA and TCI from the non-lesioned to lesioned M1 predicted a unique amount of variance in upper limb impairment. Those with less FA in anterior sub-regions of the corpus callosum and less TCI were those presenting with greater upper limb impairment (189). Another recent study combined fMRI, DTI, and TMS in the assessment of hemispheric balance between ipsilesional and contralesional cortices (229). Task-based fMRI lateralization to the ipsilesional hemisphere was associated with better TCI and stronger ipsilesional motor-related area output via DWI tractography. These studies demonstrate the usefulness of combining multiple methods of neuroimaging along with measures of TMS in order to more comprehensively assess and predict motor function and impairment. Utilizing multimodal neuroimaging can be used in future investigations to aid in identifying optimal biomarkers of stroke recovery and to predict response to rehabilitation in order to maximize treatment outcomes.

A novel multimodal neuroimaging approach combines TMS with EEG. TMS and EEG may be used in combination in real-time in order to directly characterize local and distributed cortical activity, providing a rich source of temporally specific data to determine causal mechanisms of cortical responses to TMS in humans *in vivo* (235–238). Another advantage of this approach is the ability to stimulate any cortical regions and record the evoked activity using EEG, subverting the need to record peripheral responses via surface EMG, which can prove difficult in the ipsilesional hemisphere. Although this has not been utilized in stroke, the combined technique of TMS-EEG may provide new insights into cortico-cortical connectivity in sensorimotor recovery after stroke due to spontaneous recovery and with interventions (behavioral, stimulation, pharmacological, etc.) not able to be captured before.

Multimodal Neuroimaging: TMS and MRI Assessment of rTMS-Based Interventions After Stroke

Very few studies have utilized multimodal neuroimaging with TMS to identify the underlying neurobiology of sensorimotor recovery from stroke (31, 188, 189, 227, 229, 233), and research is scarce in the investigation of an intervention using multimodal imaging with rTMS (24, 35). These studies have demonstrated the usefulness of combining imaging of cortical and subcortical structures with neurophysiological data acquired from TMS in order to better predict aspects of upper limb motor recovery and the potential response to rTMS (24, 34, 35). Carey et al. demonstrated that those with greater structural integrity of the posterior limb internal capsule of the ipsilesional hemisphere demonstrate greater response to contralateral rTMS and the behavioral improvements associated with rTMS (34). Transcallosal FA was shown to correlate with the degree of behavioral improvements due to contralateral rTMS, indicating the DTI-derived measures may aid in individually tailored interventions when considering using contralateral rTMS to potentially induce transcallosal neuroplasticity (35). Recently, we have shown that increased ipsilesional S1 white matter volume was associated with the degree of skill learning improvement when 5-Hz rTMS was applied over S1 before motor skill practice (24). These studies suggest that data acquired from structural and functional imaging may be used to categorize those who respond to rTMS in order to personalize application in a rehabilitation setting.

THE FUTURE OF MULTIMODAL NEUROIMAGING FOR PERSONALIZED THERAPY

Recently, there have been several models proposed to categorize individuals for personalized treatment based on multi-neuroimaging methods (227, 239). The “predicting recovery potential (PREP) algorithm” has been introduced and suggested that patients who present with an ipsilesional MEP have the best prognosis for recovery, and intensive unilateral therapy of the paretic limb is recommended. However, those who do not present with an ipsilesional MEP are divided into two categories: (1) low asymmetry in FA of the corticospinal tract (greater integrity of the ipsilesional corticospinal tract), with a prognosis of limited functional improvement and (2) high asymmetry in FA of the corticospinal tract (less integrity of the ipsilesional corticospinal tract) with the poorest prognosis for functional improvement. Those with low corticospinal tract asymmetry are recommended to receive “primed” ipsilesional brain stimulation and augmented training of the paretic upper limb. However, if there is an absence of an MEP after stimulating the ipsilesional M1 with a relatively high hemispheric asymmetry of FA, the recommendation of therapeutic intervention is modified to include stimulation of the contralateral M1 along with augmented bilateral therapy to engage the contralateral and ipsilesional cortices (31, 227). Di Pino and colleagues (239) similarly have suggested a bimodal balance-recovery model that

proposes a personalized application of rTMS (or other types of non-invasive brain stimulation) depending on structural reserve of the central nervous system, along with clinical and neurophysiological data from multiple imaging sources. This bimodal balance-recovery model attempts to account for the possibility of interhemispheric competition and the fact that the contralateral hemisphere may serve to support recovery of function after stroke (239).

These studies suggest that a combination of neuroimaging methods will likely benefit in the assessment of stroke-related damage and personalized treatment strategies, particularly when using rTMS (or other types of non-invasive brain stimulation) for individuals following stroke. However, there will always be a risk of mislabeling participants, resulting in a substandard care. For this reason, we must continue to utilize new technologies to broaden our understanding of stroke recovery, improving both diagnostic abilities and interventions. For instance, in an individual who does not present with an ipsilesional MEP perhaps simultaneous TMS-EEG could be used to test if cortical activity is evoked by ipsilesional TMS, making it possible to narrow down the site of impairment. This could be very useful information, giving a more accurate prognosis and identifying the ideal pathway to target for recovery. As advancements in neuroimaging continue to impact research in stroke recovery, personalized therapy will become more reliable and utilized, and new interventions will become possible.

TABLE 1 | Cost/availability are ranked relative to the other imaging methods.

Method	Benefits	Limitations	Cost/availability
Volumetric analysis	Quantification of brain volumes from basic (T1) structural scans	Limited accuracy in individuals with lesions	\$\$+++
DW-MRI	Assesses microstructural characteristics of white matter	Tractography results are variable across methods and sensitive to movement	\$\$\$/++
MRS	Quantification of neurotransmitter levels in defined area	Requires technical expertise and expensive coil for acquisition	\$\$\$/+
fMRI	Identifies patterns of brain activation at high spatial resolution	Poor temporal resolution and is limited to participants who can complete task	\$\$\$/++
Resting-state fMRI	Not dependent on task completion	Sensitive to movement	\$\$\$/+
TMS	Assessment and modulation of cortical excitability and plasticity	Requires specialized equipment and trained personnel	\$\$/+
EEG	Provides information on functional integrity of cortex and has high temporal resolution	Poor spatial resolution and is limited to cortical activity	\$/+++

Increasing price is associated with more \$ signs, and greater availability is indicated by more + signs.

DW-MRI, diffusion-weighted MRI; MRS, magnetic resonance spectroscopy; fMRI, functional MRI; EEG, electroencephalography; TMS, transcranial magnetic stimulation.

CONCLUSION

The information provided above strongly suggests the potential for multimodal imaging in future neuroplasticity and rehabilitation studies after stroke. Structural and functional imaging and physiological assessments have all provided important insights into both the pathology of stroke and mechanisms underlying neurological recovery. **Table 1** lists the major benefits/limitations of each imaging method covered in this review. Additionally, we have also highlighted the potential of non-invasive brain stimulation as an important therapeutic approach. Although many studies have found rTMS improves recovery an increasing number are failing to find benefit. Numerous technical factors affect rTMS interventions, including the site targeted, type of stimulation, and number of stimulation sessions. However, the variability in response to rTMS also highlights the importance of understanding individual

differences in response, which likely depend on a variety of biological factors, such as, age, time after stroke, lesion size, and location, which in turn impact patterns of functional and structural connectivity. Advances in neuroimaging are improving the ability to predict the patterns of structural and functional connectivity best suited to specific interventions. In the near future, novel-individualized interventions will be able to optimize recovery after stroke.

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The Virtual Brain: modeling biological correlates of recovery after chronic stroke

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There currently remains considerable variability in stroke survivor recovery. To address this, developing individualized treatment has become an important goal in stroke treatment. As a first step, it is necessary to determine brain dynamics associated with stroke and recovery. While recent methods have made strides in this direction, we still lack physiological biomarkers. The Virtual Brain (TVB) is a novel application for modeling brain dynamics that simulates an individual's brain activity by integrating their own neuroimaging data with local biophysical models. Here, we give a detailed description of the TVB modeling process and explore model parameters associated with stroke. In order to establish a parallel between this new type of modeling and those currently in use, in this work we establish an association between a specific TVB parameter (long-range coupling) that increases after stroke with metrics derived from graph analysis. We used TVB to simulate the individual BOLD signals for 20 patients with stroke and 10 healthy controls. We performed graph analysis on their structural connectivity matrices calculating degree centrality, betweenness centrality, and global efficiency. Linear regression analysis demonstrated that long-range coupling is negatively correlated with global efficiency ($P = 0.038$), but is not correlated with degree centrality or betweenness centrality. Our results suggest that the larger influence of local dynamics seen through the long-range coupling parameter is closely associated with a decreased efficiency of the system. We thus propose that the increase in the long-range parameter in TVB (indicating a bias toward local over global dynamics) is deleterious because it reduces communication as suggested by the decrease in efficiency. The new model platform TVB hence provides a novel perspective to understanding biophysical parameters responsible for global brain dynamics after stroke, allowing the design of focused therapeutic interventions.

Keywords: stroke, brain dynamics, graph theory, computational biophysical modeling, connectome, brain networks, imaging, MRI

INTRODUCTION

Heterogeneity of functional outcomes following stroke remains a major limitation to stroke rehabilitation. While the majority of stroke survivors suffer from motor impairment, particularly in the upper extremities (1), the degree and type of this impairment and the level of recovery following rehabilitation are highly variable (2). The functional basis for variation in patient deficits is still poorly understood, and there is no consensus on a theoretical or empirical framework for linking brain injury to functional deficits (3). In order to address this issue, recent approaches in stroke rehabilitation have aimed at the development and the optimization of individualized treatments that maximize long-term functional gains (4, 5).

To this end, different theoretical approaches have been used. The most general method has probed stratification measures based on patient demographics, behavioral outcomes, affective states, brain function, and lesion characteristics (4–6). None have been shown as a reliable biomarker. Particularly noticeably has been the presence of an inconsistent relationship between brain lesion and the resulting functional deficits (6), likely due to the inherent complexity of damage in a highly interconnected brain.

Researchers have thus turned to network analysis to understand stroke (7–9). In this approach, one of the goals is to explain the observed variations after stroke and predict recovery. Interestingly, the initial efforts with network analysis focused on alterations to specific pathways as the key links to understand behavior (8, 10). For example, while some functional connectivity studies showed that lesions within the motor areas can cause dysfunction of remote brain regions (11–13), others showed a relationship between improved motor function and strengthening interhemispheric and intrahemispheric connectivity involving the primary motor cortex (14). An important issue in interpreting such relationships is that the changes may reflect either the abnormal functioning of a damaged network or the formation of a different network that results in new behavioral patterns.

Furthermore, while these initial studies have been an important development, their main limitation is that they assume stable, localized changes within specific sub-networks, obliterating global changes, with the consequence that these potential biomarkers have been very adequate as descriptors at the group level but not in individual patients (15).

Recently, the neuroimaging community has begun to focus on connectomics, or the mapping of all connections at the whole-brain level. These connectomes, derived from structural [diffusion tensor imaging (DTI)] or functional outputs (fMRI and EEG), have recently been termed “big data,” referring to datasets that require the generation of large amounts of multimodal imaging data, (including raw, preprocessed, and intermediate data), for a high number of subjects (16). These initiatives span normal function [Human Connectome Project (17), CONNECT (18), Brainnetome (19), development [National Institutes of Health (NIH) Pediatric Database] and brain disorders such as Alzheimer’s disease (Alzheimer’s Disease Neuroimaging Initiative)].

In order to help interpret such large datasets, graph theory is increasingly used to distinguish inherent patterns that likely correlate with brain networks at the whole-brain level. Using

connectomics and graph theory, specific brain regions can be understood as nodes (20), and lesions can be understood as damage to nodes and/or the connections among them. With these methods, stroke has been shown to produce changes in both structural and functional network connectivity, particularly related to the organization of “hubs,” or highly interconnected nodes (21, 22). Graph theory provides an assessment of the changes at an organizational level. However, this approach still suffers from some limitations, mainly the inability to determine dynamical changes in a constantly changing brain and the lack of concrete biophysical substrates for understanding those dynamics. Consequently, according to Smith et al., one of the major challenges in the field of functional connectomics “will be to enable application of biologically interpretable models using large numbers of nodes in a robust and practical way” (9).

In other words, although tackling questions about brain network dynamics in both healthy and stroke populations requires a great deal of data, simply collecting more data is not itself an answer. While these efforts provide the necessary empirical foundation, they lack a computational and theoretical framework with quantitative tools to link these multiple datasets to “reconstruct” the brain and provide the link between these data and the brain function of individuals.

In this context, novel theoretical perspectives have been proposed based upon the nature of the brain as a large-scale network (3, 23–25). The implementation of the framework has been significantly accelerated by The Virtual Brain (TVB), a novel large-scale neural modeling platform (26–28). TVB uses neuroimaging data to parameterize a model and because individual data is used, the individual person’s brain can become a “virtual brain.”

The Virtual Brain (thevirtualbrain.org) was developed as a platform for modeling the dynamics of large-scale neural systems (3, 29). TVB integrates structural long-range connectivity generated from empirical DTI data with mesoscopic, or local level models [at each node or region of interest (ROI)]. By combining these two scales (global connectivity with local dynamics), TVB is able to predict and simulate an individual’s brain activity, essentially modeling a virtual representation of their brain. TVB thus lies at the intersection of experimental and theoretical neurosciences, making it well positioned to provide a link between population and individual datasets.

The models available in TVB integrate the anatomical connectivity between parts of the brain (provided by DTI) and the dynamics of local neural populations (embedded in the platform). Using these models, TVB has the flexibility to generate simulated data ranging from local field potentials to EEG and fMRI BOLD signals, allowing for a multimodal link between simulated and empirical data. The scalable architecture of TVB allows us to include neurophysiological information (e.g., receptor distributions and ion channels) adding another level of detail and bringing the model’s behavior closer to the real brain. Spatiotemporal motifs as present in empirical EEG/fMRI data can be reproduced to a large degree (29, 30). Because biophysical parameters are invisible to brain-imaging devices, TVB acts as a “computational microscope” that allows the inference of internal states and processes of the large-scale model.

The Virtual Brain therefore serves as a powerful research tool that has the potential to utilize big data and to develop and test advanced theories of brain dynamics. The individualization of TVB allows the creation of one model per person and systematically assesses the modeled biophysical parameters related to individual differences. The natural extension of this approach goes further into clinical applications, deriving parameters that both relate to biophysics and predict clinical outcome, making TVB an ideal tool for addressing limitations in stroke research.

The objective of this manuscript is twofold:

- (1) To give a thorough overview of the modeling method employed using TVB as it pertains to stroke, with the goal of providing details for those interested in using it in the context of stroke.
- (2) To provide a link between one of the TVB parameters (long-range coupling) to current whole-brain analytical approaches based on graph analysis.

MATERIALS AND METHODS

Subjects

Twenty individuals with ischemic stroke in the middle cerebral artery territory (41.13 ± 23.78 months postonset) and 10 age-matched controls were recruited for the study. Demographics for all stroke subjects are shown in **Table 1**.

Imaging Acquisitions

Magnetic resonance images were collected using a 3-T Philips scanner and an eight-channel SENSE head coil for signal reception and body coil transmitter for signal excitation. The following sequences were used:

1. High-resolution anatomical images (T1-w): three-dimensional (3D) Magnetization Prepared Rapid Gradient Echo sequence, FOV = 250×250 , resolution = $1 \text{ mm} \times 1 \text{ mm} \times 1 \text{ mm}$, SENSE reduction factor = 1.5, TR/TE = $7.4/3.4$ ms, flip angle = 8, sagittal orientation, and number of slices = 301 covering the whole brain.
2. Diffusion Tensor Imaging (DTI): FOV = 224×224 , TR/TE = $13,030/55$, 72 slices, slice thickness = 2 mm, resolution = $0.875 \times 0.875 \times 2$, $b = 1,000 \text{ s/mm}^2$ (and $b = 0$), 32 diffusion directions.
3. Functional imaging acquisition at rest (rsfMRI): whole brain (37 slices), single-shot echo-planar MR (EPI), slice thickness = 4.0 mm, FOV = 230×230 , voxel size = $2.8 \text{ mm} \times 2.8 \text{ mm}$, TR/TE = $2,000/20$ ms, and duration = 5 min.

Resting State fMRI Preprocessing

Resting state fMRI (rsfMRI) preprocessing analysis was performed using AFNI functions (31) and included the following steps:

1. Motion correction using a six-parameter 3D registration of functional and anatomical data sets (32).
2. Three-dimensional spatial registration to a reference acquisition from the first fMRI run.
3. Registration of functional images to the anatomical volume.
4. Despiking of the time series.
5. Mean normalization of the time series.
6. Inspection and censoring of time points occurring during excessive motion (>1 mm) (33).
7. Regression of cerebrospinal fluid and white matter signals to remove slow drifts in the fMRI signal.

TABLE 1 | Demographics and stroke characteristics of the stroke cohort.

Subject	Age	Sex	Handedness	Affected hemisphere	Affected hand	Stroke location	Stroke volume (mm ³)
1	41	F	Right	Right	ND	Cort	22,495.0
2	54	F	Right	Left	D	Cort/subcort	49,078.0
3	57	M	Right	Left	D	Cort/subcort	17,411.0
4	57	M	Right	Left	D	Cort/subcort	38,703.0
5	54	F	Right	Left	D	Subcort	27,677.0
6	50	M	Right	Right	ND	Subcort	3,570.0
7	23	M	Right	Left	D	Subcort	560.0
8	55	F	Right	Right	ND	Cort	6,781.0
9	68	M	Right	Left	D	Subcort	1,988.3
10	56	F	Right	Left	D	Subcort	6,239.7
11	46	M	Right	Left	D	Subcort	325.0
12	56	F	Left	Right	D	Cort/subcort	60,669.0
13	37	M	Right	Left	D	Cort/subcort	83,406.2
14	62	M	Right	Left	D	Subcort	22,154.8
15	57	M	Right	Right	ND	Cort/subcort	25,392.0
16	66	M	Right	Left	ND	Cort/subcort	19,927.0
17	61	M	Right	Left	D	Subcort	978.0
18	74	M	Right	Left	D	Cort/subcort	63,642.0
19	67	F	Right	Right	ND	Subcort	588.0
20	74	F	Right	Left	D	Cort/subcort	44,892.0

D, dominant hemisphere; ND, non-dominant; Cort, cortical; subcort, subcortical.

Preprocessing: Structural Connectivity

Brain Parcelation

Parcellating image data that contain lesions with the use of semiautomated schemes produce inaccurate results due to the absence of tissue and consequent mechanical deformation. We therefore developed The Virtual Brain transplant (VBT). This method effectively replaces the lesion produced by the cortical stroke with T1-w images of brain tissue from the contralateral hemisphere from the same subject (34). This method allows us to use a semiautomated parcelation scheme subsequent to the transplant. The VBT process consisted of the following steps (**Figure 1**):

1. Lesion segmentation by hand.
2. The high-resolution anatomical T1-w brain images and lesion masks were uploaded to a transplantation pipeline, which dissected the MRI brain tissue from the non-lesioned hemisphere homologous to the lesion, and transplanted it into the lesioned hemisphere at the site of the lesion, filling in the missing portions of the brain.
3. After the initial transplant was done, manual corrections in the interface between the native and transplanted T1-w images were performed.
4. The brain was segmented into 83 cortical and subcortical regions using the Lausanne 2008 (Freesurfer) parcelation scheme within the Connectome Mapper Toolkit (35, 36).

T1-w to DTI Alignment

The T1-w anatomical image was then aligned to a reference $b = 0 \text{ s/mm}^2$ DTI image, using a six degrees of freedom linear transformation with FSL's FLIRT function (37). This transformation was also applied to the Freesurfer parcellations.

DTI Tractography

We performed the following steps:

1. DWI was aligned to the same reference $b = 0 \text{ s/mm}^2$ image used to align the corrected T1-w via VBT to DTI. Distortions caused by eddy currents and head motion were corrected using the FSL eddy current correction (12 degrees of freedom linear transformation), and the diffusion gradient vectors rotated accordingly (38). That is, the T1-w images with the “transplanted masks” are used to supply the region of interest landmarks for tractography but do not directly impact the tractography algorithm as the transplant is not performed in the DWI space.
2. The diffusion-weighted images were resampled to 2mm isotropic resolution (39).
3. White matter deterministic tractography of DTI data was performed in Trackvis software (39) using the FACT algorithm (40). Threshold values of a maximum of 60° turning angle and a minimum of 0.20 fractional anisotropy (FA) were used as stopping criteria for the tracking algorithm. These thresholds take into account the decrease in signal in regions with the lesion. The FA threshold is particularly useful in

terminating tracks before they enter regions containing the lesion. These regions, filled with CSF, have FA values close to zero. Therefore white matter pathways ordinarily connecting two ROIs will not be tracked if the ROI is completely lesioned, despite appearing intact in the transplanted T1-w image from which the parcelation is made. If a parcelation is partially compromised by the lesion then white matter pathways will also be partially tracked as reflected by a lesser number of streamlines.

Generation of Structural Connectivity Matrices

Using the Connectome Mapper Toolkit, two connectivity metrics were extracted for each pathway in order to generate two structural connectivity matrices that quantify connectivity between all pairs of the cortical regions for each subject:

1. Weights, defined as $\text{FA} \times \text{number of streamlines in the pathway}$ (note that per the white matter deterministic tractography of DTI data, pathways connecting regions impacted by the lesion will show a decreased number of streamlines and potentially altered FA). This metric reflects the maximum rate of transmission of information through edges (41). The number of streamlines in the pathway was assessed using the deterministic FACT algorithm.
2. Lengths of the individual tracts, defined in millimeters, were derived after smoothing the tractography with a B-Spline filter (39).

These matrices are symmetrical, as connections using DTI are considered unidirectional (30).

Modeling with TVB

Modeling with TVB involves three initial steps, namely the import of individual structural connectivity matrices (obtained as described earlier), the selection of a biophysical local model, and the choice of relevant biophysical parameter values. TVB has several types of local models available, each one taking into account different biophysical parameters. Hence, whereas some are focused on field potentials [Stefanescu–Jirsa two dimensional (2D) and Stefanescu–Jirsa 3D (SJ3D)], others are focused on firing rates (Wilson–Cowan, Brunel–Wang, and Jansen–Rit) or are phenomenological (Generic 2D, Kuramoto, and Epileptor). In our previous efforts, since we simulated the BOLD response, the mesoscopic model used was the SJ3D, one of the more complex and refined models in the repertoire of TVB.

The reasoning behind this choice was not only the obvious relationship between the BOLD response and local field potentials (42–44) but the additional fact that the BOLD signal has poor time resolution and the model does not rely heavily on synaptic delays. Concretely, the SJ3D model is a reduced form of the Hindmarsh–Rose model (43), which forecasts individual neuronal behavior. The SJ3D model predicts local dynamics using six differential equations that include variables representing *physiological properties* such as neuron membrane potentials, transport of ions across the membrane through fast and slow ion channels, and the dynamic coupling of excitatory and inhibitory neuronal populations.

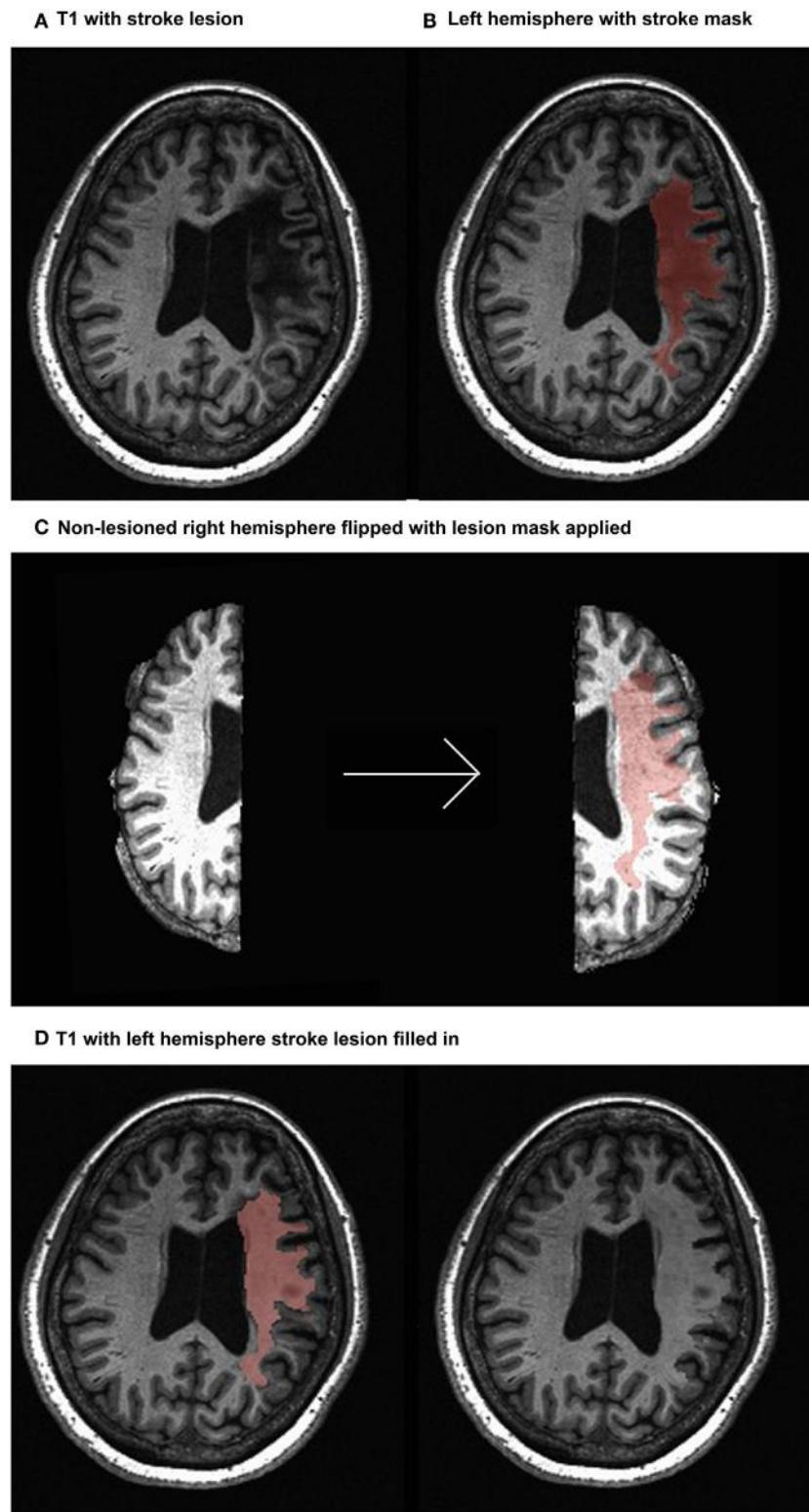


FIGURE 1 | Virtual brain transplant method. Virtual brain transplant is done in stroke cases with cortical damage with the goal of being able to parcellate the brain. This graphic representation summarizes the process of replacing the damaged portion of the brain with the homologous non-stroke tissue. **(A)** T1-w image showing the lesion (left hemisphere) of one subject. **(B)** Close-up of the left hemisphere, demarcating the lesion mask in red. **(C)** Segregation of the right and left hemispheres (left) and after the right hemisphere has been flipped having the lesion mask applied (right). **(D)** Depiction of the tissue from the right hemisphere applied to the lesion in the left hemisphere (left) and the resulting transplanted brain volume (right).

The sequential steps for modeling in TVB are as follows (graphical depiction can be found in **Figure 2**):

1. Importing the two metrics derived from individualized SC matrices [weights ($FA \times$ number of fibers) and lengths] representing connections between regions, along with the T1-w structural data providing individual brain topology.
2. Parameter space exploration: the goal of this process is the optimization of the model parameters. When applying TVB methodology to stroke, one can classify the numerous parameters included in the modeling into two categories: global parameters that will model brain dynamics between nodes, and local parameters that will describe brain dynamics within nodes. In the first category, the two main parameters to optimize are conduction velocity and long-range coupling. Likewise the biophysical parameters within the SJ3D model to be used are those providing the coupling between excitatory and inhibitory populations within the local regions: K_{11} (excitatory on excitatory), K_{12} (excitatory on inhibitory), and K_{21} (inhibitory on excitatory). This exploration systematically explores the entire range of available values for each parameter and identifies the value with the highest overall distribution of variance (**Figure 3**) as the optimal parameter value to be used on each individual for the actual signal simulation. The order of optimization can be done as follows:

- a. Long-range coupling and conduction velocity: starting ranges are 0.001–0.1 global coupling and 1–100 conduction velocity.
- b. K_{12} and K_{21} : starting ranges are 0–1.0 for both. K_{12} is optimized first, and the identified value is then used when optimizing K_{21} .
- c. K_{11} : starting range is 0–1.0.
3. Simulating the BOLD response: based on the values obtained in the parameter exploration, simulation of the BOLD time series should reflect the same duration (4 min) and sampling rate ($TR = 2$ s) of the empirical MRI acquisition. Noise is added to each node. The noise to be used is white with Gaussian amplitude (mean = 0, standard deviation = 1). Numerical integration of the system is performed using stochastic Heun's method (45), with an integration step size of 0.0122 ms.
4. Validating the simulated brain signals: this is done by comparing the simulated and empirical time series in terms of their amplitude, frequency, and phase.
 - a. Amplitude: the range is calculated by identifying the highest and lowest peaks present in the time series across all regions. The overall mean is calculated by averaging the mean amplitude per region across all regions. Mean amplitudes should be similar. An example is shown in **Figure 4A**.
 - b. Frequency is computed via fast Fourier transforms of the time series with Matlab's "fft" function with an fs of 0.5 Hz

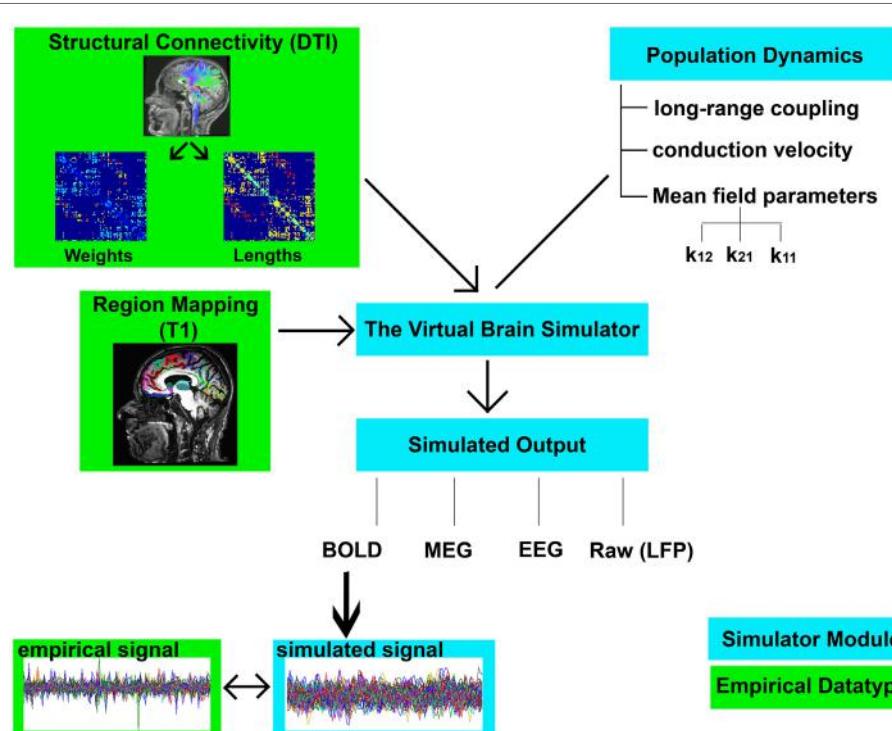
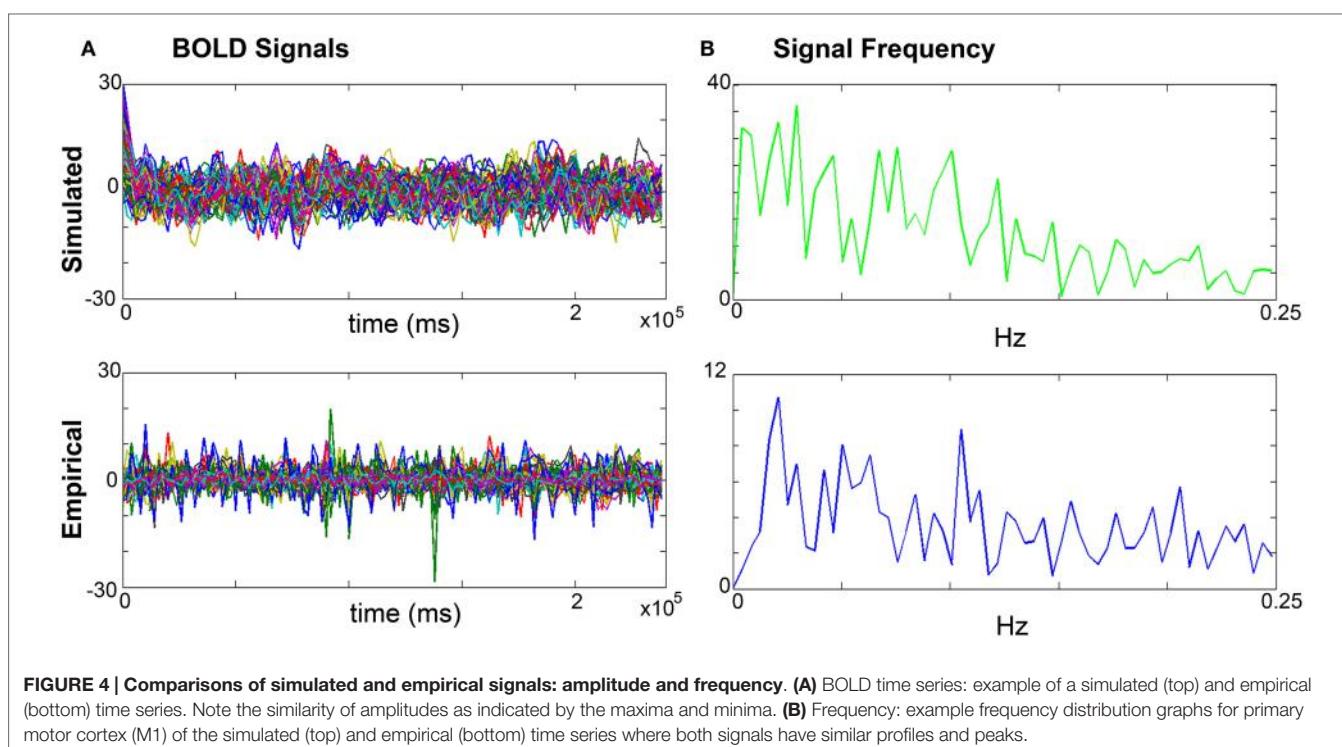
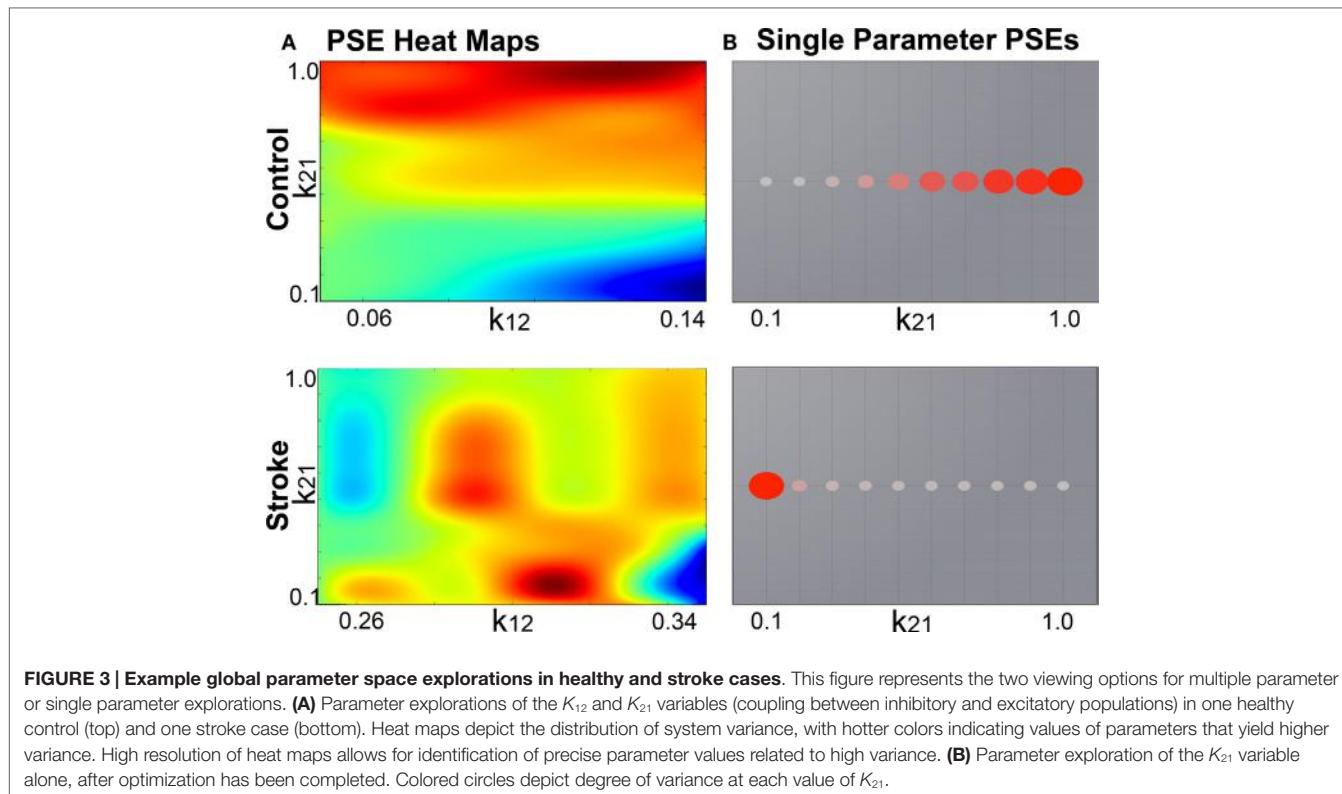


FIGURE 2 | Flowchart of TVB modeling. Graphic representation depicting the elements involved in TVB modeling. Components shown in green boxes represent empirically collected data. Elements shown in blue boxes represent modeling components within the TVB platform. Empirical input to the TVB consists of two structural connectivity matrices (weights and lengths) derived from DTI and a brain parcelation derived from T1-w acquisition. Modeling within TVB includes both global and local parameters resulting in the simulation of biological signals including BOLD. Finally, the reliability of the simulation is then compared to the empirical signals.



to determine the range, profile, and peak frequencies. The maximum frequency for simulated signals should be around 0.25 Hz that coincides with the empirical BOLD responses. An example is shown in **Figure 4B**.

- Phase can be done by calculating the pair-wise covariance of the time series for each region for each subject (30) using the “corr” function in Matlab, which results in a functional connectivity matrix for each subject. In order to smooth the

data, one can average all matrices from groups of interest to obtain a group control matrix and then calculate the pairwise linear correlation coefficient between the simulated functional connectivity matrix for each individual to the group (**Figure 5**). Results from this analysis should reveal similar phases between empirical and simulated signals. Significance of the correlation can be achieved via Fisher Z-transformation.

Comparison Between Healthy Controls and Stroke

We found an increase in long-range coupling in the stroke group compared to healthy controls. The meaning of long-range coupling is not intuitive, especially when compared to other parameters more closely linked to biophysical features, such as conduction velocity, channel dynamics, and the coupling between excitatory and inhibitory neuronal populations. The long-range coupling function is applied to the activity propagated between brain region regions by the structural pathways before it enters the local dynamic equations of the model. Its primary purpose is to rescale the incoming activity to a level appropriate to model. At a more intuitive level this parameter describes the balance between the global and the local dynamics. In other words, an increase in long-range coupling suggests a preponderance of local over long-range brain dynamics.

In order to put this parameter in the context of current network analytical approaches, in this study we determined the relationship between the modeled long-range coupling in stroke cases with structural network metrics derived from graph analysis including degree centrality, betweenness centrality, and global efficiency.

Graph Analysis

Graph Analysis Metrics

Based on the deterministic tractography performed for each individual subject, a binary adjacency matrix A_{ij} was generated

whose elements represent the connections (edges) between nodes i and j (46–48). From these matrices, three measures of functional integration were obtained: average degree centrality, average betweenness centrality, and global efficiency as others have done (49–51), using the NetworkX software (52) [mathematical notation adapted from (20)]:

1. Average degree centrality is the number of nodes adjacent to node i , averaged across all nodes in the graph (53):

$$k_{av} = \frac{1}{n} \sum_{i \in N} k_i = \frac{1}{n} \sum_{i,j \in N} a_{ij}$$

where n is the number of nodes in the graph, and N is the set of those nodes; k_i is the degree centrality for node i , and a_{ij} equals 1 when nodes i and j are the nearest neighbors and zero otherwise. This is the simplest measure of centrality and is commonly used to discriminate between well-connected nodes (hubs) and less well-connected nodes (51).

2. Average betweenness centrality refers to the fraction of shortest paths between any pair of nodes in the network that travel through a given node averaged across all nodes (54):

$$b_{av} = \frac{1}{n} \sum_{i \in N} b_i = \frac{1}{n} \sum_{i \in N} \frac{2}{(n-1)(n-2)} \sum_{\substack{h,j \in N \\ h \neq j, h \neq i, j \neq i}} \frac{p_{hj}(i)}{p_{hj}}$$

where b_i is the betweenness centrality for node i ; p_{hj} is the number of shortest paths between nodes h and j , and $p_{hj}(i)$ is the number of shortest paths between h and j that pass through node i . This is the oldest and most commonly used measure of centrality (51) where “shortest” refers to the path between two nodes that contains the least number of intermediate nodes.

3. Global efficiency is the average of the inverse of the shortest path length between all nodes (minimum number of edges traversed to connect one node to another) (21, 53):

$$E = \frac{1}{n} \sum_{i \in N} E_i = \frac{1}{n} \sum_{i \in N} \frac{\sum_{j \in N, j \neq i} d_{ij}^{-1}}{n-1}$$

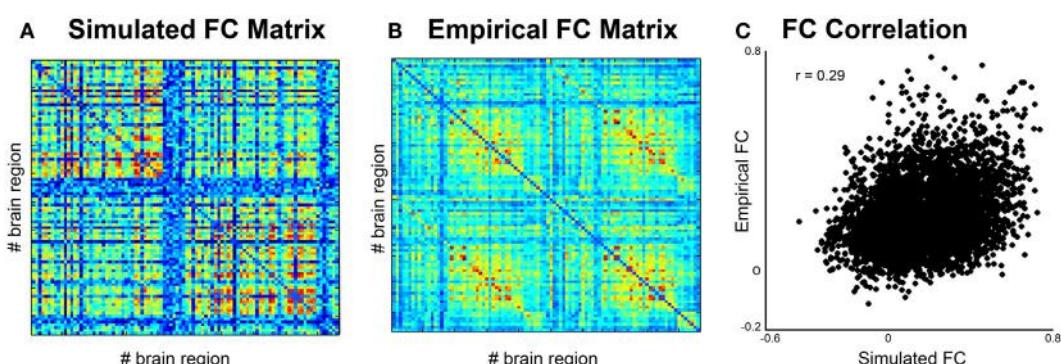


FIGURE 5 | Comparison of simulated and empirical signals: phase. **(A)** Functional connectivity matrix from simulated data modeled from one subject. **(B)** Average functional connectivity matrix from empirical data from all healthy subjects. **(C)** Correlation of functional connectivity between simulated (x-axis) and empirical (y-axis) time series.

where d_{ij}^{-1} is the inverse of the shortest path length between nodes i and j . For binary matrices, a network where each node has a direct connection to all other nodes in the graph has maximal global efficiency, equal to 1, while a partially disconnected network has lower global efficiency (49).

Comparison of Graph Analysis Metrics Between Groups

To test for differences in degree centrality, betweenness centrality, and global efficiency between healthy and stroke cases, we used the Wilcoxon-rank sum test. Significance threshold was set to $P = 0.017$ (Bonferroni correction). A simple linear regression analysis was used to correlate TVB long-range coupling (independent variable) with graph analysis metrics (dependent variables).

RESULTS

Comparison of Graph Analysis Metrics Between Stroke Cases and Healthy Controls

Results from the Wilcoxon-rank sum test showed no significant differences between healthy controls and stroke cases in degree centrality ($P = 0.11$), betweenness centrality ($P = 0.86$), or global efficiency ($P = 0.0822$). However, the distributions of each graph analysis metric between the two groups showed differences (Figure 6). Specifically, global efficiency showed a trend toward lower values in stroke cases compared to controls ($P = 0.04$) but not degree centrality ($P = 0.22$) nor betweenness centrality ($P = 0.95$). While there was not a statistical difference in distribution of

degree centrality between healthy and stroke populations, a large amount of subjects showed lower values of degree centrality.

Correlation Between Long-Range Coupling and Graph Analysis Metrics

Linear regression analysis showed that the only graph analysis metric associated with the TVB long-range coupling parameter was global efficiency (Figure 7). That is, higher values of global coupling were correlated with lower values of global efficiency ($t = -2.19$, $P = 0.038$). There was no significant correlation between global coupling and degree centrality ($P = 0.7$) or betweenness centrality ($P = 0.6$).

DISCUSSION

We have demonstrated that TVB can be a novel tool for identifying biophysical biomarkers of stroke recovery, showing that (1) the parameters associated with TVB modeling directly link structural imaging data to biophysical processes associated with brain dynamics; (2) the models are individualized, as they are based on the specific structural connectome from each person; and (3) TVB parameters can be correlated with other metrics not currently associated with biological parameters (i.e., graph analysis metrics). Importantly, this study harnessed the relationship between TVB and graph analysis, wherein the latter supplies an additional description of changes in relationships between different brain regions, while TVB supplies the neurobiological mechanisms responsible for them. The outlined steps using TVB offer a unique method, providing a new dimension to the study of stroke.

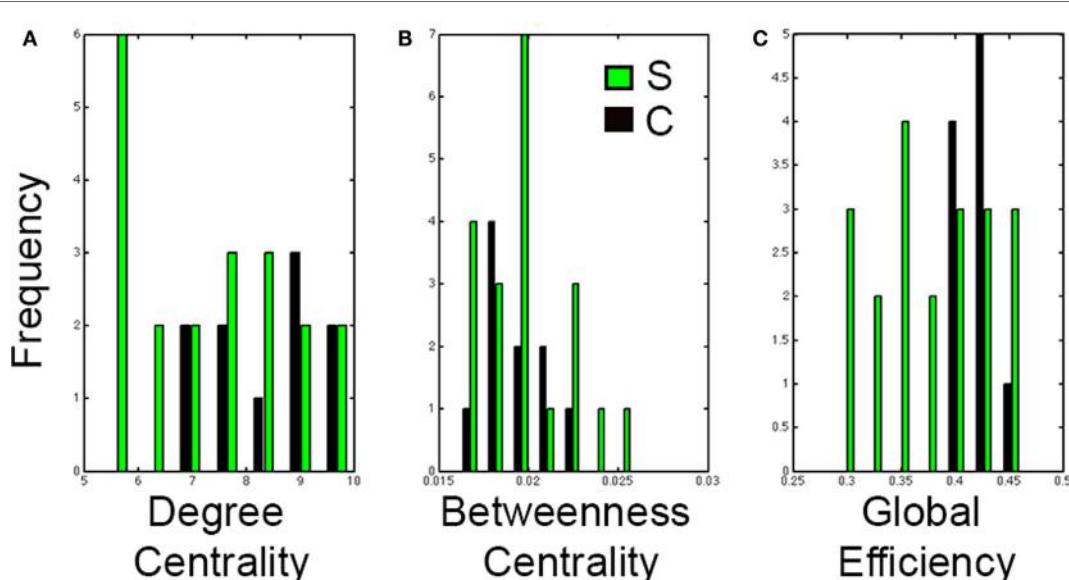
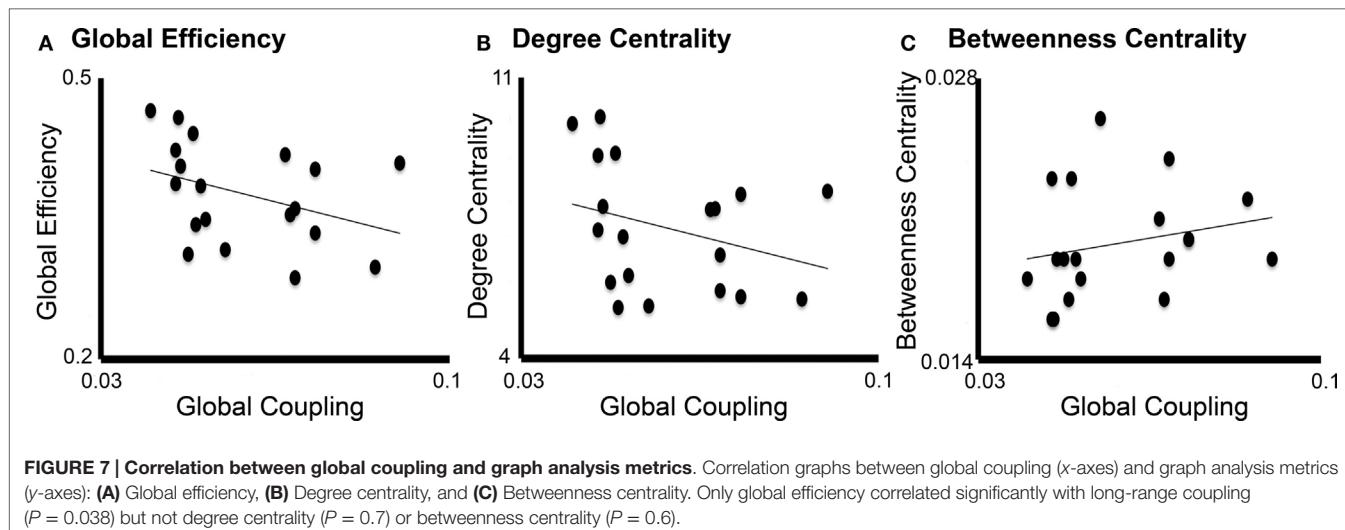


FIGURE 6 | Distributions of graph analysis metrics in control and stroke cases. Distribution graphs comparing the control (black) and stroke (green) cases for (A) Degree centrality, (B) Betweenness centrality, and (C) Global efficiency. Note that distributions in stroke shift to the left for global efficiency but not for degree centrality nor for betweenness centrality.



TVB Integrates Macroscopic and Mesoscopic Levels to Predict Brain Dynamics

There is currently no way to directly measure the local parameters modeled in TVB in humans, whereas global measures derived from imaging data have been used as potential biomarkers of stroke recovery (6, 55), the parameters considered within TVB at the local level represent a dimension reduction derived from processes at the cellular or even molecular levels. That is, the mesoscopic level represents the transitional state between the macro- and microscales (56). Thus, these parameters better inform us of underlying brain mechanism responsible for brain dynamics that current imaging analyses are unable to access, such as dynamics between excitatory and inhibitory neuronal populations and ion channel properties. In this way, TVB can assist to generate hypotheses associated with basic mechanisms that are responsible for the changes in brain dynamics associated with stroke.

In this context, it is important to mention that TVB can have wide applicability in the clinical setting because the input required for its operation can be minimal. In ideal circumstances, the experimental data needed are T1-w, fMRI (EEG or MEG), and DTI. However, some of these categories may not be necessary when only physiological data are available (e.g., EEG) without anatomical or connectivity data. In these cases, TVB platform includes normalized anatomical data (a parcellated cortical surface based on the MNI atlas) and a theoretical structural connectome based on the CocoMac database (3, 57). For stroke cases, while it is preferable to have anatomical data, it is still possible to run accurate simulations by manually modifying this provided structural connectome to exemplify the individual lesions.

The Resulting TVB Models are Individualized

There is large consensus on the importance of individualized medicine as one of the means to improve medical care. In this

sense, a central feature of TVB is its direct focus on individual subjects' brain dynamics. The structural connectivity matrix of each individual drives the modeling producing the individualized simulated brain activity, whereas the applicability of previous studies has been at the group level (15). By generating reliable simulations, the system provides a window into the state of biophysical parameters associated with it in each person and hence enables the development of customized, individualized therapies and treatments.

There are a myriad of stroke therapies currently under investigation, including constraint-induced motor therapy (58–60), action observation therapy (61, 62), neurostimulation (e.g., transcranial magnetic stimulation and transcranial direct-current stimulation) (63, 64), robotic therapy (65, 66), and cellular-based (e.g., stem cell) therapies (67), that have shown limited degrees of effectiveness, due perhaps to the fact that they are not specifically targeting brain mechanisms responsible for individual dysfunction. This is a reflection of the paucity in our understanding of basic mechanisms generating individual brain dynamics. Having new hypotheses applicable to each patient will enable us to generate new therapeutic interventions that specifically target the elements producing particular brain states. Furthermore, the more we learn about basic processes based on animal studies for instance, the more we can modify current TVB local models and hence, obtain more sophisticated simulations.

TVB Parameters can be Related to Other Network Metrics

An additional feature of parameters derived from TVB is that they can be contrasted with other measures. Our results showed a trend toward decreased global efficiency in stroke that measures the network's capacity for communication, with greater efficiency indicating better overall communication (20, 49). In other words, network communication is impaired after stroke. Interestingly,

degree centrality and betweenness centrality after stroke were not different from healthy controls probably due to the large variance of stroke size.

The negative correlation between global efficiency and the modeled long-range coupling provides unique insight into the network structure of the brain following stroke. We have previously observed increased long-range coupling after stroke, intuitively indicating a higher influence of local dynamics on brain activity than long-range dynamics. In this context, it is important to remember that the global model is derived from the structural connections between nodes, and hence, one would expect that shorter (direct) paths that originate from damaged nodes should be compromised. The graph analysis results suggest that the post-stroke connectivity between nodes is done through less efficient, longer paths (20). Therefore, decreased global efficiency and increased long-range coupling after stroke suggest a breakdown in the ability to transfer information between regions, weighting the activity toward local dynamics. Our findings thus highlight the global impact of stroke, despite its relatively focal damage. This novel finding in stroke is consistent with studies in other neurological diseases, such as schizophrenia, where imbalances between local and global dynamics, specifically a breakdown of local structure and a shift toward global dynamics have been suggested (68).

Limitations

The Virtual Brain as any modeling approach is laden with limitations. Among them:

1. The fact that TVB simulations depend on structural connectivity assumes the structural matrices having reasonable reliability. This is very relevant in stroke because the damage can produce mechanical distortions of tissue. In our case, we have used TVB transplant to minimize these issues. Additionally, there are many definitions of “weights” of connections (69, 70) although novel approaches promise at least high intraindividual reliability in the reconstruction (71). In our case, we used a surrogate measure reflecting the “number of fibers per pathway.” This is the reason why we normalized the number of streamlines between nodes by the FA of the particular pathway.
2. The weights of connections are currently based on the size (number of streamlines) of the pathways, yet the particular features of the synaptic connections are not taken into consideration. For example, the penetrance of a smaller pathway could be larger than a bigger pathway if the former establishes the synaptic contact with more proximal versus distal dendrites. This type of information is available for other species but is not yet known in humans.

Future Directions and Clinical Impact

The ability of generating a virtual brain from any individual opens up an interesting venue for therapeutics. Once a hypothesis is

derived from the biophysical parameters affected by the stroke, the effects on brain dynamics can be tested within the TVB platform by modifying the parameters for an individual case. In this way, TVB can be used as a test for potential therapeutic interventions before they are tested in animal models or individual patients.

The Virtual Brain thus has the potential to revolutionize stroke treatment in the future, by allowing for:

1. The application to “big data.” While the current study used a smaller sample size, once we have parameter changes, future studies can more readily utilize TVB in a large number of patients.
2. The ability to study longitudinal brain changes in stroke, from acute and sub-acute to chronic stroke. Because of the predictive potential of TVB, the inclusion of patients at early stages can provide the identification of powerful biomarkers for recovery.
3. The individualization of treatment with minimal input: one single MRI scan including the anatomical scan, DTI, and resting state fMRI.
4. The ability to perform whole-brain modeling, integrating the particular intercommunication between nodes (DTI derived) to local biophysical models associated with concrete basic functional parameters.
5. The opportunity to identify tangible targets for treatment that are testable within the application itself.
6. An open source platform: it is possible to add new, more sophisticated mesoscopic and microscopic models via the open source nature of TVB. Therefore, new developments on basic physiological knowledge can be easily integrated in the future.
7. Allowing the simulation of resting state brain activity, as was done in this study, but also of evoked responses through a built-in feature that allows for the stimulation of brain areas, with features determined by the modeler.

AUTHOR CONTRIBUTIONS

All authors had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: AS, VJ, and MF. Analysis and interpretation of data: AS, MF, JR, VJ, EC, ADS, and AM. Drafting of the manuscript: MF, AS, VJ, and ADS. Critical revision of the manuscript for important intellectual content: AS, VJ, JR, and AM. Statistical analysis: EC. Obtained funding: AS, VJ, and AM. Study supervision: AS and VJ.

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Role of the contralesional hemisphere in post-stroke recovery of upper extremity motor function

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Identification of optimal treatment strategies to improve recovery is limited by the incomplete understanding of the neurobiological principles of recovery. Motor cortex (M1) reorganization of the lesioned hemisphere (ipsilesional M1) plays a major role in post-stroke motor recovery and is a primary target for rehabilitation therapy. Reorganization of M1 in the hemisphere contralateral to the stroke (contralesional M1) may, however, serve as an additional source of cortical reorganization and related recovery. The extent and outcome of such reorganization depends on many factors, including lesion size and time since stroke. In the chronic phase post-stroke, contralesional M1 seems to interfere with motor function of the paretic limb in a subset of patients, possibly through abnormally increased inhibition of lesioned M1 by the contralesional M1. In such patients, decreasing contralesional M1 excitability by cortical stimulation results in improved performance of the paretic limb. However, emerging evidence suggests a potentially supportive role of contralesional M1. After infarction of M1 or its corticospinal projections, there is abnormally increased excitatory neural activity and activation in contralesional M1 that correlates with favorable motor recovery. Decreasing contralesional M1 excitability in these patients may result in deterioration of paretic limb performance. In animal stroke models, reorganizational changes in contralesional M1 depend on the lesion size and rehabilitation treatment and include long-term changes in neurotransmitter systems, dendritic growth, and synapse formation. While there is, therefore, some evidence that activity in contralesional M1 will impact the extent of motor function of the paretic limb in the subacute and chronic phase post-stroke and may serve as a new target for rehabilitation treatment strategies, the precise factors that specifically influence its role in the recovery process remain to be defined.

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Introduction

With the introduction of relatively sophisticated neuroimaging techniques, such as positron emission tomography (PET) and functional and structural magnetic resonance imaging (MRI), and novel electrophysiological techniques, such as transcranial magnetic stimulation (TMS), studying the underlying mechanisms of motor recovery after stroke in humans have become increasingly feasible. In 1991, Chollet et al. (1) reported for the first time the activation of bilateral sensorimotor cortices in stroke patients moving their affected hand and suggested that ipsilateral motor projection may play

a role in recovery. This claim was further substantiated in 1993 by Carr et al. (2) who used TMS of the primary motor cortex (M1) to probe the functional integrity of the corticospinal tract (CST) after stroke. He reported that, in patients with poor motor outcome, TMS applied to the motor cortex of the hemisphere affected by stroke (ipsilesional M1) did not produce detectable motor-evoked potentials (MEPs), indicating disrupted function of the CST. However, when TMS was applied to the motor cortex of the hemisphere spared by the stroke (contralesional M1), MEPs were detected in both the hands. These findings suggested abnormal corticospinal projections from the contralesional M1 to muscles of the affected hand (see below for more detailed discussion).

In the following years, the role of the contralesional M1 in motor recovery after stroke and its potential as new target for rehabilitation efforts have been a topic of intense research efforts in humans and animal stroke models (3–5). As this field moved forward, it became apparent that several factors may impact the role of contralesional M1 in the control of the paretic hand movements and that even in healthy intact brain the ipsilateral M1 (corresponding to the contralesional M1 in paretic hand movements) is active in the control of strictly unilateral hand movement (6–11). In the context of the incomplete understanding of the ipsilateral M1 in motor control, the interpretation of findings pertaining to the role of contralesional M1 (corresponding to the ipsilateral M1 in intact human) in motor recovery after stroke remains problematic.

In this review, the evidence for contralesional M1 activity in recovery of hand function after stroke will be discussed. In the first part of this review, I will summarize the advances in our understanding of motor control of hand movements as they pertain to a better understanding of contralesional M1 function in motor recovery of hand movements. There is emerging evidence that ipsilateral M1 (corresponding to contralesional M1 in stroke patients) is active even in healthy subjects, depending on age and motor task demands (11–14). Motor task-dependent activity of ipsilateral M1 and the interaction between M1s may contribute to the contradicting data in contralesional M1 in stroke patients, where stroke-related motor impairment impacts the demand of a given motor task. In the second part of the review, I will discuss data available from animal stroke models and humans after stroke pertaining to the role of contralesional M1 reorganization in post-stroke recovery. Finally, I will discuss in which way neurorehabilitation science can leverage on the knowledge of contralesional M1 reorganization to develop new and effective rehabilitation treatment strategies.

Ipsilateral M1 and Interhemispheric Interaction in the Control of Hand Movements in Intact Man

The Contribution of Ipsilateral M1 and its Corticospinal Connections in the Control of Hand Movements

In fMRI studies of unilateral hand motor performance in intact man, strictly contralateral M1 activation was demonstrated by some investigators (15, 16) while bilateral M1 activation was

observed by others (6, 11, 17–19). Increased ipsilateral M1 was demonstrated in tasks with higher accuracy or complexity demands (6–8, 11, 17, 20). However, the interpretation of these neuroimaging data was limited by measuring qualitatively different movements where the tasks were not being matched for their kinematics (e.g., force, amplitude, and frequency) and by lacking the verification of a strictly unilateral execution of the motor task during the acquisition of imaging data. Measuring unilateral performance is important as without it, the presence of bilateral upper extremity activity with increasing difficulty of the task referred to as “mirror movements” cannot be ruled out and may contribute to observed bilateral M1 activation. In our recent study of healthy middle-aged people ($n = 13$, 10 females, age 55.4 ± 10.9 years), subjects performed a pointing task with a joy stick. By decreasing the size of the target, the demand on accuracy was parametrically increased while participating muscle groups and movement kinematic were kept the same. Unilateral performance was verified with electromyographic (EMG) recording from upper extremity muscles. As illustrated in **Figure 1**, performance of the pointing task (collapsed across different target sizes) resulted in extensive activation of bilateral sensorimotor cortex in the precentral and postcentral gyri/sulci (**Figure 1**, red). This contrasts with activation arising from the qualitatively different finger tapping task (**Figure 1**, green/yellow), which resulted in activation restricted to contralateral sensorimotor areas and the corresponding ipsilateral cerebellum. Of note is that ipsilateral M1 activation in the pointing task is largely anterior to the activation arising from the tapping task executed by the contralateral hand.

While there is evidence for ipsilateral corticospinal projections in humans, evidence for the control of the hand movements via ipsilateral corticospinal connections is weak. In intact humans, stimulation of M1 using TMS elicits MEPs in ipsilateral hand muscles but these are difficult to obtain and require high stimulation intensity and pre-innervations of the target muscle (21). In non-human primates, recording of ipsilateral M1 neurons during upper limb movements demonstrate that cells in iM1 are modulated by the task but that the timing of this activity is best correlated with weak muscle activity in the contralateral non-moving arm (22). Alternatively, task-related effects in the ipsilateral M1 could be mediated by corticoreticulospinal connections. In contrast to corticospinal connection, corticoreticulospinal projections are bilateral and are thought to be involved in the execution of selective finger movements (23). The involvement of this pathway is supported by TMS-derived evidence of longer latencies of MEPs elicited in the ipsilateral hand muscles (21). One could also argue that this M1 area may be concerned with the integration of afferent input from other motor areas. Recent evidence of bilateral M1 projections from posterior parietal (24, 25) and dorsal premotor areas, likely conveying some task-related information such as visuospatial and motor planning information, support a more indirect effect and the notion that M1 functions at a higher level in motor control by integrating afferent information and then generating a descending motor command that defines the spatiotemporal form of the movement (26). A higher level role for M1 in motor control is also supported by the results of a recent repetitive TMS (rTMS)

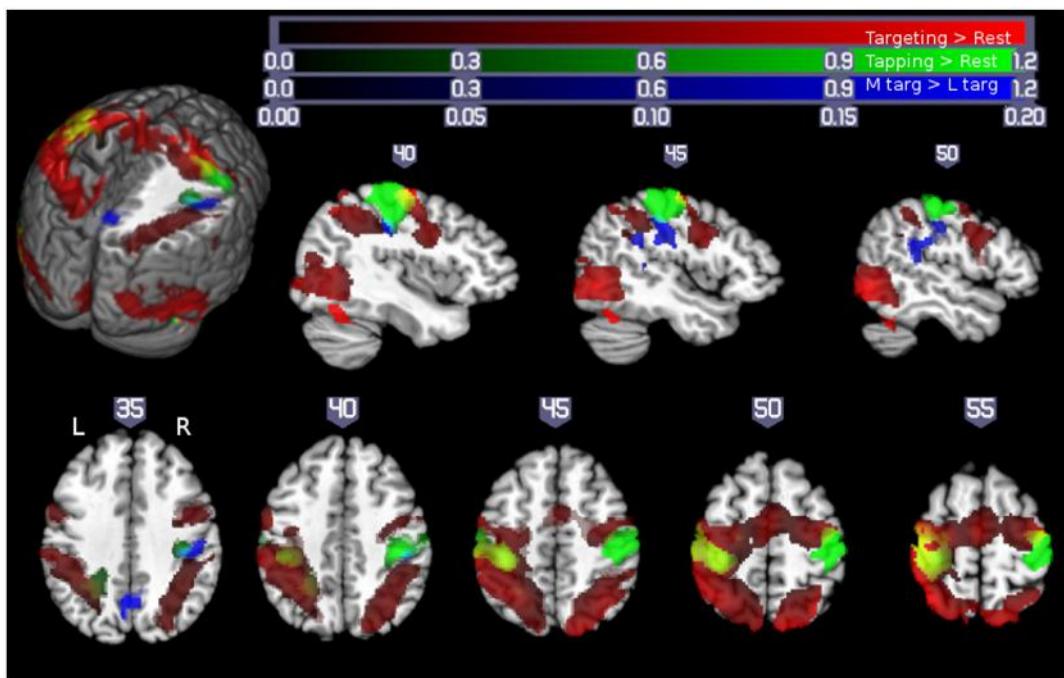


FIGURE 1 | Motor demand-dependent activation of motor cortices using a pointing task: pointing and finger tapping tasks related brain activation: Activity related to the pointing task (collapsed across XL, L, and M targets) is indicated in red. Activation related to right- and left-handed finger tapping is indicated in green, with overlap between finger tapping and pointing task performance shown in yellow. Note that while there was extensive bilateral activation for the pointing task, M1 activation in the finger tapping tasks was only seen contralateral to the performing hand, so that the left hemisphere is solely due to right-handed finger tapping (with left hemisphere yellow areas show overlap between right-handed finger tapping and right-handed pointing task performance) and the right hemisphere activity is solely due to left-handed finger tapping (yellow colors in the right hemisphere show overlap between activity due to the right-handed targeting task and left-handed finger tapping task, outlined with a yellow border for ease of visualization). Significant activation related to increasing motor demand (M targets > L targets) is indicated in blue (overlap between this region and left-handed finger tapping shown in cyan, outlined for clarity). All activations are shown overlaid on the Colin27 template in standard space, thresholded at a corrected $p < 0.05$ (uncorrected threshold $p < 0.005$ and cluster size $>2360 \text{ mm}^3$). Increased color intensity corresponds to higher estimates of percent signal change. Cuts in the three-dimensional rendering are shown at $x = 0$, $y = -15$, and $z = 35$. The right hemisphere is depicted in the upper panel. The right (R) and left (L) side of the brain are indicated in the lower panel. Numerical labels above each slice show slice coordinates in the x dimension (sagittal sections) or z dimension (axial sections) (11).

study where low-frequency rTMS applied to left M1 improved performance in both hands for the task with the highest demand on precision while performance remained unchanged for the tasks with lower demands (14).

Interhemispheric Interaction in the Control of Hand Movements in Intact Humans

In addition to the corticospinal projections and ipsilateral corticocortico connections, motor areas of the two hemispheres are interconnected to each other and interact in the execution of motor tasks. Improved performance after transiently inhibiting the ipsilateral M1 by means of low-frequency rTMS (14, 27, 28) could indicate that there may be a need for suppression of task performance related ipsilateral M1 excitatory activity. Because the relationship between the two primary motor cortices is impacted by stroke (4, 5, 29) and topic of great interest in neuromodulation treatment approaches targeting the contralesional M1 (3), this topic will be reviewed for the intact brain.

The main structure connecting the motor areas is the corpus callosum. Connections between primary motor areas are less

abundant than premotor areas and primarily excitatory [for detailed review, see Ref. (5)]. Interhemispheric inhibition (IHI) can be demonstrated with TMS by applying a conditioning stimulus (CS) to one M1 and a test stimulus (TS) to the homotopic area of the other M1 (30) (**Figure 2**). The CS inhibits the size of the MEP produced by the TS. The amount of inhibition is expressed as a percentage of the mean MEP amplitude evoked by a single TS. While resting IHI is measured with the subject at rest, active IHI is measured during movement preparation. In healthy subjects executing a hand motor task, the inhibitory effect of one M1 on the other M1 decreases (31) depending on the movement kinematics (32, 33). In a study by Talelli et al. (20), a relationship between resting IHI and task-related ipsilateral M1 activity as measured by fMRI was demonstrated. Specifically, peak forces for a hand grip were positively correlated with increases in ipsilateral M1-blood oxygenation level-dependent (BOLD) response when IHI between motor cortices was weak. This positive correlation changed to a negative correlation when IHI was strong. This would indicate that activity in ipsilateral M1 is controlled to some extent by the inhibitory effect of the contralateral M1.

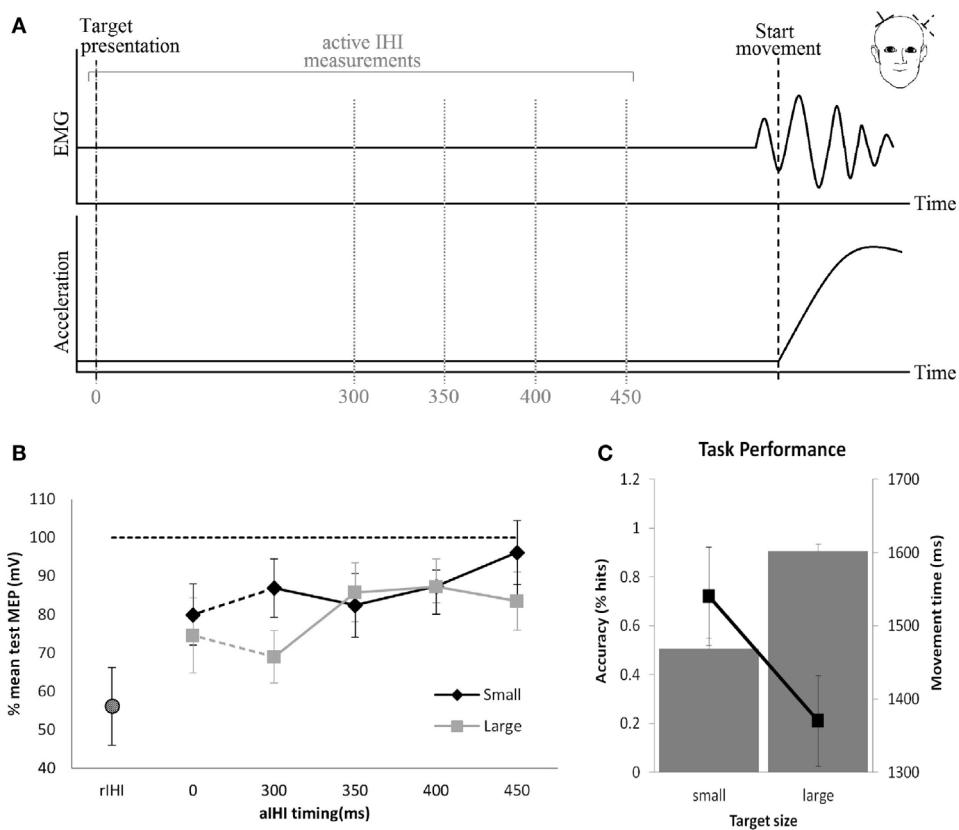


FIGURE 2 | Resting and active interhemispheric inhibition (IHI): (A) IHI can be demonstrated by applying a conditioning stimulus to M1, which inhibits the size of the motor-evoked potential (MEP) produced by the test stimulus applied to the homotopic area of the opposite M1. These measures are obtained during rest (resting IHI, rIHI) or in the pre-movement period during preparation of a movement (active IHI). (B) During rest, there is significant rIHI (round symbol) from one M1 on the other M1. Active IHI (rectangular symbol) decreases immediately prior to the movement onset depending on kinematics of the movement (B,C). (B,C) Pointing to a large target with less demand on accuracy (square) results in less reduction of active IHI compared to pointing at a small target (diamond) with high demand on accuracy (33).

Contralesional M1 Reorganization in Post-Stroke Recovery

Reorganization of Contralesional M1 in the Post-Stroke Recovery Period (fMRI Evidence)

In task-related functional imaging studies of stroke patients, the activation of contralesional motor areas (corresponding to ipsilateral motor areas in healthy subjects) have been consistently reported (34). Cross-sectional studies of stroke patients moving the affected hand revealed a shift from an initially (abnormal) bilateral activation of motor areas in the subacute stroke patients (1, 9, 16, 35–40) toward a more normal unilateral activation pattern of ipsilesional motor areas in chronic stroke patients (40). Importantly, in a longitudinal study of stroke patients, this activation shift to the ipsilesional hemisphere was associated with good recovery, whereas persistence of the bilateral activation pattern was associated with poor outcome (40). On the basis of these studies, it was concluded that greater involvement of contralesional M1 predicted poorer motor outcome. (34, 40). However, in several studies, mirror movements of the non-affected hand were reported during the performance with the affected hand during imaging (34). This raised the possibility

that some contralesional M1 activity is, in fact, related to mirror movements of the non-affected hand (41, 42). As mirror movements and coactivation of the non-affected hand are seen more frequently in patients with poor motor outcome (41, 43), the presence of these movements may have confounded the findings of increased contralesional M1 activation in patients with poor outcome.

In our own fMRI study of subacute stroke patients with excellent recovery, strictly unilateral performance resulted in activation of bilateral motor cortices (16). In this study, eight stroke patients underwent fMRI of the brain to test M1 activity related to the performance of a non-sequential finger opposition task with their paretic hand. EMG activity of bilateral arm muscles was recorded during the scanning. All patients showed excellent recovery. Their results were compared to age-matched normal volunteers. While overt mirror movements were absent in all patients, three patients showed substantial EMG activity of the non-affected arm when performing the task with the affected hand. Their data were excluded from further analysis. As demonstrated in Figure 3, in the remaining five patients with strictly unilateral performance, bilateral activation of premotor and primary motor cortices was evident. In contrast, the age-matched controls showed a strictly

unilateral activation of the corresponding contralateral M1. These results support the notion that activation in contralesional M1 most likely reflects a reorganizational process in these patients. However, based on the findings in healthy subjects, where ipsilateral M1 is activated as the task becomes more demanding, increased activity could also be explained by a relatively higher demand on motor skill in stroke patients when compared to healthy controls (i.e., because of the compromised hand function due to stroke, the execution of the task is more challenging for the patient compared to the controls). Schaechter and Perdue (44) studied chronic stroke patients with good recovery of hand function and demonstrated that cortical activation during performance of the unskilled and skilled movement was increased in the patients relative to controls in the contralesional primary sensorimotor cortex. These findings suggest that in the chronic phase after stroke the neuronal substrate supporting affected hand function includes contralesional M1. The question whether this abnormal contralesional M1 activity is related to recovery-related regenerative responses as demonstrated for the subacute stroke patients or whether these changes reflect degenerative responses to the stroke remains to be determined as both processes are to some extent activity dependent, interact and impact similar circuitries (4).

Mechanisms Underlying Reorganization of Contralesional M1 in the Post-Stroke Recovery Period

The interpretation of task-related fMRI results is limited by the fact that changes in inhibitory and excitatory activity cannot be distinguished and the functional relevance of these changes in M1 activity is unclear. Specifically, task-related increases in BOLD in

contralesional M1 could result from increases of inhibitory or excitatory activity or any combination of these.

In rodent stroke models, functional and structural reorganizational changes in contralesional M1 have been reported [for detailed review, see Ref. (4, 5)]. Briefly, in these models, small focal cortical lesions led to long-lasting changes in contralesional M1, such as down-regulation of GABA_A-receptor function (45, 46) and up-regulation of NMDA-receptor function (47, 48), both mechanisms operating in increases of synaptic efficacy such as long-term potentiation (LTP). In contrast to human studies (see below), excitability in contralesional M1 was transiently increased but returned to the original values within hours. Similarly, representation of the rodent forelimb expanded in the contralesional M1 but returned to normal dimensions over the following days [for review, see Ref. (5)]. From a structural perspective, increase in neuropil volume (49), use-dependent dendritic growth followed by dendritic pruning, synapse formation, and changes in the specific structure of synaptic connections have been described (49–51).

In humans, increased intracortical excitability of contralesional M1 has been demonstrated in subacute and chronic stroke patients (29, 52–54) when explored with the paired pulse TMS technique. In this paradigm, a suprathreshold TS is preceded by a subthreshold CS at an interstimulus interval (ISI) of 2 ms. In the M1 of healthy subjects, CS inhibits the MEP produced by the subsequent TS, referred to as short interval intracortical inhibition (55). This effect is mediated by GABA_A-receptors (56) and arises in close proximity to the stimulated area (57). By varying the intensity of CS, the effects mediated by inhibitory and excitatory networks can be separated in more detail (29, 54) (**Figures 4A,B**). In a study of subacute stroke patients, the inhibitory effect of CS at

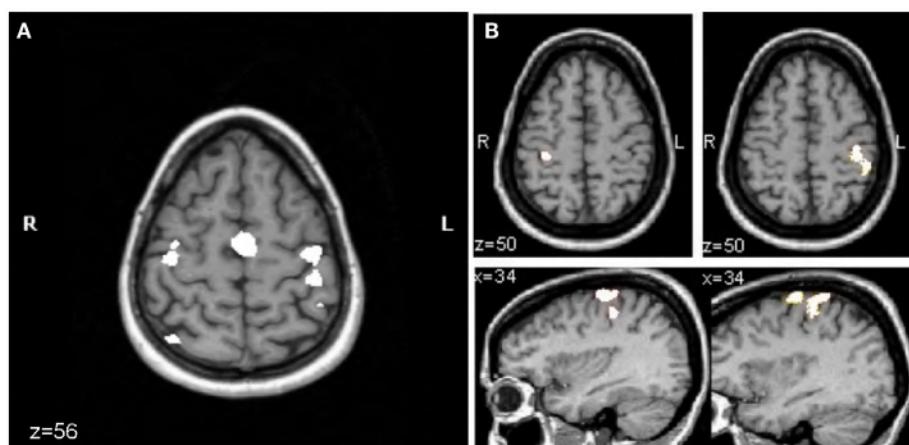


FIGURE 3 | Mean fMRI activation map of the performance of a finger sequence with the affected hand in patients ($n = 5$) (A) and with either hand in the age-matched control group ($n = 9$) (B). For both groups, the activation map is superimposed on the T1-weighted MRI of the same healthy control subject. (A) In patients, right in the axial slice of brain ($z = 56$) corresponds to the lesioned hemisphere and left to the contralesional hemisphere. Activation of contralesional precentral gyrus is evident (corrected $p < 0.05$). (B) For the control group performing the finger sequence with the left (lower left image, corrected $p = 0.05$) or right (lower right image, uncorrected $p < 5.8e-12$) hand, there was activation in the precentral gyrus of the hemisphere that is contralateral to the performing hand. Initially, the significance level was set as low as corrected $p = 0.05$ to pick up any activity in the motor cortex ipsilateral to the moving hand (shown for left hand movement, lower left image). At this significance level, massive activation was seen in the pre- and postcentral gyrus contralaterally when moving the right hand. To separate clusters of activity in pre- and postcentral gyrus, the significance level was increased until the two clusters became distinct (uncorrected $p < 5.8e-12$, lower right panel) (16).

low intensity was similar to values found in healthy age-matched controls while the inhibitory effect was abnormally reduced at higher intensities. This may indicate that the balance of excitatory and inhibitory activity in neuronal circuits was shifted toward excitatory activity (29, 54). Alternatively, abnormal function of the high threshold GABAergic inhibitory interneurons may result in a decreased inhibitory effect of CS at higher intensities. These findings suggest that regulation of excitatory and inhibitory neurotransmitter systems may play a role early in the reorganization process in contralesional M1 (48, 58) and may support functional recovery early after stroke. This notion is supported by the finding in patients in the subacute phase of stroke involving M1 or its corticospinal projections where a close association between increased excitability of contralesional M1 and good recovery of hand function was demonstrated (54). However, whether these findings hold up and can be applied to patients with other lesion locations has to be determined in larger longitudinal studies.

Relationship Between Contralesional M1 and Ipsilesional M1 (Interhemispheric Inhibition) in the Post-Stroke Recovery Period

As described for the intact brain, the two motor cortices inhibit each other through connections via the corpus callosum (5). In addition to the discussed mechanisms underlying contralesional M1 reorganization, stroke-related changes in the inhibitory drive between motor cortices could play an important role in reorganizational changes of contralesional M1. While increased contralesional M1 excitability was demonstrated in multiple studies (29, 31, 53, 54, 59), very few studies have examined the relationship between increased contralesional M1 excitability and resting IHI. It was concluded that loss of inhibitory drive of the lesioned M1 on the contralesional M1 through interhemispheric connections may contribute to the reorganizational processes observed for this motor cortex. Increases in contralesional M1 excitability may result in an excessive inhibitory effect on the ipsilesional M1, which may interfere with its reorganization and related recovery (31, 53, 59). In our study of 23 subacute stroke patients with documented ongoing recovery of motor function, contralesional M1 excitability was increased as demonstrated by paired pulse TMS technique (29) (see above for detailed description of the methods). Resting IHI from ipsilesional M1 on contralesional M1 was reduced in both cortical and subcortical location of the stroke while IHI from contralesional M1 on ipsilesional M1 was normal (**Figures 4C,D**). In patients with cortical stroke, there was an inverse correlation between inhibitory effect from contralesional on ipsilesional M1 and contralesional M1 excitability. This relationship was not seen in patients with subcortical stroke. This would indicate that in subacute patients recovering from stroke, the demonstrated increased contralesional M1 excitability is not causally related to abnormally reduced IHI from ipsilesional M1 on contralesional M1. Further, because IHI of the contralesional on ipsilesional M1 was normal and measures of contralesional M1 excitability were increased, there was no evidence in this study to support the hypothesis that an abnormally increased contralesional M1 excitability results in abnormally increased IHI of contralesional on ipsilesional M1 with subsequently decreased activity or excitability of ipsilesional M1 in this patient population.

However, when IHI was measured in the pre-movement interval (active IHI, see above for details of the methods) contralesional on the ipsilesional M1 was abnormally increased in chronic stroke patients when compared to healthy age-matched controls (31). The role of abnormally increased active IHI and the relationship between abnormal active IHI, measures of M1 excitability, and recovery of hand function in stroke needs to be determined in more detail and is currently a topic of active investigations.

There is some evidence regarding the relationship between the ipsi- and contralesional M1 in rodent stroke models. Specifically, an ischemic lesion of M1 leads to partial denervation of the contralesional M1, which has a tendency to sprout into the peri-lesional neuronal tissue of ipsilesional M1 (60, 61). Moreover, learning a new motor skill with the non-affected limb reduces spontaneous recovery and limits rehabilitation-related functional improvements of the affected limb (62–64). These findings underscore the importance of interhemispheric connections between and ipsi- and contralesional M1 and their potential involvement in mediating reorganizational effects on the ipsilesional M1.

Factors that Determine the Role of Contralesional M1 in the Post-Stroke Recovery Period

The factors that determine involvement of contralesional M1 are currently not known. In non-human primate stroke models, progressively larger M1 hand lesions were associated with a proportional expansion of ipsilesional ventral premotor (PMv) (65, 66) and supplementary motor area (SMA) (67) hand representation.

In rodent stroke models, reorganizational changes in contralesional M1 depend on the lesion size (68) and rehabilitation treatment (64, 69) and include long-term changes in neurotransmitter systems, dendritic growth, and synapse formation (45, 46, 50, 51, 70, 71). Inhibiting the contralesional hemisphere in rats that recovered from large ischemic infarcts generates more behavioral deficits of the impaired forelimb in comparison to control animals (72).

In humans, Schaechter and Perdue (44) demonstrated in chronic stroke patients a linear relationship between abnormally increased affected hand movement-related contralesional M1 activity and extent of CST damage. Further, the observed differential effect on contralesional M1 excitability and the relationship between contralesional M1 excitability and IHI (**Figure 4**) (29) supports the notion that location of the stroke seems to impact reorganizational processes. These differential remote effects of the lesion are also consistent with the findings that contralesional M1 seems to support function in a subset of patients after stroke (18) but may interfere with recovery or affected hand function in others (73, 74).

Interventions in Stroke Rehabilitation Treatment Targeting Contralesional M1

Several reports have demonstrated that non-invasive cortical stimulation can enhance functional reorganization, motor cortical excitability, and the beneficial effects of motor training on performance (75–80). Either ipsi- or contralesional M1 are target of these interventional approaches (3). In this

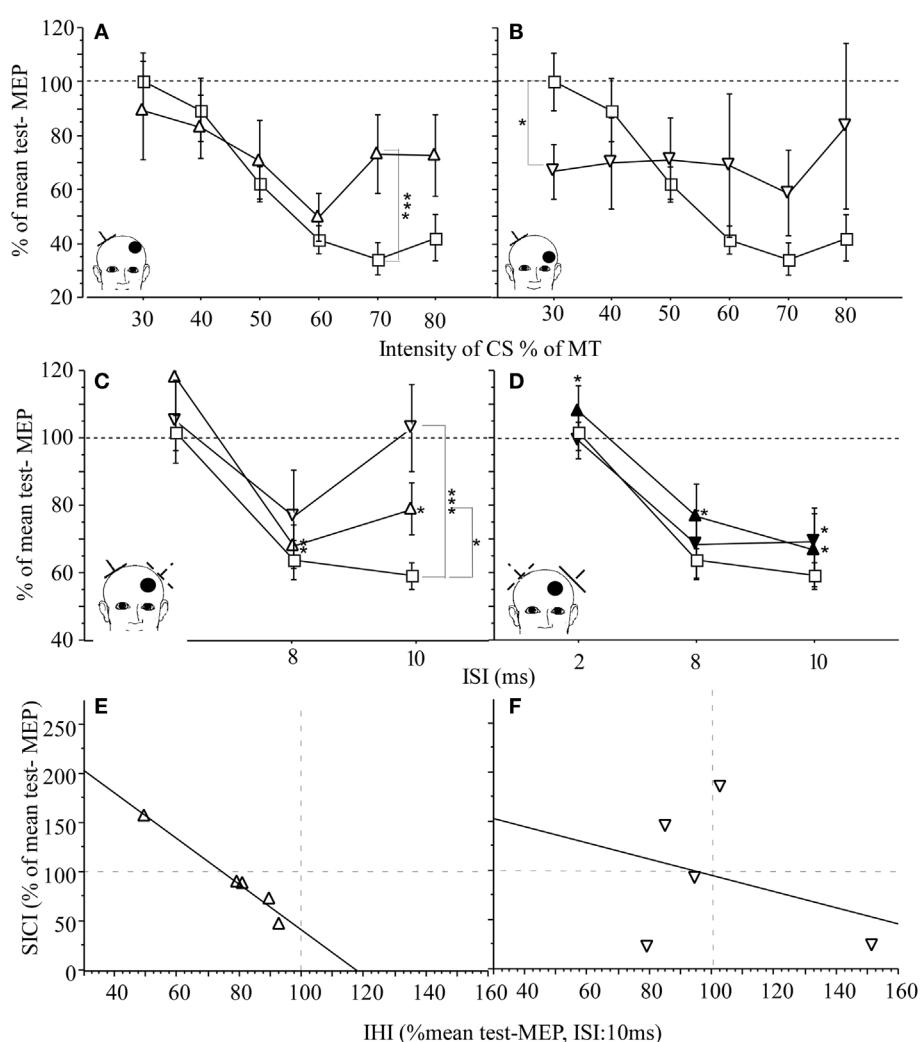


FIGURE 4 | M1 excitability and IHI in patients with subacute stroke ($n = 23$) and healthy age-matched controls ($n = 20$): EMG was recorded from the first dorsal interosseus muscle (FDI). (A,B) Effect of lesion location on SICI in patients. Control (square) and contralesional M1 of patients with cortical [open triangle (A)] and subcortical location of infarction [open inverted triangle (C)]. IHI of the lesioned M1 on the contralesional M1 is reduced in patients with cortical (open triangle) or subcortical infarction (open inverted triangle) when compared to healthy controls (square). (D) IHI from contralesional M1 on the lesioned M1 was intact for cortical infarction (black triangle) and subcortical infarction (black inverted triangle). The conditioned MEP amplitude is expressed as percentage of the mean test-MEP. (E,F) Relationship between M1 excitability, SICI (CS at 80% MT), and IHI in patients with cortical infarction (triangle) and subcortical infarction (inverted triangle). For each patient (each point represents one subject), SICI of the contralesional M1 was plotted against IHI from lesioned on the contralesional M1 (open symbols). Regression was calculated. For cortical location of the infarction, there was an inverse linear relationship between SICI of the contralesional M1 and IHI from lesioned on the contralesional M1 [(E) $r^2 = 0.972$, $p = 0.002$]. Although there is a similar trend in the subcortical group (F), the relationship was more variable [(F) $r^2 = 0.105$, $p = \text{ns}$]. The insert indicates the position of the coil for application of CS (dotted lines) and the TS (solid lines). The location of the lesion is indicated by the bullet. CS = intensity of conditioning stimulus, MT = motor threshold. The scattered lines indicate the cutoff between facilitation (>100) and inhibition (<100). Mean \pm SE. * $p < 0.05$, ** $p < 0.02$, and *** $p < 0.01$ (29).

review, I will focus on non-invasive cortical stimulation targeting the contralesional M1.

Down-regulation of excitability in one motor cortex influences corticomotor excitability in the opposite motor cortex. Several reports of studies in healthy subjects have now demonstrated that 1 Hz rTMS applied to M1 of one hemisphere results in increased corticomotor excitability in the opposite M1 (81, 82) and improved performance in the corresponding hand (14, 83) depending on the level of motor demand (14). As discussed

in the previous sections, although the extent to which the contralesional M1 contributes to motor recovery is not known, many currently employed rTMS protocols are designed with the assumption that following stroke, ipsilesional M1 is hypoactive while contralesional M1 is hyperactive and should be inhibited (3, 80). Accordingly, stimulation of contralesional M1 has been used to inhibit its hyperactivity (3, 74, 78, 84–86). Meta-analyses on the effectiveness of repetitive transcranial magnetic stimulation (rTMS) or transcranial direct current stimulation (tDCS)

in stroke rehabilitation therapy do not agree on the available evidence to either support or reject it (87–90).

Summary

Taken together, there is evidence from human and animal studies that activity in contralesional M1 will impact motor function of the paretic limb differently in different patients. However, currently employed treatment strategies are geared toward inhibiting its function. There is a great need to identify the precise factors

that specifically influence the role of contralesional M1 in the recovery process. A better understanding of those factors is critical to the development of effective therapies tailored to its specific role in the recovery process to improve outcome post stroke.

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Motor Recovery After Subcortical Stroke Depends on Modulation of Extant Motor Networks

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Introduction: Stroke is the leading cause of long-term disability. Functional imaging studies report widespread changes in movement-related cortical networks after stroke. Whether these are a result of stroke-specific cognitive processes or reflect modulation of existing movement-related networks is unknown. Understanding this distinction is critical in establishing more effective restorative therapies after stroke. Using multivariate analysis (tensor-independent component analysis – TICA), we map the neural networks involved during motor imagery (MI) and executed movement (EM) in subcortical stroke patients and age-matched controls.

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Methods: Twenty subcortical stroke patients and 17 age-matched controls were recruited. They were screened for their ability to carry out MI (Chaotic MI Assessment). The fMRI task was a right-hand finger-thumb opposition sequence (auditory-paced 1 Hz; 2, 3, 4, 5, 2...). Two separate runs were acquired (MI and rest and EM and rest; block design). There was no distinction between groups or tasks until the last stage of analysis, which allowed TICA to identify independent components (ICs) that were common or distinct to each group or task with no prior assumptions.

Results: TICA defined 28 ICs. ICs representing artifacts were excluded. ICs were only included if the subject scores were significant (for either EM or MI). Seven ICs remained that involved the primary and secondary motor networks. All ICs were shared between the stroke and age-matched controls. Five ICs were common to both tasks and three were exclusive to EM. Two ICs were related to motor recovery and one with time since stroke onset, but all were shared with age-matched controls. No IC was exclusive to stroke patients.

Conclusion: We report that the cortical networks in stroke patients that relate to recovery of motor function represent modulation of existing cortical networks present in age-matched controls. The absence of cortical networks specific to stroke patients suggests that motor adaptation and other potential confounders (e.g., effort and additional muscle use) are not responsible for the changes in the cortical networks reported after stroke. This highlights that recovery of motor function after subcortical stroke involves preexisting cortical networks that could help identify more effective restorative therapies.

Keywords: motor imagery, functional imaging, fMRI, mental imagery, brain mapping

INTRODUCTION

Stroke remains a leading cause of long-term disability and carries a significant social and economic cost (1, 2). After stroke, functional imaging studies of movement report widespread changes in activation of the cortical networks (3–8). The precise cognitive processes that determine these changes remain unclear. In this study, we used a data-led method to explore if the changes in movement-related networks are a result of processes specific to stroke patients (i.e., use of additional muscles) or whether they represent modulation of extant movement-related networks. Understanding this distinction in neuroplasticity is likely to help establish the driver of fMRI changes reported after stroke and help establish the most effective restorative therapies for patients (9–11).

Using a variety of tasks, numerous groups have reported changes in movement-related networks – importantly these remote changes relate to the recovery of motor performance. Movement-related fMRI activation in the ipsilesional primary motor cortex is associated with better recovery (4, 7, 8, 12, 13). Indeed it is on this model that many restorative intervention studies are based (14) changes in movement-related networks are being used to predict response to therapies (15). Yet it is possible that the changes in movement-related networks may represent an epiphenomenon of the increased difficulty involved in carrying out the task after a stroke (6).

There are several caveats when considering comparisons of patients with healthy volunteers (6). For instance, the kinematics of movements, EMG patterns, motor strategies (adaptation versus relearning), and whether movement involved different body parts in different subjects have not been monitored consistently in the MRI. In other words, it is possible that the differences reported represent a composite of cognitive processes specific to stroke patients that may not be directly related to the recovery process as such.

Understanding whether there are networks specific to stroke patients will greatly aid the understanding of the recovery process after stroke. It may allow a more targeted approach to rehabilitation as it could identify the most appropriate training programs. We explored the extent to which the widely described changes in motor networks after stroke are a result of specific processes (i.e., motor adaptation or use of different muscle) or whether they represent modulation of extant motor performance. There are two key aspects to our study.

First, to remove any biases produced by subtle differences in motor performance, we studied both motor imagery (MI) and executed movement (EM). MI is intrinsically linked to the motor system and can be used to study the motor system without actual movement (16–19). In stroke patients with normal activations during EM, we have reported abnormal hemispheric lateralization during MI that related to recovery of motor function. In other words, by studying MI as well as EM, we are able to identify aspects of task-dependent activation that relate to motor execution and those more “upstream” (20).

Second, we use a data-led approach using tensor-independent component analysis (TICA) (21). Using TICA, we examine

the cortical networks that are common to stroke patients and aged-matched controls or exclusive to either. Unlike the conventional mass univariate approach, TICA is a powerful data-led approach that explores similarities as well as differences in cortical networks. Importantly, both tasks (MI and EM) from both groups (stroke and aged-matched controls) are considered the same. We are able to use a “blinded task” during the production of the independent components (ICs) as they have the same temporal profile. In other words, we make no prior assumptions as to the extent of overlap, if any, between the task-related networks in stroke patients and controls or between the MI and EM. If the widely reported changes in movement-dependent networks are related to a stroke-specific cognitive process, then this analytic approach will likely produce separate components.

We hypothesize that in recovered subcortical stroke patients, the task-related motor networks identified for both EM and MI are shared with the age-matched controls. In keeping with our reports from healthy volunteers, we expect to find networks related exclusively to EM and others that are shared with MI. Finally, we expect that in stroke patients, the task-related networks would correlate with measures of motor recovery.

MATERIALS AND METHODS

Subjects

Twenty subcortical stroke patients were recruited (six females; mean age, 66 ± 8.8 years). Inclusion criteria were the following: (i) first-ever ischemic or hemorrhagic stroke with initial motor deficit lasting at least 2 weeks; (ii) ability to perform the motor activation task; and (iii) right-handedness. They had no past medical history of any neurological, psychiatric, or musculoskeletal disorders and were not taking regular medication. Seventeen age-matched control subjects (nine males) aged 40 years (mean, 57.6 ± 8.5 years) were recruited through local advertisement. Subjects had no history of medical disorders and were not taking regular medication. All subjects were right handed as assessed by the Edinburgh scale (22) and gave written consent in accordance with the Declaration of Helsinki, and the protocol was approved by the Cambridge Regional Ethics Committee.

All subjects underwent assessment with the Chaotic Motor Imagery Assessment (CMIA). They were excluded if unable to perform MI adequately. Chaotic Motor Imagery is defined as an inability to perform MI accurately or, if having preserved accuracy, the demonstration of temporal uncoupling (23). The full-assessment is described in detail in Ref. (24). Briefly, the assessment has three components performed in order. Where appropriate, subjects were given specific instructions to perform first-person kinesthetic MI. They were instructed not to view the scene from the third person and not to count or assign numbers or tones to each finger.

The stroke patients were assessed with the NIH Stroke Scale (NIHSS), the Action Research Arm Test (ARAT), Stroke Impact Score (SIS), and the Motricity Index. Thumb to index finger tapping over 15 s (TIT ratio) (25) and mirror synkinesia were measured. Transcranial Doppler was used to assess vasomotor reactivity and was preserved in all.

Functional MRI

Motor (Imagery) Paradigm

The fMRI tasks was a block design (20, 26) of a right-hand finger-thumb opposition sequence (paced at 1 Hz; sequence 2, 3, 4, 5, 2...) and rest. There were two separate runs acquired (MI and rest and EM and rest). Subjects were instructed to keep their eyes closed throughout the session. We used bilateral fiber-optic gloves (Fifth Dimension Technologies, SA) to monitor finger movements and exclude inappropriate movement. The gloves were also used to confirm the performance of MI – after each MI block (24). Post MR subjects rated the vividness of MI performance on a seven-point scale.

Data Acquisition

A 3-T Brucker MRI scanner was used to acquire both T2-weighted and proton density anatomical images and T2*-weighted MRI transverse echo-planar images sensitive to the BOLD signal for fMRI ($64 \times 64 \times 23$; FOV $20 \times 20 \times 115$; 23 slices 4 mm, TR = 1.5 s, TE 30 ms, voxel size $4 \times 4 \times 4$).

Image Analysis

Analysis was carried out using TICA (21) as implemented in MELODIC (Multivariate Exploratory Linear Decomposition into Independent Components) Version 3.09, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). Only the affected hand in stroke patients was assessed. Where necessary images were flipped, the hand studied was always contralateral to the left hemisphere matching the right-hand tasks of the age-matched controls. Contralateral is therefore ipsilesional in stroke patients.

The first 12 volumes were discarded to allow for T1 equilibration effects. Preprocessing involved masking of non-brain voxels, voxel-wise de-meaning of the data, and normalization of the voxel-wise variance. Subject movement was less than 2 mm.

The preprocessed data were whitened and projected into a multidimensional subspace using probabilistic principal component analysis where the number of dimensions was estimated using the Laplace approximation to the Bayesian evidence of the model order (27). The whitened observations were decomposed into sets of vectors which describe signal variation across the temporal domain (time courses), the session/subject domain, and the spatial domain (maps) by optimizing for non-Gaussian spatial source distributions using a fixed-point iteration technique (28). Estimated component maps were divided by the standard deviation of the residual noise and thresholded by fitting a mixture model to the histogram of intensity values. The time course of each IC was then entered into a general linear model of the convolved block design of Task versus Rest.

An IC was considered to be involved in MI or EM if a one-way *t*-test found the subject scores to be significantly different from zero across subjects. When an IC was significantly involved in both tasks, then a paired *t*-test ($p < 0.05$ corrected for multiple comparisons) was performed on the subject score for each task. In the stroke group, the subject scores of each remaining component were correlated (Spearman $p < 0.05$ corrected for multiple comparisons) with the impairment scores.

RESULTS

Behavioral Results

Four control subjects and eight stroke patients were excluded because of chaotic motor imagery. Twelve stroke patients remained [eight left hemisphere; four females; for full demographic details see Sharma et al. (24)]. There was no difference in score between the stroke group and control subjects.

All subjects suppressed movement and all were compliant during the fMRI task. Median post-MRI MI vividness score was 6 (range, 4–7).

fMRI Data

No distinction was made between tasks until the final stage of processing. As 25 subjects performed two tasks, MI and EM, 50 “blinded” tasks were processed. As no distinction was made between imagery and EM during the generation of the ICs, we use the term “blinded.”

A subject score for each IC is produced that includes the effect size for the 50 blinded tasks (13 controls subjects, EM and MI, 12 stroke patients) for the associated spatiotemporal process shown in the spatial map.

Twenty-eight ICs were defined by TICA. ICs that identified artifact recognized by previously published patterns and high frequency were excluded by visual inspection. ICs driven by outliers or were not significant across either task were also excluded. Therefore, only components in which the subject scores were significantly different from zero (for either the stroke or control group for either task) were included.

Seven ICs remained. Each component was significantly involved in both the stroke group and the control group. As hypothesized, some ICs were shared between EM and MI (subject scores significantly greater than zero for both tasks in both groups) and some were exclusive to EM (subject score greater than zero for EM only in both groups).

Figures 1 and 2 show the whole brain activations and deactivations, the time course (BOLD), subject scores, and percentage of total variance explained. **Table 1** summarizes the areas involved [labeled using the Jülich Atlas (29)].

Independent Components (IC 1, 2, 4, 5, 8) Shared by Executed Movement and Motor Imagery

Five components (IC 1, 2, 4, 5, 8; **Figures 1 and 2**) were significantly involved in both age-matched controls and stroke patients and were common to both EM and MI (subject scores > 0 for both tasks in both groups). Together these five ICs explained 33% of the total explained variance. All of the components significantly correlated with the active blocks of the task.

In three of the components (IC1, 2, 5), the subjects score was greater during EM than during MI in the age-matched controls only – no such difference was found in the stroke group. IC1 involved activation of the contralateral motor areas and bilateral involvement of premotor and parietal areas. More specifically, there was contralateral activation of BA4a, SMA, BA3b, and

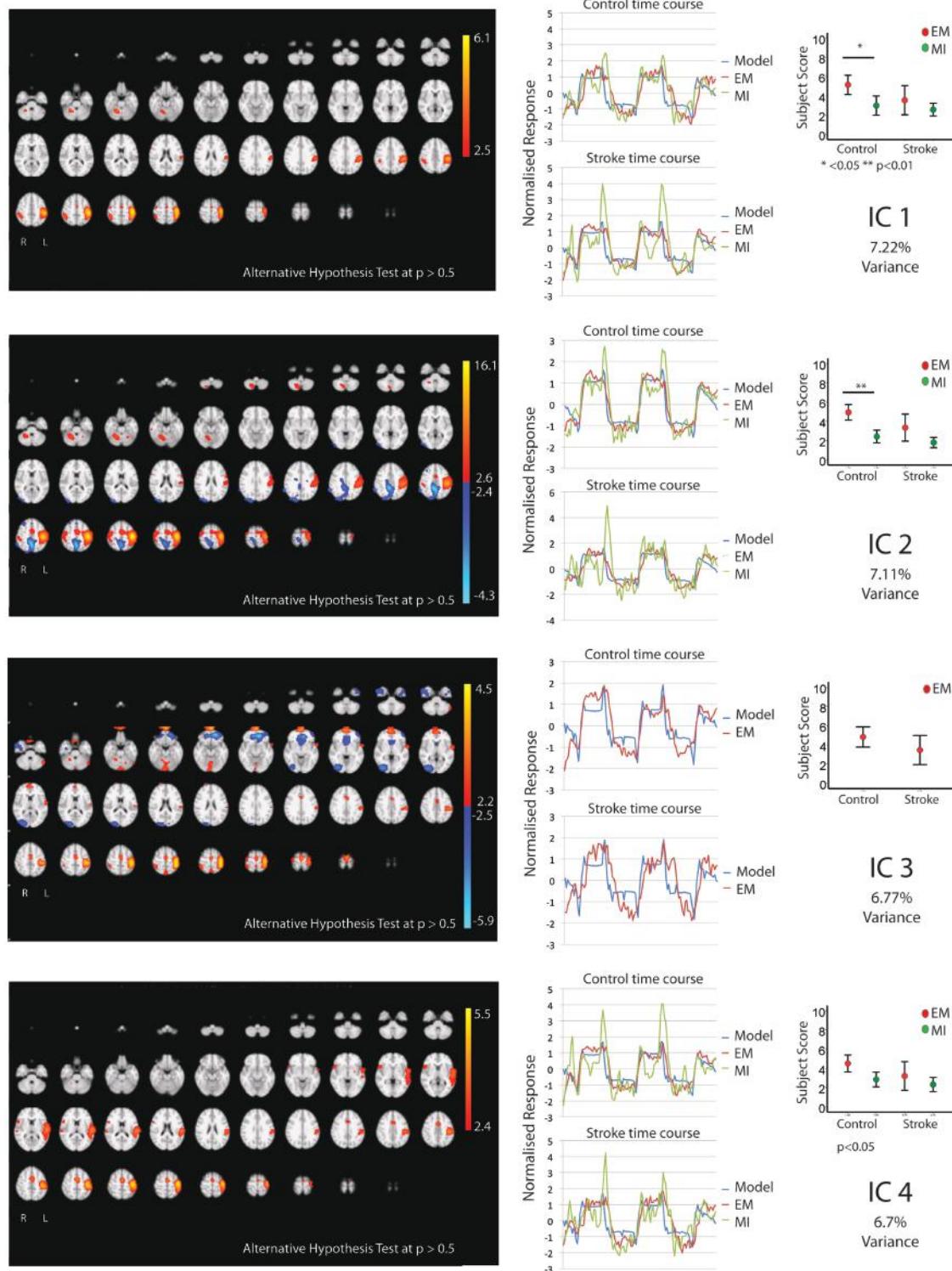
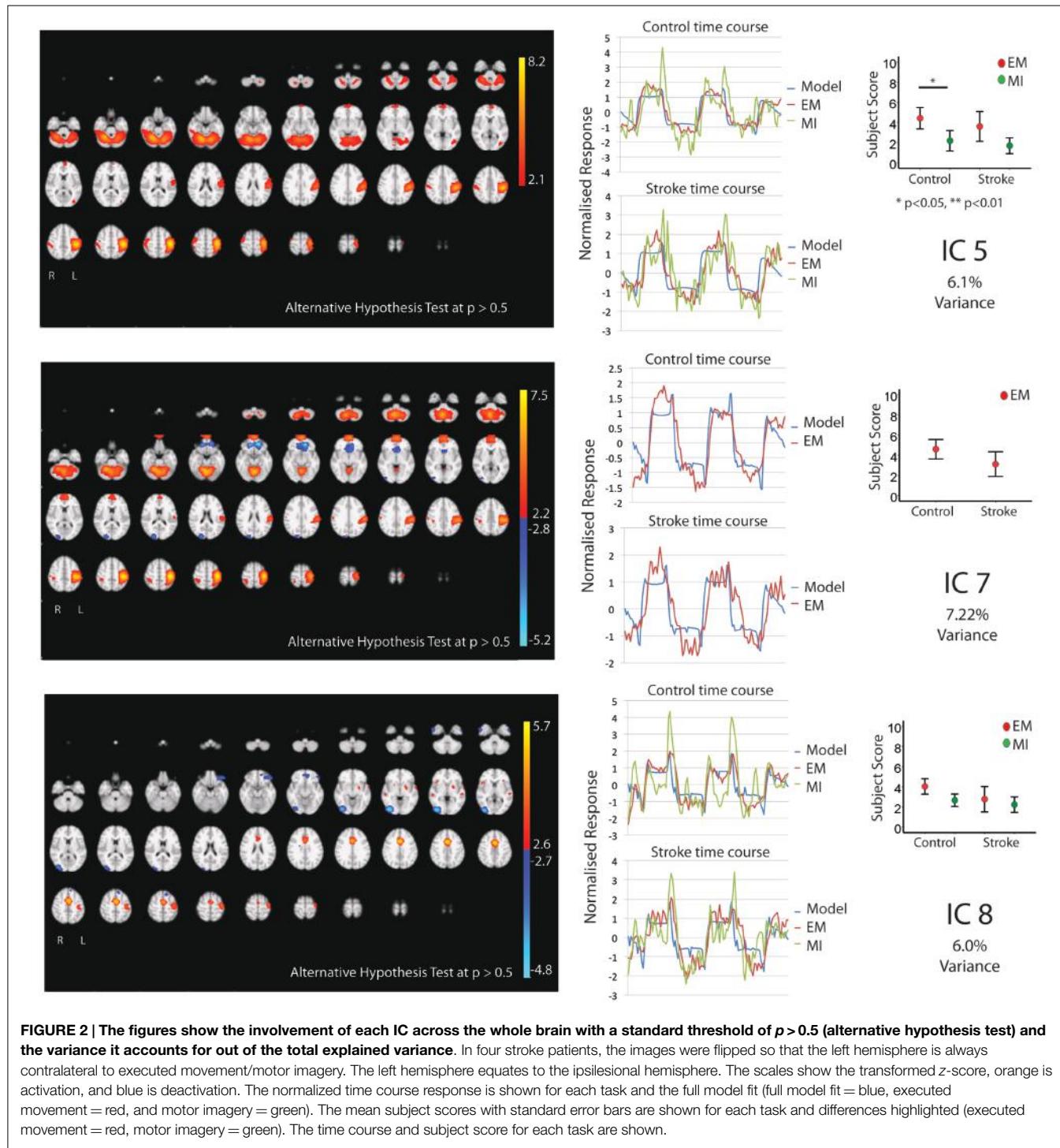


FIGURE 1 | The figures show the involvement of each IC across the whole brain with a standard threshold of $p > 0.5$ (alternative hypothesis test) and the variance it accounts for out of the total explained variance. In four stroke patients, the images were flipped so that the left hemisphere is always contralateral to executed movement/motor imagery. The left hemisphere equates to the ipsilesional hemisphere. The scales show the transformed z-score, orange is activation, and blue is deactivation. The normalized time course response is shown for each task and the full model fit (full model fit = blue, executed movement = red, and motor imagery = green). The mean subject scores with standard error bars are shown for each task and differences highlighted (executed movement = red, motor imagery = green). The time course and subject score for each task are shown.



parietal areas [IPC(PFo)]. There was bilateral activation of PMd, both SI and SII, and parietal areas (hIP2,3 and 7PC). There was ipsilateral activation of the parietal areas [hIP1, IPC (Pft)] and cerebellum.

Similarly IC 2 predominantly showed contralateral activation of BA4, parietal lobe [IPC (Pfo)], and bilateral activation of PMd, SI, SII, parietal lobe (hIP2), and contralateral cerebellum. However, in a different topographical location

(more dorsal), there was a small degree of deactivation of the contralateral BA4a and ipsilateral parietal lobe [IPC (Pfm)].

Independent component 4 was exclusively contralateral. While sensory motor areas (BA4, SMA, PMd, SI, SII, BA3a,3b) and parietal areas [both SPL(7PC) & IPC(Pfop)] were involved, it was the only IC to involve BA44. Notably there was no cerebellar activation.

TABLE 1 | Regions activated or deactivated in each independent component.

	Activated in both executed movement and motor imagery										Executed movement only			
	IC1		IC2		IC4		IC5		IC8		IC3		IC7	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
BA44						↑								
BA4	↑		↑ ^a	↓	↑		↑		↑		↑		↑	
Pre-SMA														
SMA	↑				↑						↑	↑		
PMd	↑	↑	↑	↑	↑			↑	↑		↑	↑		↑
Area 1	↑	↑	↑	↑	↑		↑	↑			↑	↑		↑
Area 2	↑	↑	↑	↑	↑		↑	↑	↑		↑	↑		↑
3a			↑		↑				↑		↑		↑	
3b	↑		↑	↑	↑		↑		↑		↑	↑	↑	
hIP1			↑											
hIP2	↑	↑	↑	↑			↑				↑			↑
hIP3	↑	↑						↑					↑	
SPL(7A)														↑
SPL(7PC)	↑	↑	↑		↑		↑				↑	↑		
IPC(PFop)	↑		↑		↑						↑		↑	
IPC(PFt)		↑		↑				↑			↑			↑
IPC(PFm)				↓										
IPC(Pga)														
IPC(PF)														
Thal_premotor														
Thal_motor														
Thal_Somatosensory														
Caudate														
TE														
CB		↑		↑			↑	↑			↑	↑	↑	↑

^aSmall area of deactivation in a more dorsal area.

Independent component 5 shared many features of IC1 and IC2, with involvement of primary and secondary motor areas as well as parietal areas. More specifically, there was contralateral activation of BA4, BA3b, parietal areas [SPL(7PC)], bilateral activation of SI, SII, and cerebellum, and ipsilateral parietal areas [IPC(PF)]. Notably, it was the only component with only ipsilateral involvement of PMd and parietal area (hIP3).

Independent component 8 was similar to IC4 with predominantly contralateral activation (except for SMA). This involved BA4a, BA3a. In contrast, it was the only component with contralateral PMd, SII activation.

Independent Components Involved During Executed Movement Only (IC 3, 7)

Two components, IC 3 and 7, were involved during EM only explaining 6.77 and 7.22% of total variance, respectively. IC3 involved activation of the contralateral BA4, BA3a, and IPC, with bilateral activation of SMA, PMd, S1&2, BA3b, parietal area (SPL), and cerebellum. There was ipsilateral activation of parietal area (hIP2). IC7 activated the contralateral BA4, PMd, S1, BA3a, and parietal areas [HIP3 SPL (7A)], with bilateral involvement of BA3b, parietal area [IPC (PFop)], and cerebellum. There was ipsilateral activation of SII, hIP2, and parietal area [IPC (Pft)].

Relationship of Motor Imagery and Executed Movement ICs in Stroke Patients to Motor Performance and Time Since Stroke (IC 1, 3, 7)

In the stroke group, there were two ICs (1 and 3) that related to motor performance. While IC 3 was exclusive to EM, it is notable that IC1 – a component common to both EM and MI – is also related to motor performance.

As there was no significant difference between the IC1 subject scores for each task, both tasks were explored together. There was a significant positive correlation between this combined IC1 subject score and the Motricity (Arm) scores ($\rho = 0.581$; $p < 0.05$), i.e., the greater the activity within this network the better the recovery. The same overall pattern of correlation was mirrored with SIS ($\rho = 0.501$; $p < 0.05$) and Motor Activity Log ($\rho = 0.540$; $p < 0.05$).

Independent component 3 (EM only) was positively correlated with SIS ($\rho = 0.648$; $p < 0.05$). In other words, greater activation of IC3 was associated with better recovery.

Finally, IC7 was negatively correlated with time since stroke ($\rho = -0.592$; $p < 0.05$), i.e., this activation within this network reduced with time since stroke.

Figure 3 summarizes these findings.

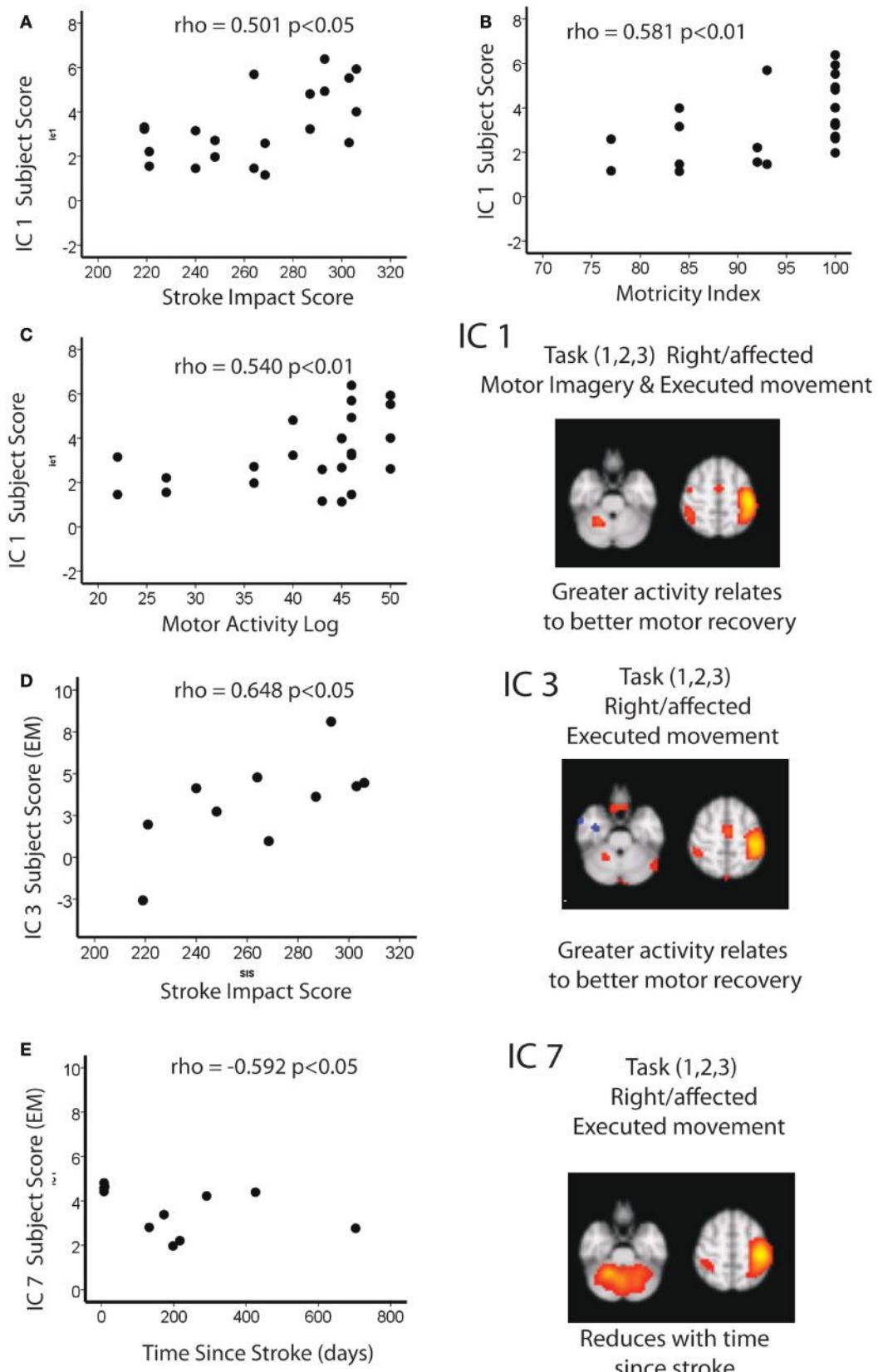


FIGURE 3 | Spearman correlations between IC1 and (A) stroke impact score (B) motricity index and (C) motor activity log. Spearman correlations between IC3 and (D) stroke impact score and (E) time since stroke.

DISCUSSION

We report that the cortical networks that relate to recovery of function are not specific to stroke but instead represent modulation of existing networks. As expected, most cortical networks were shared between EM and MI (accounting 33.13% of the total explained variance), with only two networks that were exclusively found during EM (accounting for 13.99% of the explained variance). The absence of any cortical networks specific to stroke patients suggests that the changes in cortical networks reported after stroke are not a result of a subtle biases exclusive to stroke patients – this may have included a motor behavior like adaptation (adjusting movement to new demands) or other potential confounders such as effort or attention. This work emphasizes that recovery of motor function involves preexisting cortical networks that may help identify more effective restorative therapies for stroke patients.

This study further extends the close similarities between MI and EM. We report that the first IC (IC1 accounting for 7.22% of the total explained variance) was involved in both groups and in both tasks (EM and MI). It involved activation of the contralateral motor areas and bilateral involvement of premotor and parietal areas. The involvement of the motor cortex – an area pivotal to motor learning (30) – strengthens the rationale for using MI training after stroke. We found that greater involvement of IC1 was associated with better recovery of motor performance after stroke. As this IC is shared between tasks, it suggests that a key aspect of the recovery process occurs “upstream” from motor execution. Importantly, this network is shared with age-matched controls, implying that it is not exclusive to stroke.

Consistent with our previous findings in healthy volunteers (31), we report two networks that are exclusive to motor execution (IC 3 and 7 explaining 6.77 and 7.22% of total variance, respectively). The areas common to both are the contralateral primary and secondary motor areas (although IC7 was largely bilateral with marked cerebellar involvement). This is likely explained by the differences between EM and imagery. First, EM involves discharge via the corticospinal tract (CST) that we have previously suggested dominates the movement-related activation (6). Second, the resultant movement produces afferent sensory feedback to the motor system.

We postulate that the IC3 is responsible for the discharge via the CST, given the near exclusive activation of the primary motor cortex. In support of this view, greater subject score of this network is associated with better recovery of motor performance (as assessed with the SIS). This is consistent with the findings from transcranial magnetic stimulation (TMS) studies that suggest that preservation of the CST is associated with a better recovery of motor performance after stroke (32–34).

It is likely that IC7 is related to the sensory feedback during motor execution, given the significant bilateral cerebellar activation. We found that this network reduces with time since stroke, similar to other reports that use network analysis of resting-state fMRI (35). Remarkably, both of these movement-related networks are shared with age-matched controls, again consistent

with the idea that recovery of motor performance after subcortical stroke involves modulation of extant networks rather than stroke-specific networks.

The interactions between the primary motor cortices are the foundation for numerous interventions after stroke (4, 14, 15, 25). These interventions can include but are not limited to TMS [see Cramer et al. (7) for an overview]. Overall, there is growing support for this model (13, 36). In addition to the contralateral motor cortex activation, we identify an area of deactivation within the more dorsal aspect of the ipsilateral/contralateral motor cortex (IC2). While there are complex interactions between the motor cortices during movement, the topographical distributions of these areas, i.e., away from the “hand area” make interpretation difficult. Of course, the model previously suggested (4, 14, 15, 25) is an oversimplification and fails to capture the existence of multiple cortical networks that are involved in the recovery process. It may also apply to certain stages and degrees of recovery only. Importantly, future work needs to address the effect of interventions like TMS and tDCS on multiple cortical networks (37, 38) as their effects may be more nuanced than simply increases or decreases activation. This highlights the importance of selecting the most appropriate training that should be combined with TMS or tDCS (39).

This study has a number of limitations. The patients included were relatively well recovered and whether similar results would be found in a more severely affected group is unknown. We studied only subcortical stroke. It is feasible that our findings may not apply to cortical strokes. We studied both right- and left-hemisphere strokes in right handers and flipped the MR images to one side in order to carry out the TICA on a meaningful sample size. Again, we cannot rule out that findings for dominant and non-dominant hemisphere stroke may differ. We excluded stroke patients who were performing chaotic motor imagery, and it is therefore possible that these patients may have used alternative cognitive processes that could have been interpreted as being stroke specific – though one would not expect these networks to relate to the recovery of motor performance as such. Although TICA can examine cortical networks that are shared between tasks, it has limitations (40). By considering EM and MI together in TICA analysis, we must assume that the tasks have the same temporal profile. It is entirely possible that this approach has overlooked cortical networks that have different temporal profiles – this limits the use of TICA-based fMRI as a biomarker for patient selection. However, if that was the case, then one would expect those areas to have been highlighted by earlier mass univariate fMRI studies.

CONCLUSION

In summary, we find that in our sample of well-recovered subcortical stroke patients, cortical networks associated with recovery of motor performance include some cognitive processes upstream from actual movement while others are exclusively dependent on execution. Importantly, all of these networks were

present in age-matched controls, suggesting that recovery of motor performance after stroke requires existing cortical motor networks rather than recruiting additional areas. These results also imply that the models of motor recovery after stroke [suggested by Ward and Cohen (14)] should be updated to consider movement as a combination of distinct cortical networks, each of which may have a separate contribution to recovery. Finally, we need to explore how each of these networks is affected by non-invasive stimulation to fully exploit their therapeutic potential.

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A thalamic-fronto-parietal structural covariance network emerging in the course of recovery from hand paresis after ischemic stroke

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Aim: To describe structural covariance networks of gray matter volume (GMV) change in 28 patients with first-ever stroke to the primary sensorimotor cortices, and to investigate their relationship to hand function recovery and local GMV change.

Methods: Tensor-based morphometry maps derived from high-resolution structural images were subject to principal component analyses to identify the networks. We calculated correlations between network expression and local GMV change, sensorimotor hand function and lesion volume. To verify which of the structural covariance networks of GMV change have a significant relationship to hand function, we performed an additional multivariate regression approach.

Results: Expression of the second network, explaining 9.1% of variance, correlated with GMV increase in the medio-dorsal (md) thalamus and hand motor skill. Patients with positive expression coefficients were distinguished by significantly higher GMV increase of this structure during stroke recovery. Significant nodes of this network were located in md thalamus, dorsolateral prefrontal cortex, and higher order sensorimotor cortices. Parameter of hand function had a unique relationship to the network and depended on an interaction between network expression and lesion volume. Inversely, network expression is limited in patients with large lesion volumes.

Conclusion: Chronic phase of sensorimotor cortical stroke has been characterized by a large scale co-varying structural network in the ipsilesional hemisphere associated specifically with sensorimotor hand skill. Its expression is related to GMV increase of md thalamus, one constituent of the network, and correlated with the cortico-striato-thalamic loop involved in control of motor execution and higher order sensorimotor cortices. A close relation between expression of this network with degree of recovery might indicate reduced compensatory resources in the impaired subgroup.

Keywords: stroke recovery, structural covariance network, fronto-parietal network, thalamocortical loop, tensor-based morphometry

Introduction

As both cross-sectional and a few longitudinal observational studies have demonstrated, behavioral recovery from hemiparesis after ischemic stroke shows marked between-subject variability (1, 2). This variability is thought to be determined not only by general demographic or clinical factors – such as age, gender or medical comorbidities – but also by neurobiological processes prompted by damage to critical nodes of functional and structural brain networks (3, 4). Activation studies using functional MRI (fMRI) have contributed considerably in the past to current knowledge of these processes (5–7); moreover, resting state fMRI and structural MRI have provided complementary insights in recent years (8). The improved understanding of stroke provided by neuroimaging could impact neurorehabilitative therapies (9–11).

Activation studies performed with fMRI have shown that successfully recovered subjects show almost normal cerebral patterns, exhibiting change during recovery from attention demanding controlled processing of motor performance in the subacute stage to more fluent and automatic processing in the late chronic stage (12). This suggests recovery at the synaptic and/or neuronal level in the perilesional zone. In contrast, individuals presenting impaired recovery retain ineffective motor patterns and may not regain fully the specific motor function (13, 14); they, thus, possibly require cognitive control and concentrated effort to maintain motor execution (15). Accordingly, volitional and emotional effort are means to enhance output in a diseased, low-efficient motor system, as indicated by the enhanced activation of motor networks observed in fMRI-studies of patients with chronic motor impairment (12). An additional aspect of the recovery process evidenced by studies at varying stages post-stroke is the influence of the contralesional hemisphere, functionally rather supporting motor activity in the early acute phase and mainly inhibiting it in the chronic stage (16, 17).

In the following, we utilize structural MRI to study stroke recovery in a patient cohort of 28 patients selected for first cortical sensorimotor stroke and associated initial hand paresis or plegia. The analysis employs a relatively new method, tensor-based morphometry (TBM), to quantify gray matter volume (GMV) changes during recovery (18, 19). While indicating structural neuronal plasticity, the changes cannot be assigned *in vivo* to a specific mechanism, e.g., axon sprouting, dendritic branching or synaptogenesis (20). Requiring high-resolution MRI [3D modified driven equilibrium Fourier transform (3D-MDEFT)] imaging, TBM evaluates the transformations relating one acquisition to a second in a single subject. In our longitudinal study, the first acquisition was performed after 3 months in the subacute phase and the second after 9 months in the chronic phase. In all patients, initial diffusion-weighted MR images (21) delineated impacted critical brain lesions. High-resolution T1 (3D-MDEFT)-MRIs were acquired in 28 patients 3 and 9 months after stroke (22). An example of multimodal imaging in a wider sense (23), the acquisition protocols provided two non-redundant data sets from the same MR instrument in the same study population: bright tissue contrast for lesion delineation in the acute phase and GMV changes derived from the high-resolution T1 images by TBM analysis.

Accompanying the imaging was an array of clinical, motor and sensory assessments performed regularly during the 9-month study. Of the behavioral assessments, picking small objects (PSO), a lateralized motor skill requiring a particular precision grip, showed the greatest variance over the 9-month trial period (21). Response feature analysis (RFA) using Akaike's information criterion applied to the 9-month recovery trajectories of the individual patient tests partitioned the patient cohort into three subgroups showing fast linear, slow exponential or impaired recovery (24). A multivariate analysis, principal component analysis (PCA), of the PSO task confirmed the partitioning among the 28 patients and characterized each patient's expression of the principal recovery trajectory by a single coefficient (22). This expression coefficient served as correlate to identify the neural pattern, represented as a principal component image of a PCA of the 28 TBM images, most closely associated with recovery. We have shown previously in the context of PET regional cerebral blood flow (CBF) images that PCA provides a powerful tool for elucidating disease-related abnormalities and post-lesional reorganization of neural networks in the human brain (25).

A previous mass-univariate analysis of these TBM images yielded three findings: (i) most striking, impaired patients with chronic disturbed hand motor skills showed the most prominent GMV increase in the ipsilesional medio-dorsal (md) thalamus, including also the head of the caudate nucleus; (ii) all patients evidenced GMV decreases within the contralateral anterior cerebellum at a location typical of cerebellar diaschisis after sensorimotor cortical stroke; and (iii) patients showing fast recovery exhibited a slight GMV increase in the perilesional premotor cortex (PMC). These results stimulated several questions: Does the significant GMV increase of md thalamus in these patients represent an isolated, local effect or does it implicate an extended gray matter network involved in recovery after a sensorimotor cortical stroke? Does the extended network show a structural covariance pattern that discriminates among classes of recovery process? How does the network relate to the initial lesion pattern?

These questions led to the hypotheses examined in the current study: the prominent GMV changes in the md thalamus relate to the dorsolateral prefrontal circuit of Alexander et al. (26) as proposed in our previous paper and may have access to the dysfunctional sensorimotor network post-stroke (22). A posited distributed neuronal network including the md thalamus is specifically related to sensorimotor hand skill. This network manifests a structural covariance pattern that may distinguish among patient subgroups according to recovery class. The structural covariance pattern shows a correlation with the initial lesion pattern.

Participants and Methods

Patients and Healthy Controls

We prospectively recruited patients at two comprehensive stroke centers (Departments of Neurology, University Hospital Bern and Kantonsspital St. Gallen, Switzerland) from January 01, 2008 through July 31, 2010. Inclusion criteria were (1) first-ever stroke, (2) clinically significant contralesional sensorimotor hand function impairment as leading symptom, and (3) inclusion of the pre- and/or post-central gyri within the ischemic

lesion confirmed on acute diffusion-weighted (DWI) and fluid attenuated inversion recovery (FLAIR) MRI scans. Patients were excluded if they presented (1) aphasia or cognitive deficits that precluded understanding the study purposes or task instructions, (2) prior cerebrovascular events, (3) occlusion or stenosis >70% of the carotid arteries in MR-angiography, (4) purely subcortical stroke, and (5) other medical conditions interfering with task performance. We recruited 36 patients, seven of which dropped out (three withdrew consent, two were too frail for repeated testing, one was shown to have no cortical stroke after enrollment, one was lost to follow-up). The final sample consisted of 29 patients (five female). As a control group for the analyses of behavioral and clinical data, we recruited 22 healthy older adults (11 female) from the local community. Groups were matched for age (unpaired two-tailed *t*-test: *t*(49) = 3.4, *p* < 0.12) and handedness according to the Edinburgh Handedness Questionnaire (unpaired two-tailed *t*-test: *t*(49) = 0.36, *p* < 0.30). The study received ethical approval from both research centers [Ethikkommission des Kantons St. Gallen (EKSG), Kantonsspital St. Gallen, 9007 St. Gallen and Kantonale Ethikkommission Bern (KEK), 3010 Bern, Switzerland]. All participants gave written informed consent before enrollment according to the Declaration of Helsinki. The same cohort was used for our previous publications (21, 22, 27).

Data Acquisition

Study Timeline

We performed a baseline examination within the first 2 weeks after stroke (median 5 days, range 1–18 days) with extended measurements of clinical and behavioral data (see below). The same measurements were taken 3 months (91 days, 80–121 days) and 9 months (277 days, 154–303 days) after stroke. During each of these two visits, we acquired high-resolution anatomical imaging data. Patients were additionally seen at monthly intervals in-between these examinations to evaluate recovery of dexterous hand function.

Clinical and Behavioral Data

Clinical stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS) (28). Hand motor function was assessed with two outcome variables, grip force and dexterity. Grip force was measured by hand dynamometry (HD) with a Jamar Dynamometer (29, 30). Dexterous hand function was measured using the modified Jebsen Taylor Test (JTT), a standardized quantitative assessment that consists of five timed subtests that simulate everyday activities (31). For our current analysis, we relied on data from the JTT subtest “PSO”, which consists of picking six common objects (two paper clips, two bottle caps, two coins) and dropping them into an empty can as fast as possible. As previously shown by our group, PSO explains by far most of the longitudinal variance in JTT scores and allows accurate classification of patient subgroups (see Supplementary Material for details) (21). The two motor tasks measure complementary aspects of hand motor function. Behaviorally, HD is performed with a simple power grip using the whole hand, whereas PSO necessitates precision grip characterized by opposition of the thumb against one or two fingers (32); and furthermore a proper coupling of grasping and lifting phases of objects performing this task which has been shown to be specifically vulnerable in the case

of lesioned dorsolateral PMC (33). Neuroanatomically, each grip form is controlled by different components of the sensorimotor network: power grips are mainly controlled by the primary sensorimotor cortices, whereas precision grip control includes the premotor and posterior parietal cortices (34, 35). As a measure of sensorimotor integration, we included a tactile object recognition (TOR) task, which consisted in discriminating 30 everyday objects with either hand (36). This task was administered at the same time as the NIH evaluation. Further details on measurement procedures can be found in the Supplementary Material.

Imaging Data

All patients underwent acute phase imaging at admission according to local stroke imaging protocols. This included a diffusion-weighted imaging (DWI) scan and T1-weighted (T1w) anatomical image. At 3 and 9 months after stroke, each patient underwent high-resolution T1w imaging using a 3D-MDEFT with following imaging parameters (37): repetition time TR = 7.92 ms, echo time TE = 2.48 ms, flip angle = 16°, inversion with symmetric timing (inversion time 910 ms), 256 × 224 × 176 matrix points with a non-cubic field of view (FOV) of 256 mm × 224 mm × 176 mm, yielding a nominal isotropic resolution of 1 mm³ (i.e., 1 mm × 1 mm × 1 mm), fat saturation, 12 min total acquisition time. Identical prescription of MR images was achieved by use of the Siemens auto-align sequence that automatically sets up consistent slice orientation based on a standard MRI atlas.

Data Analysis

Synopsis

Longitudinal clinical and behavioral data were analyzed with a variant of RFA (24). This is a technique that uses summary measures to simplify analysis of serial measurements [cf. Ref. (24) for clinical examples]. As described below and in Ref. (21), we proceed in two levels: at the single-subject level, we summarize each patient's *z*-transformed longitudinal data using linear and non-linear curve fitting. At the group level, we then calculate a PCA of these curves to derive a number that summarizes each patient's recovery relative to the whole cohort. The analysis of structural high-resolution imaging data was performed similarly. At the single-subject level, we calculated TBM maps that encode (longitudinal) local GMV change between 3 and 9 months after stroke, as previously described (22). At the group level, we again calculated a PCA to identify regions with co-varying GMV change across time. In analogy to previous work analyzing structural covariance in the human brain, we refer to these maps as *longitudinal structural covariance networks* (38, 39).

Response Feature Analysis of Clinical and Behavioral Data

First, each patient's PSO task data were transformed to *z*-scores using the mean and SD of a healthy control group of 22 age-matched subjects; normal performance was defined as *z* ≤ 0 ± 2.5 units. Then, each patient's recovery trajectory was identified by fitting a set of linear and exponential models to the *z*-scores, and the best fitting model was selected using Akaike's information criterion. Patients were classified in three recovery subgroups according to their recovery model: fast (linear recovery trajectory), slow (exponential recovery

trajectory converging to $z \geq -2.5$) and impaired recovery (exponential recovery trajectory converging to $z < -2.5$). The principal component analyses of the PSO and TOR task, and NIH evaluation were performed with Matlab program, *princomp* (The Mathworks, Inc., Natick, MA, USA). The PSO task yielded ten principal component time courses and variances (one per visit); the TOR task and NIH evaluation, three time courses and variances. Each produced 36 patient expression coefficients (or “scores”). The Kaiser–Guttmann criterion was used to select salient principal components (40). Missing data, arising when patient did not show or could not perform task, were replaced by means over all patients at the time point of the missing data; 10 out of 280 planned visits yielded missing data. The present study uses the expression coefficients of the subset of 28 patients for which TBM images were acquired.

Lesion Mapping

Lesions were manually traced on DWI images using MRIcron,¹ as described in Ref. (21). Lesion volumes were calculated by summing all voxels within the resultant binary lesion masks. The latter were used to exclude lesioned voxels during normalization of all images into the stereotaxic Montreal Neurological Institute (MNI) space (see below). Additionally, we built summary lesion maps for each recovery subgroup, which we thresholded at >20% lesion density for comparison with structural data (see below).

Tensor-Based Morphometry

Tensor-based morphometry maps were calculated as described in Ref. (22) using SPM8 (version 4667²) running on MATLAB (R2009a, MathWorks, Natick, MA, USA). Briefly, we first realigned 3D-MDEFT images from both acquisition time points to correct for position differences. We next used segmentation with cost-function masking to derive gray matter tissue partitions (41, 42). We then calculated in each subject the Jacobian determinants (first derivatives) of high-dimensional deformation fields that transform voxel-by-voxel the T1w image from month 3 onto the T1w image from month 9. Multiplication of the first derivatives with the matter segmentation from month 3 results in a map that encodes matter volume expansion or contraction per voxel across time. These maps were transformed into the stereotaxic MNI space using normalization parameters derived from segmentation. Normalized GMV change maps were finally smoothed with a 12 mm × 12 mm × 12 mm isotropic 3D Gaussian kernel, motivated by previous studies that show a reduction of false positives for this kernel size in voxel-based morphometry studies (43). These smoothed maps were entered in the covariance analysis as described below. Based on our previous study, we used an unbiased region of interest analysis to extract local GMV changes from ipsilesional thalamus, ipsilesional dorsal PMC and contralesional cerebellum (22).

Structural Covariance Using Principal Component Analysis

The PCA of the TBM images was performed on a subset of 28 patients representing the volume changes between months 3 and

9 (of the 29 patients retained for the study 1 had to be excluded because of MR motion artifacts). PCA was executed on the images data using in house software written in MATLAB based on the algorithm described by Alexander et al. and Moeller et al. (44, 45). Extracerebral voxels were excluded from the analysis using a mask derived from the gray matter component yielded by segmentation of the anatomical image volume into gray matter, white matter and cerebrospinal fluid followed by the calculation of residual matrices for each of the 28 scans. From matrices whose rows corresponded to the 28 scans and columns to the 132407 relevant voxels in a single image volume were subtracted from each element (i) the mean of voxel values of its column and (ii) the mean of voxel values of its row, and (iii) added to each element the grand mean of all voxel values in the original matrices. The row, column, and grand means of the resulting residual matrices vanish. Using the singular value decomposition implemented in Matlab, each residual matrix was then decomposed into 28 components. Each component consisted of an image volume, i.e., eigenimage, a temporal expression coefficient, i.e., eigenvariate, and an eigenvalue. The squared eigenvalue is proportional to the fraction of variance described by each component; the subject expression coefficients describe the amount that each scan contributes to the component; and the component image displays the degree to which the voxels co-vary in the component in the course from months 3 to 9. The subject expression coefficients and voxel values of a principal component are orthonormal and range between −1 and 1; the orthogonality reflects the lack of statistical correlation among the principal components. Significant clusters were delineated by applying a height threshold at the first and ninety-ninth percentile of voxel values and an extent threshold of 32 voxels (corresponding to the minimal resolution element of the TBM maps). These clusters were localized using the Jülich cytoarchitectonic probabilistic atlas (SPM Anatomy toolbox, Version 1.8, made available through the Human Brain Mapping division at the Forschungszentrum Jülich at http://www.fz-juelich.de/inm/inm-1/DE/Forschung/_docs/SPMANatomyToolbox/SPMANatomyToolbox_node.html). Furthermore, we calculated the overlap between each network cluster and subgroup lesion density maps.

Statistical Analysis

We used median and range for descriptive statistics. We first assessed the relationship of structural covariance component expression, clinical and structural variables, e.g., lesion volume and regional GMV change, using Pearson's correlation coefficient in order to identify the network related to hand function recovery. Next, we assessed differences with respect to subgroups in network expression and behavioral variables. To do so, we first applied the Shapiro–Wilk test and inspected Q–Q plots for each variable to assess deviations from normality. We used then non-parametric tests to compare scalar variables where appropriate, i.e., the Kruskal–Wallis one-way analysis of variance by ranks to assess differences in the central tendency among any of the three subgroups, and the Mann–Whitney *U* test to compare pairs of subgroups against each other. Finally, we used robust (multiple) regression within the framework of the general linear model to test the relationship of network expression, clinical and structural

¹<https://www.nitrc.org/projects/mricron>

²<http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>

variables to hand function recovery and their interaction across the whole patient cohort. The criterion for significance was set at $p < 0.05$, Bonferroni corrected for multiple comparisons.

Results

Clinical and Behavioral Data

Clinical characteristics of the patient cohort are summarized in **Table 1**. Representative sections of each subjects' ischemic lesion can be found in Figure S1 in Supplementary Material. The behavioral data was incorporated in two principal component analyses. The first principal components of PSO and NIH assessments were chosen for further analysis because they explained the greatest fractions of variance, 70 and 90%, respectively, of the corresponding PCAs. RFA of the PSO task indicated that eight patients showed normal motor performance at baseline (subgroup "fast recovery"), ten patients exponential recovery that converged to normal motor performance ("slow recovery") and eight whose recovery trajectories followed exponential recovery curves that did not reach normal performance ("impaired recovery") (21).

Selection of Longitudinal Structural Covariance Networks

Table 2 characterizes three principal components of the TBM images (structural covariance networks) that correlated with clinical and behavioral variables across the whole patient cohort. The first component ($PC1_{TBM}$) correlated with GMV reduction in the cerebellum contralateral to the affected hemisphere. The second component ($PC2_{TBM}$) correlated with lesion size, GMV volume increase in the md thalamus, clinical ($PC1_{NIHSS}$ expression) and hand function specific recovery ($PC1_{PSO}$ expression). The fourth principal component correlated exclusively with $PC1_{NIHSS}$ expression. None of the other PCs surviving the Kaiser–Guttmann criterion correlated with any of the external variables.

Thalamocortical Network Related To Hand Function Recovery

Effects Across the Patient Cohort

Since the second structural covariance network $PC2_{TBM}$ correlated with our specific measure of hand function recovery, we focused further analysis on its critical clusters (or nodes, **Figure 1A**). Clusters that co-varied with the thalamus fell within the first percentile of voxel values, and were labeled as "positive" clusters since the thalamus showed gray matter increase. These clusters (ordered by size) included insular and peri-insular cortex, dorsolateral prefrontal and ventral premotor cortices, thalamus, posterior parietal cortices and two smaller clusters in the temporal and occipital cortex. A single cluster fell within the ninety-ninth percentile and included pre- and post-central cortex. **Table 3** summarizes localization, statistics and functional correlates of all clusters that survived thresholding ($PC1_{TBM}$ and $PC4_{TBM}$, are summarized in Tables S1 and S2 in Supplementary Material, respectively). Functional interpretation was done in the context of motor hand function, based on current literature. The expression of this network had a strong correlation with thalamic GMV change across the whole cohort (**Figure 2A**).

Effects Within Patient Subgroups

Having identified a structural network related to hand function recovery (**Table 4**), we next analyzed its relationship to lesion topography within recovery subgroups. Lesion analyses are summarized in **Figure 1B**. Projection of subgroup lesion density maps onto $PC2_{TBM}$ clusters showed that the thalamic cluster was spared across all subgroups, but that the other clusters showed varied involvement. A detailed volumetric analysis (**Table 5**) showed that only a small fraction of each lesion density map affected network clusters (median and range 0.95%, 0–6.7%), indicating that GMV density changes occurred either in perilesional or more distant areas. When analyzing the percentage of each cluster affected by the lesion, there were notable differences: lesions in the fast recovery subgroup affected mostly the parietal-opercular and insular cluster (Cluster 1+), whereas lesions in the impaired subgroup affected mostly the ventral premotor cortex and intraparietal sulcus (IPS) (Cluster 3+ and 4+). The slow recovery subgroup showed no clear lesion profile. Affection of the pre/post-central cluster (Cluster 1-) increased across subgroups.

We further compared the patients subgroups presenting normal motor performance after 9 months (fast and slow recovery) with the subgroup that did not achieve normal performance (impaired recovery). As expected from the RFA, the latter group yielded the highest expression coefficients in $PC1_{NIHSS}$ ($p < 0.01$) and specifically in $PC1_{PSO}$ ($p < 0.0001$). This group had also the largest GMV expansion in the medio-dorsal thalamus and the highest lesion volumes (both $p < 0.05$). **Figure 2** shows the relationship between $PC2_{TBM}$ expression and thalamic GMV change (panel A) and hand skill recovery as reflected by PSO (panel B), respectively. Considering all individuals, GMV change correlated with expression coefficients of the structural covariance network of $PC2_{TBM}$ ($R = 0.72$ and $p < 0.5$ after correction for multiple comparisons). $PC2_{TBM}$ expression could also distinguish between subgroups: When dividing patients into subgroups with positive versus negative network expression coefficients (without regard to recovery subgroup assignments), we found that the positive subgroup has significantly higher thalamus GMV change (median 1.35% with range 0.83–1.79%), whereas the negative subgroup shows no significant change (median 0% with range 0.04–0.04%, Mann–Whitney U test $p < 0.001$).

However, only the impaired recovery subgroup showed a linear relationship between the expression of structural covariance network of $PC2_{TBM}$ and recovery (**Figure 2B**): the slope estimate (and SE) was 53.1 ± 22.7 ; adjusted $R = 0.74$ with $p < 0.05$. Note that one patient of this subgroup showed a negative $PC1_{PSO}$ expression score. Inspection of the raw data indicated that this particular subject showed a secondary deterioration of skilled hand function during the last 2 months of the study, after an initially favorable course. Removal of this outlier did not change results. A few individuals of the recovered subgroups exhibited high GMV changes in the medio-dorsal thalamus, representing exceptions to the group trend.

Multivariate Linear Regression

To further test the specificity of the association between $PC2_{TBM}$ and hand function recovery, we calculated a multivariate linear

TABLE 1 | Descriptive statistics of clinical and demographic data of stroke patients at baseline, month 3 and month 9.

No.	Id	Age	Gender	Side	Etiology	NIH B	NIH M3	NIH M9	mRS B	mRS M3	mRS M9	HD B	HD M3	HD M9	PSO B	PSO M3	PSO M9	TOR B	TOR M3	TOR M9
1	p01	77	M	L	UN	4	2	1	2	1	1	31	40	41	9.7	7.9	5.7	30	30	30
2	p02	50	M	R	OC	7	1	0	4	1	0	6	54	63	0.0	6.0	6.2	25	28	30
3	p03	78	M	R	LAD	5	5	3	3	2	2	15	17	42	13.5	11.1	9.1	28	29	27
4	p05	80	M	L	LAD	2	3	1	2	1	1	42	42	37	10.6	6.5	8.4	30	30	30
5	p06	53	F	R	LAD	6	3	3	3	2	1	11	9	19	29.9	10.1	14.9	0	0	0
6	p07	78	F	R	CE	4	2	2	2	1	1	18	21	21	14.0	7.5	7.1	0	12	24
7	p09	70	F	R	CE	3	2	0	2	1	0	21	31	34	9.1	8.5	6.0	29	30	30
8	p11	41	F	L	LAD	3	2	0	1	0	0	32	37	39	5.6	4.0	5.11	24	30	30
9	p12	54	M	R	UN	4	2	1	3	1	0	14	33	38	8.5	5.5	5.2	30	30	30
10	p15	54	M	L	LAD	6	4	1	3	1	1	10	24	33	38.8	13.1	11.1	0	6	10
11	p16	73	M	R	OC	4	2	0	2	1	0	51	55	55	7.3	4.9	5.3	26	29	30
12	p17	58	M	L	CE	4	2	0	3	0	0	20	39	48	11.5	4.3	4.7	30	29	30
13	p20	70	M	L	CE	6	4	2	3	1	1	24	35	42	12.9	9.7	9.3	0	6	10
14	p24	74	M	R	CE	4	1	0	1	0	0	34	49	50	14.3	6.9	5.1	28	30	30
15	p25	49	M	R	CE	3	2	1	2	1	0	49	59	67	12.3	5.3	5.9	0	6	10
16	p26	44	M	L	CE	3	1	0	1	0	0	9	33	50	11.5	6.0	5.1	30	30	30
17	p30	63	M	L	CE	4	1	1	3	0	0	43	41	45	10.6	6.3	6.3	30	30	30
18	p31	63	M	L	UN	5	0	0	2	0	0	30	48	44	5.3	4.2	4.7	30	30	30
19	p33	75	M	R	LAD	3	2	2	2	1	1	3	14	22	0.0	18.8	11.5	12	28	30
20	p35	78	M	L	LAD	5	3	2	3	1	1	23	48	40	10.1	6.8	6.1	30	30	30
21	p36	60	M	L	CE	4	1	1	3	1	1	31	40	41	18.2	8.0	6.6	30	30	30
22	p37	75	M	R	OC	4	2	1	2	1	1	0	27	32	0.0	8.6	10.4	4	23	25
23	p38	77	M	L	LAD	5	2	2	3	1	1	10	21	23	26.9	10.9	8.3	29	30	30
24	p41	51	M	R	CE	2	1	0	2	1	1	36	41	52	7.1	5.1	4.8	30	30	30
25	p42	64	M	R	LAD	1	0	0	2	0	0	14	33	35	18.9	7.1	7.4	29	30	30
26	p43	82	M	L	LAD	3	3	2	2	2	1	17	10	18	16.8	21.4	13.9	20	22	25
27	p44	67	M	R	UN	11	10	9	4	3	3	15	15	41	52.3	45.1	12.3	3	4	2
28	p45	53	M	R	LAD	11	9	4	5	3	2	0	10	17	0.0	45.5	19.9	0	1	3
Median		65.5	24 M	13 L	11 LAD, 10 CE, 4 UN, 3 OC	4	2	1	2	1	1	20	35	41	11.0	7.3	6.5	28	29	30
Range		41, 82	4 F	15 R		0, 11	0, 10	0, 9	1, 4	0, 3	0, 3	0, 51	9, 59	17, 67	0.0, 52.3	4.0, 45.5	4.7, 19.9	0, 30	0, 30	0, 30
Median (z)												-1.3	-0.2	0.4	-5.0	-1.2	-0.4	0.6	0.6	0.6
Range (z)												-2.8,	-2.3,	1.6, 2.6	-38.9,	-33.2,	-11.7,	-7.5,	-6.5,	-4.5,
												1.3	1.9	0.5	1.6	1.0	0.6	0.6	0.6	0.6

M, male; F, female. Etiology is classified according to the Trial of ORG 10172 in acute stroke treatment (TOAST): LAD, large artery disease; CE, cardioembolism; OC, other determined cause; UN, undetermined cause. NIH, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; HD, hand dynamometry (in kilograms); PSO, picking small objects task (in seconds); TOR, tactile object recognition (in numbers of recognized objects); z, z-scores using mean and standard deviation of healthy controls for each task.

TABLE 2 | Correlation of longitudinal structural covariance networks across all patients (*n* = 28).

Component	Variance (%)	Parameters with significant correlations ^a	Values of parameters ^b	Correlation coefficient (<i>r</i>)
PC1 _{TBM}	19.9	GMV change ant. cerebellum	-0.2 (-1.3, 0.6) %	-0.57
PC2 _{TBM}	9.1	Lesion volume	9.0 (0.6, 141.7) cc	0.61
		PC1 _{NIHSS} expression	-3.65 to 11.9	0.61
		PC1 _{PSO} expression	-20.99 to 64.26	0.51
		GMV change md Thalamus	0.4 (-0.6, 4.0) cc	0.72
PC4 _{TBM}	8.1	PC1 _{NIHSS} expression	0 (-3.65, 11.9)	0.54
Cumulative Variance	37.1			

NIHSS score, National Institute of Health Stroke Scale-score; PSO, picking small objects, MI primary motor cortex, SI primary sensory cortex.

^aSignificant correlations after correction for eight multiple comparisons: 0.05/8 = 0.006 yields significant entries. This probability corresponds to a correlation coefficient of 0.466.

^bValues of parameters are indicated as median, including range, expression coefficients are indicated as range due to normalization (median of 0).

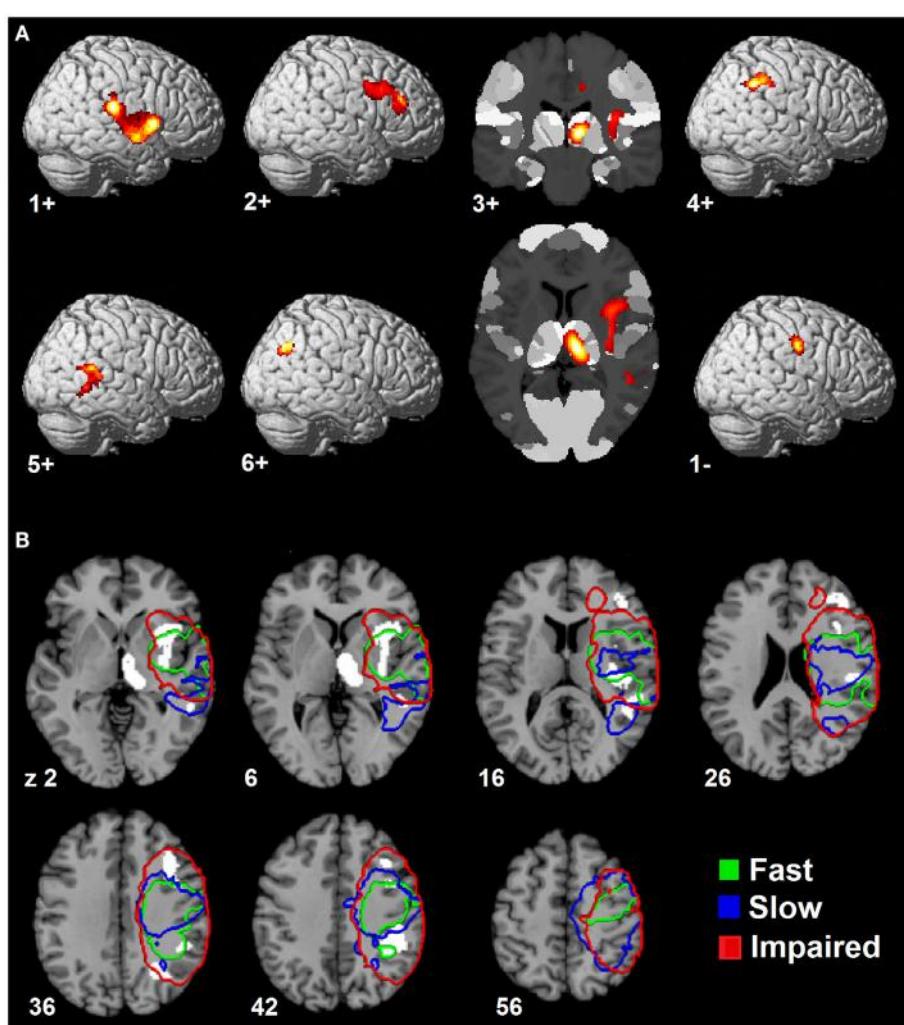
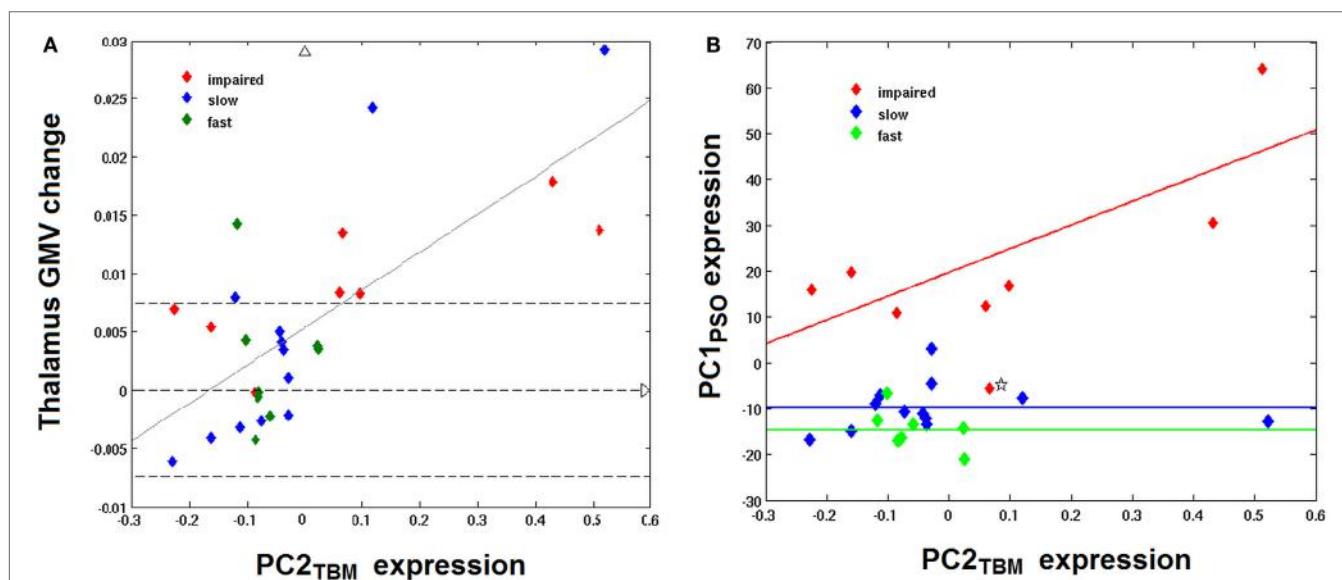


FIGURE 1 | Spatial topography of longitudinal structural covariance network correlating with hand function recovery. (A) shows the six largest clusters of supra-threshold voxels for the second principal component (PC2_{TBM}) projected onto a standard three dimensional brain and onto a cytoarchitectonic atlas (cluster 3+) in MNI space. Clusters are labeled according to their (positive or negative) correlation with gray matter volume expansion in the medio-dorsal thalamus. The threshold for positive clusters corresponds to the first percentile of voxel values (absolute value 0.0064), the threshold for the negative cluster to the ninety-ninth percentile (absolute value 0.0095). (B) shows the spatial relationship between the covariance network clusters and lesion maps of patient subgroups. Color-coded contours define areas with ≥20% lesion probability in each subgroup. Size, localization, cytoarchitectonic assignment, and functional correlates of the individual clusters are summarized in **Table 3**.

TABLE 3 | Clusters of the longitudinal structural covariance network (PC2_{TBM}) related to hand function recovery: size, localization, cytoarchitectonic assignment, and functional correlates.

Cluster	Size (n vox.)	MNI (max.)	Anatomical area	Cytoarchitectonic area	Functional correlate (references in brackets)
First-percentile voxels (height threshold: 0.0064, extension threshold: 32 voxels)					
1+	1362	38/-28/16	R. parietal operculum	OP1, OP2, OP3	Tactile working memory, stimulus discrimination and perceptual learning (41–44)
			R. insula	Ig1, Ig2	Multisensory processing (36, 50–53)
		54/-26/28	R. inferior parietal lobule	PFcm, PFop, PFt	Action observation and imitation (47–49)
2+	653	43/26/26	R. DLPFC (dorsal-posterior part)	n.a.	Action execution and working memory (34, 35)
		40/10/34	R. ventral premotor cortex	n.a.	Motor hand skill related to intrinsic objects properties (83)
3+	502	10/-20/6	R. thalamus	Thal: prefrontal Thal: temporal Thal: parietal	MD nucleus to prefrontal cortex (33–35) MD nucleus to temporal lobe (33–35) LP/Pu complex to parietal lobe (33–35)
4+	408	42/-38/42	R. intraparietal sulcus R. post-central gyrus R. inferior parietal lobule	hlp1, hlp2, hlp3 BA2 PFt, PFm	Spatial attention, visuomotor transformation (57, 66–68) Primary somatosensory information processing (56) For PFt see above; for PFm non-spatial attention (49)
5+	271	52/-48/2	R. superior (and middle temporal) gyrus	n.a.	Spatial awareness (69)
6+	158	30/-62/36	R. middle occipital gyrus	n.a.	Spatial processing of tactile stimuli (70)
Ninety-ninth-percentile voxels (height threshold: 0.0095, extension threshold: 32 voxels)					
1-	179	54/-14/38	Pre- and post-central gyrus	BA 4p, 3b, 1, 2	Voluntary and passive finger motion (BA 4p) (71) Somatosensory information perception (3b) and processing (1, 2, 73)

**FIGURE 2 | Correlation between PC2_{TBM}, thalamic GMV change, and longitudinal hand function recovery.** (A) shows the relationship between thalamic gray matter volume (GMV) change and expression of the structural covariance network PC2_{TBM}: thalamus GMV change = $0.0325 \times \text{PC2}_{\text{TBM}} + 0.0053$; $R = 0.72$, $p < 0.001$. Dashed transversal lines indicate the interval of reliable GMV change ($\pm 0.75\%$), as determined in previous studies. (B) shows the correlation between expression of the structural covariance network PC2_{TBM} and the first component of longitudinal behavioral recovery of skilled hand function, PC1_{PSO}. Only impaired patients show a strong correlation between network expression and hand function recovery; $\text{PC1}_{\text{PSO}} = 51.9 \times \text{PC2}_{\text{TBM}} + 19.76$; adjusted $R = 0.74$, $p < 0.05$. The recovered subgroups are characterized by a constant of differing magnitude. One patient with negative expression coefficients of PC1_{PSO} (marked by an asterisk) has been identified as outlier (see text).

regression of PC1_{PSO} onto covariance network expression, age, volume, and thalamic GMV change: it showed significant effects of the model intercept ($p = 0.036$), PC2_{TBM} expression ($p = 0.048$) and lesion volume ($p = 0.037$). The significant intercept indicated

residual variance not modeled by our predictors. We, therefore, investigated a reduced model that included PC1_{PSO} as dependent variable, and only the significant predictors from the first model, i.e., PC2_{TBM} expression, lesion volume and their interaction

TABLE 4 | Clinical and structural variables across recovery subgroups.

	Fast recovery n = 8	Slow recovery n = 12	Impaired recovery n = 8	Kruskal–Wallis, p	Mann–Whitney, impaired versus recovered p (2-tailed)
Network of gmv change between months 3 and 9					
PC2 _{TBM} expression coeff.	−0.079 (−0.117, 0.025)	−0.04 (−0.23, 0.52)	0.06 (−0.22, 0.51)	0.55	n.a.
Parameters tested for correlation					
Age	63 (41, 73)	75 (49, 80)	68.5 (53, 82)	0.31	n.a.
Lesion size (cc) ^a	7.80 (0.76, 75.52)	3.48 (0.57, 70.39)	42.84 (2.72, 141.71)	0.08	<0.05
PC1 (NIH) expression coeff. ^a	−2.18 (−3.07, 0.61)	−1.31 (−3.65, 2.12)	1.33 (−1.40, 11.90)	<0.01	<0.01
PC1 (PSO) expression coeff. ^a	−15.3 (−21.0, 6.7)	−10.8 (−16.8, 3.1)	16.5 (−5.6, 64.3)	<0.0001	<0.0001
PC1 (TOR) expression coeff.	13.9 (−0.45, 14.3)	13.9 (11.0, 14.3)	−29.3 (−34.3, 13.7)	<0.001	<0.001
GMV premotor area	0.0043 (−0.0010, 0.0092)	0.0017 (−0.0013, 0.0078)	0.0011 (−0.0020, 0.0083)	0.69	n.a.
GMV thalamus ^a	0.0017 (−0.0043, 0.0143)	0.0023 (−0.0061, 0.0292)	0.0083 (−0.0002, 0.0179)	0.06	<0.05
GMV cerebellum	−0.0019 (−0.0130, 0.0031)	−0.0029 (−0.0089, 0.0060)	−0.0012 (−0.0121, 0.0051)	0.43	n.a.

All values are given as median (range).

NIH, National Institutes of Health Stroke Scale; PSO, picking small objects; PC1, first principal component of longitudinal data of corresponding clinical or behavioral variable;

PC2_{TBM}, second principal component of tensor-based morphometry data; GMV, gray matter volume.

^aSignificant correlations after correction for multiple comparisons: at a nominal alpha level of 0.05 and eight correlations, a p-value of 0.05/8 = 0.006 yields significant entries. This probability corresponds to a correlation coefficient of 0.466.

TABLE 5 | Overlap between subgroup lesion density maps and longitudinal structural covariance network related to hand function recovery.

	Cluster 1+ pOP	Cluster 2+ vPMC	Cluster 3+ Thal	Cluster 4+ IPS	Cluster 5+ STG	Cluster 6+ MOG	Cluster 1– PCG
Raw volume (cc)	10.9	5.2	4.0	3.3	2.2	1.3	1.4
Fast	105.7	7.10	0.3	1.0	0.3	0	0.3
Slow	113.9	1.82	0.5	0	1.0	1.0	0.2
Impaired	239.7	8.7	3.3	0	3.3	1.1	0.9
<i>Percent of lesion on cluster</i>							
Fast	6.7	0.3	0	0.9	0.3	0	0.3
Slow	1.6	0.4	0	0.9	0.9	0.2	0.5
Impaired	3.6	1.4	0	1.4	0.5	0.4	0.6
<i>Percent of cluster affected</i>							
Fast	61.5	5.8	0	30.3	13.6	0	21.4
Slow	14.7	9.4	0	29.7	46.4	17.7	44.3
Impaired	33.0	62.7	0	98.8	51.4	69.2	100.0

Cluster labels correspond to **Table 3**, additionally including main anatomical region within each cluster. Volumes are calculated for subgroup density maps in **Figure 1B** and each cluster separately.

pOP, parietal operculum; vPMC, ventral premotor cortex; Thal, thalamus; IPS, inferior parietal sulcus; STG, superior temporal gyrus; MOG, middle occipital gyrus; PCG, pre- and post-central gyrus.

(PC2_{TBM} expression × lesion volume) as independent variables. The interaction term significantly predicted PC1_{PSO} scores ($\beta = 1.1$, $t(24) = 3.83$, $p < 0.001$) over and above the other variables (both $p > 0.1$). The interaction term explained a significant portion of variance in hand function recovery ($R^2 = 0.639$, $F(3, 24) = 14.18$, $p < 1.6e-5$). Full model parameters are summarized in the Table S3 in Supplementary Material.

Discussion

In this study, we have identified structural covariance networks deduced from GMV changes during the recovery of patients suffering from hand paresis after ischemic sensorimotor stroke. These networks correspond to the first, second, and fourth

principal components determined from a PCA of TBM images and explained 19.9, 9.1, and 8.1% of the variance, respectively. Implied by the correlation of its expression coefficients with GMV-decrease in the anterior cerebellum contralateral to pre- and post-central infarction in all patients, the first component PC1_{TBM} appears to reflect a neuronal network caused by diaschisis from sensorimotor cortex (46). The second component PC2_{TBM}, associated with a specific manual skill, i.e., precision grip, as implied by its correlation with PC1_{PSO} represents a neuronal network involving GMV-increase in the md thalamus. Finally, the correlation of the fourth component expression coefficients with the NIHSS scores summarized in PC1_{NIHSS} suggests that the corresponding network reflects general neurological deficit. A third behavioral parameter of sensory information processing, TOR,

showed no significant correlation with a principal component, although a deficit persisted in the impaired subgroup.

Finally, a multivariate linear regression approach verified (i) the unique relationship of PC_{1PSO} to the structural covariance network of PC_{2TBM}; and furthermore, that this relationship is related specifically to the network expression but not to a single constituent, e.g., md thalamus. Since PC_{2TBM} relates directly to hand function recovery and thus to our study aim, we will discuss this network in more detail in the following.

Associations of the Structural Covariance Network With External Variables

This study represents important progress following our recent paper on “Gray matter volumetric changes related to recovery from hand paresis after cortical sensorimotor stroke” (9) as it relates the most prominent finding of gray matter increase in the md thalamus in patients after a first-ever stroke to a large distributed structural covariance network including a cortico-striato-thalamic loop and diverse sensorimotor cortices.

Irrespective of the clinical and behavioral course, this PC_{2TBM} network distinguishes clearly within the study cohort since the subgroup with positive expression coefficients is associated with large GMV increases in the md thalamus between months 3 and 9, while the subgroup with negative expression coefficients did not exhibit a recognizable GMV change. The GMV increases in the former subgroup exceed the measurement uncertainty and are consistent with the few comparable studies, e.g., in the paper of Gauthier et al. (32). As **Table 2** shows, the neural network represented by PC_{2TBM} is significantly related to the recovery of motor hand skill in the patient cohort; however, only the impaired recovery subgroup shows a strong linear regression, while the fast and slow recovery groups show little correlation with PC_{1PSO} (**Figure 2B**). A multivariate linear regression positing the dependence of PC_{1PSO} on the three salient principal components as well as on age, lesion volume, and GMV change in the thalamus showed significant effects only in PC_{2TBM} and lesion volume. A refined analysis showed a significant interaction between these two variables, and revealed that the interaction was the only significant explanatory variable. The fast and slow recovery groups indicated an inverse relationship between PC_{2TBM} and lesion volume; the greater lesion volumes were accompanied by smaller component expression coefficients, and vice versa. In contrast, the members of the impaired group exhibiting the largest interaction expressed most strongly PC_{1PSO}.

Network Topography and Suggested Functions

The salient regions of the second principal component PC_{2TBM} are summarized in **Table 3**; the regions characterized by voxel intensities of the first percentile contain the thalamic cluster. Using a probabilistic atlas of white matter connections, we found that this thalamic cluster was located on regions of the md thalamus that are preferentially connected to prefrontal, temporal and parietal cortex (33, 34). These three cortical regions were also found in the set of regions belonging to the first percentile, underscoring the importance of the thalamic gray matter increase. The implicated md thalamus and dorsolateral prefrontal cortex are constituents

of the subcortico-cortical, dorsolateral prefrontal loop (35). The involvement of this dorsolateral prefrontal-striato-thalamic loop suggests a compensatory mechanism to maintain motor execution by cognitive control once the primary (more automatic) sensorimotor network of hand motor skill is dysfunctional (47).

Both parts of posterior medial thalamus and dorsal-posterior subarea of the dorsolateral prefrontal cortex are interconnected with the posterior parietal cortex (PPC) (48), which our previous VLSM studies (8) have shown to be seriously affected in the impaired subgroup.

Densely interconnected structures of ventral PMC, PPC, SII and posterior insula are represented in the component image of PC_{2TBM}, representing possible sub-networks engaged in higher order sensorimotor information processing and spatial awareness (see below). In the PPC locally functional processed information, e.g., space and action perception, is transmitted via feedback loops to ventral PMC (34, 49, 50). The areas co-varying positively with the thalamus represent a complex neuronal network consisting of functional and dysfunctional nodes. The functional nodes outside of the lesions comprise the dorsolateral prefrontal loop for motor execution (26), whereas the dysfunctional nodes include various higher order sensorimotor cortices within the lesions. Performance of sensorimotor hand skill, especially in the impaired recovery group, is related to lesion size and extension into network nodes in ventral PMC, PPC, SII, and posterior insula.

A remarkable feature of the structural covariance pattern is the appearance of the parietal operculum subarea OP1 in the absence of OP4. OP4 plays a role mainly in basal sensorimotor integration processes, e.g., incorporating sensory feedback into motor actions which are the basis for information processing during tactile exploration (51, 52). The involved OP1 seems to support more complex information processing demanded during tactile working memory, stimulus discrimination, and perceptual learning (53–56). These differing functional roles are reflected by the distinct connectivity profiles of the areas: OP4 is connected to fronto-parietal areas, while OP1 is connected predominantly to the inferior parietal cortex (IPC) (57). In a three-region model in humans, the rostral IPC, including PFcm, PFop, Pf, has been shown to be involved in reaching and grasping (58). The very rostral part (PFop) seems to be activated specifically during observation of tool use. Moreover, meta-analyses indicated the participation of Pf in action observation and imitation networks (59–61). In humans somatosensory activation of the posterior insula has been observed during simple stimulation paradigms, e.g., estimation of the roughness of gratings and TOR, suggesting a role in somatosensory processing (62–65). Multisensory processing in the posterior insula has also been observed in primate experiments with responses also to auditory, baroreceptive and painful stimuli (66, 67).

As has been shown in primates, while area 2 is activated by fine grained proprioceptive sensory information obtained by transitive finger movements (68), specific neuron populations within anterior IPS (AIP) are activated by grasping and manipulation of 3-D objects as well as by visual fixation of objects (69). Analogously, in humans area 2 is involved in the perception

of geometrical and texture characteristics like edge length and roughness. This function contrasts to the putative human homologue of the IPS, which responds to shape perception, including somatosensory discrimination, visuo-tactile matching, and, together with premotor cortices, skilled motor manipulation of 3-D objects (50, 70–73). The human IPS has been characterized using cytoarchitectonical techniques (74, 75). Functional connectivity analyses have shown these sub-areas along the IPS to be distinguished by distinct connections (76). The AIP ROIs (hIP1 and hIP2) connect mainly to frontal attentional regions, whereas posterior IPS (hIP3) connects mainly to posterior occipital regions. Analog connections have been shown in macaque anatomical studies, e.g., the strong connections between the AIP and ventral PMC and the posterior IPS (CIP) to visual cortices (77). This explains also visuomotor coordination via the AIP and the implication of the posterior IPS in peripersonal visual representations (78–80). Karnath et al. found that in patients free of lesions in visual as well as subcortical structures, the critical site for spatial awareness was located in the superior temporal gyrus (BA 22 and 42) (81). Using fMRI it could be shown that the right middle occipital gyrus processes spatial rather than non-spatial auditory and tactile stimuli (82). In a review, Rizzolatti et al. conclude that the ventral PMC executes both motor and cognitive functions: motor functions comprise hand actions related to intrinsic object properties and head and arm actions related to spatial locations, whereas cognitive functions include space perception, action understanding and imitation (83). In the context of our study the observation of Ehrsson et al. is of importance as they found that precision grip showed more extending activations compared to power grip, involving ventral PMC in both hemispheres (35).

Of the salient regions of the second principal component PC2_{TBM}, a single cortical cluster contains voxels belonging to the ninety-ninth percentile, which presumably characterizes fast and slow recovered individuals. It includes a sub-network within pre- and post-central gyrus, ventral to the center of gravity of the lesion in the slowly recovering subjects as described in our previous paper (21). The isolated involvement of 4p, but not of 4a, substantiates the double representation of the motor system in the precentral gyrus, the former activated in simple motor tasks, whereas the latter responds to more complex and self-initiated tasks (84). In activation studies of healthy individuals, voluntary and passive finger motion stimulated areas 4p and 3a, simple sensory stimulation areas 3b, 1 and 2 and complex sensory stimulation area 4a (85).

Limitations

This study comprises a detailed evaluation and discussion of structural covariance networks associated with hand motor skill. At the outset, the number and composition of recovery subgroups in the patient cohort was unknown. Thus, the number of patients in each subgroup is relatively small. Larger cohorts would be desirable to assign subjects reliably to subgroups characterized by distinct patterns of structural reorganization associated with varying degrees of recovery. Besides subgroup specific patterns,

especially in the subgroup with slow but complete recovery, the assessment of idiosyncratic aspects, e.g., exceptions to the involvement of the dorsolateral prefrontal-striato-thalamic loop, is another challenge. Meeting it would necessitate detailed protocols, including a comprehensive neuro-rehabilitation program, reporting of targeting interventions and physiological measures of movement efforts versus efficiency of motor activity. As the existence of the subgroup with fast complete recovery indicates, an earlier begins after stroke of the study might help to assess structural plasticity in the first 3 months when most recovery occurs. The incomplete gender matching must also be taken into consideration, because women have been shown to perform dexterity tasks (nine-hole peg test) faster than men depending on age, and upper limb kinesthetic asymmetries in contralateral reproduction of elbow movements, elicited by tendon vibration, were prevalent in males (86, 87).

Conclusion

As posited in Section “Introduction”, our study confirms that the md thalamus, distinguished by significant gray matter increase after first-ever stroke, is a constituent of an extensive structural covariance network encompassing (i) a cortico-striato-thalamic loop involved in motor execution and (ii) higher order sensorimotor cortices affected to varying degrees in the study cohort. Positive expression coefficients of the network are associated with significant GMV increases in the md thalamus in contrast to negative expression coefficients. This brain structural covariance pattern reflects a specific structural covariance network related to recovery of motor hand skill and may distinguish among patient subgroups according to recovery class. The surrogate marker for motor hand skill, PSO, depends on an interaction between the expression of the network and lesion volume. Related to this condition, the impaired group exhibiting the largest interaction expressed most strong PC1_{PSO} and inversely were limited in the expression of the structural covariance network of PC2_{TBM}. To conclude, our application of tensor-based morphology has shown it to be a powerful method for studying gray matter changes after stroke; it is capable of revealing both local changes and in associated extensive neural networks. Regarding its future use application, TBM will be potentially of interest in the study of targeted treatment effects in the long-term.

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Supplementary Material

The Supplementary Material for this article can be found online at <http://journal.frontiersin.org/article/10.3389/fneur.2015.00211>

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Improvement in touch sensation after stroke is associated with resting functional connectivity changes

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Background: Distributed brain networks are known to be involved in facilitating behavioral improvement after stroke, yet few, if any, studies have investigated the relationship between improved touch sensation after stroke and changes in functional brain connectivity.

Objective: We aimed to identify how recovery of somatosensory function in the first 6 months after stroke was associated with functional network changes as measured using resting-state connectivity analysis of functional magnetic resonance imaging (fMRI) data.

Methods: Ten stroke survivors underwent clinical testing and resting-state fMRI scans at 1 and 6 months post-stroke. Ten age-matched healthy participants were included as controls.

Results: Patients demonstrated a wide range of severity of touch impairment 1 month post-stroke, followed by variable improvement over time. In the stroke group, significantly stronger interhemispheric functional correlations between regions of the somatosensory system, and with visual and frontal areas, were found at 6 months than at 1 month post-stroke. Clinical improvement in touch discrimination was associated with stronger correlations at 6 months between contralateral secondary somatosensory cortex (SII) and inferior parietal cortex and middle temporal gyrus, and between contralateral thalamus and cerebellum.

Conclusion: The strength of connectivity between somatosensory regions and distributed brain networks, including vision and attention networks, may change over time in stroke survivors with impaired touch discrimination. Connectivity changes from contralateral SII and contralateral thalamus are associated with improved touch sensation at 6 months post-stroke. These functional connectivity changes could represent future targets for therapy.

Keywords: stroke recovery, somatosensory disorders, neuronal plasticity, magnetic resonance imaging, tactile, intrinsic functional connectivity

Introduction

Somatosensory impairment is common after stroke, occurring in 50–80% of stroke survivors (1, 2). However, investigations of the neural correlates of clinical somatosensory improvement after stroke are scarce (3). In particular, knowledge of how brain networks are interrupted is limited, but is critical to better understand the nature of the clinical deficit and post-stroke recovery (4).

Stroke impacts not only the focal lesion site but also on remote brain regions (5, 6). Lesions have important remote effects on the function of connected neural networks that are structurally intact, i.e., physiological changes in distant but functionally related brain areas (4, 7, 8). These remote effects contribute significantly to the observed behavioral deficits and recovery potential (4, 8). Further, changes in brain networks (across both hemispheres and function-specific networks) have been shown to be important in recovery of motor and attention functions (4, 6). A significant challenge is to identify the brain networks and processes that mediate functional improvement so that rehabilitation strategies can be aimed at the appropriate targets (9).

Only a few studies have investigated changes in the brain over time in association with somatosensory recovery (3, 10–13). These studies have primarily involved identification of brain regions associated with task-related brain activation. A few studies have reported that somatosensory recovery is associated with patterns of activation in primary somatosensory (SI) cortex that resembles those seen in healthy controls. For example, return of ipsilesional SI activation has been shown to be associated with improved somatosensory perception (10–12). Staines et al. (12) found that enhanced primary somatosensory cortex activation using functional MRI in the stroke-affected hemisphere occurred in conjunction with improved touch detection in four patients with thalamocortical strokes. Likewise, Wikström et al. (10) reported that increased amplitude of early somatosensory evoked fields in the ipsilesional SI in response to median nerve stimulation was associated with recovery of two-point discrimination (the ability to discern that two nearby objects touching the skin are truly two distinct points, not one) in stroke patients.

While relative “normalization” of brain activity in primary and secondary (SII) somatosensory regions in both hemispheres seems to underlie good clinical recovery, patients with more severe impairments have been shown to recruit attention and multisensory brain regions to a greater degree than that seen in healthy controls, in order to accomplish successful task performance (3, 11, 14–17). In an early positron emission tomography (PET) study of five patients after subcortical stroke, Weder et al. (14) reported activation across bilateral sensorimotor cortex and distributed regions, such as premotor cortex and cerebellum, with worse performance on a tactile shape discrimination task found to correlate with bilateral sensorimotor cortex activation. Tecchio et al. (16) used magnetoencephalography (MEG) to study 18 patients at the acute (5 days) and post-acute (6 months) stages after stroke. They reported that excessive interhemispheric asymmetry correlated with a greater degree of clinical improvement over time in those patients who showed partial recovery. Taskin et al. (15) reported reduced activation of ipsilesional SI with preserved responsiveness of SII in six patients who had

suffered thalamic strokes. More recently, in 19 patients, a study into the relationship between touch impairment and interruption to cortical and subcortical somatosensory areas revealed that the neural correlates of touch impairment in patients with interruption to subcortical somatosensory areas (e.g., thalamus), involved a distributed network of ipsilesional SI and SII, contralateral thalamus, and attention-related frontal and occipital regions (3).

Use of task-based brain activation paradigms can be challenging for stroke patients who may have difficulty performing a given task, and inability to perform the task may impact on the validity of the results (18). Resting-state functional connectivity analysis of functional magnetic resonance imaging (fMRI) data has more recently been employed as a way of assessing activity in the brain over time and across different networks of the brain (19, 20). Resting-state functional connectivity reveals intrinsic, spontaneous networks that elucidate the functional architecture of the human brain at rest (task-independent). Functional connectivity is defined as the statistical association (or temporal correlation) among two or more anatomically distinct regions (21). Data are analyzed for coherence across the whole brain and/or in relation to particular regions of interest (ROIs). Evidence suggests that this measure is indicative of behaviorally relevant brain networks without requiring task performance (22). Consistent resting-state networks, with sharp transitions in correlation patterns, are reliably detected in individual and group data (23, 24).

In stroke patients, use of this technique has revealed disruption of functional connectivity of brain networks, even within structurally intact brain regions (6, 25, 26). Changes in functional connectivity have been described in motor recovery under resting-state and task-related conditions (27). Further, changes in functional connectivity over time have been found to occur in conjunction with behavioral change, both in healthy individuals (22) and in stroke patients (7, 25). For example, He and colleagues (25) reported that in patients with spatial neglect, dorsal attention network connectivity was disrupted early after stroke, but appeared to have improved to similar levels as controls by 9 months post-stroke, in conjunction with behavioral improvement. This supports the interpretation that different networks or areas of the brain may dynamically change and assume different roles to allow behavior to occur.

The aim of the current study was to identify longitudinal changes in functional connections of the somatosensory network in stroke patients with somatosensory impairment, and to establish if and how these correlations are associated with improvement in touch discrimination.

The importance of interhemispheric functional connectivity in behavioral performance and recovery has been highlighted from studies using resting-state fMRI (rsfMRI) with animal and human stroke populations (7, 25, 28). The most consistent finding is of changes in interhemispheric functional connectivity between homotopic areas, such as ipsilesional and contralateral primary motor cortex (7). Longitudinal changes have also been reported. Decreased interhemispheric functional connectivity of the ipsilesional sensorimotor cortex has been reported early after stroke, with return to more normal levels during the recovery process (7, 29, 30). These findings are not surprising given that

interhemispheric connections are implicated in sensory (31) and cognitive processing (32) and in models of motor and somatosensory recovery (33–37). Thus, changes in interhemispheric functional connectivity in stroke patients and associations between these changes and behavioral improvement are expected. We hypothesized that over time, stroke patients would exhibit return to a more “typical” pattern of interhemispheric functional connectivity between homologous cortical somatosensory regions, and that stronger interhemispheric resting-state functional correlations between homologous SI and SII regions at 6 months than at 1 month post-stroke would be associated with clinical improvement.

Increased connectivity with distributed networks has also been reported in recovery after stroke. First, the visual system drives human attention and planning (38, 39), and a rich history of evidence for cross-modal plasticity between the visual and somatosensory systems exists (40). Recruitment of visual areas has been reported in previous studies of motor recovery after stroke (30, 41) as well as in patients with somatosensory impairment after stroke (3). Second, greater recruitment of attention systems is known to be necessary (42) to compensate for the impairment of function-specific brain areas due to aging or injury (43, 44). In stroke patients, increased attention has been shown to be required to accomplish previously simple tasks, such as walking, and attention skills have been shown to predict outcome after stroke (42, 45). Increased activation of frontoparietal attention areas, such as inferior parietal cortex (IPC), has been reported to occur in recovering stroke patients with motor problems (46–48). Thus, greater functional connections with frontoparietal attention networks could be expected in stroke patients with somatosensory impairment. As such, we predicted that stronger thalamocortical and cortico-cortical functional correlations with frontoparietal visual attention networks at 6 months post-stroke would be associated with clinical improvement.

Materials and Methods

Participants

Ten stroke patients with impaired touch discrimination of the upper limb were assessed at 1 and 6 months post-stroke. Inclusion criteria were as follows: first episode infarct, medical stability, ability to give informed consent and comprehend simple instructions, and right-hand dominance. Exclusion criteria included the following: brain-stem infarct or hemorrhagic stroke, previous neurological dysfunction, medical history impairing hand function or precluding MRI, or evidence of neglect based on standard neuropsychological tests. We also studied 10 age-matched, right-hand dominant healthy controls (4 male, mean age 60.60 years, range 23–79 years) without any history of neurological or somatosensory impairment. The relevant university and hospital human ethics committees approved the study and written informed consent was obtained from each participant.

Demographic and Clinical Profile

Background information included age, gender, and premorbid hand dominance (49). For the stroke patients, a clinical profile

obtained within 48 h of the MRI study included the following: severity of neurological impairment, using the National Institute of Health Stroke Scale (NIHSS) (50); severity of global disability, using the Barthel Index (51); and upper limb function, using the action research arm test (ARAT) (52). Severity of somatosensory impairment was quantified across several modalities, including touch (see below); limb position sense, using the wrist position sense test (WPST) (53); tactile object recognition, using the functional tactile object recognition test (54); and temperature discrimination, using the Rolyan® hot and cold discrimination kit. Age-matched healthy controls were also assessed on measures of somatosensation.

Quantification of Touch Impairment

The primary somatosensory outcome measure was the tactile discrimination test (TDT) (55), a psychophysical measure of touch discrimination of plastic gratings using the fingertip. Participants discriminate differences in finely graded plastic texture surfaces using the method of constant stimuli and a three-alternative forced-choice design. Five surface sets, which span the Weber function of texture differences, are each presented 10 times. The test score is the probability of correct discrimination response across all stimuli presented ($n = 50$) and represents the area that subtends the psychometric function after accounting for chance. The TDT has high test-retest reliability, age-appropriate normative standards, and excellent discriminative properties (55). Touch detection of the fingertips was assessed using the Weinstein enhanced sensory test (WEST) hand monofilaments and the rapid threshold procedure (56).

Image Acquisition

Functional Imaging Sequences

Whole-brain fMRI studies were performed using a 3-T GE Horizon LX Sigma MRI scanner with quadrature head coil (GE Medical Systems, WNL, USA). Five minutes of resting-state data (100 volumes) were acquired for all participants. Images were acquired in 25 axial slices spanning cerebellum to the apex of the cerebrum using a gradient-echo, echoplanar (EPI) sequence [repetition time (TR) = 3000 ms; echo time (TE) = 40 ms; flip angle = 75°; field of view (FOV) = 240 mm; 128 × 128 matrix; slice thickness = 4 mm; interslice gap = 1 mm; in-plane voxel size = 1.95 mm × 1.95 mm; bandwidth = 100]. The participants were instructed to close their eyes and perform no particular task. Participants’ arms rested comfortably on their chest, but not touching each other or anything else. The data were collected immediately after performing an in-scanner somatosensory task involving perception of a plastic texture grating, the results of which have been reported elsewhere (3). The participants were monitored during the scanning session to ensure that they were awake and alert. They were debriefed after resting-state data collection and none of them reported falling asleep.

Structural Imaging Sequences

Whole-brain anatomic and angiographic images were acquired at the same session and included the following: a high-resolution 3D anatomical image, 2D T1-weighted and axial 2D T2-weighted images in the same plane as EPI, and 2D angiographic images.

Data Analysis

Pre-Processing of fMRI Data

Pre-processing for each participant's data included image conversion, slice timing correction, determination of optimum realignment target (median center-of-within-brain intensity), motion detection and realignment (rigid body with six degrees of freedom), normalization to a customized EPI brain template (see below), Gaussian smoothing (8 mm full width at half maximum), and automated creation of within-brain mask of normalized images, using Statistical Parametric Mapping, SPM2 (www.fil.ion.ucl.ac.uk) and iBrain™ software (57). Motion correction parameters were included as covariates of no interest. Data from each imaging run were scaled to a grand mean of 100. The statistical analysis of the resting-state data employed an Autoregressive AR (1) model to account for temporal autocorrelation in the data.

For group analyses, fMRI data were brought into standard space. The spatial normalization target used was a custom template, approximating the EPI template in Montreal Neurological Institute (MNI) space supplied with SPM2. The custom template was created in an iterative fashion from a larger group of participants ($N = 33$) involved in the overall study. Images of patients with right hemisphere lesions were flipped such that all infarcts were in the left hemisphere.

Pre-Processing for Connectivity Analysis

Several processing steps were used to optimally prepare the functional data for analysis of voxel-based correlations. Data were high-pass filtered (using SPM8) (www.fil.ion.ucl.ac.uk) with a high-pass cut-off of 0.01 Hz and low-pass filtered in iBrain™ (57) using a finite impulse response filter to remove the effect of high-frequency noise ($f < 0.08$ Hz) (58).

Construction of Seed Regions of Interest

To measure interregional functional connectivity of the somatosensory system, we identified functionally and anatomically defined regions of interest (ROIs) representing nodes in the somatosensory system. These ROIs for functional connectivity analysis were determined by identifying regions of maximal activation from somatosensory fMRI task-related brain activation data in healthy controls (59). Significant activation clusters were restricted to the *a priori* determined cortical ROIs, the hand regions of SI, and bilateral SII, using cytoarchitectonic maps (60). The thalamic clusters were restricted to regions of the thalamus previously reported to show high probability of connectivity to somatosensory cortex, based on a thalamic connectivity atlas (61).

Six seeds were selected, and comprised clusters in the left and right primary and secondary somatosensory cortices and left and right somatosensory ventroposterior lateral thalami. Each cortical seed ROI was approximately 100 voxels in size (voxels were 1.95 mm × 1.95 mm × 4 mm in size). The cortical seed regions were constructed to make the ROIs relatively uniform in size and were anatomically verified. As the thalamic seeds were based on the thalamic atlas (61), the size was determined by that template (141 and 168 voxels). Seeds were placed on the normalized images for each individual.

rsfMRI Correlation Analysis

The first step in all rsfMRI analyses was to extract BOLD signal time courses from each of the six ROIs by averaging timecourses over voxels within each region for each individual at each time point. For each individual, to compute functional connectivity maps corresponding to a selected seed ROI, the average BOLD signal timecourse of the voxels within the ROI was correlated against all other voxels within the brain, as originally described by Biswal et al. (62). Several potential sources of spurious variance along with their temporal derivatives were included in the design matrix as confounds: (1) six parameters obtained by rigid body correction of head motion; (2) the average whole-brain signal; (3) signal from a ventricular cerebrospinal fluid (CSF) ROI; and (4) signal from a region centered in the white matter (63). Regions in the CSF and white matter were identified manually using MRIcro software (64). The regression of these factors as variables of no interest was aimed at removing fluctuations unlikely to be involved in specific regional correlations (63). The analysis was performed using Statistical Parametric Mapping, SPM8 (www.fil.ion.ucl.ac.uk), with the individual functional connectivity maps thresholded at p -value <0.001 (uncorrected) at the voxel level.

Second Level Imaging Analysis

In the group analysis, the contrast (con*.img) images from the individual analyses of each individual participant were combined in a second level, random-effects model. To test for differences in patterns of functional connectivity between the healthy and stroke groups, *between-group* differences were evaluated using two-sample t tests. To test for differences in patterns of functional connectivity within the stroke group between the 1-month and 6-month time points, *within-group* differences were evaluated using paired t tests. In order to identify how differences in functional connectivity over time might be associated with changes on clinical test scores, individual changes in TDT scores over time were included as a regressor in subsequent correlation analyses in the group-level random-effects analysis of change in functional connectivity for the stroke group. Only clusters with p -values <0.05 (false discovery rate, FDR, corrected) are reported as significant. Anatomical localization of significant clusters was defined using the anatomy toolbox in SPM8, which is based on probabilistic cytoarchitectonic maps (60).

Lesion locations were outlined on axial slices of the 3D anatomical images obtained at 6 months post-stroke, plotted into stereotactic space, as described previously (65), and displayed on a template. The percentage overlap between lesion location and the seed regions was defined for each participant.

Results

Demographic, Lesion, and Clinical Data

Ten stroke survivors (4 male, mean age 58.96 years, range 18–79 years) were studied at approximately 1 month ($M = 4.56$, $SD = 1.58$ weeks) and 6 months ($M = 26.99$, $SD = 1.69$ weeks) post-stroke (Table 1). All were right-hand dominant with a median hand laterality quotient of 100 (49). The left hemisphere was infarcted in six patients (Figure 1). Five patients had lesions

TABLE 1 | Background and clinical characteristics and lesion details of stroke patients ($N = 10$).

ID	Age	Gender	Side of lesion	Site of lesion	Lesion volume (voxels)	Overlap with seed regions (%)	Weeks since stroke		NIHSS	
							1 month	6 months	1 month	6 months
S10	71	M	L	Lateral thalamus (vpl, vpm)	345	0	7.57	25.29	1	0
S13	71	F	L	Lateral thalamus (vpl, vpm)	253	5 – L Th	3.29	28.57	2	1
S14	56	M	L	Multiple lesions in hemispheric white matter	22,761	2 – L Th	6.14	25.86	6	6
S16	76	M	R	Posterior insula, inferior parietal lobule, adjacent hemispheric white matter	14,728	19 – R SII	6.00	30.86	1	1
S17	40	F	R	Posterior insula, inferior parietal lobule, postcentral gyrus	3998	19 – R SII	3.71	26.86	3	1
S18	79	M	L	Putamen/caudate nucleus, parietal/cortical	21,939	87 – L SII	3.86	26.00	4	1
S19	18	F	R	Thalamus (lp), hippocampus, fusiform gyrus	12,465	0	2.43	25.29	4	3
S20	55	F	L	Supramarginal gyrus, parietal operculum, superior parietal lobule, postcentral gyrus	6593	0	3.57	27.14	4	1
S21	63	F	L	Thalamus (vpl), occipital periventricular white matter, lacunar lesion in head of right caudate nucleus	10,107	10 – L Th	5.00	27.29	3	2
S22	59	F	R	Postcentral gyrus, superior parietal lobule, anterior portion	8990	10 – R SII	4.00	26.71	2	2
Median (IQR) 25th–75th	59.00 (55.00–71.00)	4M; 6F 6L; 4R			8990 (2435–13,597)		3.93 (3.61–5.75)	26.77 (25.89–27.25)	3.00 (2.00–4.00)	1.00 (1.00–2.00)

ID, stroke identification number; NIHSS, National Institute of Health Stroke Scale, 1–4 = minor stroke, 5–15 = moderate stroke, 16–20 = moderate/severe stroke, and 21–42 = severe stroke (50); M, male; F, female; R, right; L, left; voxel size = 1.95 mm × 1.95 mm × 4mm; vpl, ventral posterolateral nucleus; vpm, ventral posteromedial nucleus; lp, lateral posterior nucleus; Th, thalamus; SII, secondary somatosensory cortex; IQR, interquartile range.

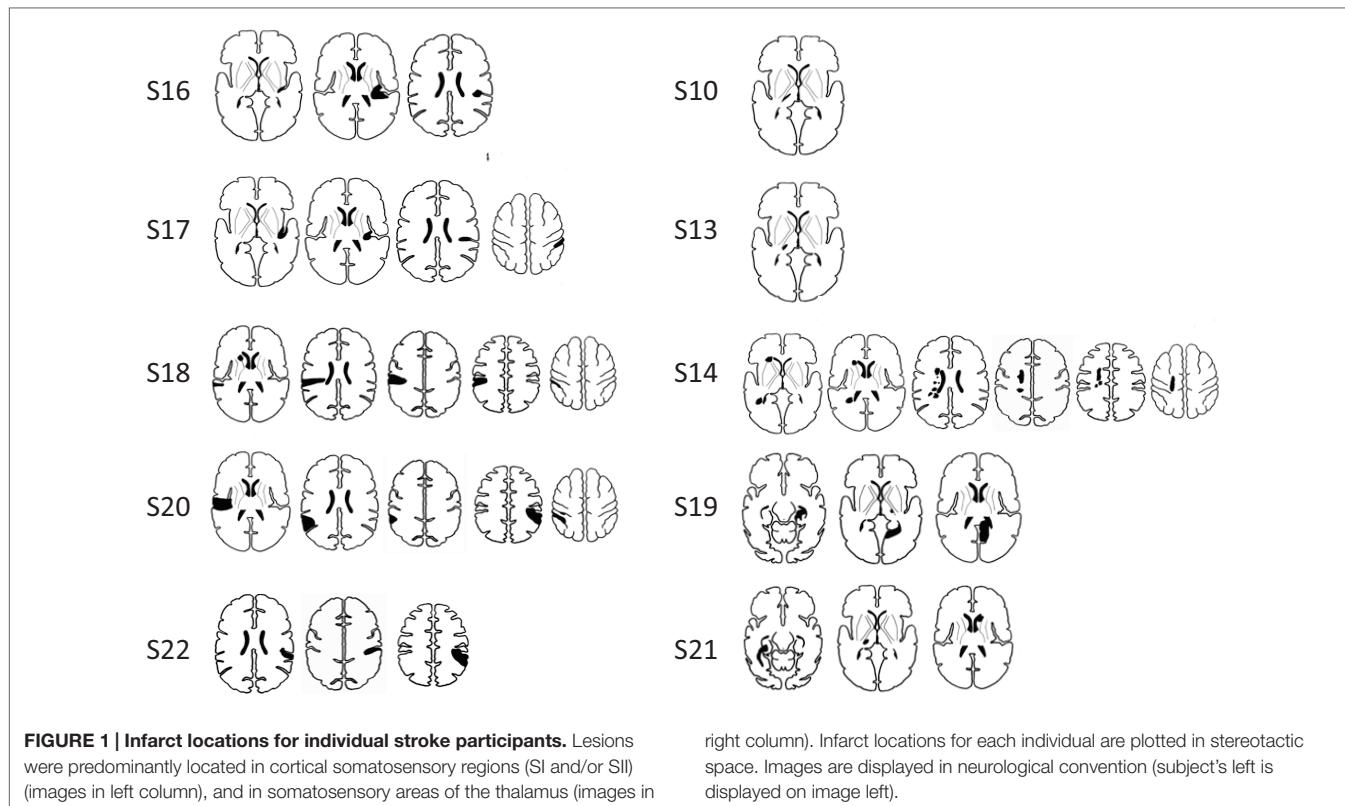
primarily involving subcortical somatosensory structures, in particular the thalamus, and five had lesions predominantly involving cortical SI and/or SII. The percentage overlap between lesion location and our pre-defined seed regions is provided in **Table 1**. All with subcortical lesions had involvement of thalamus, often including ventral posterolateral nucleus, a region known to project somatosensory information to SI. Only three had 2–10% overlap with the thalamic seed used in analysis. Those with cortical lesions primarily had involvement of postcentral gyrus ($n = 3$) and/or secondary somatosensory regions ($n = 5$) including parietal operculum and nearby regions of the insula and supramarginal gyrus. Across patients there was no overlap between lesion site and the SI seed. Four patients had lesion locations that overlapped with the SII seed; three had 10–19% overlap and a further patient with a very large lesion had 87% overlap.

Patients presented with wide variation in severity of touch discrimination (**Table 2**), ranging from -11.58 (very severe impairment) to 79.31 (just within the normal range) on the TDT (55) at the 1-month study. Several patients performed within normal limits on the TDT at 6 months post-stroke, and in three cases at the 1-month time point. Somatosensory impairment was indicated in these patients on the basis that the TDT score for the affected hand was lower than for the “unaffected” hand, they demonstrated impairment on other clinical somatosensory tests, and/or they reported a “hyper-sensitivity” profile of heightened sensitivity to somatosensory stimuli.

For the stroke group, mean affected-hand score on the TDT at the 1-month time point was 35.98 ± 33.13 SD (median 35.47 percentage correct area under the curve), compared to 79.85 ± 8.11 SD (median 77.09) for healthy controls in the matched hand. TDT scores were significantly higher in the healthy control group than in the patient group (Mann–Whitney $U = 11.00, p = 0.002$). The stroke group demonstrated significant improvement in TDT scores with the affected hand between the 1- and 6-month time points ($Z = -2.293, p = 0.022$). Clinical scores and demographic and clinical information for the stroke patients are presented in **Table 1**.

Functional Connectivity During the Resting State Functional Connectivity of Stroke Patients Compared to Healthy Controls

Within the healthy control group, the SI seeds for both hemispheres showed significant functional connectivity with bilateral SI and motor (Brodmann Area, BA 4a, 6) regions (**Figure 2**). In contrast, at 1 month post-stroke the stroke group exhibited a lack of interhemispheric connectivity for both of the SI seeds, with each SI seed functionally connected only with surrounding SI and motor areas. At 6 months post-stroke, there appeared to be some return of interhemispheric SI connectivity for the stroke group (**Figure 2**). For example, the ipsilesional SI seed showed significant functional connectivity not only with surrounding SI and motor areas but also with contralateral SI, contralateral visual and motor areas, and with ipsilesional SII. Similarly, the



contralesional SI seed remained functionally connected with surrounding SI and motor regions, and showed connections not present at the 1-month time point with ipsilesional SI and SII, and contralesional middle occipital gyrus. At the 1-month time point, the healthy group exhibited significantly greater functional connectivity than the stroke group between the contralesional SI seed and a cluster in the contralesional occipital lobe and contralateral cerebellum (MNI = 30/-56/16; $k = 99$ voxels; $z = 4.76$).

In the healthy control group, SII seeds of each hemisphere exhibited significant functional connectivity with bilateral SII and SI, as well as with medial supplementary motor area (SMA, BA 6). The stroke group demonstrated a similar pattern of connectivity for the ipsilesional SII seed at 1 month post-stroke. For the contralesional SII seed, significantly connected clusters also extended into bilateral SI. At 6 months post-stroke, the ipsilesional SII seed showed functional connectivity only with surrounding SII and SI. The contralesional SII seed was again functionally connected with bilateral SII and contralesional SI, with additional small clusters in contralesional SMA (BA 6) and medial visual areas (BA 17, 18, commonly referred to as human V4 and V2).

In the healthy control group, thalamus seeds of each hemisphere were functionally connected to a statistically significant extent with bilateral thalamus (thalamus surrounding the seed region in the same hemisphere, as well as contralateral thalamus) and SII/insula in the same hemisphere (Figure 2). At 1 month post-stroke, the patient group showed significant functional connectivity from both thalamus seeds with bilateral thalamus,

although to a less extent than that seen in the healthy control group. In addition, the contralesional thalamus was functionally connected with small clusters in contralesional inferior and superior frontal gyri, and contralesional cerebellum. At 6 months, ipsilesional thalamus in stroke patients still showed significant functional connectivity with thalamus in both hemispheres, whereas the contralesional thalamus was only functionally connected with surrounding contralesional thalamus and with a small cluster in the left putamen.

Longitudinal Functional Connectivity Changes in the Stroke Group

To test for differences in patterns of functional connectivity within the stroke group between the 1- and 6-month time points, within-group differences were evaluated using paired t tests (Table 3). For the contralesional SI seed, there was significantly greater functional connectivity at 1-month than at 6-month post-stroke between contralesional SI and a cluster falling in the contralesional cerebellum and hippocampus. At 6 months, there was significantly greater functional connectivity between contralesional thalamus and a cluster in ipsilesional middle cingulate cortex.

Functional Connectivity Changes Associated with Somatosensory Improvement

In subsequent correlation analyses, changes over time in clinical scores, as measured using the TDT, were included as a regressor in the group-level random-effects analysis of change in functional

TABLE 2 | Scores on somatosensory and hand function tests in the stroke group ($N = 10$).

ID	TDT - Aff (/100)		TDT - Unaff (/100)		WEST - Aff		WEST - Unaff		fTORT - Aff (/42)		Temp Aff (/10)		WPST Aff (error)		ARAT Aff (/57)	
	Initial	6 months	Initial	6 months	Initial	6 months	Initial	6 months	Initial	6 months	Initial	6 months	Initial	6 months	Initial	6 months
S10	43.10	77.59	75.37	85.22	1.1	0.2	0.135	0.035	41	40	5	9	15.40	10.90	57.0	56.0
S13	72.91	72.66	81.03	72.41	1.1	0.135	0.035	0.07	41	40	10	10	7.85	4.45	55.0	55.5
S14	30.54	47.29	67.98	81.03	102	0.07	0.07	0.07	29	40	7	9	21.75	20.30	0.0	0.0
S16	75.12	76.85	86.45	93.60	0.135	0.14	0.135	0.135	38	42	4	4	14.20	7.10	56.5	57.0
S17	8.87	40.39	52.71	77.59	300	1.1	0.035	0.035	34	42	0	2	16.65	13.90	42.0	53.5
S18	40.39	67.73	83.99	89.41	200	1.1	0.035	0.07	40	41	8	8	15.20	13.85	54.5	57.0
S19	79.31	66.99	84.73	73.64	0.035	0.07	0.035	0.07	40	42	10	10	11.15	5.35	57.0	57.0
S20	-11.58	78.57	87.93	81.53	102	0.2	0.07	0.135	40	41	0	0	17.70	12.30	49.0	57.0
S21	29.56	33.74	68.72	85.47	2	0.07	0.035	0.035	37	41	8	7	16.25	12.50	55.0	56.5
S22	-8.37	32.02	63.55	57.88	2	1.1	0.07	0.035	31	39	8	4	15.60	15.70	11.0	42.0
Median	35.47	67.36	78.20	81.28	2.00	0.17	0.07	0.05	39.0	41.0	7.5	7.5	15.50	12.40	54.75	56.25
(IQR)	(14.04– 25th–75th	(42.12– 65.46)	(88.17– 75.80)	(74.63– 84.55)	(1.10– 85.41)	(0.09–0.88)	(0.035– 102.00)	(0.07)	(34.8– 40.0)	(40.0–41.8)	(4.3–8.0)	(4.0–9.0)	(14.45– 16.55)	(8.05– 13.89)	(43.75– 56.13)	(54.00– 57.00)

TDT, tactile discrimination test (55), score is percentage correct area under the curve (2). The 95th percentile criterion for abnormality is a score <6.1. Minus values indicate scores with touch discrimination less than chance; WEST, Weinstein enhanced sensory test monofilaments touch detection score for the finger tip used in the TDT; threshold in grams pressure (56); fTORT, functional tactile object recognition test, score out of maximum 42; 5th percentile criterion of abnormality is 37 (54); Temp, Hot/cold temperature discrimination, number correct out of 10; WPST (average error), wrist position sense test, average error in degrees, 95th percentile of abnormality is 9.5°; average error (53); ARAT, action research arm test, score out of maximum 57 (52); IQR, interquartile range.

connectivity for the stroke group. The functional connectivity changes significantly associated with changes in TDT scores are shown in **Table 3** and **Figure 3**. Greater improvement in TDT scores was associated with greater functional connectivity at 6-month than at 1-month post-stroke between the contralesional SII seed and clusters in the contralesional IPC and contralesional middle temporal gyrus. Greater connectivity at 6-month than at 1-month post-stroke between the contralesional thalamus seed and a cluster in contralesional cerebellum was also associated with greater improvement. Greater functional connectivity at 1-month than at 6-month post-stroke between the contralesional SI seed and contralesional cerebellum was associated with greater improvement in TDT scores over time. Conversely, relative to the 6-month recovery time, greater improvement in TDT scores may be viewed as being associated with less functional connectivity at 6 months than at 1 month between the contralesional SI seed and contralesional cerebellum (**Figure 3**).

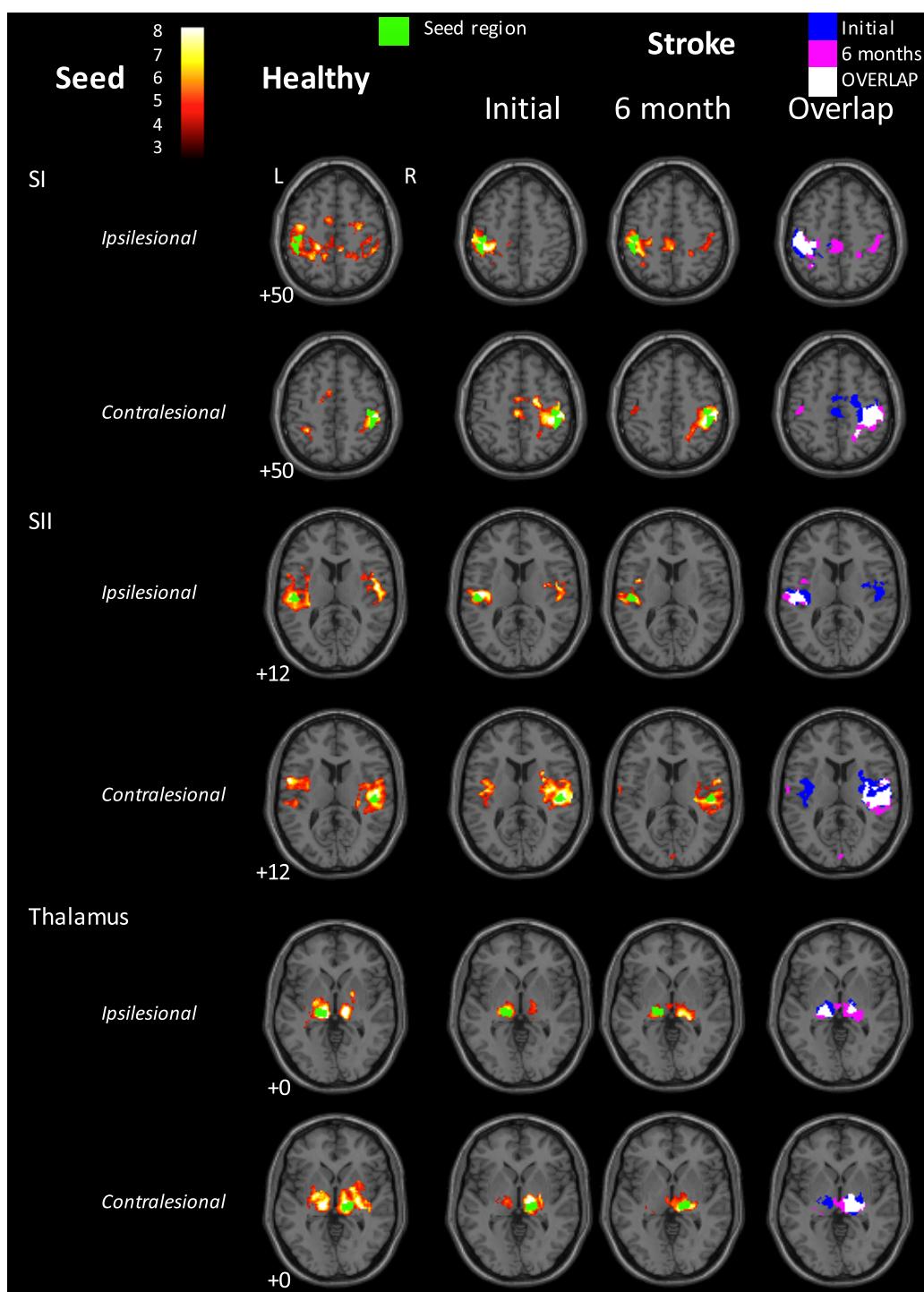
Discussion

Interhemispheric Functional Connectivity is Disrupted at 1 month After Stroke and Shows Some Recovery Toward Normal Levels at 6 months

Our findings of functional connectivity extend previous findings of changes in activation of brain regions with somatosensory impairment and add to the growing body of literature on the role of interhemispheric connectivity in stroke recovery across a range of functions. One month post-stroke, patients with impaired touch sensation and lesions predominantly located in somatosensory areas of the thalamus, and/or in cortical somatosensory regions (SI and/or SII), exhibited disruption of interhemispheric functional connectivity of homologous SI regions relative to age-matched healthy controls. At 1 month post-stroke, the stroke group only exhibited SI functional connectivity with SI within the same hemisphere; at 6 months, there was some return of interhemispheric SI connectivity.

Our finding of less interhemispheric connectivity in the stroke patients with impaired touch sensation relative to healthy controls early post-stroke is consistent with evidence of disrupted interhemispheric functional connectivity in stroke patients for other functions, such as movement and attention (7, 25, 27, 28). The disruption observed is likely to be behaviorally relevant, given that activity in both hemispheres has been shown to be important in sensory processing (31, 66) and in activation studies of somatosensory and motor recovery (33–36). Further, previous studies using rsfMRI in stroke recovery across functions have highlighted the importance of interhemispheric functional connectivity in behavioral performance and in recovery over time (7, 25, 28).

Evidence of SI interhemispheric connectivity at 6 months in stroke patients with less severe touch impairment is consistent with growing evidence from related studies. Activation studies of motor recovery indicate “return to more normal patterns” is associated with better recovery in the post-acute and chronic phase (e.g., 6 months) post-stroke (67, 68). In addition, a recent review of rsfMRI studies in motor recovery



Images are displayed in neurological convention (subject's left is displayed on image left). The left hemisphere represents the ipsilesional hemisphere – images of patients with right hemisphere lesions were flipped such that all infarcts are represented in the left hemisphere. Healthy controls were individually matched and images flipped accordingly. Slice numbers represent axial slice position in Montreal Neurological Institute (MNI) space. Color scale represents Z-values of group functional connectivity maps. SI, primary somatosensory cortex; SII, secondary somatosensory cortex. Analyses are based on contrast maps with an individual voxel height threshold level of $p < 0.001$. Results are displayed for significant clusters with $p < 0.05$ (false discovery rate, FDR, corrected).

TABLE 3 | Functional connectivity changes in the stroke group between the 1- and 6-month time points, and changes associated with improvement in TDT scores.

Seed region	Cluster size (voxels)	Z-value	MNI maxima coordinates (x, y, z)	Cluster anatomical location of significantly correlated regions
Regions showing greater functional connectivity at 1 month				
Contralesional SI	60	4.14	14, -32, -18 12, -28, -6 6, -34, -20	Contralesional cerebellum lobules I–V, hippocampus
Regions showing greater functional connectivity at 6 months				
Contralesional thalamus	49	4.44	-16, -22, 38	Ipsilesional middle cingulate
Regions showing greater functional connectivity at 6 months than 1 month post-stroke in association with improvement in touch discrimination				
Contralesional SII	53	4.55	52, -58, 26	Contralesional IPC
	30	5.02	58, -26, -10	Contralesional middle temporal gyrus
Contralesional thalamus	35	4.27	12, -42, -40 20, -44, -34	Contralesional cerebellum lobule IX
Regions showing greater functional connectivity at 1 month than 6 months post-stroke in association with improvement in touch discrimination				
Contralesional SI	42	3.89	28, -44, -24	Contralesional cerebellum lobules V, VI

Anatomical definitions are based on the anatomy toolbox in SPM8, which is based on probabilistic cytoarchitectonic maps (60).

MNI, Montreal Neurological Institute; SI, primary somatosensory cortex; SII, secondary somatosensory cortex; IPC, inferior parietal cortex.

found that reorganization of motor networks encompasses a restoration of interhemispheric functional coherence in the resting state, particularly between the primary motor cortices (27). While we do not report a significant longitudinal change in connectivity between contralesional and ipsilesional SI, we did observe significant interhemispheric connectivity for both SI seeds at 6 months that was not present at 1 month. Together, our findings resonate with studies illustrating the role of inhibitory influences from intact hemisphere in stroke recovery (33) and highlight the need to re-establish a balance of activity across hemispheres in association with improvement (35).

Disruption and Resolution of Functional Connectivity with Occipital Visual Areas

Another key finding was the role of functional connectivity with primary visual occipital regions. At the 1-month time point, functional connectivity between contralesional SI and the occipital lobe was significantly less in the stroke group compared to the matched healthy control group. At the 6-month time point, the stroke group demonstrated functional connections with visual occipital areas that were not present at 1 month post-stroke, including between ipsilesional SI and contralesional visual areas (BA 17, 18), between contralesional SI and contralesional middle occipital gyrus, and between contralesional SII and bilateral visual areas (BA 17, 18). Together, these findings suggest a pattern of disruption of functional connections between somatosensory and visual areas at 1 month post-stroke, which showed some return after 6 months.

Supporting the suggestion of less connectivity with visual occipital regions early post-stroke is Park et al.'s (30) finding that one month after stroke, patients with motor impairment demonstrated decreased functional connectivity between primary motor regions and occipital cortex. Similarly, Carey et al. (3) reported that in a group of stroke patients with thalamic lesions studied at 1 month post-stroke, touch discrimination correlated negatively with task-related activation in occipital

regions. Connectivity with occipital regions at 6-month are also consistent with Seitz et al.'s (41) study of the functional networks related to motor recovery, which found that improved motor function after stroke was associated with involvement of distributed areas including extrastriate visual areas. Thus, there seems to be a pattern of disrupted interactions between sensorimotor and visual occipital systems around 1 month after stroke, with some resolution over time that may be clinically relevant.

Functional Connections to Frontoparietal Attention Regions

In stroke patients, functional connections to frontoparietal attention regions (69), involving middle cingulate and IPC, were significantly greater at 6 months than at 1 month post-stroke, and these differences between time points were in part associated with changes in behavioral performance. Functional connectivity between contralesional thalamus and ipsilesional middle cingulate cortex was significantly greater at 6 months than at 1 month post-stroke. Furthermore, behavioral improvement on the TDT was associated with greater functional connectivity 6 months post-stroke between contralesional SII and a cluster in contralesional IPC. In addition, the individuals who showed thalamocortical functional connectivity with frontal regions at the 1-month time point also had relatively low TDT scores, while those who showed this connectivity pattern at 6 months had better TDT scores.

Activation of distributed attention networks has been observed in previous task-based studies of stroke recovery, including in relation to somatosensory recovery (3, 36). Involvement of frontoparietal attention networks in association with behavioral outcome has been a common finding in stroke patients in the motor domain (46–48). Further, longitudinal changes in rsfMRI include changes in frontal and parietal cortices during motor recovery (30). Here, we extend this finding of functional connectivity to somatosensory recovery post-stroke. Baseline brain activity in the medial thalamus and the frontoparietal network is important

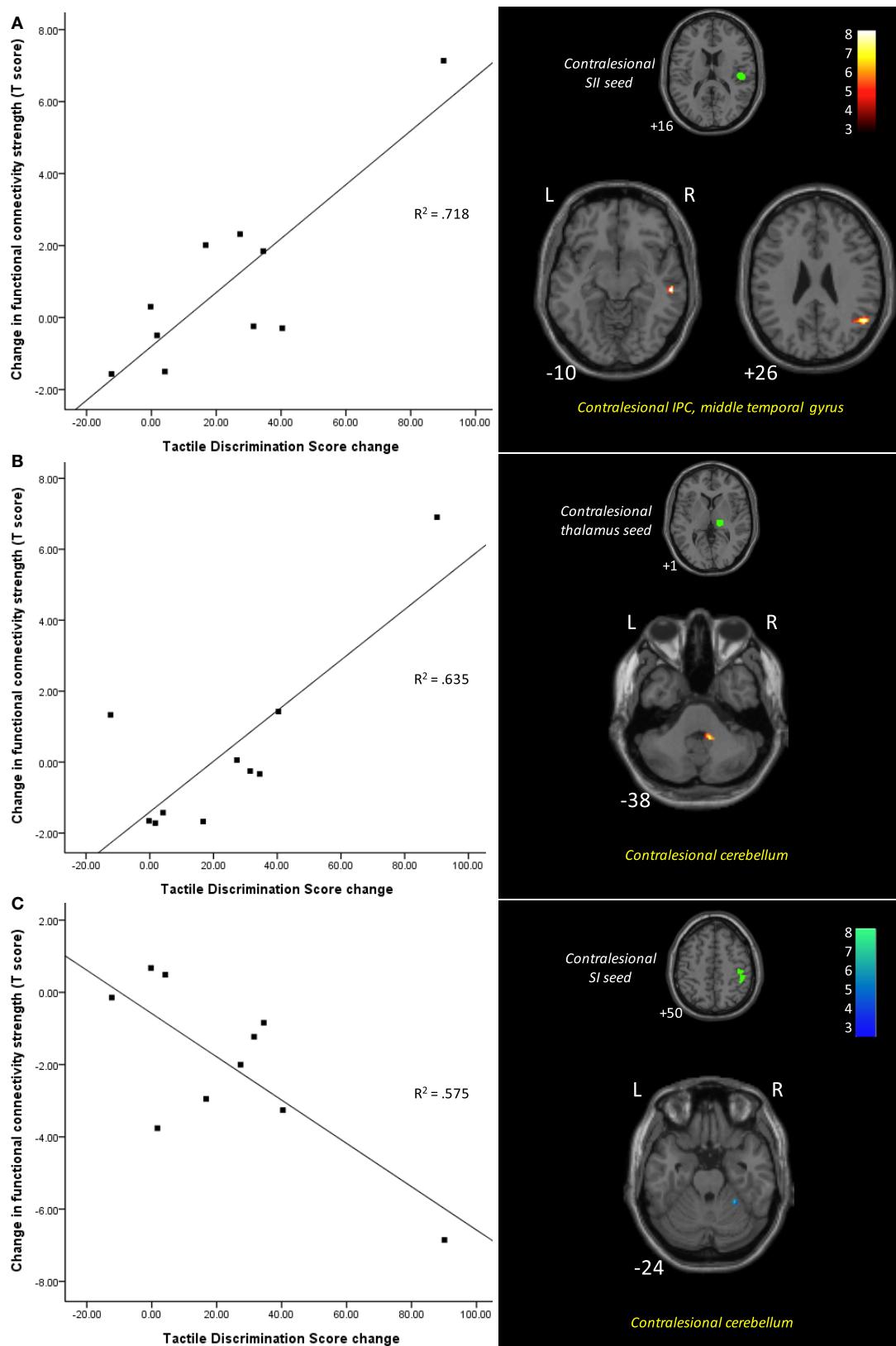


FIGURE 3 | Functional connectivity differences between 1 and 6 months post-stroke associated with changes in tactile discrimination test (TDT) scores.

(Continued)

FIGURE 3 | Continued

Scatter plots illustrate change in TDT score plotted against the difference between the two time points in connectivity strength between the seed and the cluster (shown in images on the right), for each individual. Improvement in TDT scores was associated with **(A)** greater functional connectivity at 6 months between the contralesional SII seed and clusters in the contralesional inferior parietal cortex and contralesional middle temporal gyrus; **(B)** greater functional connectivity at 6 months between the contralesional thalamus seed and a cluster in contralesional cerebellum; and **(C)** less functional connectivity at 6 months between the contralesional SI seed and contralesional cerebellum. Images are displayed in neurological convention

(subject's left is displayed on image left). The left hemisphere represents the ipsilesional hemisphere – images of patients with right hemisphere lesions were flipped such that all infarcts are represented in the left hemisphere. Slice numbers represent axial slice position in Montreal Neurological Institute (MNI) space. Color scale represents Z-values of functionally connected clusters associated with TDT score change. SI, primary somatosensory cortex; SII, secondary somatosensory cortex; IPC, inferior parietal cortex. Analyses are based on contrast maps with an individual voxel height threshold level of $p < 0.001$. Only clusters with p -values <0.05 (false discovery rate, FDR, corrected) are reported as significant and displayed.

in perception (70) and may affect information processing following sensory impairment. In addition, focal attention involved in perception of pain, processing of reward, and error detection, has been associated with activity in medial frontal/anterior cingulate (69). Attention is essential to any perception or learning (69, 71), and has been identified as a key element of recovery from brain injury (25, 42, 45). It could be speculated that our findings reflect stroke patients' use of higher-level attention and behavioral processes to supplement previously more automatic somatosensory perceptual functions.

Involvement of Contralesional Hemisphere

Changes in functional connectivity between the 1- and 6-month time points and in association with improvement in TDT over time were all seeded within the contralesional hemisphere, i.e., contralesional SI at 1 month and contralesional thalamus and SII at 6 months. We did not find significant changes over time in connectivity from our ipsilesional seeds. Further, the regions showing relatively increased correlation were also primarily in the contralesional hemisphere, with the exception of ipsilesional middle gyrus at 6-month >1 -month post-stroke. These findings highlight a role for change in connectivity of the "intact" contralesional hemisphere, in particular somatosensory SI, SII and thalamus regions, in individuals with impaired touch sensation post-stroke. Further, the observation that increased connectivity from these contralesional somatosensory seeds was associated with improvement in touch discrimination scores over time suggests a role for the contralesional somatosensory network in facilitating touch discrimination perception. While previous task-based fMRI studies typically show an initial increase in activation of contralesional sensorimotor cortex early followed by restoration of activation in the ipsilesional cortex, our finding suggests that disruption of the initial interhemispheric connectivity at resting state may lead to ongoing alterations in the activity (functional connectivity) of contralesional hemisphere. These relative increases in connectivity, observed both early and late, may help in achieving a more balanced interhemispheric connectivity in association with greater improvement in patients with partial recovery.

At 1-month, increased connectivity between contralesional SI and contralesional cerebellum was associated with greater improvement in touch sensation over time. In comparison, at 6 months, the relatively greater connectivity associated with better touch discrimination was between contralesional SII and IPC and contralesional thalamus and cerebellum. Interestingly, contralesional cerebellum had changed connectivity to somatosensory

seeds associated with improvement at both times, but via different nodes of the network. A role for increased connectivity between contralesional SI and cerebellum, at 1-month associated with improvement, is consistent with our observation of greater connectivity between these regions in the healthy group, compared to stroke patients, at 1-month. Longitudinal changes in rsfMRI during motor recovery have also involved bilateral thalamus and cerebellum, with involvement of cerebellum persisting over the 6-month period post onset (30). A large proportion of cerebellum maps to association areas (72). In addition, the cerebellum has connections with SI, although preferentially with the contralateral cerebrum (72). Afferent projections first synapse in the deep cerebellar nuclei and then project to a second synapse in the contralateral thalamus that in turn serves as a relay to the cerebral cortex, consistent with involvement of thalamus at 6 months. Co-observation of greater functional connectivity of contralesional thalamus with ipsilesional middle cingulate at 6 months, suggests an increased interhemispheric connectivity. Involvement of contralesional thalamus has been reported in association with touch impairment in a sample of 19 stroke survivors at 1-month post-stroke (3). Contralesional thalamus has potential to be accessed irrespective of lesion location (3), has an influence on bilateral SI via its prefrontal connections (73), and may have a role in gating of sensory information and in large-scale reorganization in the somatosensory cortex and thalamus after sensory loss (74, 75).

Limitations

The major limitation of this study was the small and heterogeneous sample of stroke patients. Replication of these preliminary findings in larger samples is required. Use of a larger sample would also allow investigation of these changes without the need to flip individual brain maps into common space. This would permit inferences about the role of lateralized frontoparietal attention networks in facilitating post-stroke behavioral improvement (8). While it is recognized that functional connectivity may be influenced by the participants recent experience (76), the sequence of acquisition was common for all participants, i.e., it was immediately preceded by a touch discrimination task. Further, our stroke findings may be interpreted with reference to healthy controls who underwent the same protocol sequence, and our longitudinal findings with reference to connectivity studies in the same individual over time.

Application of rsfMRI analyses in stroke patients presents issues that need to be considered in the interpretation of our findings. The potential impact of lesion location on pre-defined seed ROIs is an unavoidable issue. This was in part minimized through

application of individual lesion masks during the normalization phase. In addition, we quantified the percentage overlap between the lesion and seed region for each participant to monitor the presence of this potential limitation. All but one participant had <20% overlap. There was no overlap with the ipsilesional SI seed and only 10% or less overlap with the thalamic seed. The seed with most overlap was the SII seed, with 4 of 10 patients having overlap. Our major findings of change in connectivity were evident for contralesional seeds, and thus, can be interpreted with confidence. Further, lack of evidence of significant change for ipsilesional SI and thalamic seeds is unlikely explained by analysis method and seed overlap, as this was minimal. Interpretation of functional connectivity from ipsilesional SII may be impacted by overlap between lesion and seed. Although we did not find a significant change over time, we did observe significant connectivity from ipsilesional SII at 1 and 6 months, suggesting presence of lesion overlap with this seed is an unlikely explanation. A recent investigation of overlap between lesion location and seeds between stroke and healthy groups suggests that the percent of infarct-related overlap to any ROI was not related to connectivity strength in connections that included those damaged seeds (77). While this finding is based on a larger sample ($n = 32$) and multiple correlations, it does provide some support for interpretation of seed-based connectivity data in stroke patients. Finally, even if the differences in connectivity observed between stroke and healthy controls is due to impaired anatomic connections from these regions, our findings still inform us of the key functional connections involved in somatosensory impairment, the impact of lesion on the function, and the changes in functional connectivity associated with clinical improvement in touch discrimination.

Use of the BOLD signal in fMRI studies of stroke patients has been a highly debated issue given the potential impact of vascular compromise. The BOLD signal provides an indirect indication of neural activity, and changes in resting-state activity can reflect a complex combination of neural, vascular, and metabolic factors (78). Connectivity analysis methods have the advantage that they do not rely on BOLD signal stability, nor assume a common hemodynamic response function (79). However, they are not immune to issues associated with abnormal neurovascular coupling in stroke patients. Indeed, it is unclear how potential vascular latency differences between brain regions impact interpretation following stroke. For example, changes in peri-infarct regions, such as hypoperfusion and potential decoupling of the neurovascular response (80), may impact the signal. It has been suggested that differences across regions may confound studies of whole-brain connectivity (81). A few studies have therefore adjusted for non-neural vascular latency differences prior to resting-state connectivity analyses in healthy controls with only a minor impact on their findings (81). However, we should exercise caution when interpreting findings in stroke patients, particularly in locations close to the lesion border. Further, it is important to recognize that changes observed with rsfMRI may reflect an interaction between neural activity and vascular changes over 1–6 months. It should also be noted that we did not exclude patients with conditions that may impact the BOLD signal, such as leukoencephalopathy and/or carotid artery disease, and thus the impact of these conditions if present is unknown.

Implications and Future Directions

In summary, stroke patients showed changes in functional connectivity over a period of recovery under non-specific rehabilitation conditions. Further, most changes in functional connections from 1 to 6 months post-stroke were shown to relate to improvement in touch discrimination scores over time, in patients with partial recovery. There appeared to be some return of functional connections over time in patients between homologous SI regions, and between somatosensory and visual occipital areas, although not to the levels seen in age-matched controls. Change in connectivity over time and/or in association with improvement was observed in relation to contralesional somatosensory seeds, and primarily involved frontoparietal attention regions and cerebellum. Change in contralesional SI connectivity was important at 1-month in relation to improvement over time, while changes in connectivity of contralesional SII and thalamus become important at 6 months.

These changes in connectivity could represent future targets for therapy. In particular, increase in strength in connections between somatosensory regions and attention and vision regions is consistent with pre-existing connections with these networks and suggest targets for neuroscience-based rehabilitation approaches designed to access viable brain networks (36). While our findings indicate that some individuals spontaneously access these regions in association with improved performance, the potential exists for knowledge of these individual differences to guide access in other stroke survivors through therapy. For example, the effective sensory discrimination training approach described by us to achieve stimulus specific improvements in touch discrimination (82) employs training strategies to achieve cross-modal calibration of perceived texture roughness across touch and vision, as well as use of attentive exploration of textured stimuli and deliberate use of anticipation trials (36, 82). These strategies may be helpful in accessing vision and attention networks in survivors who may not otherwise make these connections.

Targeting of contralesional and distributed networks via secondary somatosensory cortex and thalamus is also suggested. Our findings first highlight the role of the contralesional hemisphere in post-stroke performance and recovery. The seed-based change in contralesional functional connectivity is consistent with structural and functional connectivity studies of sensorimotor training that suggest global network efficiency is influenced by long-range connections across hemispheres, in addition to ipsilesional integrity (83). Changes in connectivity of contralesional SII and thalamus at 6 months suggest a role for nodes that have connections within the somatosensory network and beyond. SII has strong connections with SI, thalamus, and homologous SII, as well as with frontal and parietal networks (84). SII has more dense bilateral connectivity than SI (85), is involved in tactile working memory, discrimination, and perceptual learning (86–88), and is regarded as an integration node of the somatosensory network. Enhanced SII connections with IPC and middle temporal gyrus at 6 months highlight connectivity with distributed networks. The potential exists to influence this highly connected node of the somatosensory network through rehabilitation designed to access discriminative and tactile

learning functions. The thalamus is also implicated. It has an important role in gating somatosensory input and deactivation of contralateral thalamus is associated with touch discrimination performance in stroke survivors at 1 month post-stroke (3). Involvement of thalamus is consistent with evidence from animal studies (74, 75) that gating of sensory inputs, rather than cortical representation alone, is important in recovery. In addition, increased connectivity between thalamus and cerebellum suggests short-range functional connectivity of subcortical networks (89). Thalamus and cerebellum are two of three major subcortical network hubs identified (89). Involvement of both long-range and short-range functional connectivity changes may reflect not only the individual variation in recovery and underlying mechanisms but also the potential to drive one or other through appropriately targeted therapy. While connectivity-based research is still in its infancy post-stroke, it has great potential to guide the development of scientifically informed rehabilitation interventions.

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Determinants of concurrent motor and language recovery during intensive therapy in chronic stroke patients: four single-case studies

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Despite intensive research on mechanisms of recovery of function after stroke, surprisingly little is known about determinants of concurrent recovery of language and motor functions in single patients. The alternative hypotheses are that the two functions might either “fight for resources” or use the same mechanisms in the recovery process. Here, we present follow-up data of four exemplary patients with different base levels of motor and language abilities. We assessed functional scales and performed exact lesion analysis to examine the connection between lesion parameters and recovery potential in each domain. Results confirm that preservation of the corticospinal tracts (CSTs) is a neural predictor for good motor recovery while preservation of the arcuate fasciculus (AF) is important for a good language recovery. However, results further indicate that even patients with large lesions in CST, AF, and superior longitudinal fasciculus, respectively, are able to recover their motor/language abilities during intensive therapy. We further found some indicators of a facilitating interaction between motor and language recovery. Patients with positive improvement of motor skills after therapy also improved in language skills, while the patients with no motor improvements were not able to gain any language recovery.

Keywords: stroke, motor, language, hemiplegia, hemiparesis, aphasia, recovery

Introduction

It is a common clinical observation that in patients with both initial hemiparesis and aphasia after stroke, motor and language recovery may take different courses. Interestingly, scientific research has primarily focused on the examination of the course of recovery regarding either motor or language abilities, but only few studies addressed both. Aphasia has even been a criterion for exclusion in several studies of motor recovery (1, 2).

To our knowledge, there is only one multiple single-case study that addressed the issue of language recovery going parallel to a therapy of motor functions of the upper limb. Harnish et al. (3) examined

five stroke patients during the course of 6 weeks of motor therapy. They assessed not only the recovery of motor functions of the upper limbs and functional motor reorganization but also changes in their language abilities. The authors report that in the three subjects showing the largest motor improvements they could also observe significant language improvements. In the individual fMRI measurements, where the patients had to tap the fingers of the paretic hand within the scope of their capacities, a shift of activation to the right hemisphere during the course of motor treatment could be observed in these three patients. Harnish et al. concluded that language changes seem to co-occur with motor changes after motor therapy. Anatomical analyses of the patients' lesions were not carried out.

The finding of Harnish et al. that motor recovery can foster language recovery is very interesting for the current state of discussion about common mechanisms in motor and language processing. Especially the theory of cognitive embodiment has gained broad attention and kindled a whole line of research. In the light of embodiment theory, cognitive functions like language are grounded in the sensorimotor experiences and to the underlying systems (4, 5). For example, Hauk et al. (6) were able to show that language processing and comprehension activate motor regions, while Glenberg et al. (7) found that first- and second-grade children who manipulate images of toys on a computer screen develop improved comprehension skills in reading – a comprehension benefit was evoked by the conduction of motor tasks. There are numerous imaging studies demonstrating activation of the sensorimotor systems by listening to language with motor content [for example, see Ref. (8, 9)]. Recently anatomic correlates for common motor speech and motor (10) as well as language and motor processing (11) have been postulated on the basis of imaging data.

With the theoretical and experimental background that motor and language activity are not functionally independent, interdependencies regarding the course of recovery of these two domains can be assumed as well. These relations might result in two possible interactions between motor and language recovery processes: either competitive or additive effects may occur. Competitive rehabilitative interactions might be characterized by a "fight" for resources between the language and motor recovery capacities. In this case, a good motor recovery may limit or even prevent the course of language recovery and vice versa. The inverse assumption of an additive interaction between both domains during recovery implies that a positive course of motor recovery would influence language recovery positively, and vice versa. The results of Harnish et al. (3), which are in line with the findings concerning embodiment, seem to support the second hypothesis.

The identification of determinants of motor and language recovery after stroke is within the main stream of research on neurorehabilitation. There are several studies evaluating the role of lesion parameters as well as brain activation for complete or poor recovery for language and motor domain separately. We will briefly highlight the most relevant results in order to establish the backdrop for our study.

As to the motor domain, the lesion location is an important predictor for motor rehabilitation (12), whereas the size of the brain lesion seems to be no predictor for motor function recovery

after stroke (12–14). Shelton and Reding (15) found that the probability of recovery of the upper limbs after stroke seems to diminish in dependence of the lesion location in the following order: cortex, corona radiata, and internal capsule. The dimension of impairment of the corticospinal tract (CST) is another indicator for good rehabilitation of hand motor function after stroke; severe damage of the CST has mainly been assessed in more severely affected patients (12–15).

Similarly, in the language domain, lesion location may play an important role in sufficient language recovery. Meinzer et al. (16) found that language rehabilitation after intensive language therapy was correlated with the integrity of the left hippocampus and the surrounding white matter. Marchina et al. (17) were able to show that the extent of impairment of the left arcuate fasciculus (AF) is a predictor for language recovery. The global lesion size does not have an influence on language rehabilitation after stroke [e.g., see Ref. (16, 18)].

Functional imaging has resulted in inconsistent results for both recovery of motor [e.g., see Ref. (19–23)] and language abilities [e.g., see Ref. (24–29)]. The heterogeneity of the results in the language and motor domain can possibly be attributed to different methods and objectives that were used in previous studies as well as different types of strokes (e.g., subcortical vs. cortical). Therefore, it is hardly possible to combine the mentioned results of the two different domains for predicting recovery patterns in patients with concurrent impairments in both domains.

Therefore, neural correlates for simultaneous recovery in the language and motor domain after stroke remain unclear. The results of Harnish et al. (3), which were investigated through fMRI and behavioral measurements, give a first hint for an additive interaction between both domains during the course of rehabilitation. To our knowledge, there are no studies with the aim to explore lesion characteristics of different ways of concurrent motor and language recovery. Therefore, it remains unclear if an additive interaction between motor and language recovery processes through therapy can be linked to specific structural lesions in the brain.

The aim of the present study was to investigate systematically the determinants of language and motor recovery in four exemplary patients with different base levels of motor and language abilities. Alongside the clinical assessment of motor and language abilities, we focused on (1) the examination of lesion characteristics at pre-test and (2) possible interactions of motor and language recovery processes following the 7-week language and motor therapy phase (i.e., outcome at the post-test).

Apraxia of speech is a clinically known influence factor to the possibilities of improving language skills in aphasic patients. Furthermore, since anatomic correlates for common motor speech and motor processing have been described (10), motor speech could be considered a "link" between motor and language processing functions. Therefore, in addition to motor and language processing functions, we considered the phenomenon of apraxia of speech independently for the patients in our patient group. Since we aimed to discuss motor speech functions on a purely exploratory level, no precise hypotheses were formulated.

Over all, four hypotheses were formulated concerning both lesion characteristics and possible therapy-induced interactions:

Regarding (1), lesion characteristics, the following hypotheses were addressed:

- (i) In line with current research (12–14, 16, 18), we assume that global lesion size is not a correlate for sufficient concurrent motor and language recovery.
- (ii) We expect that patients with smaller lesions in function-specific white matter tracts for motor (CST) and language processing [AF, superior longitudinal fasciculus (SLF)] show good recovery potential in the particular domains as opposed to patients with extensive lesions to these tracts.

Regarding (2), possible interactions of motor and language recovery processes, our hypotheses are as follows:

- (iii) In line with Harnish et al. (3), we assume that patients with an increase in motor abilities after therapy phase will also show positive language recovery (i.e., an increase in language abilities at the post-test) and vice versa. This would indicate an additive interaction between motor and language domains during simultaneous motor and language therapy.
- (iv) Complementarily, we anticipate that patients who do not profit from motor therapy do not show an increase in language abilities at the post-test after therapy phase and vice versa.

Materials and Methods

Patients

Four patients suffering from subacute to chronic stroke with different base levels of motor and language skills at the beginning of the study (see **Figure 1**) were selected. The selection of patients with opposing base levels in motor and language skills was conducted in order to include previous individual recovery processes into the evaluation of the current recovery process. Clinical records documented that at the acute stage of the stroke, all patients were described as non-fluent to globally aphasic and had paresis of varying degrees, ranging from mild hemiparesis (4/5) to full hemiplegia. The different base levels resulted from the patients' individual recovery processes prior to the participation in the study.

At the beginning of the study, language skills were classified as "good" (Base: L+) or "poor" (Base: L-) according to the patients' individual profile height in the language assessment of the Aachener Aphasia Test [AAT; (30) (see **Table 1**)]. Correspondingly, the classification of "good" vs. "poor" motor skills (M+ vs. M-) was based on the raw score of the Wolf Motor Function Test [WMFT (31), see **Table 2**]. This resulted in four possible baseline profiles: Base: M+/L+, M-/L+, M+/L-, and M-/L-, denoting good functions in both motor and language domains, the dissociations between the domains, and finally the combination of both severely impaired motor and language function at the pre-test of the study.

Apart from different performance patterns in the language and motor domain, the patients had to meet the following criteria for inclusion into the study: (1) general MRI compatibility, (2) native German speakers, (3) right-handed according

to the Edinburgh Inventory of Handedness [Laterality coefficient ≥ 80 ; (32)], (4) normal or corrected-to-normal vision, (5) no hearing loss, (6) no pregnancy, (7) single stroke in the left hemisphere, (8) subacute or chronic stage of stroke (at least 6 weeks post onset), (9) clinically diagnosed aphasia or residual symptoms of aphasia and clinically diagnosed hemiparesis, and (10) no history of dementia or other CNS or psychiatric diseases.

The patients were recruited from the Aphasia Rehabilitation Ward of the Neurological Clinic, Uniklinik RWTH Aachen. Informed written consent for participating in the study was obtained from each patient prior to the participation in the study. The study was approved by the local ethics committee and conducted according to the Declaration of Helsinki. Patient characteristics are displayed in **Figure 1**.

Research Design

All patients were recruited during their 7-week stay at the Aphasia Rehabilitation Ward of the Department of Neurology, Uniklinik RWTH Aachen. A pre-post test design was used to assess both motor and language abilities prior and after the 7-week therapy phase. The pre-test took place during the first week of the treatment. Deficits were quantified using standardized assessment tests and applied by trained personnel (speech and language therapists, physiotherapists, and neurologists). Structural MRI scans were conducted in the first week of the patients' stay at the hospital. The post-test took place during the seventh (i.e., last) week of the stay at the Aphasia Rehabilitation Ward. Again, the functional language and motor scales were used to evaluate patients' development during the intensive treatment. MRI measurements were not repeated. Between pre- and post-test, the patients participated in 7 weeks of motor and language therapy (for an overview of the research design, see **Figure 2**).

Clinical Examinations

The following tests were applied:

Functional Language Scales

The "Aachener Aphasia Test" [AAT (30)], a robust and highly validated test of language in multiple domains, was conducted to assess the patients' overall linguistic abilities. Additionally, five subtests of the standard neurolinguistic test battery "Lexikon Modellorientiert" [LEMO (33)] were employed: subtest 5 – "lexical decision making," subtest 25 – "finding synonyms," subtest 30 – "oral naming," and subtest 32 – "finding rhymes."

Functional Motor Scales

The Wolf Motor Function Test [WMFT (31)] was applied to evaluate the quality and duration of the patients' arm and hand movements. In addition, the Dynamic Gait Index [DGI (34)] was conducted in order to assess gait and balance.

Additional Scale

Three subtests from the "Aachener Materialien zur Diagnostik Neurogener Sprechstörungen" [AMDNS (35)] were used in order to screen for neurogenic speech disorders: "duration of phonation," "variability of speech intensity," and "articulatory dia-dochokinesis." These subtests were used to control the influence

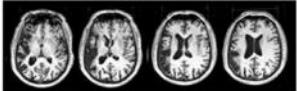
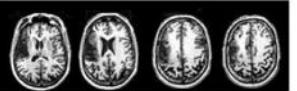
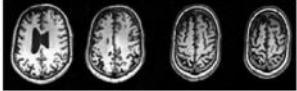
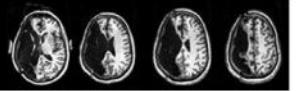
Base Levels	Language +	Language -
Motor +	<p>Patient 1 (M+/L+; 53 y/o, m)</p> <ul style="list-style-type: none"> - ischemic stroke, left MCA territory, 17 months p.o. - Base Level abilities: amnestic aphasia, mild apraxia of speech, mild disorder of fine motor skills - Lesion characteristics: lesion mostly covered frontal, temporal and parietal regions, the insular cortex and putamen 	<p>Patient 3 (M+/L-; 55 y/o, m)</p> <ul style="list-style-type: none"> - cardioembolic stroke, left MCA territory, 10 months p.o. - Base Level abilities: global aphasia, apraxia of speech, mild residual symptoms of hemiparesis - Lesion characteristics: lesion included the frontal and parietal lobe 
Motor -	<p>Patient 2 (M-/L+; 43 y/o, m)</p> <ul style="list-style-type: none"> - ischemic stroke, left MCA and ACA, 7 months p.o. - Base Level abilities: mild residual symptoms of aphasia, hemiparesis - Lesion characteristics: frontoparietotemporal, also including insular cortex, putamen, thalamus and caudate nucleus 	<p>Patient 4 (M-/L-; 47 y/o, f)</p> <ul style="list-style-type: none"> - ischemic stroke, left MCA; 31 months p.o. - Base Level abilities: global aphasia, apraxia of speech, severe hemiparesis - Lesion characteristics: spacious lesion with subsequent hemicraniectomy including nearly the whole left hemisphere 

FIGURE 1 | Overview of the four patients' base levels upon inclusion into the study, including T1-weighted images of the patients' lesions, optimized for displaying the position of the lesion. Abbreviations: "+" = good; "-" = poor motor/language skills; p.o., post onset; MCA, middle cerebral artery; ACA, anterior cerebral artery.

TABLE 1 | Results of the patients in the AAT and LEMO.

Pat. (base)	AAT				LEMO					
	Profile height		LD		FS		FR		ON	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1 (M+/L+)	57.9	58.7	78	79	38	39	11	7°	19	20
2 (M-/L+)	72.5	73.3	80	80	40	40	20	20	18	18
3 (M+/L-)	41.9	43*	45	61*	34	36	10	6	—	—
4 (M-/L-)	40.9	41.3	70	74	35	37	—	—	9	9

AAT, Aachener Aphasia Test; LEMO, Lexikon Modellorientiert; Pat., patient; pre, pre-test; post, post-test; LD, Lexical Decision; FS, Finding Synonyms; FR, Finding Rhymes; ON, Oral Naming; *significant improvement [AAT: calculated with AATP; LEMO: McNemar Test, $p < 0.05$; (*)]; °, significant deterioration (McNemar Test, $p < 0.05$).

of the patients' motor speech function on motor and language ability and recovery.

In addition, subtest "Articulation" (spontaneous speech) of the AAT was considered separately, since it is specially related to motor speech functions.

Analysis of Behavioral Data

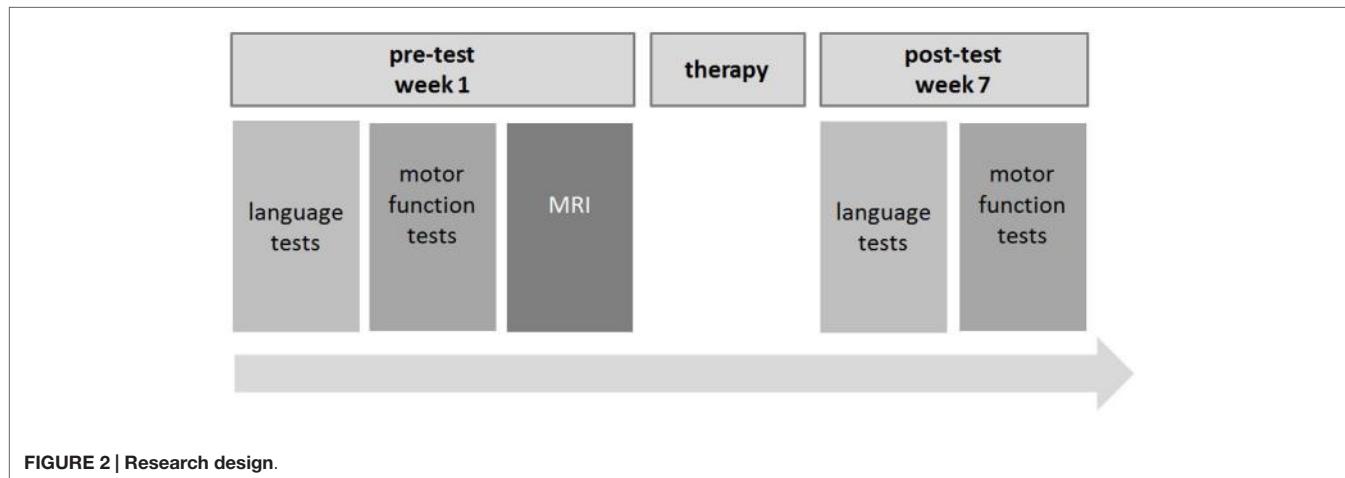
The single-case characteristics of our study put some restraints on the statistical tests that are available. For the AAT, significant improvements and deteriorations were differentiated. To test for significant changes in the patient's performance between pre- and post-test, the computer program "AATP" (36) was employed. This program automatically calculates significant changes using the psychometric single-case diagnosis (37) with $p < 0.1$, an alpha-level that is common for single cases. In reference to LEMO, significant changes between pre- and post-test were calculated

TABLE 2 | Results of the patients in WMFT and DGI.

Pat. (base)	WMFT		DGI	
	Pre	Post	Pre	Post
1 (M+/L+)	70	73	24	24
2 (M-/L+)	34	40*	20	24**
3 (M+/L-)	69	74**	21	23
4 (M-/L-)	5	5	11	13

Pat., patient; pre, pre-test; post, post-test; WMFT, Wolf Motor Function Test; DGI, Dynamic Gait Index; *significant improvement (Wilcoxon signed rank test, $p < 0.05$); **significant improvement (Wilcoxon signed rank test, $p < 0.1$).

for each subtest conducting the McNemar test ($p < 0.1$ or $p < 0.05$). Concerning the WMFT and DGI, significant changes were calculated with the Wilcoxon signed rank test ($p < 0.1$ or $p < 0.05$). In the additional scale AMDNS, only notable changes

**FIGURE 2 |** Research design.

were evaluated. They were defined as a positive or negative change of severity comparing the degrees of severity on a 4-point scale (3 = severe impairment, 0 = no impairment).

Imaging Acquisition

Structural MRI measurements (T1, FLAIR) were conducted for lesion analyses using a Philips 3T scanner at the Brain Imaging Facility at University Hospital, RWTH Aachen. All images were made using SENSE (Sensitivity Encoding) technology conducting an eight-channel phase array head coil. A three-dimensional isotropic T1-weighted sequence (MPRAGE) was performed in the sagittal plane. Acquisition parameters were: repetition time/echo time = 9.9/4.6 ms; flip angle = 8; field of view = 256 mm; matrix = 256 × 256; slice thickness = 1 mm; voxel size = 1 mm × 1 mm × 1 mm. Acquisition parameters for the FLAIR measurement were: repetition time/echo time = 11,000/125 ms; field of view = 224 mm; matrix = 312 × 157; slice thickness = 3 mm; voxel size = 0.72 mm × 1.13 mm × 3 mm.

Analysis of Imaging Data

All data were analyzed on an individual subject basis. For the analysis of lesions, all lesions were marked within the FLAIR image using MRIcron (38). Afterwards, the lesion maps were normalized via FLIRT (39) and transformed into standard MNI space. Anatomical masks of interest from the atlases supplied with FSL [MNI Structural Atlas (40) and JHU White-Matter Tractography Atlas (41)] were extracted. The right hemisphere in the MNI Structural Atlas was masked out by zeroing all voxels with x -coordinates 0–45; anatomical structures of interest were already lateralized in the JHU White-Matter Tractography Atlas. No thresholding was applied. The size of each structure was determined by counting the number of non-zero voxels in each map. Then, an intersection of the patient-specific lesions (in standard space) with the respective anatomical maps was created by multiplying them with each other using FSL command line tools (fslmaths). This yielded a map representing the damage to the particular map inflicted by the patient's lesion. The size of this map was determined by counting the non-zero voxels inside this map. Afterwards, the calculation of the percentage of the entire anatomical structure affected by the lesion followed by dividing

the voxel count of the intersection by the voxel count of the anatomical map.

Lesions of the patients were analyzed according to their localization in the following cortical and subcortical structures: frontal lobe, parietal lobe, temporal lobe, occipital lobe, insula, putamen, thalamus, and caudate. Concerning white matter tracts, the lesion analysis procedure previously described was conducted for the CSTs, SLF and AF. All fiber tracts were included due to their previously described role in motor and language processing.

Results

Behavioral Data

The patients' overall behavioral outcome (changes of performance after the 7-week therapy phase) in the functional scales showed heterogeneous results both for motor and language assessments (see **Tables 1** and **2**; Tables S1–S3 in Supplementary Material).

Additional Scale

Motor speech abilities (AMDNS) showed heterogeneous results with both notable improvements and deteriorations across all patients' performances. However, none of the measured changes occurred on a significant level. An overview of the results in these tests is given in **Table 3**. As described above, subtest "Articulation" of the AAT was considered separately and showed heterogeneous results with notable improvements in Patient 1 (Base: M+/L+) and Patient 3 (Base: M+/L−), one notable deterioration [Patient 2 (Base: M−/L+)] and one stable result [Patient 4 (Base: M−/L−; see **Table 4**)].

Synoptical Analysis of Behavioral and Lesion-Related Data

As shown in **Tables 5** and **6**, all patients had lesions in the frontal and parietal lobe, as well as white matter tract injury in the SLF and AF (see also **Figures 3** and **4**). Concerning further cortical and subcortical structures, patients did not show a homogeneous pattern of their lesions. In the following tables, we demonstrate an overview of the patients' recovery outcome following the 7-week therapy phase together with the patients' lesion characteristics in

cortical and subcortical (**Table 5**) as well as white matter tract areas (**Table 6**).

Discussion

The present study explored if there are determinants for concurrent motor and language recovery during intensive therapy in four exemplary chronic stroke patients with different base levels of language and motor abilities. In particular, we examined if (1) concerning lesion characteristics (i) the global lesion size is a correlate of sufficient concurrent motor and language recovery

and if (ii) the extent of damage of the function-specific white matter tracts for motor and language is predictive for the recovery potential in the respective domains.

In the further analysis of (2) possible interactions of motor and language recovery processes, we investigated if (iii) an additive interaction between motor and language domains during simultaneous motor and language therapy occurs and if (iv) there will be a lack of interaction between both domains when there is no recovery progress in at least one domain.

The four patients had different motor and language base levels and were systematically examined in this study to evaluate the relation of their therapy outcome in both domains (i.e., recovery process that was measured from pre- to post-test) and lesion parameters. To explore predictors for (iii) concurrent motor and language recovery, various functional scales in the motor and language domain and also in the motor speech domain were applied. Concerning the lesion analysis, cortical and subcortical lesion characteristics as well as white matter tract damage were explored.

One major finding of this study is that we could detect some indicators for an additive behavior of motor and language recovery. It seems that motor and language recovery co-occur in a sense that motor recovery facilitates the possibility of a positive therapy-induced language recovery. In addition, lesion size *per se* is not determining a sufficient motor and language recovery. However, the specific lesion areas play an important role for a sufficient recovery. Another main finding was that large damage in important fiber structures for motor or language processing allows no prediction about the recovery of the fiber-induced function at a single subject level.

Lesion Characteristics

Global Lesion Size

Considering the global lesion size in our four patients, Patient 3 (Base: M+/L-) was the only participant who was able to improve significantly in both motor and language functions at the post-test. In addition, this patient had the second largest overall lesion size. In comparison, Patient 2 (Base: M-/L+), the patient showing the

TABLE 3 | Results of the patients in the AMDNS (degree of impairment).

Pat. (base)	AMDNS					
	DIA		DU		INT	
	Pre	Post	Pre	Post	Pre	Post
1 (M+/L+)	18	15	9	5	0	3
2 (M-/L+)	18	18	3	3	3	0
3 (M+/L-)	6	6	6	6	0	2
4 (M-/L-)	9	9	16	17	3	3

Cumulative dysarthria score: degree of impairment: 0 = no impairment; 3 = severe impairment. Pat., patient; pre, pre-test; post, post-test; DIA, diadochokinesia; DU, duration of phonation; INT, variability of speech intensity.

TABLE 4 | Results of the patients in the subtest "Articulation" (AAT; degree of impairment).

Pat. (base)	Articulation (AAT)	
	Pre	Post
1 (M+/L+)	3	4
2 (M-/L+)	5	4
3 (M+/L-)	2	3
4 (M-/L-)	3	3

Degree of impairment: 5 = no impairment; 1 = severe impairment (i.e., cannot be evaluated due to lack of intelligibility).

TABLE 5 | Overview of the patients' functional recovery (post-test) in both domains, lesion volume, and percentage of damaged tissue in defined cortical and subcortical brain areas.

Pat. (base)	Lesion volume to specific areas										
	Outcome after 7-week therapy phase		Total lesion volume	Cortical (Lobar)				Subcortical			
	Motor recovery	Language recovery		Fro	Par	Tem	Occ	Ins	Put	Tha	Cau
1 (M+/L+)	Non-responder WMFT (o), DGI (o)	Non-responder AAT (o), LEMO-	10,325	2,403	6,368	2,409	335	1,903	437	-	-
2 (M-/L+)	Strong responder WMFT+, DGI+	Non-responder AAT (o), LEMO (o)	6,852	5,815	3,193	-	-	-	-	-	-
3 (M+/L-)	Partial responder WMFT+, DGI (o)	Strong Responder AAT+, LEMO+	14,406	8,422	4,171	1,255	-	2,764	732	23	25
4 (M-/L-)	Non-responder WMFT (o), DGI (o)	Non-responder AAT (o), LEMO (o)	50,472	18,747	16,884	9,692	7,556	3,340	1,237	87	12

+, Significant improvement; (o), no change; -, significant deterioration. Non-responder, patient showed no positive response to motor or language therapy; partial responder, partial positive response, i.e., significant improvement in one of the applied tests; strong responder, strong positive response, i.e., significant improvement in both applied tests; -, no lesion measured; Fro, frontal lobe; Par, parietal lobe; Tem, temporal lobe; Occ, occipital lobe; Ins, insula; Put, putamen; Tha, thalamus; Cau, nucleus caudate.
Lesion volume was calculated within the FLAIR data (voxels).

TABLE 6 | Overview of the patients' functional recovery (post-test) in both domains, lesion volume, and percentage of damaged tissue in particular white matter tracts.

Pat. (base)	Outcome after 7-week therapy phase		Lesion volume to specific white matter tracts		
	Motor recovery	Language recovery	CST	SLF	AF
1 (M+/L+)	Non-responder WMFT (o), DGI (o)	Non-responder AAT (o), LEMO–	–	5,115	1,196
2 (M-/L+)	Strong responder WMFT+, DGI+	Non-responder AAT (o), LEMO (o)	1,057	1,916	736
3 (M+/L–)	Partial responder WMFT+, DGI (o)	Strong responder AAT+, LEMO+	568	7,188	3,944
4 (M-/L–)	Non-responder WMFT (o), DGI (o)	Non-responder AAT (o), LEMO (o)	1,643	16,462	7,458

+, Significant improvement; (o), no change; –, significant deterioration. Non-responder, patient showed no positive response to motor or language therapy; partial positive response, i.e., significant improvement in one of the applied tests; strong responder, strong positive response, i.e., significant improvement in both applied tests; –, no lesion measured; CST, corticospinal tract; SLF, superior longitudinal fasciculus; AF, arcuate fasciculus.
Lesion volume was calculated within the FLAIR data (voxels).

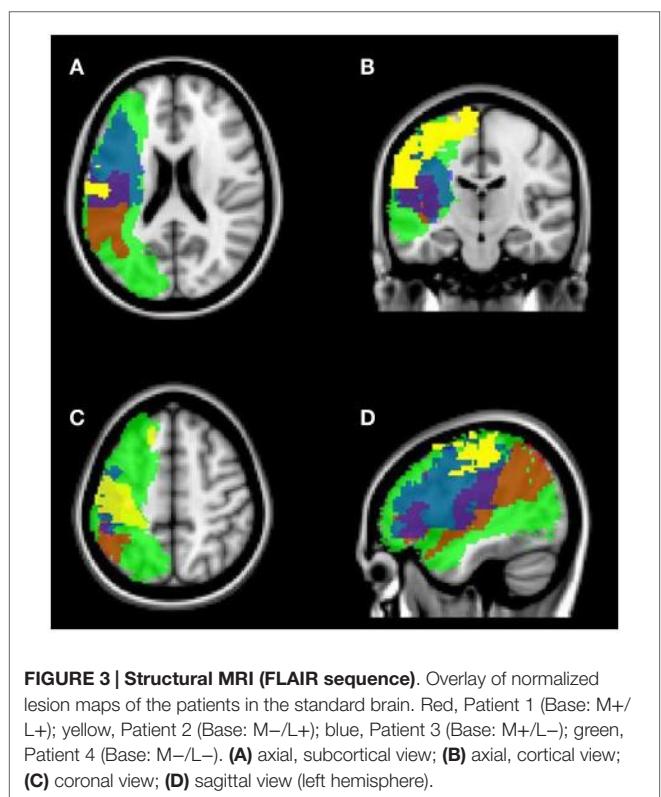


FIGURE 3 | Structural MRI (FLAIR sequence). Overlay of normalized lesion maps of the patients in the standard brain. Red, Patient 1 (Base: M+/L+); yellow, Patient 2 (Base: M-/L+); blue, Patient 3 (Base: M+/L-); green, Patient 4 (Base: M-/L-). **(A)** axial, subcortical view; **(B)** axial, cortical view; **(C)** coronal view; **(D)** sagittal view (left hemisphere).

smallest global lesion size, was able to improve in motor but not language scales at the post-test, whereas Patient 1 (Base: M+/L+) and Patient 4 (Base: M-/L–, the patient with the largest global lesion size), did not show improvement in any scale. The fact that Patient 3 (Base: M+/L–) was able to improve on such an extensive level shows that the global lesion size cannot be the single determinant regarding recovery potential. This finding is in line with the current state of research [e.g., see Ref. (12–14, 16, 18)].

White Matter Tracts

Concerning white matter tracts, lesion characteristics seem to be less distinct. Although Patient 1 (Base: M+/L+) was the only

patient who did not show a lesion of the CST, he also did not improve in motor therapy, most possibly due to a high motor base level and a ceiling effect. Patient 3 (Base: M+/L–) showed the smallest lesion of all patients (i.e., of all patients with lesions of the CST) and was able to improve in one motor test. Whereas Patient 2 (Base: M-/L+) with the second largest lesion of the CST was a strong responder to motor therapy with improvements in both motor function tests. Patient 4 (Base: M-/L–) had the most extensive CST lesion and was a non-responder to motor therapy.

Especially the distinction between Patients 2 (Base: M-/L+) and 3 (Base: M+/L–) is of further interest: although fiber damage of the CST in Patient 2 (Base: M-/L+) was about two times larger than that in Patient 3 (Base: M+/L–), probably leading to his worse baseline profile, Patient 2 (Base: M-/L+) actually showed better abilities to recover in the motor domain than Patient 3 (Base: M+/L–; strong responder vs. partial responder, see Tables 5 and 6). This difference could be attributed to the fact that the measurable extent of the lesion in Patient 2 is primarily caused by the location of the lesion at the level of the primary motor cortex, whereas Patient 3's smaller lesion mainly affects the part of the pyramidal tract further down in the corona radiata (see Figure 4). It is possible that this specificity of the anatomical lesion site in Patient 3 leads to a higher amount of damage to fibers that are relevant to motor recovery.

In summary, among our patient group, Patient 1 (Base: M+/L+) showed no lesion of the CST and no therapy-induced improvement due to ceiling effects and an already high level of motor functions at the pre-test. Patient 2 (Base: M+/L–) showed an extensive overall lesion, however, damage was more related to cortical structures than to lesions in the CST. This patient showed good recovery potential with improvements in both motor function tests. In comparison, Patient 3 (Base: M+/L–) showed a smaller lesion, however, he only recovered to a smaller degree than Patient 2 (Base: M-/L+). His lesion location in the corona radiata probably led to a reduction in recovery potential. Last, Patient 4 (Base: M-/L–) with the most extensive lesion of the CST was not able to improve in motor therapy at all. This result is supportive to the finding that strategic lesion location, rather than

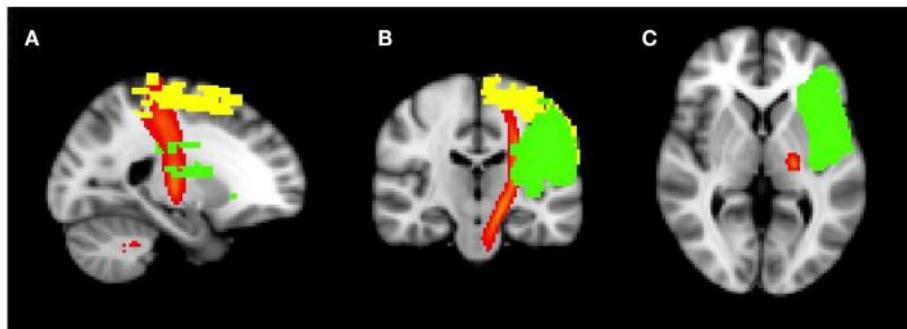


FIGURE 4 | Structural MRI (FLAIR sequence). Overlay of normalized lesion maps of patients 2 and 3 in the standard brain. Yellow, Patient 2 (Base: M-/L+); green, Patient 3 (Base: M+/L-); red, corticospinal tract. **(A)** sagittal view; **(B)** coronal view; **(C)** axial, subcortical view.

lesion volume, is an important determinant to recovery potential [e.g., see Ref. (15)].

Concerning the lesion of the AF, similar results could be found. Patient 2 (Base: M-/L+) who showed the smallest lesion of the AF did not show therapy-induced language improvement at the post-test, as well as Patient 1 (Base: M+/L+) who presented with the second smallest lesion. Patient 4 (Base: M-/L-) who showed the most spacious lesion of the AF was also not able to profit significantly from intensive therapy. Only Patient 3 (Base: M+/L-) was able to improve strongly in both language scales, although he showed the second largest lesion of the AF.

These findings seemingly point toward the assumption that the specific lesion size of the CST and/or AF does not directly influence the outcome of motor and/or language recovery. However, we feel that this assumption would be too shortsighted since at this point, the individual base levels, i.e., the level of motor and language skills that the patients presented with at the pre-test, need to be considered: regarding the lesion of the CST, we pointed out that Patient 1 (Base: M+/L+) did not show motor recovery although he did not have any lesion of the CST. However, Patient 1 already showed a comparatively high level of motor skills at the pre-test (see Table 2), leaving him with only small possibilities for significant improvements at the post-test. The same holds for Patient 2 (Base: M-/L+) regarding the extent of the AF lesion. As described, Patient 2 showed the smallest AF lesion of all patients but did not show language recovery. This could be attributed to possible ceiling effects. However, even after eliminating those two patients with possible ceiling effects from our considerations, in our patient group still neither the patient with the (then) smallest CST lesion [Patient 3 (Base: M+/L-)] nor the patient with the (then) smallest AF lesion [Patient 1 (M+/L+)] are the patients showing most motor and language recovery, respectively. This observation points strongly toward the conclusion, that even patients with large lesion of the CST/AF are able to recover motor/language abilities during intensive therapy.

Interactions of Motor and Language Recovery Processes

Based on the results that were published by Harnish et al. (3), we assumed that the patients with an increase in motor abilities

after the 7-week therapy phase would show positive language recovery (i.e., an increase in language abilities at the post-test), indicating that an additive interaction between motor and language domains during simultaneous motor and language therapy occurs. We also anticipated that patients who do not profit from motor therapy do not show an increase in language abilities at the post-test after therapy phase and vice versa. Regarding the data of our four patients, two of our patients, namely Patient 2 (Base: M-/L+) and Patient 3 (Base: M+/L-), were able to profit from motor therapy, leading to a significant improvement of motor functions at the post-test. Of these two patients, Patient 2 (Base: M-/L+) did not show improvements in the language domain while Patient 3 (Base: M+/L-) was a strong responder to language therapy also (see Tables 5 and 6). However, Patient 2 (Base: M-/L+) already showed a comparatively high level of language skills at the pre-test with a mean profile height of 72.5 in the AAT (see Table 1) as well as even the maximum possible raw scores at LeMo, indicating only mild residual symptoms of aphasia even at the beginning of the therapy phase.

As to Patient 1 (Base: M+/L+) and Patient 4 (Base: M-/L-), none of them were able to improve motor function skills and, in addition, none of them were able to profit from language therapy. Of the two patients, Patient 1 (Base: M+/L+) already showed a relatively high language profile at the pre-test, however, with a mean profile height of 57.9 in the AAT, he clearly could have improved significantly in that scale. Additionally, the raw scores indicate that significant improvement of the subtest “Finding Rhymes” (LeMo) would also have been possible (see Table 1). Therefore, the existence of ceiling effects in this patient can be excluded and the lack of positive therapy outcome has to be considered as a “real” effect.

In none of our four patients improvements in the motor or the language domains were bound to measurable deteriorations in the other domain. This lack of dissociation between the recovery processes of the two domains hints toward the assumption that a “fight for resources” could not be observed in our patient group.

In conclusion, only one patient with a positive response to motor therapy [Patient 3 (Base: M+/L-)] was able to improve significantly in language functions at the pre-test, whereas Patient 2 (Base: M-/L+), who also improved significantly in

motor functions, could not have achieved measurable improvements due to ceiling effects in the language domain but did show numerical improvements of language skills. The evident motor recovery in the case of Patient 3 (Base: M+/L-) might have been a facilitating factor for a good response to language therapy. The two patients who could not benefit from the intensive motor therapy program [Patient 1 (Base: M+/L+) and Patient 4 (Base: M-/L-)] could also not improve significantly concerning language skills. Therefore, we assume that these results are suggestive of a positive interaction operating between motor and language domains during recovery in the sense that a positive therapy-induced motor recovery is a prerequisite to the possibility of recovering language skills through language therapy. This finding is in accordance with Harnish et al. (3). Regarding the oppositional outcome (positive language therapy outcome leading to improved motor outcome), no such interactions could be observed, therefore, due to our small sample size, it is not possible to formulate a conclusion concerning the possibility of contrary recovery dynamics.

Apraxia of Speech

Interestingly, dissociations in the recovery of apraxia of speech became apparent in the additional functional Scale AMDNS and in the subtest “articulation” of the AAT.

Only Patient 1 (Base: M+/L+), who had the smallest amount of lesioned voxels in the frontal lobe (see **Table 5**), was able to improve notably in “articulatory diadochokinesis” and “duration of phonation” (AMDNS) and showed a notable improvement in the communication parameter “articulation” (AAT; see **Tables 3 and 4**). Patient 2 (Base: M-/L+), showing a larger lesion in the frontal lobe, showed stable performances regarding motor speech. Patient 3 (Base: M+/L-) and 4 (Base: M-/L-) had the highest amount of lesioned voxels in the frontal lobe and stable or inferior results in the post-test [except of a notable improvement in the communication parameter “articulation” (AAT, Patient 3)]. Patients 3 (Base: M+/L-) and 4 (Base: M-/L-) were also not able to conduct complex articulatory diadochokinesis tasks at the pre- and post-test, probably due to the severe apraxia of speech. These two patients demonstrate larger affection of the insular cortex by the lesion in comparison to Patient 2 (Base: M-/L+; no insular lesion) and Patient 1 (Base: M+/L+; see **Table 5**). The insula is associated with articulatory coding/motor programming and motor control [e.g., see Ref. (42, 43)] and its left precentral gyrus forms also an anatomical correlate for the development of apraxia of speech (44, 45). Therefore, preservation of the insula appears to be a necessary, but not exclusive predictor for motor speech recovery. Lesions in other cortical or subcortical regions may also play a role for developing recovery potential in motor speech coordination. This assumption would be in accordance with the findings of Ogar et al. (45). They pointed out that patients showing a severe apraxia of speech had larger lesions in neighboring regions like Broca’s area or basal ganglia. To conclude, the described literature and our findings suggest that the overall amount of lesioned voxels in the frontal lobe *per se* is able to predict motor speech recovery in our sample of patients. This finding has to be tested in a larger number of patients and, in addition, distinctive

subcortical parts of the frontal lobe like insular or basal ganglia should be analyzed precisely in reference to their predictive value for recovery.

Limitations

The present multiple case study provides a new approach in analyzing concurrent motor and language recovery as well as the interaction behavior between these domains during recovery. On the one hand, our findings provide some first indicators, given the fundamental research gap in this field. On the other hand, the data in this study are of limited generalizability as only single cases were examined. In addition, a more specific analysis of specific brain areas is needed. It was also not possible to control the time of onset/duration of aphasia and motor dysfunction in the patients. This is a variable of potential influence due to different restitution processes in different time intervals after stroke [e.g., restitution in the early subacute vs. chronic stage of aphasia; see Ref. (46)]. A group study would be necessary to elucidate if these first results are transferable to a larger sample of subjects.

Conclusion and Perspectives

To conclude, we show that primarily the strategic location of the lesion is a determinant of functional recovery in the motor and language domain. Another main finding was that large damage to important white matter structures for motor or language processing is not a single predictive factor for the recovery of the affected function. Regarding motor speech, the extent of damage to the frontal lobe (especially insula) seems to be a neural correlate for a good motor speech (apraxia of speech) recovery. Poor motor speech abilities, often associated with an apraxia of speech, play a special role in the recovery of language skills and are distinguished by large frontal lesions.

With respect to the interaction of the motor and language domain during recovery, first hints for additive effects were found. Those patients with good base levels in motor skills improved in language abilities. Therefore, motor and language improvement seem to co-occur, as stated before by Harnish and colleagues (3), rather than to compete for recovery resources.

Concerning the mechanisms of recovery, we were not able to find evidence for a “fight for resources,” since motor or language recovery was not associated with a loss of abilities in the other domain, respectively. But it was clearly visible that there is no prospect of recovery in the language domain if there are no resources and abilities available in the motor domain. This is indicative for an additive, synergistic recovery mechanism as described by Harnish and colleagues (3).

A further important finding was that the characteristics of the lesion (specific area, overall size) are no obligatory determinant or predictor for the success of motor or language therapy. We could show that a patient with large CST damage exhibited positive motor recovery while a patient with large AF/SLF damage improved well in the language testing.

In this study, only single cases were analyzed. A larger group study will investigate recovery mechanisms and correlates supported by a higher statistical power as well as additional fMRI measurements. The results, together with the findings in this

paper, will add to the knowledge about recovery processes in this clinically relevant patient group.

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Supplementary Material

The Supplementary Material for this article can be found online at <http://journal.frontiersin.org/article/10.3389/fneur.2015.00215>

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The Right Supramarginal Gyrus Is Important for Proprioception in Healthy and Stroke-Affected Participants: A Functional MRI Study

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Human proprioception is essential for motor control, yet its central processing is still debated. Previous studies of passive movements and illusory vibration have reported inconsistent activation patterns related to proprioception, particularly in high-order sensorimotor cortices. We investigated brain activation specific to proprioception, its laterality, and changes following stroke. Twelve healthy and three stroke-affected individuals with proprioceptive deficits participated. Proprioception was assessed clinically with the Wrist Position Sense Test, and participants underwent functional magnetic resonance imaging scanning. An event-related study design was used, where each proprioceptive stimulus of passive wrist movement was followed by a motor response of mirror copying with the other wrist. Left (LWP) and right (RWP) wrist proprioception were tested separately. Laterality indices (LIs) were calculated for the main cortical regions activated during proprioception. We found proprioception-related brain activation in high-order sensorimotor cortices in healthy participants especially in the supramarginal gyrus (SMG LWP $z = 4.51$, RWP $z = 4.24$) and the dorsal premotor cortex (PMd LWP $z = 4.10$, RWP $z = 3.93$). Right hemispheric dominance was observed in the SMG (LI LWP mean 0.41, SD 0.22; RWP 0.29, SD 0.20), and to a lesser degree in the PMd (LI LWP 0.34, SD 0.17; RWP 0.13, SD 0.25). In stroke-affected participants, the main difference in proprioception-related brain activation was reduced laterality in the right SMG. Our findings indicate that the SMG and PMd play a key role in proprioception probably due to their role in spatial processing and motor control, respectively. The findings from stroke-affected individuals suggest that decreased right SMG function may be associated with decreased proprioception. We recommend that clinicians pay particular attention to the assessment and rehabilitation of proprioception following right hemispheric lesions.

Keywords: proprioception, kinesthesia, upper extremity, functional laterality, stroke, magnetic resonance imaging, cerebral cortex

Abbreviations: BA, Brodmann area; fMRI, functional magnetic resonance imaging; IPL, inferior parietal lobe; LI, laterality index; LWP, left wrist proprioception; MI, primary motor cortex; PMd, dorsal premotor cortex; RWP, right wrist proprioception; SI, primary somatosensory cortex; SIMI, primary sensorimotor cortex; SII, secondary somatosensory cortex; SMA, supplementary motor area; SMG, supramarginal gyrus.

INTRODUCTION

Limb proprioception refers to knowledge of the spatial location of one's limb in the absence of vision. Proprioception is vital for motor control (1), particularly of the upper limbs (2). It is essential for the control of coordinated movements, especially small or precise movements, and for motor skill acquisition (3). Hence, proprioceptive deficits in the upper limbs are associated with decreased function (1). Despite the importance of proprioception for function, it remains unclear which brain regions beyond the primary sensorimotor cortices (SIMIs) are involved in the processing of proprioception and how this brain activation is altered following focal brain lesions associated with proprioceptive deficits.

Researchers studying brain activation during passive movements of the elbow (4, 5), wrist (6, 7), hand (8), and finger (9, 10) have identified activation in the contralateral primary somatosensory (SI) and motor (MI) cortices and the inferior parietal lobe (IPL). However, investigators disagreed on the pattern (contralateral, ipsilateral, or both) and exact location of activation [supramarginal gyrus (SMG) or the secondary somatosensory cortex (SII)]. In contrast, neurophysiological studies of primates, identified the superior parietal lobe as a key region for the processing of proprioception (11, 12). The ability of current brain imaging paradigms to investigate proprioceptive specific processing, and in particular the contribution from higher order brain regions, requires careful consideration and design.

Inconsistent proprioception-related brain activation has also been reported in high-order motor cortices including the supplementary motor area (SMA), cerebellum (6, 8), and the premotor cortex (PMC) (5, 6, 8). Variations in proprioception-related brain activation may have been due to the fact that brain imaging studies of passive movements varied in paradigm design. In some cases, the support of the moving limb was suboptimal and may have introduced significant tactile stimulation (6, 8, 10), thus generating confounding brain activation.

Proprioception-related brain activation has also been studied using illusory vibrations. This is vibration of a tendon at a frequency between 70 and 100Hz, which creates an illusion of movement (13). Early findings from illusory vibration studies emphasized activation in motor cortices including: MI, SMA, PMC, and the cingulate motor area (14, 15). Later, researchers also identified brain activation in the IPL (5, 16–18). However, as was the case with passive movements, reported activation varied in location, with reports of activation in the parietal operculum (5, 15, 17) or the SMG (16, 18). Hemispheric bias was also controversial with some researchers reporting bilateral activation (16, 18), while others report a right hemisphere dominance (15, 17).

Illusory vibrations provide different peripheral stimuli to passive movements. The stimulus is large phasic and of uniform frequency in the primary afferent fibers of the muscle spindles (19, 20). Minimal, if any, stimulation is produced in the secondary fibers of the muscle spindles and the joint receptors (19, 20). In contrast, passive movements produce multifrequency phasic and tonic stimulation of the primary afferent fibers in the muscle spindles (21). Secondary fibers of the muscle spindles and joint receptors are also stimulated (21–23). It is possible that different

peripheral stimuli were associated with differential brain activation (5). In such circumstances, brain activation during passive movements is likely to reflect the central processing of proprioception more accurately than illusory vibration.

An important limitation of both passive movement and illusory vibration brain imaging studies of proprioception is that participants were not required to provide accurate and measurable responses to the proprioceptive stimuli during scanning. Responses to proprioceptive stimuli are important for two reasons. First, by asking participants for accurate responses to proprioceptive stimuli (and monitoring the responses), examiners ensure that participants adequately engage in proprioceptive information processing. Second, the response requirement introduces a certain degree of difficulty to the proprioceptive task, which would not have been present if responses were not required. Increased task difficulty is desirable due to the associated increase in cortical activation (24, 25).

In healthy participants, findings from behavioral studies have suggested asymmetry in the accuracy of proprioception from the right and left limbs (26–28). Asymmetry in behavioral measures suggests hemispheric dominance and thus asymmetry in proprioception-related brain activation. Brain activation studies of illusory vibration stimulation confirmed right hemispheric dominance (15, 17, 18). Brain activation in the IPL and inferior frontal gyrus was found in all three studies, but the exact loci of activation and degree of laterality (i.e., right hemispheric or bilateral activation) varied. None of the brain imaging studies of passive movements investigated laterality of proprioception.

Quantitative behavioral measures of proprioception in stroke-affected individuals have shown deficits in about 50% of the participants (1, 29). Considering the adverse effect of proprioceptive deficits on function (1), it is important not only to understand the central processing of proprioception in healthy participants but also how it changes following brain lesions associated with proprioceptive deficits. This is because proprioception can be rehabilitated (30–32) with associated changes in brain activation (33) and improvement in function (34).

The current study was designed to investigate the brain-behavior relationship of proprioception. The research questions were:

- (1) Which high-order brain areas are important for early coding of natural proprioceptive stimuli?
- (2) Is proprioception-related brain activation lateralized, and if so in which areas?
- (3) How does proprioception-related brain activation in stroke-affected individuals with proprioceptive deficits differ from that of healthy participants?

To answer these questions, we designed an event-related functional magnetic resonance imaging (fMRI) study with a controlled proprioceptive stimulus and response paradigm. The study was exploratory with data-driven laterality analyses.

First, proprioceptive stimuli were delivered with maximal limb support and minimal tactile stimulation to eliminate confounding brain activation. Second, participants were required to respond accurately to each proprioceptive stimulus for optimal brain activation related to attended proprioceptive information processing.

Third, the paradigm and analyses were designed to show brain activation at the beginning of a proprioception task during the coding of proprioceptive stimuli. We hypothesized that coding proprioception would involve high-order somatosensory cortices in the parietal lobe including the IPL, the SII, and the superior parietal lobe. We also hypothesized that proprioception-related brain activation would be found in high-order motor cortices in the frontal lobe including the PMC, SMA, and cingulate motor cortex. The second hypothesis was that proprioception-related brain activation would be lateralized to the right hemisphere, particularly the high-order cortices. Finally, we hypothesized that laterality would decrease following stroke which affected proprioception.

MATERIALS AND METHODS

Participants

Twelve healthy right-handed participants (35) were recruited. Participants were aged 23.4 ± 3.3 years (seven females) and their age was restricted (18–30 years) to control for age-related variations in proprioception (36) and brain activation (37). Participants' proprioception was within the normative range (average absolute error below $11 \pm 4.8^\circ$) as verified behaviorally with the Wrist Position Sense Test (38).

Three participants with chronic strokes (CSs) and proprioceptive deficits were also recruited: CS1 45 years, male, 16 months post right hemisphere stroke, average absolute wrist position error on the Wrist Position Sense Test was $25.6 \pm 22.5^\circ$; CS2 65 years, female, 72 months post left hemisphere stroke, average absolute error $17.9 \pm 15.2^\circ$; and CS3 46 years, male, 68 months post left hemisphere stroke, average absolute error $20.8 \pm 18.4^\circ$.

Participants had no history of wrist injury, neurological injury (other than the three participants affected by stroke), psychiatric conditions, ongoing medical issues, diabetes, hearing impairments, or any of the standard contraindications to MRI scanning. The study was approved by the La Trobe University and Austin Health Human Ethics Committees, conforming to Declaration of Helsinki standards. Participants gave written informed consent prior to recruitment.

Experimental Design and Analysis Approach

Participants performed a limb position matching task in the scanner using an event-related study design. The experimental paradigm was carefully constructed to ensure that fMRI data were collected specifically during coding of proprioception and not during response generation. Care was also taken to ensure that other confounding stimuli were excluded. We used an exploratory approach to identify the parietal and frontal regions activated specifically at the beginning of the proprioceptive stimuli during coding of proprioception. Brain laterality analyses were data driven, and only regions that showed significant activation during coding of proprioception were then analyzed for laterality. *A priori* selection of specific brain regions for the laterality analyses was not possible due to the conflicting literature. Testing and analysis of right wrist proprioception and its

laterality were performed separately to that of the left wrist. No direct comparisons were made between left and right wrist data. Data of stroke-affected individuals were analyzed as case studies and no direct comparisons were made with data of healthy participants.

Experimental Paradigm

An event-related fMRI study was conducted in which participants performed a limb position matching task. The proprioceptive task was performed with eyes closed to eliminate the effect of vision on proprioception. Participants' hands were placed in splints attached to a lap-tray (wrist and splint axes were aligned), and their arms were supported on contoured foam cushions. Hand placement was designed to minimize confounding tactile stimulation or voluntary movement. The event-related design enabled temporal separation of brain activation related to proprioception from that related to motor response. A single trial was composed of two events: a proprioceptive stimulus event and a response event (see Figure 1). Each event was followed by a randomly varying interstimulus interval which varied between 0.5 and 12 s: 0.5–6.0 s for 70% of events, 6.0–10.0 s for 20% of events, or 10.0–12.0 s for 10% of events (i.e., jittering) (39). The purpose of the response events was to ensure participants' vigilance. Hence, the specific pattern of brain activation during response events was not relevant to the research question. The brain activation of interest took place at the beginning of the proprioceptive events, during coding of proprioception.

The investigator was visually cued to passively move the participant's hand via a lever (to minimize tactile stimulation) for a maximal duration of 3 s. In addition, the investigator was pretrained to deliver passive wrist movements at a rate of 10° a second or faster, to ensure stimulation of the main proprioceptors which are sensitive to changes in joint position, and to produce a phasic firing pattern (40). Passive movements of the wrist were presented in random order to any one of 21 predetermined positions within a 100° range of wrist flexion-extension movements. Positions were analyzed together rather than individually as the research question pertained to proprioception-related brain activation in general and not the differential processing of each position.

Response requirements were designed to ensure maximal attendance to the proprioceptive stimulus. Response events commenced with a 600 ms auditory cue of either a pink noise (random noise with an equal energy in all octaves) or a click train, and participants were allowed 3 s for their response. The pink noise cued the participant to mirror copy the wrist position with the opposite hand (70% of the events), while the click train cued participants not to copy the wrist position (30% of the events). The examiner closely monitored participants' responses during the scans and accuracy of response measurements were collected in the prescan testing. Vigilance was also monitored in the prescan testing by assessing adherence to auditory sounds that served as cues to either respond or not respond to the proprioceptive stimuli. Responses were considered non-vigilant if participants moved their response hand half way or more toward mirror copying the stimulus position when cued not to respond. Vigilance was scored as percentage

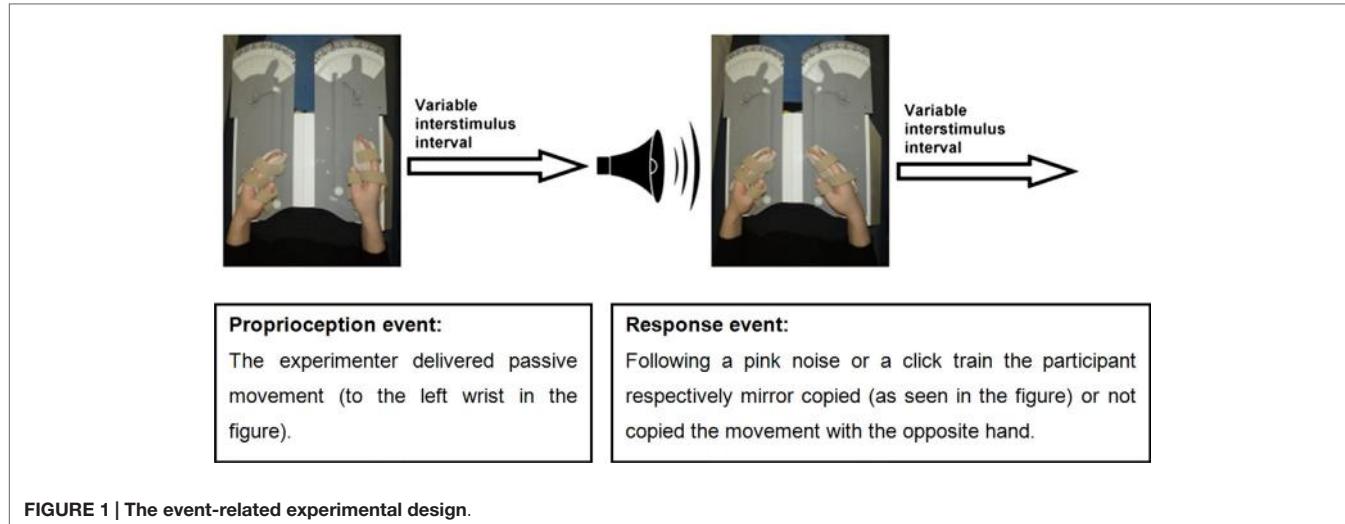


FIGURE 1 | The event-related experimental design.

of correct adherence to “do not respond” cues. Participants were first studied during left wrist proprioceptive stimuli (LWP) with right wrist responses, and then 2–6 months later during right wrist proprioceptive stimuli (RWP) and left wrist responses. The time between LWP and RWP scans was not expected to affect the results as no direct comparisons were made between the two.

Tests and Prescan Training Performed Outside the Scanner

The proprioceptive paradigm was practiced in a prescan session, 1–9 days before the scan to ensure familiarity with the task. During the prescan sessions, measurements of angular wrist displacements were taken by potentiometers attached to the wrist axes. Following familiarization, participants’ responses were measured for accuracy and vigilance.

Electromyographic recordings were taken outside the scanner only. The EMG amplifier that we used is designed to work in the electrically noisy clinical environment and therefore has an operating bandwidth of 18–370 Hz. Outside the bandwidth, signal was filtered below –3 db. Notch filter was set at 50 Hz. Rectified signal was then sampled at 10 Hz, and these samples were employed to compute the average signal for each condition: passive movements, active movements, and rest. Recordings were collected simultaneously from two channels (wrist flexors and extensors) during random 30 s blocks of passive movements, active movements, and rest. Recordings were collected over 6.5 min, and 2 s of data was trimmed from the beginning and end of each block to avoid contamination of the data. Data were then normalized in the following manner. For each participant, the median of active movement readings was multiplied by a constant that gave it the value of 100. Then all recordings from the same muscle group were multiplied by this constant. Data of all participants were then pooled, and a non-parametric Wilcoxon *T*-test was conducted to compare EMG recordings during passive and active movements. Statistically significant difference was interpreted as evidence of participants’ ability to relax their forearm muscles during passive

movements. This ensured that brain activation was not related to voluntary muscle contraction.

Data Acquisition

A scanning session contained four runs. Each run extended over 20 trials. Runs commenced with auditory instructions, which lasted for 27 s. The first 12 volumes of each run were discarded (nine volumes of instruction and three equilibration volumes). One hundred and thirty-one whole brain volumes were collected from each run. The computer program Presentation® (Version 9.70¹) was used to coordinate scanner timing with the delivery times of the visual cues to the investigator and the auditory cues to the participants. The same software served to generate log-files, which recorded event times in each run.

Data were acquired on a 3 T GE Horizon LX MRI scanner (GE Systems, Milwaukee, WI, USA). Tilted axial slices were oriented parallel to a line passing inferior to the genu of the corpus callosum and superior to the cerebellum. The tilted imaging plane served to maximize the signal from the parietal cortex. Functional scans were acquired using a T2*-weighted gradient echo echo-planar imaging sequence [imaging parameters: repetition time = 3000 ms, echo time = 40 ms, flip angle = 75°, field of view = 240 mm, matrix = 128 × 128, 25 slices, 4 mm thick, and 1 mm gap (in-plane resolution 1.875 mm × 1.875 mm)].

Anatomical axial 3D scans were acquired using a T1-weighted FSPGR imaging sequence [repetition time = 13.8 ms, echo time = 2.7 ms, inversion time = 500 ms, flip angle = 20°, field of view = 240 mm, matrix = 512 × 512, 80 slices, 2 mm thick (in-plane resolution 0.47 mm × 0.47 mm)]. Axial 2D T2-weighted image was also taken [repetition time = 3400 ms, echo time = 77 ms, inversion time = 500 ms, flip angle = 90°, field of view = 240 mm, matrix = 512 × 512, 25 slices, 4 mm thick, 1 mm gap (in-plane resolution 0.47 mm × 0.47 mm)].

¹<http://www.neurobs.com/presentation>.

Stroke Lesion Mapping

Lesion sites were identified on the non-normalized anatomical axial 3D T1 images of each stroke-affected participant. A neurologist visually mapped the lesion sites to normalized generic axial slices (41) taken from the Talairach atlas (42). A second neurologist then evaluated that the lesions were accurately mapped. While lesion mapping has a subjective element, this process minimized the risk of bias.

Data Analysis of fMRI Scans

Individual Image Processing

Data analyses were carried out using SPM 2 (Wellcome Department of Imaging Neuroscience, London, UK). Raw images were inspected for artifacts or structural abnormalities and then pre-processed: (i) correction for slice acquisition time, (ii) realignment to a target volume closest to the median value of head motion (iBrain™ Version 3² used for median image calculation), (iii) coregistration of anatomical scans to functional scans, (iv) spatial normalization into the Montreal Neurological Institute space [with masking the lesion sites for the stroke-affected participants – cost function masking (43)], and (v) spatial smoothing with a kernel size of 8 mm.

Statistical Analyses

Only the beginning of each proprioception event was modeled as the research question was related to brain activation during coding of proprioceptive stimuli. Timing of each event was entered according to time recorded in the Presentation® log-file. We used a hemodynamic response function and included an additional dispersion regressor to allow for the longer event durations in this study (up to 3 s).

It was expected that the brain regions most significantly activated during the beginning of the proprioceptive stimuli (coding of proprioception) would not be activated to the same degree during other components of each trial, namely: response generation, auditory cues, and interstimulus intervals. Therefore, contrasts were generated to identify brain activation that took place at the beginning of proprioception events above conditions of no interest (response generation, auditory cues, and interstimulus intervals). Individual data of healthy participants were analyzed using a standard unpaired *t*-test. The voxel-height threshold was set at $p < 0.001$, uncorrected for multiple comparisons. Analysis at the individual level was exploratory; therefore, a low threshold was selected to reveal trends of brain activation. The threshold used for data of stroke-affected participants was set at $p < 0.05$ corrected for multiple comparisons due to the expected bilateral brain activation (44, 45) of greater extent (44) compared to healthy participants. A high pass filter was used to remove the effect of low frequency drift on the data.

Group Analyses

Random effect analyses were used to generate *t*-contrasts for group activation maps of the LWP and RWP scans. As with individual analyses, only the beginnings of proprioception

events were modeled, and they were contrasted against all other brain activation that took place during the experiment (response events, auditory cues, and interstimulus intervals). To avoid the risks of multiple comparisons, cluster correction (minimum cluster size of 20 voxels) for multiple comparisons was used at $p < 0.05$ (contrasts entered in the analysis were at voxel-height threshold of $p < 0.001$). Anatomical loci of significant activation were identified using probabilistic maps (46) available from the SPM2 toolbox.

The probabilistic maps, however, did not specify the cyto-architectonic probability of Brodmann area (BA) 6. Thus, using the Talairach coordinates BA 6 was divided into lateral and medial parts. The area lateral to $x = 15$ was considered as the PMC and medial to it, the SMA. The PMC was divided into superior and inferior areas. The area superior to $z = 42$ was considered as the dorsal PMC (PMd), while inferior to it was the ventral PMC (PMv). The SMA was divided into anterior and posterior parts. The area anterior to $y = 0$ was considered as pre-SMA, while posterior to it was interpreted as the SMA proper [see Figure 2, (47)].

L laterality Analyses

L laterality calculations in the form of laterality index (LI) were used to quantify the hemispheric symmetries of proprioception-related brain activation during LWP and RWP separately, and no direct comparisons were made between the two. Anatomical brain regions selected for the LI calculations (regions of interest) were the primary SI and MI (based on the literature reviewed in Section “Introduction”), and more importantly high-order somatosensory and motor cortices identified in both the LWP and RWP group analyses. Outlines for the regions of interest were defined using an independent template – the Wake Forest University PickAtlas available from the SPM2 toolbox. For BA 6, outlines of subregions were generated manually using the FSLView tool (Version 3.0), in accordance with the guidelines detailed in Section “Group Analyses.”

L laterality was determined using signal extent based on the previously described protocol (48). Signal intensity of each voxel in the region of interest was determined by the statistical parametric maps of the LWP and the RWP contrasts. The average signal intensity was then calculated for the 5% of voxels showing the highest *t*-score. The LI was calculated as: $(\text{right} - \text{left}) / (\text{right} + \text{left})$. Using the top 5% of voxels showing the highest *t*-score served to reduce the risk of confounding brain activation related to inhomogeneities in the magnetic field or multiple comparisons. This risk was also reduced by contrasting brain activation during proprioceptive coding with all other experimental conditions (response generation, auditory processing, and rest), rather than contrasting with rest only.

L laterality thresholding is designed to limit type I errors. Based on the literature, we selected an *a priori* threshold of $-0.2 \geq \text{LI} \geq 0.2$ to indicate lateralized brain function (49). Thus, we expected that in the dominant region the area of the most significant brain activation showed at least 33% higher signal intensity compared to the homologous area. LIs were calculated for each ROI of each participant based on the individual analyses. Group LIs were reported as mean and standard deviation.

²<http://www.brain.org.au/software.html>.

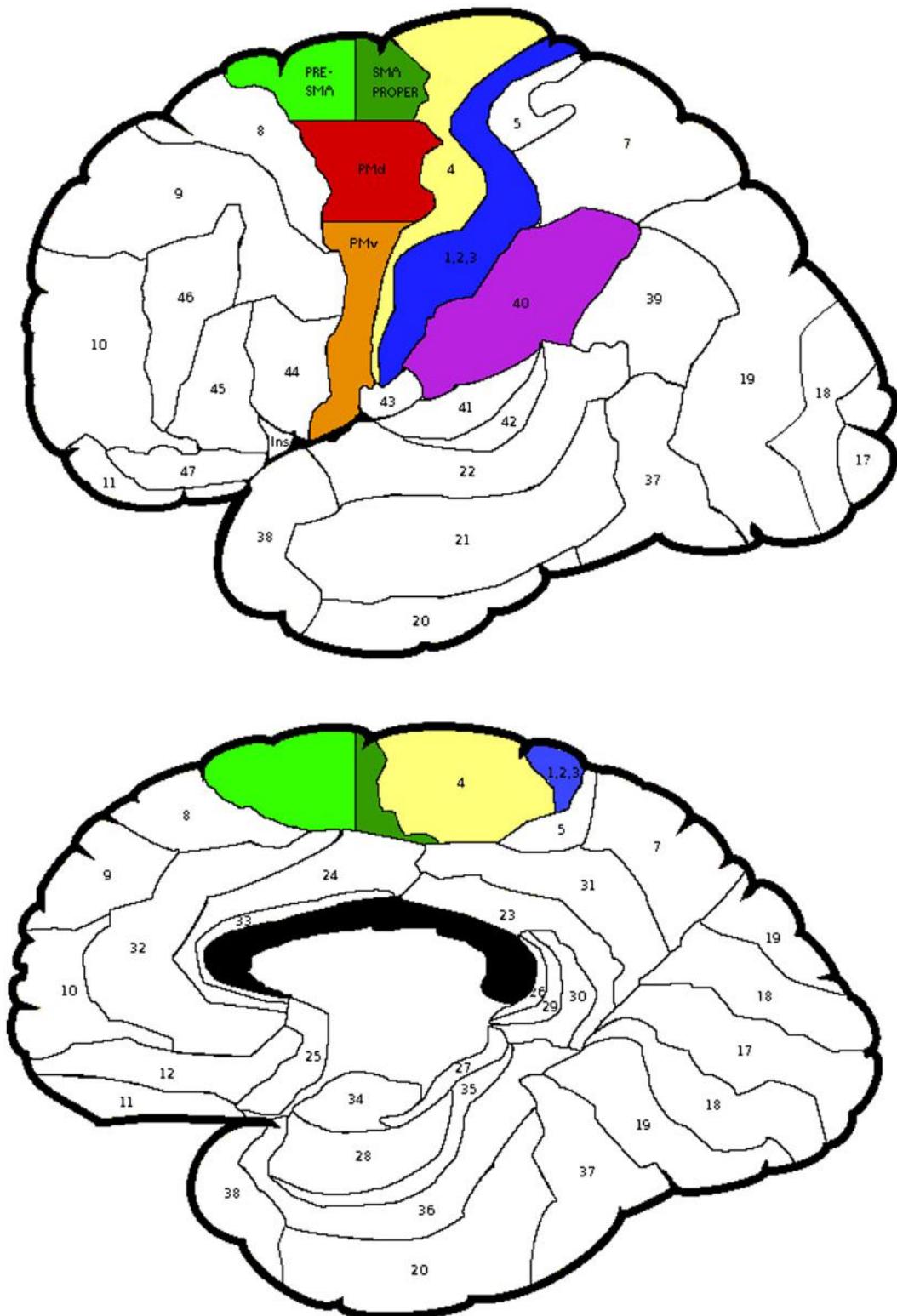


FIGURE 2 | The regions of interest selected for the laterality calculations and the subdivisions of Brodmann Area 6. Areas depicted: Brodmann Areas 1,2,3, primary somatosensory cortex; area 4, primary motor cortex; area 40, supramarginal gyrus; PMv, ventral premotor cortex; PMd, dorsal premotor cortex; subdivisions of area 6, Pre-SMA, pre-supplementary motor area and SMA proper, supplementary motor area proper.

RESULTS

Clinical and Proprioception Results

All healthy participants completed the LWP scans and six completed the RWP scans. The other six were not available to participate in the RWP study. During the prescan sessions, participants were vigilant for 96.8% of the tested trials (range: 89–100%, SD = 4.8%). The mean absolute error of participants' response accuracy for the matching task performed in the scanner was 8.6° (SD = 2.7°) for LWP and 7.5° (SD = 0.9°) for RWP.

As with the previous studies (5), forearm muscle electromyographic recordings for healthy participants during passive movements (mean 10.73, SD 7.70) were significantly lower than during active movements (116.96, 76.44) when tested with the Wilcoxon *T* test ($p < 0.001$).

Lesion sites of stroke-affected participants were subcortical, and the common lesion site was the thalamus (see **Figure 3**). The lesions of CS1 and CS3 extended to include the posterior limb of the internal capsule and the basal ganglia. During the prescan session, the mean absolute error of response for CS1 (LWP) was 17.9° (SD = 9.6°), vigilance 91.7%; for CS2 (RWP) mean absolute error of response 7.5° (SD = 7.0°), vigilance 94.4%; and for CS3 (RWP) mean absolute error of response 19.6° (SD = 13.3°), vigilance 100%.

Cortical Areas Activated During Proprioception

Group brain activation of healthy participants during the LWP task was in the right SI cortex, particularly in BA 3a, the right SMG, PMd, MI (BA 4a and 4p), superior and middle frontal gyri, SMA proper, and the middle cingulate cortex (see **Table 1**; **Figure 4**). Group brain activation during performance of the RWP task was significant in the right SMG, the left PMd, and MI (BA 4a) (see **Table 1**; **Figure 4**).

Proprioception-related brain activation varied among stroke-affected participants; however, common areas of brain activated included the IPL, SPL, and PMd (see **Table 2**).

Laterality of Proprioception-Related Brain Activation

Laterality was investigated for the SMG and PMd, high-order somatosensory and motor cortices identified in the group analyses and for the SI and MI given their well-established role in

proprioception (see **Figure 2**). Right laterality of SMG activation was observed for both the LWP and the RWP scans (see **Figure 5**; **Table 3**). Laterality calculations for the PMd illustrated a lesser degree of laterality compared to the SMG, with contralateral activation during LWP and bilateral activation during RWP (see **Figure 5**; **Table 3**). As expected, LIs of the SI and MI showed contralateral activation (see **Figure 5**; **Table 3**). For stroke-affected participants, brain activation was bilateral in both the SMG and PMd (see **Table 3**).

DISCUSSION

We investigated the brain-behavior relationship pertaining to processing of proprioceptive stimuli at the wrist. There are three novel aspects to our study design. First, natural proprioceptive stimuli of passive movements were used, and maximal effort was made to control for confounding tactile and motor stimuli. Participants were required to provide accurate and measurable response to each proprioceptive stimulus both in and outside the scanner. Second, the event-related design with its variable inter-stimulus intervals enabled temporal isolation of brain activation related to coding proprioception. Third, stroke-affected participants with proprioceptive deficits were studied with respect to the effect of pathology on proprioception-related brain activation.

Our findings indicated that proprioception-related brain activation in high-order somatosensory and motor cortices included the SMG and PMd. The right SMG was activated during both RWP and LWP, and its activity was reduced in the presence of proprioceptive deficits. Proprioception-related brain activation in the PMd was contralateral during LWP and bilateral during RWP. Thus, a certain degree of right PMd laterality was also observed during the central processing of proprioception. These findings confirm right hemispheric dominance in the processing of proprioception, but unlike other studies highlight the key role the right SMG plays in proprioception.

High-Order Proprioception-Related Brain Activation

The findings from our study suggest that the high-order proprioception-related brain activation of both the SMG and PMd is pivotal for the central processing of proprioception. Several studies have identified proprioception-related brain activation in frontoparietal networks; however, various activation loci were

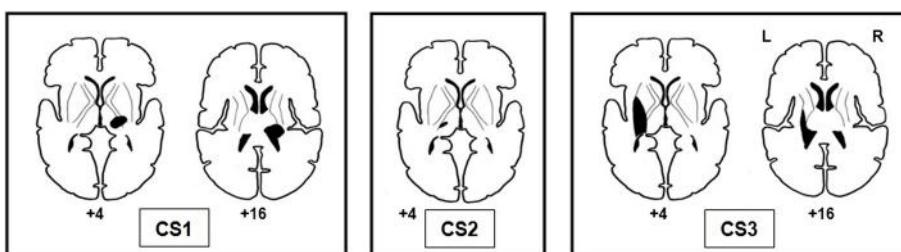


FIGURE 3 | Lesion sites of the three stroke-affected participants.

TABLE 1 | Group analyses of brain activation loci in healthy participants during proprioception.

Task	Anatomical location	BA	Cluster size	Z score	Talairach coordinates		
					x	y	z
LWP	R SI	3a	844	4.57	34	-32	45
	R SMG ^a	40		4.51	52	-40	37
	R PMd ^a	6		4.10	32	-26	69
	R MI	4a		3.96	36	-32	69
	R MI	4p		3.86	36	-22	53
	R SFG ^a	6/8		3.32	24	4	57
	R MFG	6/8		3.31	26	6	53
	R SMA (proper) ^a	6		3.75	16	-12	61
	R MCC ^a	6/24		3.19	10	-8	49
RWP	R SMG ^a	40	33	4.24	56	-38	29
	L PMd	6	29	3.93	-32	-26	64
	L MI ^a	4a		3.38	-36	-32	69

Clusters of proprioception-related brain activation are reported at the cluster-level threshold of $p < 0.05$ FDR corrected.

^aAnatomical locations of more than one maxima. Within each cluster (>20 voxels), only the most significant maximum is listed per anatomical location. BA, Brodmann area; L, left; LWP, left wrist proprioception; PMd, dorsal premotor cortex; R, right; RWP, right wrist proprioception; SFG, superior frontal gyrus; SI, primary somatosensory cortex; SMA, supplementary motor area; SMG, supramarginal gyrus; SPL, superior parietal lobe; MCC, middle cingulate cortex; MI, primary motor cortex; MFG, middle frontal gyrus.

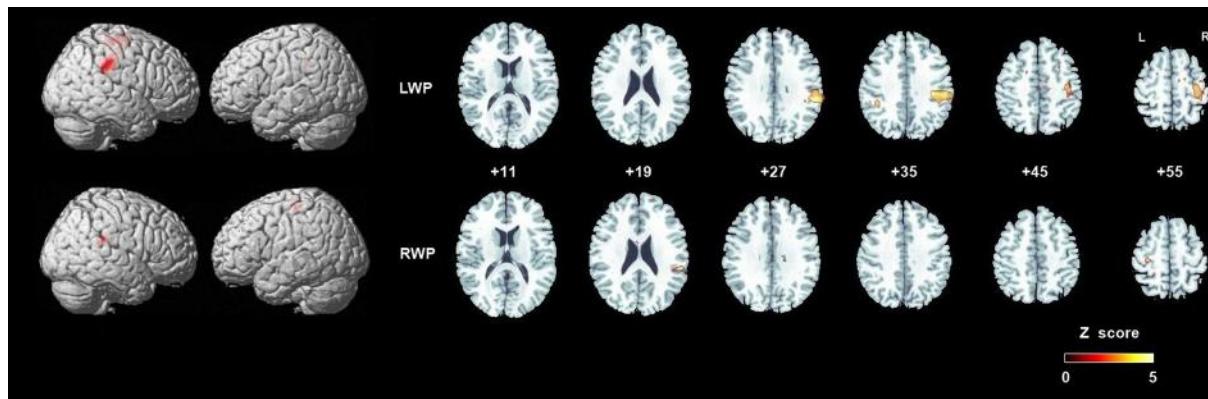


FIGURE 4 | Group analyses of brain activation in healthy participants during proprioception. Group brain activation was overlaid on a whole brain and axial sections of the Montreal Neurological Institute template. Threshold level $p < 0.05$ corrected at the cluster level. Abbreviations: LWP, left wrist proprioception; RWP, right wrist proprioception.

suggested (15, 18, 50–52). Both passive movement and illusory vibration studies identified brain activation in the IPL. Within the IPL, most studies reported proprioception-related brain activation in the parietal operculum (5, 9, 15, 17, 51, 53–55) and only a few reported brain activation in the SMG (6, 18, 56, 57). The SMG is located in the lateral aspect of the IPL whilst the parietal operculum is located medially to the SMG and in the roof of the Sylvain fissure (46). Variability across subjects in the cytoarchitectonic maps of the five areas that occupy the surface SMG has been reported (58) and may have contributed to the variable naming of regions (e.g., parietal operculum compared to SMG) in previous studies. The parietal operculum unlike the SMG is best known for its involvement in the processing of tactile stimuli (59). Tactile stimulation may have accompanied some of the passive movement stimuli in previous studies, for example, from the soles of the feet during ankle dorsiflexion (54, 55). Where tactile stimulation accompanied the proprioceptive

stimulation, it is not possible to identify which of the two stimuli generated activation in the parietal operculum.

The SMG is part of the somatosensory association cortex which has a role in interpretation of tactile sensory information as well as in perception of space and limbs location (15, 18). Previous literature suggests that frontoparietal activation in the SMG and PMC may be related to the spatial processing of stimuli around the hand (60) or the recognition of voluntary movement in the human, equivalent of the mirror neuron system (61). Such functions would rely heavily on knowledge of one's limb position. Indeed Brozzoli et al. (60) showed that the posterior parietal cortex was explicitly responsible for the hand's position sense.

Brain activation in the SMA is the commonest activation in high-order motor cortices identified in illusory vibration (15, 18) and passive movement (51, 54–56, 62, 63) studies. The SMA has been implicated in processes underlying internally guided movements (i.e., active movements). In comparison, the PMd has

TABLE 2 | Individual brain activation loci of stroke-affected participants during proprioception.

Participant and task	Anatomical location	BA	Cluster size	Z score	Talairach coordinates		
					x	y	z
CS1 LWP	L IPL ^a	40/7	272	7.44	-42	-50	53
	L Sup M Gyr ^a	6	182	7.26	-4	22	53
	L SMA (proper)	6		6.99	-4	16	61
	R ITG	37	58	7.22	58	-60	-7
	R SPL	7	111	6.99	16	-72	65
	R IPL ^a	40	149	6.96	40	-54	53
	R SMG ^a	40	126	5.94	56	-38	33
	L PMd ^a	6	87	5.91	-36	-8	65
CS2 RWP	L SPL ^a	7	458	7.26	-26	-56	73
	L SI ^a	2		7.19	-34	-40	57
	L SI ^a	1		6.64	-36	-42	73
	L IPL ^a	40	292	7.15	-54	-40	45
	L STG	41/42		5.76	-64	-42	25
	L SMG	40		5.50	-54	-48	29
	L PMd ^a	6	130	6.37	-24	-20	81
	L SMA (proper)	6		5.99	-6	-14	73
	R SMG ^a	40	80	5.74	56	-46	49
	R IPL ^a	40		5.06	58	-34	57
CS3 RWP	L PMd ^a	6	340	Inf	-32	-14	73
	L MFG	6		4.95	-24	-4	61
	R IPL ^a	40	519	Inf	32	-54	45
	R SMG ^a	40		6.50	40	-38	45
	R SPL ^a	7	421	Inf	12	-86	57
	R cuneus	18/19		Inf	12	-88	49
	L SOG	18		7.19	-10	-88	45
	L cuneus ^a	18		5.11	-6	-98	25
	L SMG ^a	40	304	Inf	-66	-38	37
	L STG	42/37		6.58	-52	-42	25
	R PMd	6	255	7.73	26	-10	69
	L IPL	40	246	7.61	-42	-56	57
	L SPL ^a	7		6.19	-38	-58	69
	L angular gyrus	39		5.46	-48	-62	45
	L ITG	37	71	7.26	-60	-56	-7
	R MOG	19	93	7.02	34	-88	33
	L calc gyrus ^a	17	55	5.48	-20	-64	9

Clusters of proprioception-related brain activation are reported at the cluster-level threshold of $p < 0.05$ FDR corrected.

^aAnatomical locations with more than one maximum. Within each cluster (>50 voxels), only the most significant maximum is listed per anatomical location. BA, Brodmann area; calc gyrus, calcarine gyrus; IPL, inferior parietal lobe; ITG, inferior temporal gyrus; L, left; LWP, left wrist proprioception; MFG, middle frontal gyrus; MOG, middle occipital gyrus; PMd, dorsal premotor cortex; PMv, ventral premotor cortex; R, right; RWP, right wrist proprioception; SI, primary sensory cortex; SMA, supplementary motor area; SMG, supramarginal gyrus; SOG, superior occipital gyrus; SPL, superior parietal lobe; STG, superior temporal gyrus; Sup M Gyr, superior medial gyrus.

been associated with externally guided movements (i.e., passive movements) (64). Given that passive movements are externally imposed, higher activation of PMd than SMA was both expected and found in our study.

Frontal activation in the PMd is important for the processing of proprioception, probably due to the tight coupling between proprioception and its use during movement. Bilateral PMd and right SMG activation was found in a brain imaging study of precision grip but not power grip (65). As proprioception is pivotal for precise motor control (3), it is likely that the frontoparietal brain activation found during precision grip included that of proprioception.

Other lines of research have also found functional association between the SMG and PMd. Anatomical studies in primates showed that proprioceptive information travels to the PMd and that extensive connections exist between the posterior parietal lobe and the PMd (66). In a brain imaging study where healthy

participants were required to integrate proprioceptive information into spatial visual or somatic sensory tasks, frontoparietal activation (especially in the right hemisphere) was found (67). Finally, lesion studies indicated that the integrity of the parietal cortex, frontal cortex, and their connections was required for recovery from spatial neglect (68).

Right Hemispheric Dominance During Proprioception

We found activation of the right SMG during both RWP and LWP, and its activity was reduced in the presence of proprioceptive deficits. Some evidence exists for left laterality of proprioception in the IPL (16, 69). Most of the evidence, however, suggests right hemispheric laterality during proprioception. Illusory vibration studies identified lateralized frontoparietal activation in the right SI (BA 2), middle frontal gyrus (BA 44, 45), parietal operculum,

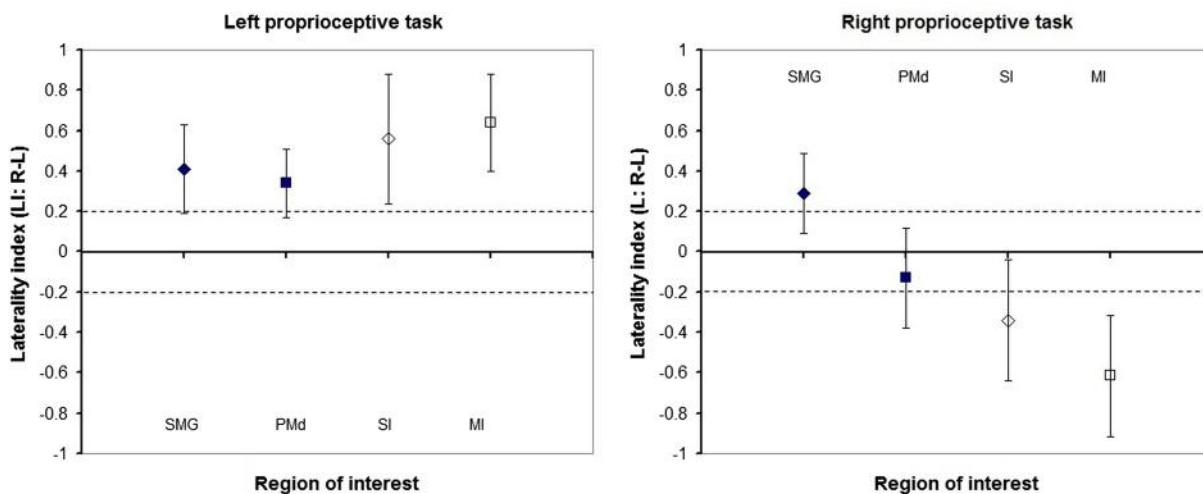


FIGURE 5 | Laterality of proprioception-related brain activation in regions of interest of healthy participants. Group mean and standard deviation of laterality indices of the: supramarginal gyrus (SMG), dorsal premotor cortex (PMd), primary somatosensory (SI), and motor (MI) cortices. Diamonds represent sensory cortices and squares motor cortices. Filled shapes represent high-order cortices, while outlined shapes represent primary cortices. Dashed lines represent absolute laterality indices of 0.2. Laterality indices higher than 0.2 represent greater cerebral activation in the right compared to left hemispheres and vice versa for values smaller than -0.2.

TABLE 3 | Lateralization calculations of brain activation during proprioception of healthy and stroke-affected participants.

Anatomical region	Healthy				CS1 LWP LI	CS2 RWP LI	CS3 RWP LI
	LWP LI (<i>n</i> = 12)		RWP LI (<i>n</i> = 6)				
	Mean	SD	Mean	SD			
SMG	0.41	0.22	0.29	0.21	-0.18	-0.19	-0.05
PMd	0.34	0.17	-0.13	0.25	-0.06	0.02	0.18
SI	0.56	0.32	-0.34	0.30	-0.56	0.42	0.19
MI	0.64	0.24	-0.62	0.30	-0.77	0.66	0.59

Positive values indicate right hemisphere activation greater than left and vice versa for negative values. Stroke-affected participants are listed as CS1–3. LI, laterality index; LWP, left wrist proprioception; MI, primary motor cortex; PMd, dorsal premotor cortex; RWP, right wrist proprioception; SI, primary sensory cortex; SMG, supramarginal gyrus.

and insula (15, 17), with one study reporting activation in the SMG rather than the parietal operculum (18). In passive movement studies of left and right limbs, right hemispheric laterality was evident in the superior temporal gyrus and the parietal operculum for ankle movements (55) or bilateral IPL and parietal operculum for wrist movements (51). Our findings provide support for right hemispheric laterality but identify the right SMG in particular as a key region activated during proprioception. The lack of brain activation in the parietal operculum is likely due to the effort made in our study to minimize confounding tactile stimulus.

Right SMG activation during proprioception may be explained by the role that this region plays in spatial processing (70). In their important work, Stephan and colleagues (70) used identical visual stimuli to perform a simple reaction time task, a lingual task or a spatial task. They found that despite the common visual stimuli only the spatial processing task activated the right SMG and the junction of the occipital, parietal, and

temporal lobes. We regard proprioception as a spatial-processing task because it involves judgments of a limb's spatial location. If proprioception is a spatial-processing task and the right SMG is a key brain region involved in spatial processing, then this could explain the significance of right SMG activation found in our study.

Studies of participants with hemispatial neglect have also demonstrated an association with right SMG lesions (71). The diagnosis of hemispatial neglect is often made based on visuo-spatial assessment (72), which involves the extrapersonal space. Committeri et al. (73) showed that lesions in the right SMG were particularly related to impaired spatial processing in the personal space studying a large sample of participants with hemispatial neglect, although proprioception as such was not tested. Our findings raise the question of whether hemispatial neglect caused by right SMG lesions not only affects personal space in general but also affects proprioception specifically.

The Effect of Proprioceptive Deficits Poststroke on the Central Processing of Proprioception

The thalamus was the common lesion site of the three stroke-affected participants included in our study. For two of the participants (CS1 and CS3), the brain lesions extended to the internal capsule, and both displayed more severe proprioceptive deficits on behavioral testing (the Wrist Position Sense Test and the prescan behavioral measures). Similar lesion sites in the thalamus and the internal capsule were found in other studies of participants with proprioceptive deficits (74–79).

We found that SMG activation was bilateral in stroke-affected participants. This was the most significant difference observed from the proprioception-related brain activation patterns in healthy participants, where right SMG laterality was found. The findings from stroke-affected individuals with proprioceptive deficits are consistent with the significance of right SMG integrity for adequate proprioceptive function. In previous brain imaging studies of stroke-affected participants where passive movement stimuli were delivered, participants with somatosensory deficits were specifically excluded (50–52, 77, 80, 81). Our findings are therefore not comparable and are novel for stroke survivors with quantified proprioceptive deficits.

Of interest is our finding of ipsilateral brain activation in SIMI. A similar pattern of ipsilateral rather than contralateral SIMI activation has been found in stroke-affected individuals with motor deficits (82, 83). Furthermore, ipsilateral SIMI activation was found in the studies of participants with tactile deficits who performed a touch discrimination task during scans (84, 85). Our findings suggest that similar to other sensory and motor modalities, proprioceptive deficits are associated with a shift of brain activation to the ipsilateral SIMI.

Study Limitations

Sample size is the main limitation for this study. Twelve participants performed the LWP and only six of them performed the RWP. Due to the smaller RWP group size, group analyses were conducted with a threshold of 0.001 uncorrected for multiple comparisons. Such a threshold increases the risk of false positives, i.e., reporting activation that did not actually occur. To assess the effect of this risk on our results, two additional analyses were conducted. First, group analysis of the LWP was performed at a threshold of 0.05 corrected for multiple comparisons (FDR). Second, a LWP group analysis was conducted for the six participants who also performed the RWP. Results of both analyses showed the same patterns of brain activation were maintained with the same anatomical loci. To minimize the risk of false positives reported in this paper only activation under the threshold of 0.05 corrected at the cluster level was reported. Thus, the additional analyses designed to address limitations related to sample size and threshold, supported the principal proprioception-related brain activation identified in this study.

Contralateral brain activation in SI was not found during RWP. The laterality calculation showed that SI activation during RWP tended to be bilateral. In another brain imaging study of

arm proprioception, bilateral SI activation was found during right stimulation compared to contralateral activation during left stimulation (15). In our study, bilateral SI activation during RWP together with the small sample size was the likely cause for activation not reaching significance level. Thus, bilateral SI activation was under represented in our study.

Clinical Implications

The presence of laterality in proprioception-related brain activation suggests differences in the central processing of proprioception arriving from the left and right limbs. Previous behavioral studies have identified smaller absolute errors for left compared to right limb proprioception (26–28). Our findings together with those of previous brain imaging studies support right hemisphere dominance of proprioception.

Right hemisphere dominance for proprioception has clinical implications for both assessment and treatment. Particular care appears necessary when assessing proprioception in people with brain lesions affecting the right hemisphere, particularly the SMG. The question of which assessment tool to use for proprioceptive assessment is beyond the scope of this paper. However, accurate quantitative tools with normative ranges such as the Wrist Position Sense Test (38) are preferred. A relevant clinical question is the relative contribution of lesions in the right SMG and PMd to proprioceptive deficits.

People with right hemispheric lesions are more likely to require specific proprioceptive rehabilitation. Furthermore, based on the studies of recovery from spatial neglect (68), recovery from proprioceptive deficits may be a function of right SMG and or PMd integrity. A future study examining the relative effect of rehabilitation on right SMG and PMd function would be useful, as would studies on whether normalization of brain activation in these regions correlate with functional recovery.

CONCLUSION

We present a novel and innovative brain imaging study of proprioception, where participants were required to provide a direct response to each stimulus, and where response accuracy was monitored. This is the first time that laterality of proprioception-related brain activation has been directly studied with a natural proprioceptive stimulus (passive movements). This is also the first time that such stimuli have been used to examine brain activation in stroke affected individuals with proprioceptive deficits. We achieved temporal isolation of brain activation during coding of proprioceptive stimuli by using the event-related study design. This activation involved high-order somatosensory and motor cortices, namely the SMG and PMd, respectively. Laterality analyses and lesion studies indicated that the right SMG plays a key role in the processing of proprioception. The results provide a novel insight into the brain–behavior system of proprioception and how it is affected by brain lesions. These insights suggest that people with right hemispheric lesions may be more susceptible to proprioceptive deficits, particularly if the right SMG is affected. As the right SMG is commonly implicated in spatial neglect, it raises important questions of whether spatial neglect and proprioceptive deficits are different or associated

impairments, and what the relative contribution of the SMG and PMd to proprioceptive function might be. If SMG and PMd lesions affect proprioception differently, then it is possible that different treatment methods may be required to address these differential impairments.

AUTHOR CONTRIBUTIONS

EB-S contributed to conception, data collection, analysis, interpretation, and manuscript preparation. TM contributed to conception, interpretation, and critical revision of the manuscript. GP contributed to conception, analysis, and critical revision of the manuscript. AB contributed to conception, analysis, interpretation, and critical revision of the manuscript. LC contributed to conception, data collection, analysis, interpretation, and critical revision of the manuscript.

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Supplementary motor complex and disturbed motor control – a retrospective clinical and lesion analysis of patients after anterior cerebral artery stroke

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Background: Both the supplementary motor complex (SMC), consisting of the supplementary motor area (SMA) proper, the pre-SMA, and the supplementary eye field, and the rostral cingulate cortex are supplied by the anterior cerebral artery (ACA) and are involved in higher motor control. The Bereitschaftspotential (BP) originates from the SMC and reflects cognitive preparation processes before volitional movements. ACA strokes may lead to impaired motor control in the absence of limb weakness and evoke an alien hand syndrome (AHS) in its extreme form.

Aim: To characterize the clinical spectrum of disturbed motor control after ACA strokes, including signs attributable to AHS and to identify the underlying neuroanatomical correlates.

Methods: A clinical assessment focusing on signs of disturbed motor control including intermanual conflict (i.e., bilateral hand movements directed at opposite purposes), lack of self-initiated movements, exaggerated grasping, motor perseverations, mirror movements, and gait apraxia was performed. Symptoms were grouped into (A) AHS-specific and (B) non-AHS-specific signs of upper limbs, and (C) gait apraxia. Lesion summation mapping was applied to the patients' MRI or CT scans to reveal associated lesion patterns. The BP was recorded in two patients.

Results: Ten patients with ACA strokes (nine unilateral, one bilateral; mean age: 74.2 years; median NIH-SS at admission: 13.0) were included in this case series. In the acute stage, all cases had marked difficulties to perform volitional hand movements, while movements in response to external stimuli were preserved. In the chronic stage (median follow-up: 83.5 days) initiation of voluntary movements improved, although all patients showed persistent signs of disturbed motor control. Impaired motor control is predominantly associated with damaged voxels within the SMC and the anterior and medial cingulate cortex, while lesions within the pre-SMA are specifically related to AHS. No BP was detected over the damaged hemisphere.

Conclusion: ACA strokes involving the premotor cortices, particularly the pre-SMA, are associated with AHS-specific signs. In the acute phase, motor behavior is characterized by the inability to carry out self-initiated movements. Motor control deficits may persist to a variable degree beyond the acute phase. Alterations of the BP point to an underlying SMC dysfunction in AHS.

Keywords: anterior cerebral artery, stroke, supplementary motor area, anterior cingulate cortex, Bereitschaftspotential

Introduction

Voluntary and involuntary movements are generated and controlled by a complex bihemispheric neuronal network involving the primary motor (MI) and supplementary motor complex [SMC; consisting of the supplementary motor area (SMA) proper and pre-SMA], cingulate cortex, and dorsolateral prefrontal cortex as well as a number of subcortical brain structures such as the basal ganglia and the cerebellum. Motor areas supplied by the anterior cerebral artery (ACA) involve the SMC, the anterior and middle cingulate cortex, and the rostral section of the corpus callosum. This part of the motor network is particularly involved in the generation of self-initiated (i.e., volitional), complex movement sequences, inhibition of purposeless movements triggered by external stimuli such as the grasp reflex, error control during motor performance, and motor learning (1, 2). An electrophysiological measure of voluntary control is available with the so-called Bereitschaftspotential (BP). The BP is a negative potential over the vertex emerging approximately 1 s before the onset of a voluntary movement. The early component most presumably originates from the SMA, while the later component is mainly assigned to the primary motor cortex and the lateral premotor cortex (3). The BP probably reflects cognitive processes preceding the initiation of volitional movements (4). According to recent computational frameworks for action, both conscious awareness of intention and a sense of agency characterize voluntary movements (5). By applying direct electrical stimulation to the SMC, a conscious intention of moving can be provoked underlining its role in generating volitional movements (6).

As previously mentioned, ACA strokes lead to a severe disruption of the above-mentioned motor network. The clinical spectrum of disturbed motor control after ACA strokes may encompass signs such as involuntary grasping of nearby objects, utilization behavior, and intermanual conflicts (i.e., the two hands are directed at opposite purposes) with absence of volitional movements (7). Underutilization of one body side in the absence of relevant weakness or sensory disturbances or deficits of reflexes, as it can be observed in ACA strokes, has been summarized under the term “motor neglect” (8). Apart from limb weakness, the above-mentioned motor signs have been acknowledged as characteristic features of the so-called alien hand syndrome (AHS) (9, 10). Its first description was rendered by Goldstein in 1908 who reported “a type of apraxia with the feeling of estrangement

between the patient and his hand” (11). In 1972, Brion and Jeydnak observed analogous symptoms in a patient with a corpus callosum tumor, which inspired them to coin the term “la main étrangère.” It was subsequently translated into the English term “alien hand” (12, 13).

It has turned out that the clinical picture of AHS is variable and reflects a spectrum of abnormalities in motor control rather than a homogeneous clinical entity (14). Dolado et al. proposed following hallmarks as essential for the diagnosis of an AHS: (i) a feeling of foreignness of the affected limb, (ii) failure to recognize ownership of it when visual clues are removed, (iii) autonomous motor activities that are perceived as involuntary and are different from other identifiable movement disorder, and (iv) attribution of an action to another subject due to lacking sense of agency (15). Lesions within the SMC, the cingulate cortex and the corpus callosum have often been implicated in the context of AHS (10).

Although AHS has been known for a very long time, there is no comprehensive clinical-anatomical correlation addressing impaired motor control in a larger number of ACA stroke patients. Hitherto, most of the published literature is restricted to case reports and case series [reviewed in (7, 16, 17)]. The only systematic approaches published suffer from methodological drawbacks, including definitions that are too wide apart with regard to disturbed motor control and/or the lack of using adequate imaging methods (16, 17). Therefore, the aim of this case series was to characterize the clinical spectrum of disrupted motor control, including signs attributable to AHS and to identify the main underlying neuroanatomical correlates (18). We hypothesized that an involvement of the SMC is essential for the occurrence of the AHS spectrum of disturbed motor control after ACA strokes.

Patients and Methods

Study Population

Over a period of 6 years, patients with arm paresis or plegia, after circumscribed ACA infarction were identified at our center and included in this case series. Conscious awareness of intention and sense of agency of volitional movements of the affected limb, both thought to be key features of an AHS, were the main focus of this study (5). On the basis of these two key features, clinical signs of disturbed motor control were classified into three different groups (7, 10, 13, 15–17, 19–31). Group A included AHS-specific signs, namely (A.I) lack of self-initiated movements, (A.II) exaggerated (not suppressible) grasping and groping behavior, and (A.III) presence of an intermanual conflict (i.e., the two hands are directed at opposite purposes). Group B included clinical signs, which did not necessarily reflect disturbed

Abbreviations: ACA, anterior cerebral artery; AHS, alien hand syndrome; BP, Bereitschaftspotential; MNI, Montreal National Institute; NIHSS, National Institute of Health Stroke Scale; SMA, supplementary motor area; SMC, supplementary motor cortex; SPM, statistical parametric mapping; VOI, volume of interest.

awareness of intention and sense of agency. These symptoms were thus considered as non-AHS-specific signs of disturbed motor control: (B.I) maintaining a particular limb position after a preceding complex motor task (i.e., motor perseveration), (B.II) Co-activation of the contralateral limb during volitional movements of the ipsilateral limb (i.e., mirror movements), and (B.III) any form of tremor. Group C included symptoms, which were signs beyond disturbed motor control of the upper limbs: (C.I) in this group, gait apraxia was expected (21). The study was approved by the ethics committee of the Kanton St. Gallen and was conducted according to GCP guidelines.

Epidemiological Data and Clinical Tests

Demographics and disease characteristics, including National Institute of Health Stroke Scale (NIHSS)-scores and stroke etiology according to TOAST criteria (32), were taken from the patients' records. All patients underwent a standard neurological examination. The following procedures were used to screen clinically for the aforementioned clinical signs of disturbed motor control: (A.I) *impaired self-initiated movements* were studied by observing volitional gestures and the interaction with the examiner during taking the history and the clinical assessment. Furthermore, patients were asked to voluntarily perform tasks such as virtual piano playing or typing on a keyboard. Testing of muscle strength was difficult in the acute phase due to the inability to perform voluntary movements, but weakness was excluded in the subacute stage in all cases. (A.II) *The presence of an intermanual conflict* was evaluated by antiphasic upper limb movements (i.e., windmill-like movements of both arms), transferring objects from one hand to the other or by performing bimanual tasks (e.g., putting on glasses). A marked shift or loss of phase, disturbances on performing coordinated bimanual tasks, and purposeless counteracting of upper limbs during bimanual tasks were attributed to the presence of an intermanual conflict. (A.III) *Exaggerated grasping behavior* was tested by moving objects nearby in the visual field and by asking them to suppress compulsive grasping. Patients were also observed when they released objects or when they transferred objects from one hand to the other. (B.I) *Motor perseveration* was defined as maintaining a particular hand position, which was clearly related to a preceding (complex) motor task. (B.II) *Mirror movements* were picked up during the assessment of the affected hand by observing the contralateral one and vice versa. (B.III) We also screened our patients for any form of resting, postural and action *tremor*. (C.I) *Gait apraxia* was assessed in those patients who were able to walk independently. They were asked to walk along the corridor and to turn toward and away from the affected side. Shuffling gait with high cadence and paroxysmal interruption of locomotion, with trembling of the feet in place and preserved (seemingly paradoxical) ability to increase step length and height when stepping over an object on the floor or when presenting cueing signals, were considered as signs of gait apraxia (33). Patients were asked if they had the feeling of their feet being glued on the floor.

Typical clinical signs of disturbed motor control in the context of an ACA stroke, as specified above, were documented according to a predefined protocol. In nine patients (with exception from patient P6), videos of the clinical examination as detailed

above were available for retrospective review. In P6, who explicitly declined video monitoring, symptoms were documented in detail in his hospital files. Symptom severity and persistence were rated by a neurologist in a semi-quantitative manner: clinical symptoms were considered as severe (+++), if they were permanently present and/or if they were a relevant source of impairment in the patient's ability to carry out the clinical test. Severity was considered as moderate (++) if symptoms were frequently present, but only mildly interfered with the patient's ability to carry out the clinical test. If there was just a hint of a particular sign or if the respective sign occurred only rarely, it was considered as mild (+). Absence of a particular sign was rated as "0." Notably, due to the lack of validated clinical scores, this scale has been designed for the purpose of this case series. To assess the reliability of this rating, a second blinded examiner rated the videos and the interrater reliability rate (IRR) was calculated by the means of kappa statistics. Calculations yielded a kappa coefficient of 0.83 ± 0.12 observed as proportion of maximum possible kappa thus indicating a good IRR.

Bereitschaftspotential (Readiness Potential)

The BP was recorded by using an EEG-EMG polygraphy. The EEG electrodes were placed over C3, C4, and Cz and the reference over Fpz according to the 10–20 EEG system. The ground electrode was fixed at the ear lobe. Patients were asked to keep their eyes closed and to repetitively perform briskly initiated middle finger extensions of 1-s duration in a self-paced manner, with an interval between each movement of approximately 6–7 s. Before the actual recording, they were instructed how to perform the finger movements while getting the sense for timing and movement initiation. To generate entirely self-initiated movements, they were instructed not to count or to pace the movement onset by using any other form of rhythmical encoding (e.g., by humming). They were also advised to fully shift their attention on the finger movement and to avoid falling asleep. Muscle activity was recorded from the long finger extensors by surface EMG. To avoid blinking, particularly at the time of movement initiation, we positioned two small sand bags over their eyelids. Eighty to 100 sequences of middle finger extensions were recorded from each hand. The BP was calculated offline using the ASA software (ENT Enschede, Netherlands). At least 50 artifact-free EEG epochs lasting from 2.0 s before to 1.0 s after motor onset were chosen and averaged for each limb separately. The BP was baseline corrected by averaging the epoch 1.5–2.0 s before motor onset. The amplitude at 0.25, 0.50, 0.75, 1.00, 1.25, and 1.25 s before and 0.25, 0.50, 0.75, and 1.00 s after motor onset as well at motor onset was calculated by averaging all data points acquired 50 ms before and after each respective time point (34). The results were then plotted against the grand average of the BP from 13 healthy controls.

Lesion Summation Mapping

Images were acquired within the first days after hospital submission (median 2.5 days; range 0–30). Isotropic diffusion weighted imaging (DWI) sequences and T1 sequences were acquired in a 1.5 T or a 3 T MRI scanner (T1: slice thickness 5 mm, DWI: $b = 1000 \text{ s/m}^2$, slice thickness 4 mm). We used DWI sequences for lesion analysis as they showed the best contrast for ischemic

brain tissue. In two patients, only CT scans were available. In both patients, however, the scans already showed a clearly demarcated ischemic brain lesion. Hence, they were feasible for reliably drawing lesion maps and were included for further imaging analysis.

For pre-processing of the scans, DWI and T1 sequences were first co-registered using Statistical Parametric Mapping 8 (SPM8)¹ (18, 35). According to the general agreement for working in the stereotaxic standard space, the anterior commissure was defined as the origin of the coordinate system in all scans (MNI-coordinates $x = 0, y = 0, z = 0$). The ischemic lesions were drawn manually on the DWI sequences using the freely available MRIcron software² and the drawings were put together to a 3D volume of interest (VOI). Both DWI and T1-weighted sequences were normalized to a MRI template and T1-weighted images were segmented by the means of the Clinical Toolbox running on SPM8³. The lesion maps were entered as masks in the algorithm for cost function masking to avoid distortion of the voxels within the ischemic lesion during spatial normalization. CT normalization routine integrated in the Clinical Toolbox was used analogously to normalize CT scans to a standard space template. Afterwards, all lesions were flipped to the left side to enhance power of the analysis. In the patient with a bilateral ACA infarction, the larger hemispheric volume defect was accordingly flipped to the left side.

Calculation of lesion maps was done in three steps. (1) Weighted summation (overlap) maps were calculated in SPM 8 for each clinical sign. Only VOIs from patients showing a particular sign were included in the retrospective calculation (see Table 1). A Kernel filter with 4 mm full-width half maximum was used to slightly smooth the summation maps. Each map was then thresholded to voxels damaged in >25% of our patients showing the respective clinical sign. (2) Summation maps for each symptom group (A, B, C) were created by using the image calculator function integrated in SPM8. The respective summation maps were calculated by summing up the summation maps of the different symptoms included in groups A and B, respectively. The summation map of group C was identical to the map for gait apraxia and therefore did not require further calculation. (3) To address the question which part of the ischemic lesions contributes to

disturbed motor control of upper limbs in general, the union set of group A and B ($A \cap B$) and the set difference of $(A \cap B) \setminus C$ were calculated. To address the question which brain section is specific for symptoms of the AHS spectrum, the set difference of $A \setminus (B \cup C)$ was calculated. Prior to calculation of all these sets, each group summation map was transferred into binary maps using the SPM8 image calculator.

In a final step, each lesion map was plotted onto the automated anatomical label (AAL) atlas using MRIcron and the involved brain areas as well as the center of gravity were identified by the respective built-in function. As the AAL does not distinguish between the pre-SMA and SMA proper, ROIs with the anterior commissural line as the border between these two areas (1, 36) were manually drawn in MRIcron and were used to determine the number of damaged voxels encompassed by each subsection.

Results

Study Populations

Information from 10 patients aged between 63 and 87 years (mean 74.2) were available. Among them were eight males and two females. Initial NIHSS ranged from 2 to 21 points (median 13.0). Seven patients were followed up from the acute stage and three patients were added after reviewing our stroke database and clinical notes from the last 2 years. All patients investigated in the acute stage had disturbed conscious awareness of intention and sense of agency. The first signs of recovery occurring within days were involuntary finger movements elicited by touching their palm. In one patient, information on these features were missing. Five patients had an ischemic lesion within the left hemispheric ACA territory, four within the right and one had large bilateral ACA infarctions. The lesion pattern ranged from circumscribed infarcts confined to the SMA to bilateral territorial infarcts within the ACA territory. According to the TOAST criteria (32), macroangiopathy was identified as a stroke etiology in 3/10 patients, cardioembolic events in 6/10, and arterial emboli secondary to aneurysm coiling in the ACA in 1/10. Detailed clinical and radiological information are summarized in Table 1.

Signs of Motor Control

Initially, all cases had marked difficulties to perform volitional hand movements. In the acute setting, 9/10 patients presented

¹<http://www.fil.ion.ucl.ac.uk/>

²<http://www.mccauslandcenter.sc.edu>

³<http://www.mricro.com/clinical-toolbox/>

TABLE 1 | Baseline demographic data and clinical findings.

No	Age (years)	Sex	First ever stroke	Stroke etiology	NIHSS-score
P1	83	Female	Total right-sided ACA stroke	Cardioembolism	15
P2	82	Male	Partial left-sided ACA	Cardioembolism	17
P3	74	Male	Partial left-sided ACA	Cardioembolism	21
P4	87	Female	Total right-sided ACA infarct	Cardioembolism	7
P5	63	Male	Total bilateral ACA infarct	Cardioembolism	15
P6	75	Male	Partial left-sided ACA infarct	Cardioembolism	16
P7	69	Male	Partial left-sided ACA infarct	Large artery arteriosclerosis	3
P8	70	Male	Total right-sided ACA infarct	Large artery arteriosclerosis	6
P9	65	Male	Partial left-sided ACA infarct	Stroke of other determined etiology (Secondary to aneurysma coiling)	11
P10	74	Male	Partial right-sided ACA infarct	Large artery arteriosclerosis	7
					2

Stroke etiology according to Toast criteria; ACA, anterior cerebral artery; NIHSS, National institute of health stroke scale.

TABLE 2 | Disturbed motor control.

No	I. Primary presentation		II. Motor signs at follow-up					
	AHS	Impaired self-initiated movements	Grasping	Intermanual conflict	Motor perseveration	Mirror movements	Tremor	Gait apraxia
P1	++	+++	+++	+	0	++	na	++
P2	++	+	++	++	+	+	0	+
P3	++	+	++	++	+	+	+	+
P4	++	+	0	++	+	+	0	+
P5	+	++	+++	++	+	na	++	na
P6	++	(+)	0	+	+	+	0	0
P7	na	(+)	0	++	0	0	0	0
P8	++	++	++	++	+	+	++	+
P9	++	0	++	+	0	+	+	0
P10	+	0	0	+	+	+	0	0

Primary presentation (scoring): AHS, ++; minor or transient AHS, +.

Motor signs at follow-up (scoring): severe presentation, +++; moderate presentation, ++; mild presentation, +; symptom not present, 0.

na, information not available.

with an apparently severe paresis or plegia of one or both upper extremities, respectively (five right-sided, three left-sided symptoms, and one bilateral symptoms). In the subacute and chronic stage for all patients, movement initiation improved but signs of disturbed motor control such as exaggerated grasping, and disturbing movements of the affected limb persisted to a variable extent. These data are summarized in **Table 2** and relate to the last control after the ischemic stroke (median duration of follow-up: 83.5; range: 7–585 days). Lack of self-initiated movements was present in 8/10 patients, intermanual conflict in 10/10 patients, exaggerated grasping and groping behavior in 6/10 patients, motor perseveration in 8/10 patients, mirror movements in 7/9 patients, tremor in 5/10, and gait apraxia in 4/8 patients. In the patient with bilateral ACA infarcts, a reliable evaluation of mirror movements was not possible due to the severe impairment of initiating movements of both limbs.

Imaging

Table 3 summarizes the size of and the anatomical location of the weighted summation maps for each clinical sign. The total centers of gravity of the specific maps for grasping, intermanual conflict, lack of self-initiated movements, mirror movements, and motor perseveration were located in the caudal tier of the anterior cingulate cortex, whereas the center of gravity of the maps for gait apraxia and tremor were located within the white matter adjacent to the anterior cingulate cortex (MNI-coordinates: lack of self-initiated movements: $x = -11, y = 9, z = 36$; intermanual conflict $x = -3, y = -3, z = 34$; grasping: $x = -10, y = 10$, motor perseveration: $x = -10, y = 10, z = 34$; mirror movements: $x = -7, y = 0, z = 42; z = 34$; tremor: $x = -3, y = -2, z = 27$; gait apraxia: $x = -13, y = 16, z = 27$) (**Figure 1**). The combined summation map for all AHS-specific motor symptoms (i.e., group A) encompassed a total lesion volume of 99,863 voxels (corresponding to a lesion volume of 99.9 ml with a center of mass in the anterior cingulate cortex ($x = -10, y = 12, z = 35$)). Similarly, the respective map for non-AHS-specific motor symptoms of the upper limbs (group B) had a lesion volume of 101,691 voxels (lesion volume: 101.7 ml) with its center of mass in the anterior cingulate cortex ($x = -11, y = 11, z = 36$) (**Figure 2**).

The union set of the maps for group A and B ($A \cap B$) had a total size of 66,132 voxels (corresponding to a total lesion volume of 66.1 ml). 4.8% of the total lesion volume was located in the SMA proper, 8.5% in the pre-SMA, 14.4% in the anterior cingulate cortex, 10.0% in the MC, 6.5% in the genu, and 5.8% in the body of the corpus callosum. The remaining 50% of the lesion volume affected various other frontal brain regions. The calculation of the set difference of $A \cap B \setminus C$ revealed a total lesion volume of 9,394 voxels (total lesion volume: 9.4 ml). 8.1% of the lesion volume was found in the SMA proper, 25.2% in the pre-SMA, 13.1% in the midcingulate cortex, and 3.0% in the body of the corpus callosum. The set difference of $A \setminus B \cup C$ encompassed 2,447 voxels (total lesion volume 2.4 ml). 0.6% of the lesion volume was located in the SMA proper, 32.3% in the pre-SMA, and 20.8% in the midcingulate cortex (**Table 4**; **Figure 3**). In between comparison showed that group A had the highest percentage of lesion load within the pre-SMA while the corpus callosum was not affected.

The Bereitschaftspotential (Readiness Potential)

The BP was recorded in two patients (P2 and P3). Both of them had a left-sided ACA infarct involving a large section of the vascular territory. Accordingly, the BP could not be detected over the contralateral hemisphere (corresponding to the electrodes C3) while performing finger movements with the affected right hand. Interestingly, a BP could not be recorded over the right hemisphere either (C4), when they performed the same task with the clinically unaffected left hand. The patients' recordings are shown in **Figure 4** (plotted against a grand average of BP recordings from 13 healthy controls).

Illustrative Cases Reflecting the Spectrum of Disturbed Motor Control in ACA Strokes

Of all cases, P1 (female, 83 years) with an ischemic lesion of the entire ACA territory, including the genu corpus callosum showed the most severe form of an intermanual conflict and exaggerated grasping. In the subacute stage, she was unable to perform bimanual tasks, e.g., putting on her glasses, as the affected limb counteracted the unaffected one. Moving objects in the nearby visual field led to compulsive grasping (magnetic hand) (**Figure 5**). After

TABLE 3 |Anatomical details on the summation maps.

	Total lesion volume			SMC			ACC			MCC			PCC			CC (genu)			CC body																		
				Center of gravity (SMap)			Volume (pre-SMA)			Center of gravity			Volume			Center of gravity			Volume			Center of gravity															
	x	y	z	Voxels	%	x	y	z	Voxels	%	x	y	z	Voxels	%	x	y	z	Voxels	%	x	y	z	Voxels	%												
AHS-specific																																					
SM	-13	16	27	104,842	100	-9	-1	47	3,577	3.4	6,845	6.5	-6	2	32	10,654	10.2	-8	1	43	11,384	10.9	-7	-40	34	463	0.4	-14	20	26	583	0.6	-14	-2	38	707	0.7
Grasping	-10	10	34	101,672	100	-10	-2	50	4036	4.0	7,852	7.5	-4	25	22	10,739	10.2	-9	-3	50	8,481	8.1	na	na	0	0	-4	28	12	583	0.6	-14	2	36	526	0.5	
IMC	-3	-3	34	47,705	100	-10	-17	54	4,330	9.1	3,734	3.6	-5	8	32	4,270	4.1	-5	-3	40	6,962	6.6	na	na	0	0	-6	22	18	276	0.3	-14	2	36	387	0.4	
Non-specific AHS																																					
Perseveration	-11	9	36	109,169	100	-6	1	47	3,910	3.6	6,864	6.5	-6	7	32	10,684	10.2	-7	-1	43	12,748	12.2	-7	-40	34	462	0.4	-14	20	26	583	0.6	-12	-4	36	708	0.7
MM	-7	0	42	35,929	100	-5	1	50	3,691	10.3	6,193	5.9	-3	14	32	1,166	1.1	-6	0	39	5,475	5.2	na	na	0	0	-6	24	16	20	0	-16	-2	38	119	0.1	
Tremor	-10	10	34	81,362	100	-10	-2	50	3,026	3.7	4,834	4.6	-4	25	22	10,737	10.2	-9	-3	50	6,659	6.4	na	na	6,659	6.4	-4	28	12	583	0.6	-14	2	36	502	0.5	
Other signs																																					
Gait apraxia	-3	-2	27	127,473	100	-9	-1	47	2,600	2.0	4,443	4.2	-7	5	32	10,920	10.4	-8	1	43	10,292	9.8	-7	-40	34	485	0.5	-14	20	26	583	0.6	-12	2	34	744	0.7

Group analysis ($N = 10$). The table shows the location of the summation maps with regard to the involvement of various brain regions of interest. The proportion of the total lesion volume is shown for each brain regions (in voxels and in percentage of the total lesion volume). Furthermore, the center of gravity is provided for each brain regions. The coordinates (x,y,z) refer to the MNI standard space where all images have been normalized to. ACC, anterior cingulate cortex; CC, corpus callosum; IMC, intermanual conflict; MCC, midcingulate cortex; PCC, posterior cingulate cortex; SMC, supplementary motor area; SMA, supplementary motor cortex; SM/C, supplementary motor cortex.

discharge from the hospital, she deteriorated progressively due to her deficient awareness of motor intention and self-agency. Therefore, she became severely impaired in managing her day-to-day life.

P2 (male, 82 years) with a large stroke in the left ACA territory did not show any volitional movements of the left upper limb on clinical testing in the acute phase. However, when he was explicitly asked to grasp the examiner's hand, he was able to develop full strengths in keeping with a severe motor neglect. He also suffered from mildly exaggerated grasping. Intriguingly, he was not aware of preserved motor function and strength of the upper limb before. At the follow-up 5 months later, apart from a slight intermanual conflict, the motor neglect had resolved and motor control of the affected limb had recovered almost completely. He was fully independent in his daily life.

P5 (male, aged 63 years) experienced bilateral ACA infarcts and accordingly showed the most severe clinical syndrome of all our cases. The onset of his neurological symptoms and clinical course during the first 3 months after admission remain unclear, as he was found after lying on the floor for 1 day and signs of disturbed motor control were not sufficiently documented by the referring hospital. We saw him 3 months after stroke onset where he presented with akinetic mutism, an almost complete inability to perform volitional hand movement despite preserved limb strength, abasia, severely disturbed initiation of volitional movements, exaggerated grasping, motor perseveration, and mild bilateral action tremor.

Of all cases, P7 (male, 69 years) showed the mildest symptoms. He experienced a sudden feeling of a not obeying hand on the right side. These symptoms persisted for a few hours, but quickly improved thereafter. In the subacute stage, he had markedly improved but still showed a slight intermanual conflict and had slight problems with writing. The MRI revealed a small ischemic lesion within the left SMA.

Discussion

In our case series, which is one of the largest imaging-based studies in AHS patients, we found a wide spectrum of clinical signs related to disturbed motor control ranging from very mild presentations with only transient impairment to very severely affected cases. Some of these symptoms, which we observed in our patients, have been well described in previous papers on AHS (7, 10, 13, 15–17, 19–31), while features such as mirror movements, motor perseveration, or the occurrence of an action tremor have so far not been reported in association with ACA strokes. In this case series, we were confronted with the interesting phenomenon of an initially apparent paresis/plegia of the upper extremity with significant recovery of muscle strength over the subsequent few weeks. The recovery curve of muscle strength, however, was more rapid and more favorable than it would have been expected in patients with weakness due to a corticospinal tract lesion. This observation points to alternative explanations of impaired upper limb function such as a severe motor neglect in the acute stage. The first signs of motor recovery in our patients were movements triggered by external stimuli such as touching their hand. Interestingly, patients were neither aware of the underlying

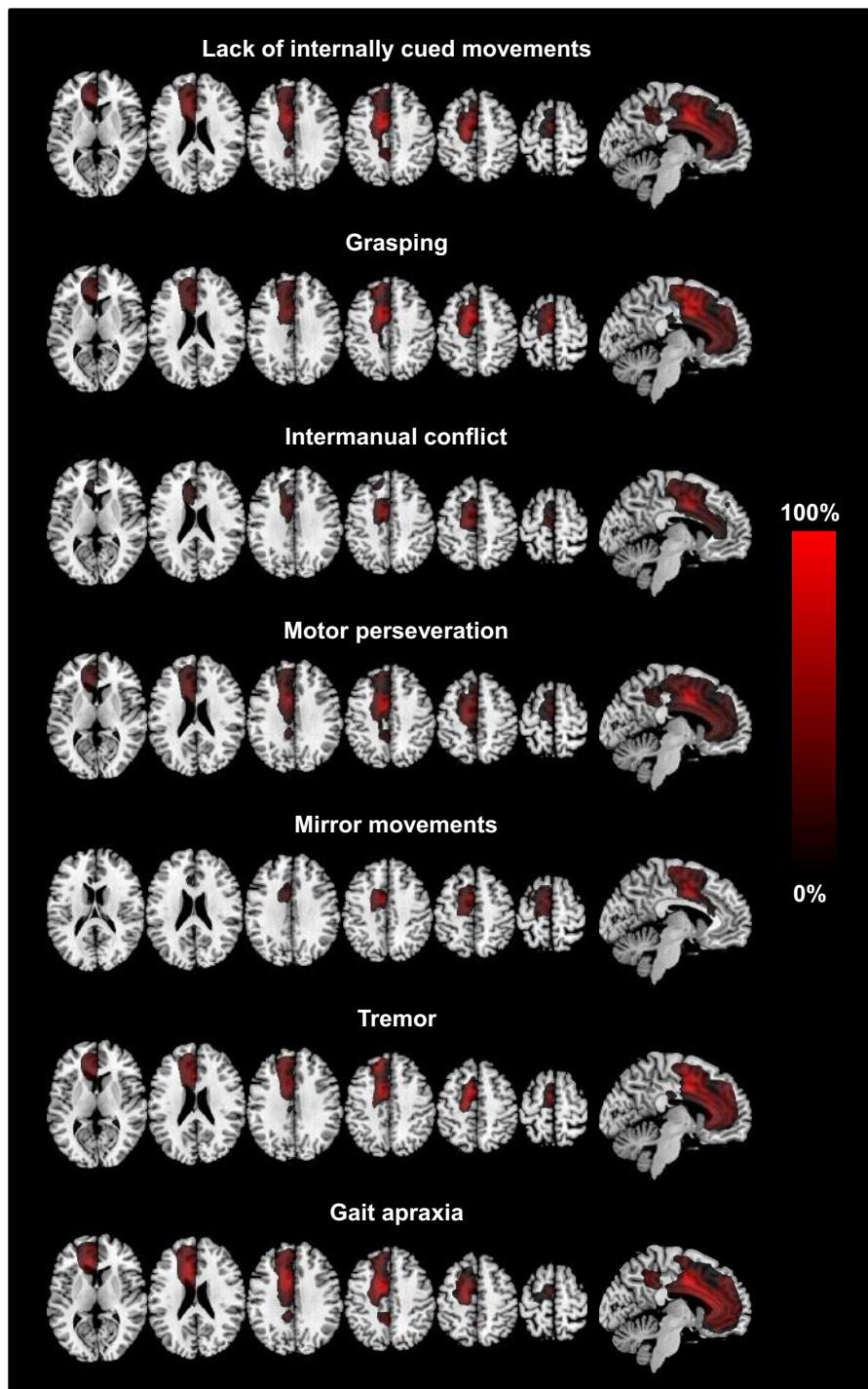


FIGURE 1 | The figure shows axial slices and a sagittal slice of a T1-standard MRI scan with the superimposed summation lesion maps for each clinical sign. Each lesion map is thresholded at voxels damaged in >25% of patients showing the respective clinical sign. The legend (provided in percentages) refers to the total number of patients showing the respective clinical sign (MNI-coordinates: $z = 8, 23, 33, 43, 53, 63$ and $x = -6$, respectively).

motor intention nor of the self-agency of their movements, thus suggesting a full-blown motor form of AHS (15, 20, 24). The dissociation between self-initiated and externally triggered movements is essential, because they are largely dependent on the medial motor system supplied by the ACA, whereas the latter

mainly rely on the lateral premotor system (supplied by the MCA) (37). A few patients presented with mild or only transient signs of AHS, as reflected by disturbed motor awareness in the acute, but not in the chronic phase. However, at the follow-up they still showed some signs of disturbed motor control as seen in the more

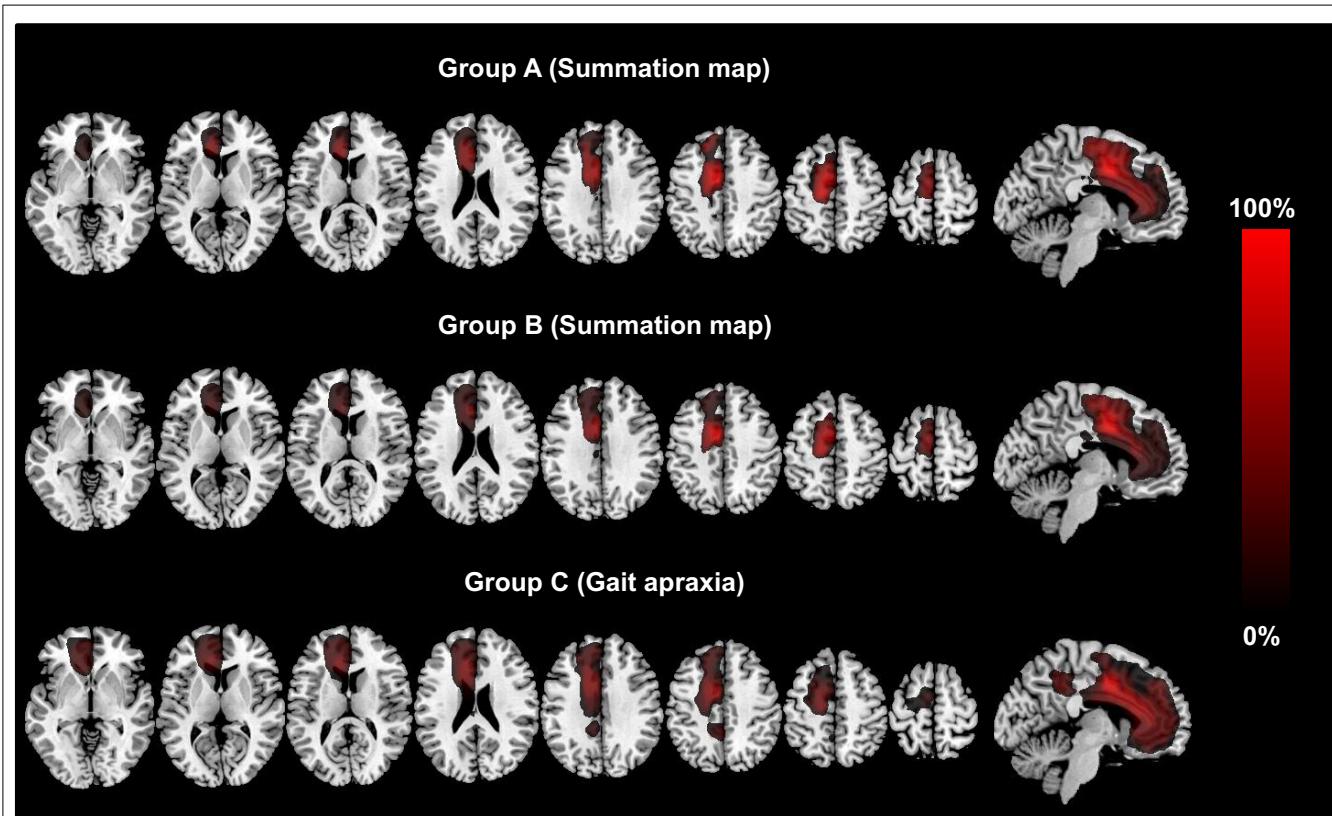


FIGURE 2 | The figure shows the lesion summation maps for group A, which included the lesion maps for all AHS-specific symptoms (grasping, intermanual conflict, impaired self-initiated movements). Group B encompasses all lesion maps for non-AHS-specific motor symptoms (motor perseveration, mirror movements, tremor). Group C (other signs) corresponds to the lesion map for gait apraxia. The legend encodes the percentage of all patients' signs. The lesion maps are thresholded to voxels damaged in at least 25% of patients' signs and are plotted on axial and sagittal slices of standard T1 MRI scan (MNI-coordinates: $z = -2, 8, 13, 23, 33, 43, 53, 63$ and $x = -6$, respectively).

TABLE 4 | Percentage of lesion on regions of interest related to AHS associated and specific symptoms.

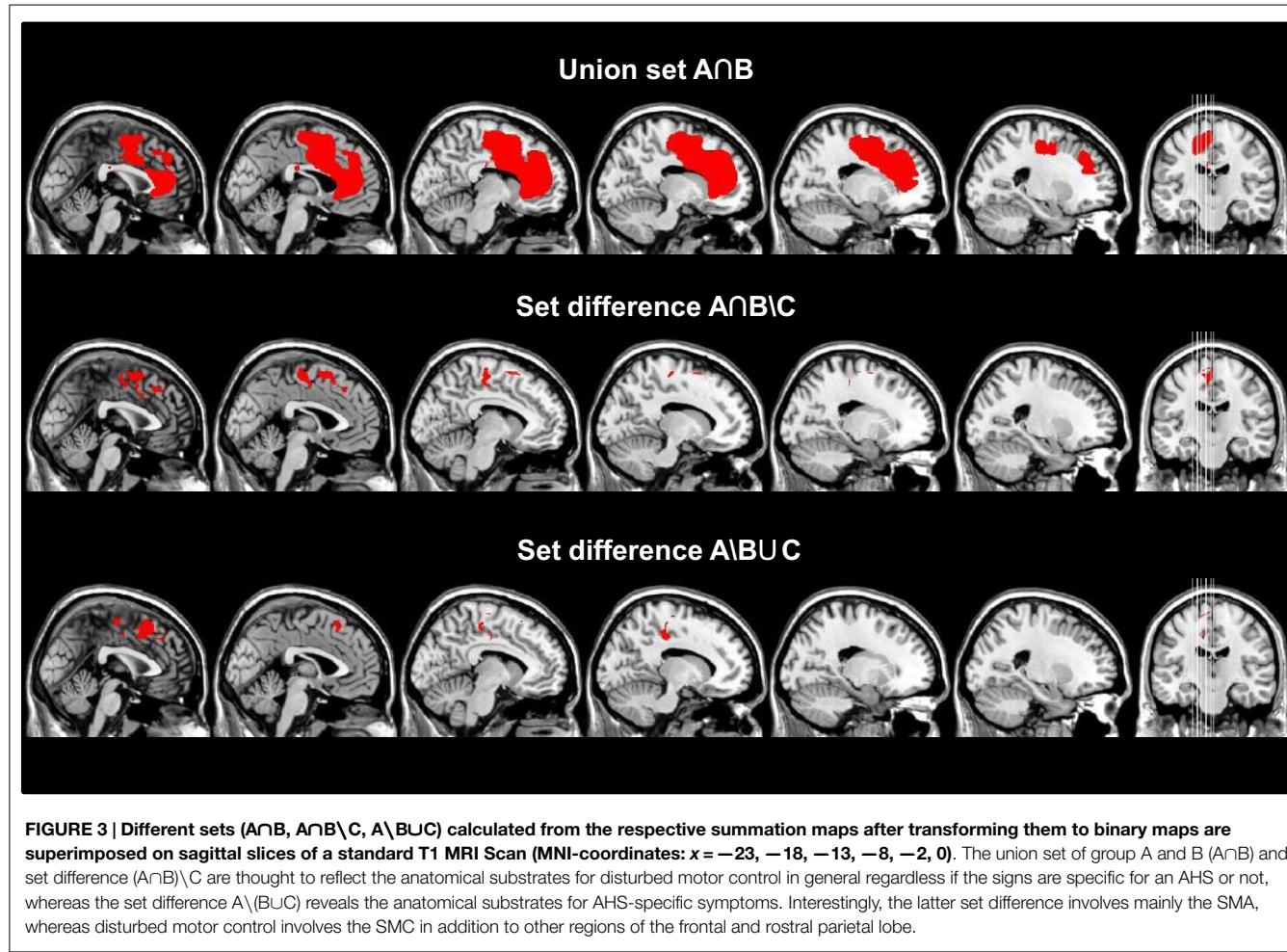
	Total lesion volume		SMC				ACC		MCC		PCC		CC (genu)		CC (body)	
	Volume		Volume (SMAp)		Volume (pre-SMA)		Volume		Volume		Volume		Volume		Volume	
	Voxels	%	Voxels	%	Voxels	%	Voxels	%	Voxels	%	Voxels	%	Voxels	%	Voxels	%
A \cap B	66,132	100	3,162	4.8	5,599	8.5	9,551	14.4	6,622	10.0	0	0.0	4,317	6.5	3,811	5.8
A \cap B\C	9,394	100	763	8.1	2,364	25.2	0	0.0	1,232	13.1	0	0.0	0	0.0	279	3.0
A\B\U\C	2,447	100	14	0.6	791	32.3	0	0.0	505	20.6	0	0.0	0	0.0	0	0.0

The table shows the location of the intersections with regard to the involvement of various brain regions of interest. The proportion of the total lesion volume is shown for each brain regions (in voxels and in percentage of the total lesion volume). A, symptom group A; AHS, alien hand syndrome; ACC, anterior cingulate cortex; B, symptom group B; C, symptom group C; CC, corpus callosum; MCC, midcingulate cortex; n.a., not applicable; PCC posterior cingulate cortex; SMA, supplementary motor area; SMap, SMA proper; SMC, supplementary motor cortex.

severely affected cases, though to a much milder degree. This underscores the notion that the presentation of an AHS has a wide clinical spectrum.

Moreover, we were interested whether clinical signs of AHS (as defined as lack of conscious awareness of intention and the sense of agency) and non-AHS-specific signs, commonly observed in association with AHS, are caused by different lesion patterns. We could demonstrate that both AHS-specific and non-AHS-specific

signs trace back to lesions within the SMC, and the anterior and medial cingulate cortex. This result was not entirely unexpected due to the important role of these brain areas in voluntary motor control (i.e., self-initiated movements and suppression of externally triggered motor subroutines) (1, 2). Our results are in line with a previously published retrospective analysis of 100 ACA strokes, which showed that motor disturbances were by far the most common signs (17).



A main finding of the present study is the predominant involvement of the pre-SMA in AHS-specific signs as shown by the approach with different set differences. This is a novel finding for ACA infarcts, but consistent with results from fMRI studies in healthy persons showing greater activations in rostral parts of the SMA after self-initiated movements (37). The pre-SMA, projects both to the lateral premotor cortex and the caudal parts of the SMA (38), although latter is not considered to play a major role in movement preparation as its projections descend directly through the pyramidal tract (39, 40). Gait apraxia was selected as a “reference”-clinical sign not associated with AHS and not affecting the upper extremities, but known to occur in lesions involving the medial frontal lobe. In line with this, gait apraxia was associated with lesions affecting the cingulate cortex in our study (21).

Previous studies of clinical-anatomical correlation in ACA stroke patients were biased mostly because of the approach with semi-quantitative analyses of predefined regions of interest. In the work of Chang and colleagues, AHS was associated with a combined involvement of the medial frontal lobe and the corpus callosum. An isolated or predominant affection of the cingulate cortex was found to result in an intermanual conflict, while medial frontal lesions were more likely to present with grasping behavior (10, 16). More recently, Sarva and colleagues published a systematic review of the literature on AHS (7). They

concordantly found that the SMC, cingulate cortex and corpus callosum were the most commonly affected structures in the “frontal” AHS variant. Predominant involvement of hemispheric structures more frequently led to involuntary grasping and groping behavior, whereas an intermanual conflict was the most frequent clinical sign in callosal lesions. Our findings, however, do not favor the same relevance of the corpus callosum for clinical signs of AHS as suggested by these authors.

There were some clinical signs, which have not yet been described as common signs in ACA strokes. Mirror movements are usually seen in early childhood due to mutations in the DCC and RAD51 genes (41), although they may sometimes also occur in patients with basal ganglia disorders and strokes, mainly of the corona radiata. However, they have rarely been described in association with ACA strokes (26, 42). Functional MRI revealed that mirror movements are paralleled by bilateral activation of M1 and the SMA (25). Mirror movements probably occur due to an insufficient interhemispheric inhibition of the motor cortex located ipsilateral to the moved limb by a network, which connects the SMC, dorsolateral PFC, and M1 (43). The unilateral tremor of the affected hand we observed in some patients also deserves further consideration. It occurred in all patients as a new clinical sign with a latency of a few weeks after stroke. To our best knowledge, there are hardly any comparable reports of

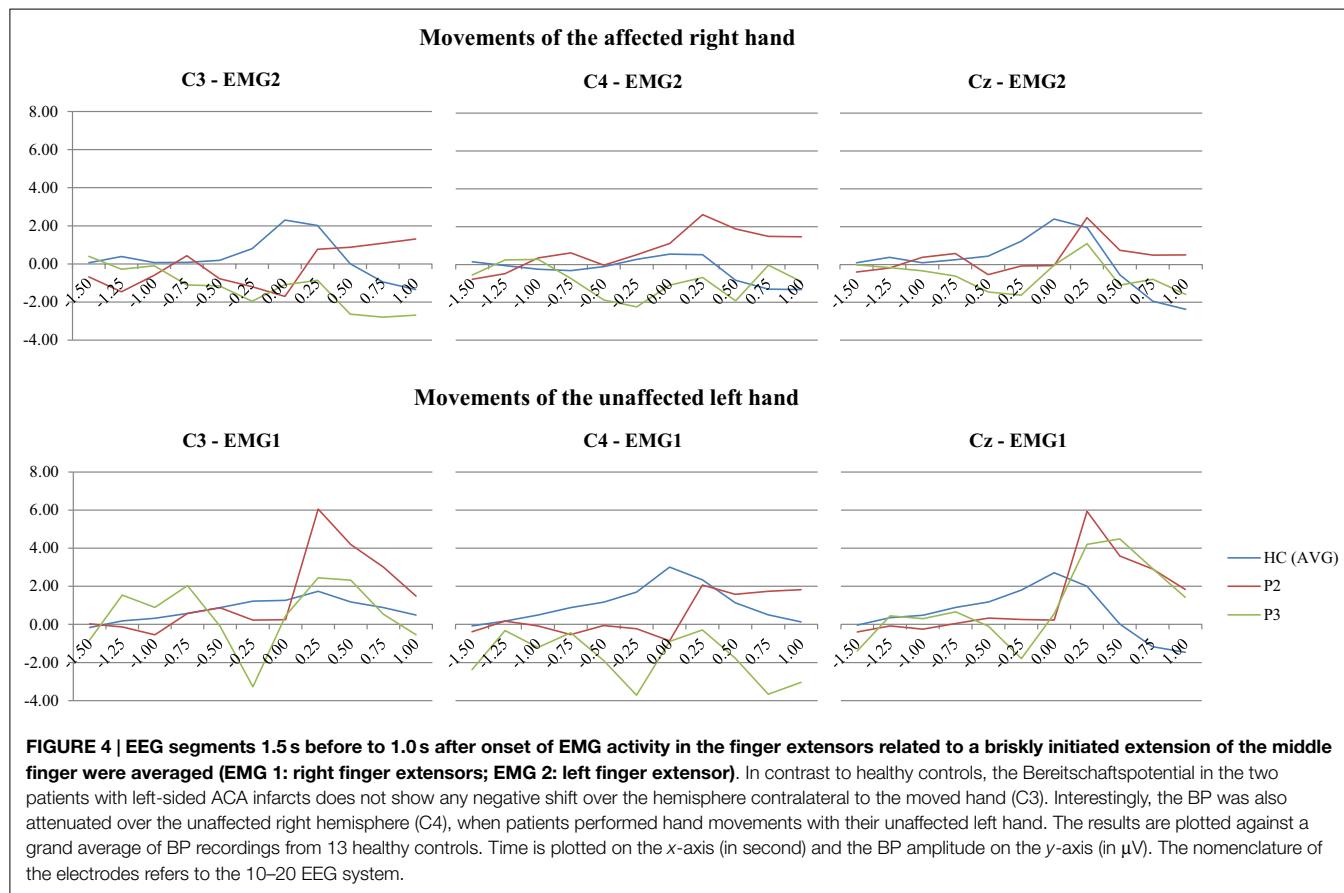


FIGURE 4 | EEG segments 1.5 s before to 1.0 s after onset of EMG activity in the finger extensors related to a briskly initiated extension of the middle finger were averaged (EMG 1: right finger extensors; EMG 2: left finger extensor). In contrast to healthy controls, the Bereitschaftspotential in the two patients with left-sided ACA infarcts does not show any negative shift over the hemisphere contralateral to the moved hand (C3). Interestingly, the BP was also attenuated over the unaffected right hemisphere (C4), when patients performed hand movements with their unaffected left hand. The results are plotted against a grand average of BP recordings from 13 healthy controls. Time is plotted on the x-axis (in second) and the BP amplitude on the y-axis (in μ V). The nomenclature of the electrodes refers to the 10–20 EEG system.



FIGURE 5 | The series of images illustrates the compulsive grasping behavior in P2. The patient was asked to avoid grasping the examiner's hand. However, the patient could not inhibit grasping. After she had taken the examiner's hand, she could not release it without support by her left hand.

a hand tremor associated with ACA ischemia in the literature (25, 31). Stroke-associated tremor has mainly been reported in lesions of the thalamus, and the striatonigral, cerebello-thalamic or dentatorubrothalamic pathways (44). Clinically, the observed tremor resembles that of a dystonic tremor with a strong tendency to occur during action (45). In line with one previous report, the tremor was mostly seen just transiently (31). An association of the tremor with SMA and cingulate cortex lesions is of interest because an abnormal overactivity of these brain regions was found in an fMRI study in essential tremor (46). Our observations may thus suggest that an impaired function of the SMC or cingulate

cortex may play an important role in the generation or suppression of pathological oscillatory network activity.

Our findings underpin the crucial role of lesions involving the SMC and cingulate cortex for disturbed motor control after ACA infarcts. Error detection and conflict monitoring have previously been attributed to the anterior cingulate cortex (2). The SMC, in turn, is more important for the generation of self-initiated movements, generation of complex motor tasks and the suppression of stimulus-driven, though, purposeless movements (i.e., grasping) (1). In this context, the BP is also of interest since it presumably originates from the SMC and reflects cognitive motor control

prior to voluntary movements (3). So far, there are only two case reports of ACA strokes, which included BP recordings. In line with our findings, the BP following movements of the affected hand was also attenuated there (28–30, 47). Notably, the BP was also attenuated in our patients when they performed finger movements with their non-affected hand. This might indicate disturbed interhemispheric activation following a unilateral SMC lesion.

This analysis has several limitations. Due to its design as in parts retrospective case series, follow-ups were not standardized and patients were seen at different latencies after their strokes. Therefore, transient neurological signs may have been missed. Furthermore, this lack of standardized time intervals between the assessment in the acute stage and the follow-up assessments does not allow drawing definite conclusions to the clinical and functional outcome of these patients. A limitation of our clinical approach is the fact that it has not been validated elsewhere and there are no validated clinical scores for AHS symptoms in the literature. Therefore, we invented a semi-quantitative rating for our case series, which was proven here to have a very good IRR. Furthermore, the fact that just two patients underwent BP recordings does not allow to draw final conclusions on the BP in ACA strokes, since this potential is quite variable despite optimal recording settings (28). We acknowledge that a larger number of patients would also have increased the statistical power here. Moreover, our imaging results may have been flawed because of the different imaging methods used. A CT scan yields a different image of the brain in terms of contrast and distortion as an MRI scan. We attempted to overcome this concern making use of validated CT and MRI templates for spatial normalization (43). Nonetheless, we

decided to include the two ACA stroke patients with CT scans, because otherwise we would have abolished two clinically very interesting patients from our series. A theoretical issue is the definition of an adequate threshold to delineate damaged brain voxels for lesion analysis. In order to avoid false negative results in this small patient cohort, we went for a rather low threshold of more than 25% of individuals to explore the defined motor signs by voxel-based symptom lesion mapping (35). Furthermore, we determined lesion location as the basis of anatomical probability atlases, which do not account for individual variability of neuroanatomy.

Conclusion

In summary, AHS is primarily characterized by disturbed conscious awareness of intention and a deficient sense of agency for voluntary hand movement. This is reflected by the inability to carry out self-initiated movements while externally cued movements are neither suppressed nor perceived by the subjects. Common motor signs following ACA strokes are disturbed self-initiated movements, grasping and groping behavior, intermanual conflict, motor perseveration, mirror movements, and coarse tremor. Their occurrence is mainly associated with lesions of SMC, as well as the anterior and midcingulate cortex. The motor signs specifically related to AHS, i.e., disturbed self-initiated movements, grasping and intermanual conflict, are mainly related to lesions of the pre-SMA and MCC. To date, little is known about the clinical course and long-term outcome of these patients or about the best approach on how to rehabilitate these patients. Therefore, further studies in these patients are warranted.

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Multi-modal imaging of neural correlates of motor speed performance in the trail making test

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The assessment of motor and executive functions following stroke or traumatic brain injury is a key aspect of impairment evaluation and used to guide further therapy. In clinical routine, such assessments are largely dominated by pen-and-paper tests. While these provide standardized, reliable, and ecologically valid measures of the individual level of functioning, rather little is yet known about their neurobiological underpinnings. Therefore, the aim of this study was to investigate brain regions and their associated networks that are related to upper extremity motor function, as quantified by the motor speed subtest of the trail making test (TMT-MS). Whole-brain voxel-based morphometry and whole-brain tract-based spatial statistics were used to investigate the association between TMT-MS performance with gray-matter volume (GMV) and white-matter integrity, respectively. While results demonstrated no relationship to local white-matter properties, we found a significant correlation between TMT-MS performance and GMV of the lower bank of the inferior frontal sulcus, a region associated with cognitive processing, as indicated by assessing its functional profile by the BrainMap database. Using this finding as a seed region, we further examined and compared networks as reflected by resting state connectivity, meta-analytic connectivity modeling, structural covariance, and probabilistic tractography. While differences between the different approaches were observed, all approaches converged on a network comprising regions that overlap with the multiple-demand network. Our data therefore indicate that performance may primarily depend on executive function, thus suggesting that motor speed in a more naturalistic setting should be more associated with executive rather than primary motor function. Moreover, results showed that while there were differences between the approaches, a convergence indicated that common networks can be revealed across highly divergent methods.

Keywords: trail-making test, motor speed, inferior frontal sulcus, voxel-based morphometry, resting state fMRI, meta-analytic connectivity modeling, structural covariance, probabilistic tractography

INTRODUCTION

Hand motor deficits are among the most common impairments following stroke (1). As a result, post-stroke assessment of motor functions is a key aspect of patient evaluation and is used to guide further therapy. In addition to fast but typically qualitative clinical assessments, this often involves neuropsychological tests of coordinated hand function. In practice, such assessments are still largely dominated by pen-and-paper tests. One example of such a simple pen-and-paper test is the motor speed subtest of the trail-making test (TMT-MS) from the Delis–Kaplan executive function system [D–KEFS; (2)]. This test measures the time that subjects take to manually trace a pre-specified trail. The TMT-MS requires the examinee to connect circles by following a dotted line, and aims to serve as a baseline measure of the motor component that should be shared by the other portions of the test. The results should thus provide information about the extent to which difficulty on the other TMT subtests probing higher, executive functions may be related to a motor deficit. However, the results of the TMT-MS cannot only be used as a baseline for other TMT subtests, but also provide information of drawing speed *per se*, and thus can be used by clinicians as an assessment of upper extremity motor function (2).

Pen-and-paper tests such as the TMT provide standardized and reliable valid measures of the individual level of functioning; however, rather little is yet known about their neurobiological underpinnings. Therefore, one aim of the current study is to investigate brain–behavior relationships with regard to upper extremity motor function, as quantified by the TMT-MS from the D–KEFS. Additionally, previous studies have demonstrated that while the brain can be subdivided into distinct modules based on functional and microstructural properties [reviewed in Ref. (3)], processes such as motor function are likely to involve the efficient integration of information across a number of such specialized regions. Due to this integrative nature of the brain, most higher mental functions are likely implemented as distributed networks (4), and it has therefore been suggested that an understanding of how a brain region subserves a specific task should require information regarding its interaction with other brain regions (3). Therefore, the current study additionally aims to investigate the networks associated with the regions we find to be related to TMT-MS performance.

A number of different approaches can be employed to investigate networks associated with a particular brain region. Task-free (seed-based) resting-state functional connectivity (RS-FC) refers to temporal correlations of a seed region with spatially distinct brain regions, when no task is presented (5, 6). Meta-analytic connectivity modeling (MACM) (7–9) investigates co-activation patterns between a seed region and the rest of the brain, by calculation of meta-analyses across many task-based fMRI experiments and paradigms stored in, e.g., the BrainMap database (10, 11). Structural covariance (SC) is based on the correlation patterns across a population of gray-matter characteristics such as volume or thickness (12, 13) that are thought to reflect shared mutational, genetic, and functional interaction effects of the regions involved (14, 15). While having conceptual differences, these three modalities all share

the goal of delineating regions that interact functionally with a particular seed region. By contrast, probabilistic tractography (PT) focuses on white-matter anatomical connectivity obtained from diffusion-weighted images (DWI) by producing a measure of the likelihood that two regions are structurally connected (16, 17). Previous studies have reported convergence between RS and MACM (18–20), between RS and SC (21, 22), RS and fiber tracking (23–26), and between RS, MACM, and SC (27, 28). However, striking differences among the different connectivity approaches have also been found (26, 27).

In this study, we first used whole-brain voxel-based morphometry [VBM; (29)] and whole-brain tract-based spatial statistics [TBSS; (30)] to investigate the association between TMT-MS performance with gray-matter volume (GMV) and white-matter integrity, respectively. Using the result of these initial analyses as the seed region of interest, we further examined and systematically compared networks obtained through RS-fMRI, MACM, SC, and PT. The aim of these analyses was twofold. First, we sought to explore the relationship of brain morphology to a simple measure of hand motor function. Second, we aimed to characterize both the divergence and convergence of four unique approaches to quantifying brain connectivity.

MATERIALS AND METHODS

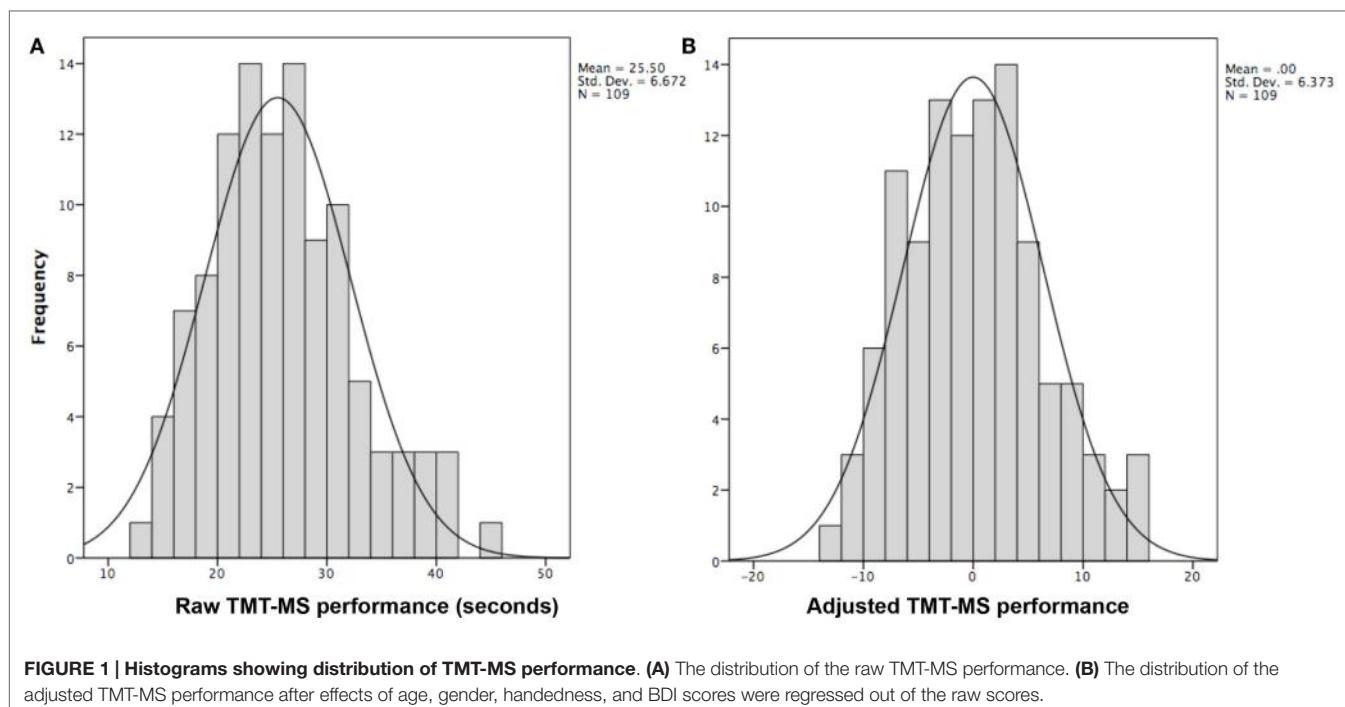
Subjects

Data from the Enhanced Nathan Kline Institute – Rockland Sample¹ (31) was used for all analyses except for meta-analytical connectivity modeling and functional characterization (where the BrainMap database was used). From this cohort, we used anatomical, RS, and DWI of subjects that had completed the TMT-MS, no current psychiatric diagnosis, a Beck depression inventory score (BDI) of less than 14 and did not exceed 3 SDs from the population mean. This resulted in a sample of 109 right-handed healthy volunteers between 18 and 75 years of age (mean age 40.39 ± 15.49 ; 37 males). First, effects of age, gender, handedness, and BDI score as known influences on hand motor speed (32, 33) were regressed out of the raw TMT-MS performance score (Figure 1A; Table 1). This resulted in an adjusted performance score, which indicated how much better or worse a subject performed than would be expected given these confounding factors (Figure 1B). The association of these adjusted scores with local GMV and white-matter integrity was then tested by carrying out whole-brain VBM and TBSS, respectively.

Delis–Kaplan Executive Function System: Trail-Making Test – Motor Speed

The Delis–Kaplan executive function system: trail-making test (D–KEFS TMT) consists of five different conditions (2). For the current study, we were exclusively interested in the TMT-MS, which requires participants to trace over a dotted line as quickly as possible while making sure that the line drawn touches every circle along the path. In particular, the participant is prompted to

¹http://fcon_1000.projects.nitrc.org/indi/enhanced



focus on speed rather than neatness but has to make sure that the line touches every circle along the path. If the line departs from the dotted line or is not correctly connected to the next circle, the participant is stopped immediately and redirected to the dotted line while keeping the stopwatch running. The scoring measure is the time (in seconds) that the participant needs to complete the task.

Relationship Between TMT-MS Performance and Gray-Matter Volume Whole-Brain VBM Analysis

The association between regional GMV and individual performance (adjusted for the potentially confounding effects of age, gender, handedness, and BDI), was investigated by performing a whole-brain VBM analysis. This analysis used the anatomical T1-weighted images of the 109 subjects described above. These scans were acquired in sagittal orientation on a Siemens TimTrio 3T scanner using an MP-RAGE sequence (TR = 1900 ms, TE = 2.52 ms, TI = 900 ms, flip angle = 9°, FOV = 250 mm, 176 slices, voxel size = 1 mm × 1 mm × 1 mm). Images were preprocessed using the VBM8 toolbox in SPM8 using standard settings, namely spatial normalization to register the individual images to ICBM-152 template space, and segmentation, wherein the different tissue types within the images are classified. The resulting normalized gray-matter segments, modulated only for the non-linear components of the deformations into standard space, were then smoothed using an 8 mm isotropic full-width-half-maximum (FWHM) kernel, and finally assessed for significant correlation between GMV and the adjusted TMT-MS performance scores. Age, gender, BDI scores, and Edinburgh handedness inventory (EHI) scores were used as covariates together with the adjusted

TMT-MS performance scores, leading to an analysis of partial correlations between GMV and TMT-MS. As we modulated the gray-matter probability maps by the non-linear components only to represent the absolute amount of tissue corrected for individual brain size, we did not include total brain volume as an additional covariate in the analysis. That is, given that the correction for inter-individual differences in brain volume was applied directly to the data it was not performed (a second time) as part of the statistical model. Statistical significance using non-parametric permutation inference was assessed at $p < 0.05$ [family-wise error (FWE) corrected for multiple comparisons].

Whole-Brain TBSS Analysis

A TBSS whole-brain analysis was performed to investigate the association between white-matter volume and adjusted TMT-MS performance. DWI from the same group of 109 volunteers acquired on a 3T TimTrio Siemens scanner (137 directions, $b = 1,500 \text{ s/mm}^2$) were used. Preprocessing was performed according to standard protocols using FSL². The DWI data were first corrected for head-motion and eddy-current effects of the diffusion gradients. The b0 images were averaged and skull-stripped using BET (34) to create the analysis mask. Within this mask, a simple diffusion-tensor model was estimated for each voxel. Finally, non-linear deformation fields between the diffusion space and the ICBM-152 reference space were computed using FSL's linear (FLIRT) (35, 36), and non-linear (FNIRT) image registration tools (37). These allow mapping between the individual (native) diffusion space and the ICBM-152 reference space; i.e., the same space

²www.fmrib.ox.ac.uk/fsl

TABLE 1 | Characteristics of the cohort.

Age	Gender	BDI	EHI	Age	Gender	BDI	EHI
26	Male	4	80	41	Male	1	70
20	Male	0	95	26	Female	7	75
53	Male	0	55	51	Female	5	75
48	Female	9	100	61	Female	0	80
62	Female	5	90	58	Male	5	80
18	Female	7	75	56	Female	0	65
54	Female	0	95	54	Female	4	95
18	Female	1	90	27	Male	5	60
21	Male	4	85	42	Female	9	70
62	Female	1	100	31	Female	7	100
53	Male	3	75	21	Female	1	100
22	Male	4	90	18	Male	3	90
62	Female	12	100	48	Female	3	85
54	Female	0	95	20	Female	5	55
24	Female	1	85	60	Female	1	100
44	Female	8	90	20	Female	1	90
57	Female	2	95	50	Female	2	90
44	Female	3	70	62	Male	7	70
51	Male	7	70	18	Male	2	85
63	Female	0	80	57	Female	1	100
26	Female	1	60	24	Female	0	95
59	Male	4	95	26	Female	0	80
30	Male	0	85	57	Female	5	85
50	Female	1	90	19	Male	2	70
26	Female	2	75	49	Male	0	60
18	Male	0	80	23	Female	2	85
24	Female	10	95	58	Female	5	55
64	Female	0	95	55	Male	4	80
47	Male	4	100	41	Female	5	100
38	Female	0	80	41	Female	0	100
23	Female	1	70	25	Female	2	75
42	Female	8	85	49	Female	0	90
59	Female	2	100	49	Female	1	100
26	Male	5	100	21	Female	6	75
18	Male	3	90	50	Male	1	85
19	Male	1	100	19	Male	3	65
27	Female	12	60	59	Male	3	85
20	Female	3	100	41	Male	0	80
56	Female	5	100	44	Male	13	100
18	Male	4	85	20	Female	13	85
30	Male	4	55	47	Male	5	90
58	Female	6	95	21	Male	2	55
52	Female	3	85	47	Female	7	55
38	Male	1	65	55	Female	1	90
64	Male	5	80	23	Female	13	100
41	Female	2	100	61	Male	1	80
49	Female	5	60	52	Female	0	100
57	Female	8	60	20	Male	10	60
40	Female	3	80	51	Female	0	65
48	Female	0	100	42	Female	0	100
36	Female	1	100	21	Female	0	80
20	Male	5	90	36	Female	8	100
60	Female	3	75	43	Female	9	85
59	Male	2	85	43	Female	5	95
52	Female	8	100				

to which also the VBM and RS (as described below) data are also registered. The FA images were hereby normalized into standard space and then merged to produce a mean FA image. This was in turn used to generate a skeleton representing all fiber tracts common to all subjects included in the study (30, 38). The maximal FA scores of each individual FA image were then projected onto

the mean FA skeleton. This projection aims to resolve any residual alignment problems after the initial non-linear registration (38). The resulting skeleton was then used to perform a multi-covariate analysis, using age, gender, BDI scores, EHI scores, and TMT-MS scores. Statistical significance using non-parametric permutation inference was again assessed at $p < 0.05$ multiple comparisons.

Seed Definition and Functional Characterization

The regions revealed by the initial VBM analysis were functionally characterized based on the behavioral domain meta-data from the BrainMap database³ (10, 11, 39), using both forward and reverse inference, as performed in previous studies (40, 41). Behavioral domains, which have been grouped for the purpose of the database, describe the cognitive processes probed by an experiment. Forward inference is the probability of observing activity in a brain region, given knowledge of the psychological process; whereas reverse inference is the probability of a psychological process being present, given knowledge of activation in a particular brain region. The results of both the forward and reverse inferences will be defined by the number and frequency of tasks in the database. In the forward inference approach, the functional profile was determined by identifying taxonomic labels for which the probability of finding activation in the respective region/set of regions was significantly higher than the overall (*a priori*) chance across the entire database. That is, we tested whether the conditional probability of activation given a particular label [$P(\text{Activation}|\text{Task})$] was higher than the baseline probability of activating the region(s) in question *per se* [$P(\text{Activation})$]. Significance was established using a binomial test [$p < 0.05$, corrected for multiple comparisons using false discovery rate (FDR)]. In the reverse inference approach, the functional profile was determined by identifying the most likely behavioral domains, given activation in a particular region/set of regions. This likelihood $P(\text{Task}|\text{Activation})$ can be derived from $P(\text{Activation}|\text{Task})$ as well as $P(\text{Task})$ and $P(\text{Activation})$ using Bayes' rule. Significance (at $p < 0.05$, corrected for multiple comparisons using FDR) was then assessed by means of a chi-squared test.

Multi-Modal Connectivity Analyses

Multi-modal connectivity analyses were used to further characterize the results from the initial VBM analysis. In particular, we investigated; (1) RS-FC, inferred through correlations in the blood-oxygen-level-dependent (BOLD) signal obtained during a task-free, endogenously controlled state (5, 6); (2) MACM, revealing co-activation during the performance of external task demands (7, 8); (3) SC, identifying long-term coordination of brain morphology (15); and (4) probabilistic fiber tracking, providing information about anatomical connectivity by measuring the anisotropic diffusion of water in white-matter tracts (16, 17).

All the analyses were approved by the local ethics committee of the Heinrich Heine University Düsseldorf.

Task-Independent Functional Connectivity:

Resting-State

A seed-based RS analysis was used to investigate the task-independent FC of the seed region (5, 6). RS-fMRI images of the 109 subjects described above were used. During the RS acquisition, subjects were instructed to not think about anything in particular but not to fall asleep. Images were acquired on a Siemens TimTrio 3T scanner using BOLD contrast [gradient-echo EPI pulse

sequence, TR = 1.4 s, TE = 30 ms, flip angle = 65°, voxel size = 2.0 mm × 2.0 mm × 2.0 mm, 64 slices (2.00 mm thickness)].

Data were processed using SPM8 (Wellcome Trust Centre for Neuroimaging, London⁴). The first four scans were excluded prior to further analyses and the remaining EPI images were then corrected for head movement by affine registration which involved the alignment to the initial volumes and then to the mean of all volumes. No slice time correction was applied. The mean EPI image for each subject was then spatially normalized to the ICBM-152 reference space by using the "unified segmentation" approach. (42). The resulting deformation was then applied to the individual EPI volumes. Furthermore, the images were smoothed with a 5-mm FWHM Gaussian kernel so as to improve the signal-to-noise ratio and to compensate for residual anatomic variations. The time-series of each voxel were processed as follows: spurious correlations were reduced by excluding variance that could be explained by the following nuisance variables: (i) the six motion parameters derived from the re-alignment of the image; (ii) their first derivatives; (iii) mean gray matter, white matter, and CSF signal. All nuisance variables entered the model as both first- and second-order terms. The data were then band-pass filtered preserving frequencies between 0.01 and 0.08 Hz. The time-course of the seed was extracted for every subject by computing the first eigenvariate of the time-series of all voxel within the seed. This seed time-course was then correlated with the time-series of all the other gray-matter voxels in the brain using linear (Pearson) correlation. The resulting correlation coefficients were transformed into Fisher's z-scores and tested for consistency across subjects by using a second-level ANOVA including age, gender, BDI scores, and EHI scores as covariates of no interest. Results were corrected for multiple comparisons using threshold-free cluster enhancement, a method that has been suggested to improve sensitivity and provide more interpretable output than cluster-based thresholding [TFCE; (43)], and FWE-correction at $p < 0.05$.

Task-Dependent Functional Connectivity: Meta-Analytic Connectivity Modeling

The whole-brain connectivity of the seed was characterized using a task-dependent approach by carrying out MACM. This method looks at FC as defined by task activation from previous fMRI studies and benefits from the fact that a large number of such studies are normally presented in a highly standardized format and stored in large-scale databases (9). Thus, MACM is based on the assessment of brain-wise co-activation patterns of a seed region across a large number of neuroimaging experiment results (7). All experiments that activate the particular seed region are first identified and then used in a quantitative meta-analysis to test for any convergence across all the activation foci reported in these experiments (9). Any significant convergence of reported foci in other brain regions as the seed was considered to indicate consistent co-activation with the seed. For this study, we used the BrainMap database to identify

³<http://www.brainmap.org>

⁴<http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>

studies reporting neural activation within our seed region⁵ (10). A coordinate based meta-analysis was then used to identify consistent co-activations across the experiments identified by using activation likelihood estimation (ALE) (44–46). This algorithm treats the activation foci reported in the experiments as spatial probability distributions rather than single points, and aims at identifying areas that show convergence across experiments. The results were corrected using the same statistical criteria as for the RS imaging data, i.e., using TFCE (43) and FWE-correction at $p < 0.05$.

Structural Covariance

Structural covariance was used to investigate the pattern of cortical gray-matter morphology across the whole brain by measuring the correlations of GMV, obtained through VBM, between different regions. This method assumes that such morphometric correlations carry some information about the structural or functional connectivity between the regions involved (13–15, 21). SC analysis was performed using the GMV estimates obtained from the VBM pipeline, as described above. Following preprocessing of the anatomical images, we first computed the volume of the seed region by integrating the (non-linear) modulated voxel-wise gray-matter probabilities of all voxels of the seed, which was then used as our covariate of interest for the group analysis. A whole-brain general linear model (GLM) analysis was applied using the GMV of the seed, along with the same additional covariates (of no interest) as for the RS-FC analysis. The results were corrected using the same statistical criteria as for the other connectivity modalities, i.e., using TFCE (43) and FWE-correction at $p < 0.05$.

Probabilistic Tractography

Probabilistic tractography was used to investigate white-matter anatomical connectivity from our seed region to the rest of the brain. The PT analysis was performed based on the same DWI as used for the TBSS analysis using the Diffusion Toolbox FDT implemented in FSL (16, 47). Fiber orientation distributions in each voxel were estimated according to Behrens et al. (48), i.e., using the BEDPOSTX crossing fiber model. Linear and subsequent non-linear deformation fields between each subject's diffusion space and the MNI152 space as the location of the seeds and subsequent output were computed using the FLIRT and FNIRT tools, respectively. For PT, 100,000 samples were generated for each seed voxel and the number of probabilistic tracts reaching each location of a cortical gray matter. Importantly, we did not investigate the number of tracts reaching specific ROIs, but rather analyzed the number of tracts reaching each gray-matter voxel of the ICBM-152 template. The distance of each target (i.e., whole-brain gray matter) voxel from the seed voxel was computed using the ratio of the distance-corrected and non-corrected trace counts [cf. (49)]. This allowed us to address a limitation of structural connectivity profiles generated by PT, namely the fact that trace counts show a strong distance-dependent decay. That is, voxels close to the region of interest will inevitably feature higher connectivity values than even well-connected distant ones. These effects were adjusted

by referencing each voxel's trace count to the trace counts of all other gray-matter voxels in the same distance (with a 5-step, i.e., 2.5 mm, tolerance) along the fiber tracts [for a detailed description see Ref. (49)]. We thus replaced each trace count by a rank-based z-score indicating how likely streamlines passed a given voxel relative to the distribution of trace counts at that particular distance. The ensuing images were tested for consistency across subjects by using a second-level ANOVA. Results were corrected using the same statistical criteria as for the other connectivity modalities, i.e., using TFCE (43) and FWE-correction at $p < 0.05$.

Comparison of Connectivity Measures

The similarities and differences amongst all the different connectivity maps were compared and contrasted. The overlap between all the four thresholded connectivity maps (RS, MACM, SC, and PT) was computed using a minimum statistic conjunction (50), in order to identify *common connectivity* with the seed across the different modalities. This was done by computing the conjunction between the maps of the main effects for each of the modalities. An additional minimal conjunction analysis was also performed across the three modalities used to investigate gray-matter regions, namely, RS, MACM, and SC. Furthermore, we looked at *specifically present connectivity* for each of the modalities. *Specifically present connectivity* refers to regions that were connected with the seed in one modality but *not* in the other three [cf. (27)]. This was assessed by computing differences between the connectivity map of the first modality and those of the other three, respectively. Then a conjunction of these three different maps was performed. For example, the *specifically present connectivity* for MACM was assessed by computing the difference between the MACM map and the RS map in conjunction with the difference between the MACM map and the SC map and the difference between the MACM map and the PT map. Conversely, *specifically absent connectivity* was investigated by computing differences between one modality and the other three in order to identify regions that were present in the latter three modalities but not in the former. A conjunction of these different maps was then performed. For example, the *specifically absent connectivity* for MACM was assessed by computing the difference between the RS and MACM maps in conjunction with the difference between the SC and MACM maps and the difference between PT and MACM. All resulting maps were additionally thresholded with a cluster extent threshold of 100 voxels.

Finally, the resulting *common connectivity*, *specifically present connectivity* and *specifically absent connectivity* networks were functionally characterized based on the behavioral domain data from the BrainMap database as previously described for the seed region.

RESULTS

Relationships Between TMT-MS Performance and Brain Structure: Whole-Brain VBM and TBSS Analyses

The whole-brain VBM analysis revealed a significant negative correlation between the adjusted TMT-MS score and the GMV of a region in the lower bank of the left inferior frontal sulcus (IFS)

⁵<http://www.brainmap.org>

Figure 2A). Since the TMT-MS score refers to task completion time, this negative correlation indicates that better performance was associated with higher GMV in this region (**Figure 2B**).

The functional profile (based on the BrainMap database) of this region showed a significant association with cognition, specifically reasoning, at $p < 0.05$ (**Figure 3**).

The TBSS analysis of white-matter associations did not yield any significant results.

Connectivity of the IFS

Whole-brain connectivity of the region showing a significant association with TMT-MS performance was mapped using RS-FC, MACM, SC, and PT. Both similarities and differences amongst all the different connectivity maps were observed.

Converging Connectivity

Connectivity of the IFS seed, as revealed through RS-FC, MACM, SC, and PT analyses, included a number of distinct brain regions (**Figure 4**). Investigation of common regions interacting with

the IFS across the different connectivity modalities (calculated through a minimum statistical conjunction analysis across the four thresholded connectivity maps) revealed convergence in the left inferior frontal gyrus (IFG) extending into the left IFS. An additional cluster was observed in the right Brodmann Area 45 (**Figure 5A; Table 2**). Functional characterization of this network found across all four connectivity approaches indicated an association with processes related to language, including semantics, phonology, and speech. Additionally, associations with working memory and reasoning were also revealed (**Figure 5B**). On the other hand, a conjunction across the modalities used to investigate gray-matter regions (RS-FC, MACM, and SC) resulted in a broader convergence, including clusters in the IFG bilaterally extending into the precentral gyrus, together with clusters in the middle cingulate cortex, middle orbital gyrus, and insula lobe of the left hemisphere (**Figure 6**).

Specifically Present Connectivity for Each Modality

In the next step, we looked at the connectivity effects that were present in one modality but not in the other three (**Figure 7A; Table 3**).

For RS-FC, we found specific connectivity between the seed region and bilaterally in the inferior parietal lobule, IFG (pars opercularis and pars triangularis), middle frontal gyrus, inferior temporal gyrus, middle orbital gyrus, and supramarginal gyrus. Additionally, areas in the right IFG (p. orbitalis), cerebellum, superior orbital gyrus, middle occipital gyrus, and angular gyrus were also revealed by RS-FC. Moreover, specific RS-FC connectivity was found in areas of the left superior parietal lobule (**Figure 7A** in red). When functionally characterized using the BrainMap meta-data (**Figure 7B** in red) the components of this network were found to be mainly associated with cognitive functions, including working memory, attention, and action inhibition. In addition, fear was also found to be associated with this network.

Connectivity exclusively found using MACM was only observed in one region in the left hemisphere, namely in the insula lobe and adjacent IFG (p. triangularis), in an area slightly more posterior position to that found in RS-FC (**Figure 7A** in green). This region was found to be mainly associated with language functions, namely semantics, speech, and speech execution. Moreover, functions such as pain perception and music were also found to be related (**Figure 7B** in green).

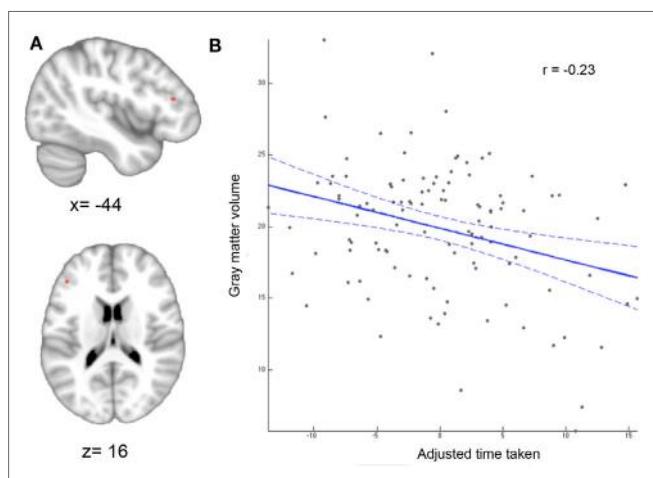


FIGURE 2 | Whole-brain VBM results. (A) Region showing significant correlation between gray-matter volume and adjusted time taken. Statistical significance using non-parametric permutation inference was assessed at $p < 0.05$ [family-wise error (few) corrected for multiple comparisons]. **(B)** Correlation between motor speed and gray-matter volume. The better (lower) the performance score the higher the gray-matter volume.

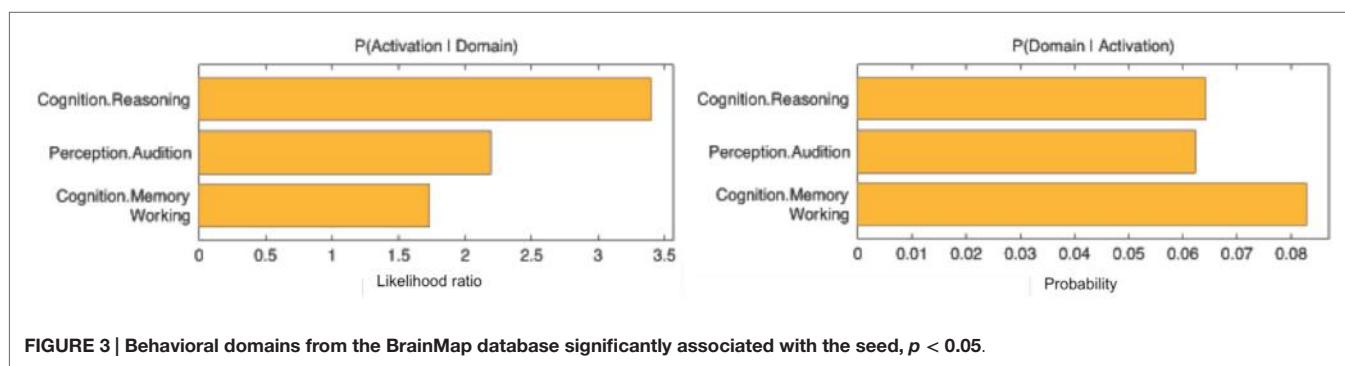


FIGURE 3 | Behavioral domains from the BrainMap database significantly associated with the seed, $p < 0.05$.

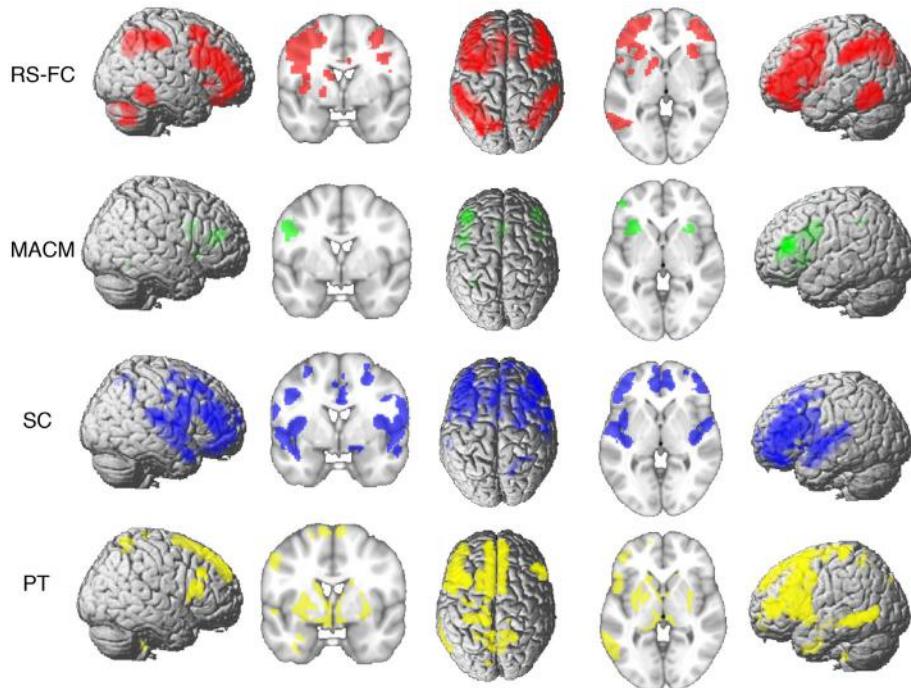


FIGURE 4 | Brain regions found to be significantly connected with the seed for each modality at $p < 0.05$, FWE corrected for multiple comparisons using threshold-free cluster enhancement (TFCE statistic).

Connectivity specific to SC was observed in the bilateral superior medial gyrus, temporal pole, superior temporal gyrus, Heschl's gyrus, rolandic operculum, supplementary motor area, superior and middle frontal gyri (more anterior to the effect found in RS-FC), IFG (p. orbitalis) (inferior to the area found in RS-FC on the right hemisphere) and middle orbital gyrus (bilaterally more anterior to the RS-FC effect). In the right hemisphere, specifically present SC connectivity included areas in the anterior cingulate cortex, insula lobe, middle temporal gyrus, supramarginal gyrus (more inferior to the area found in RS-FC), medial temporal pole, superior and inferior parietal lobules (the latter being more inferior to the area found in RS-FC), and superior orbital gyrus (more anterior to RS-FC specific connectivity in the same region). Additional connectivity was also observed in the left rectal gyrus, and left precentral gyrus (Figure 7A in blue). This network was found to be mainly functionally associated with functions related to emotion (fear, disgust, and sadness) and perception (audition and pain) (Figure 7B in blue).

The network specifically present for PT was found to be mainly functionally associated with functions related to emotion and pain. Additionally, functions such as action execution and action imagination were also found to be related (Figures 7A,B in yellow).

Specifically Absent Connectivity for Each Modality

Additionally, we looked at connectivity that was specifically absent in each modality, i.e., regions for which connectivity was absent in a particular modality but was observed in the other three (Figure 8A; Table 4). No regions were found to be specifically

absent for the RS-FC modality. By contrast, for MACM we found specifically absent connectivity with areas of the left middle and inferior frontal gyri (p. triangularis) (Figure 8A in green). These regions were found to be functionally associated with cognitive functions, namely working and explicit memory but also with phonology, semantics, and syntax (Figure 8B in green).

Conversely, for SC specifically absent connectivity was found for an area in the left precentral gyrus (Figure 8A in blue; Table 4). This region was in turn found to be mainly functionally associated with language-related functions (phonology, semantics, speech, and syntax) together with working memory (Figure 8B in blue).

Connectivity specifically absent for PT was also found to be functionally associated with language-related functions (phonology, semantics, and speech) together with working memory, reasoning, and attention (Figures 8A,B in yellow).

DISCUSSION

The aim of this study was to employ a multi-modal approach to investigate the regions and associated networks related to upper extremity motor function, as quantified by the TMT-MS. In a first step, we therefore correlated local GMV with performance in motor speed. This analysis revealed a significant correlation between TMT-MS performance and GMV in a small region in the IFS, which was functionally characterized as being involved in cognitive tasks. In turn, the TBSS analysis of local WM associations yielded no significant result. We then further investigated the connectivity of the left IFS seed using a multi-modal approach. Functional interactions with other gray-matter

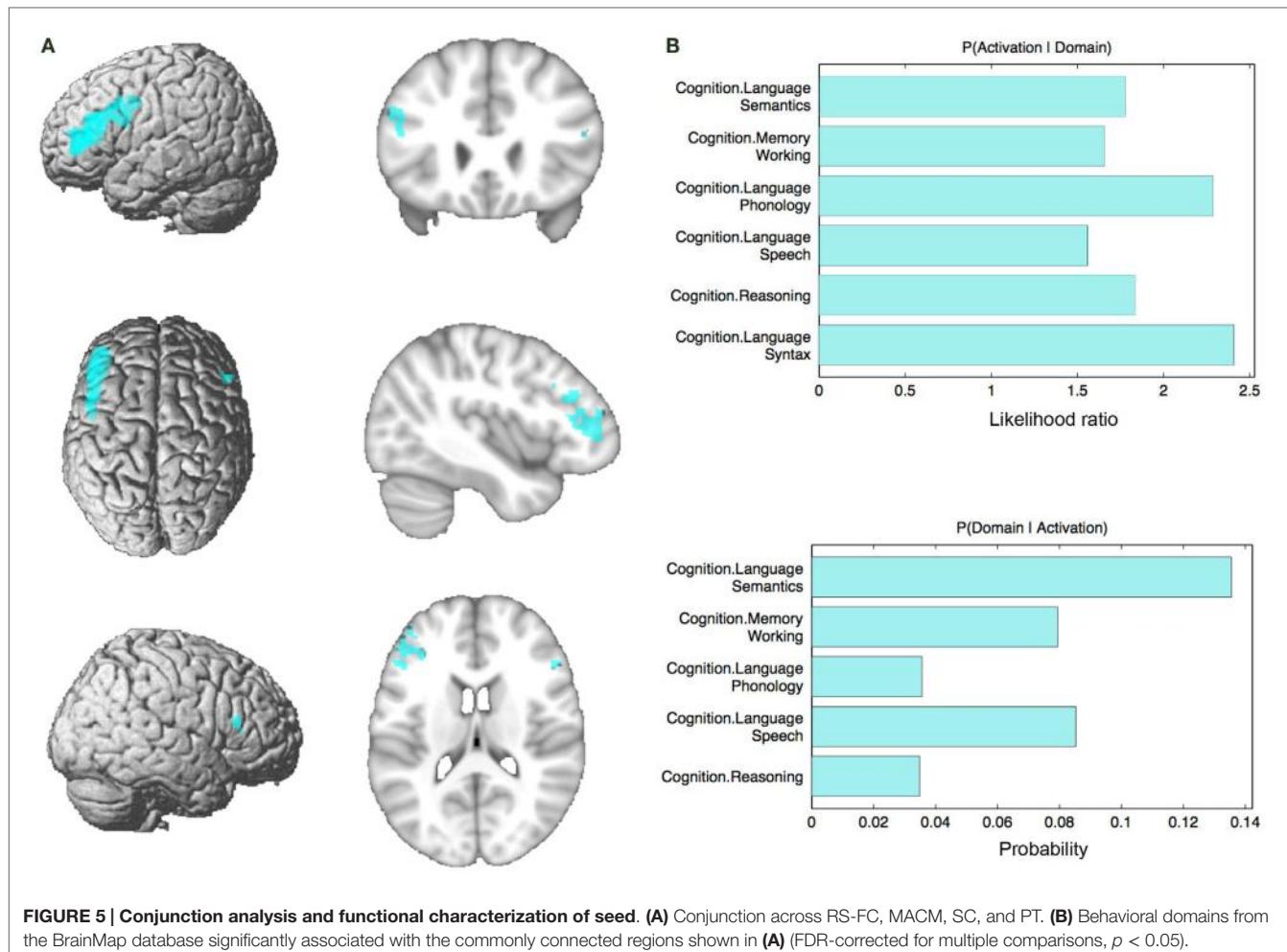


TABLE 2 | Converging connectivity of the IFS seed.

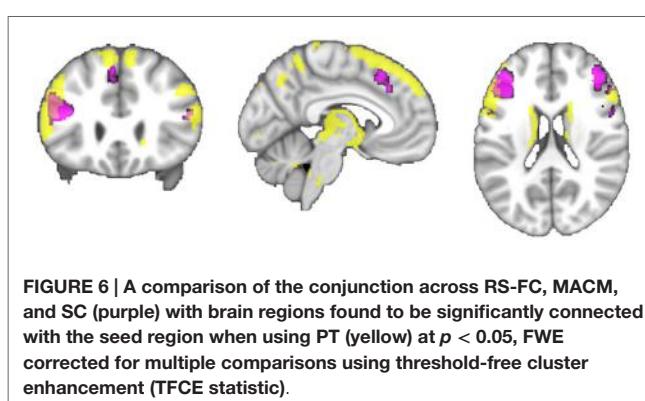
Region	x	y	z	Cytoarchitectonic assignment
Cluster 1 (780 voxels)				
L middle orbital gyrus	-46	46	-2	
Cluster 2 (1,235 voxels)				
R Inferior frontal gyrus (p. triangularis)	52	28	14	Area 45

x, y, and z coordinates refer to the peak voxel in MNI space. R, right; L, left.

regions and white-matter structural connections were assessed using RS-FC, MACM, SC, and PT approaches. The networks that emerged revealed both similarities and differences between the different modalities. A conjunction analysis between the four connectivity approaches was used to delineate a core network. Further analyses were used to investigate connectivity patterns specific to each of the modalities.

Relationships Between TMT-MS Performance and Brain Structure

In this study, we found TMT-MS performance to be specifically related to the local brain volume of a region in the lower bank of the left IFS. That is, across subjects better performance (lower



completion time) was associated with higher GMV in this cluster. The left IFG, including IFS, has been formerly described as part of a multiple-demand system responsible for multiple kinds of cognitive demand, in which goals are achieved by assembling a series of sub-tasks, each separately defined and solved (51). An objective definition of this “multi-demand network” has recently been proposed by Müller et al. (52) based on a conjunction across three large-scale neuroimaging meta-analyses to identify regions consistently involved in sustained attention (53), working

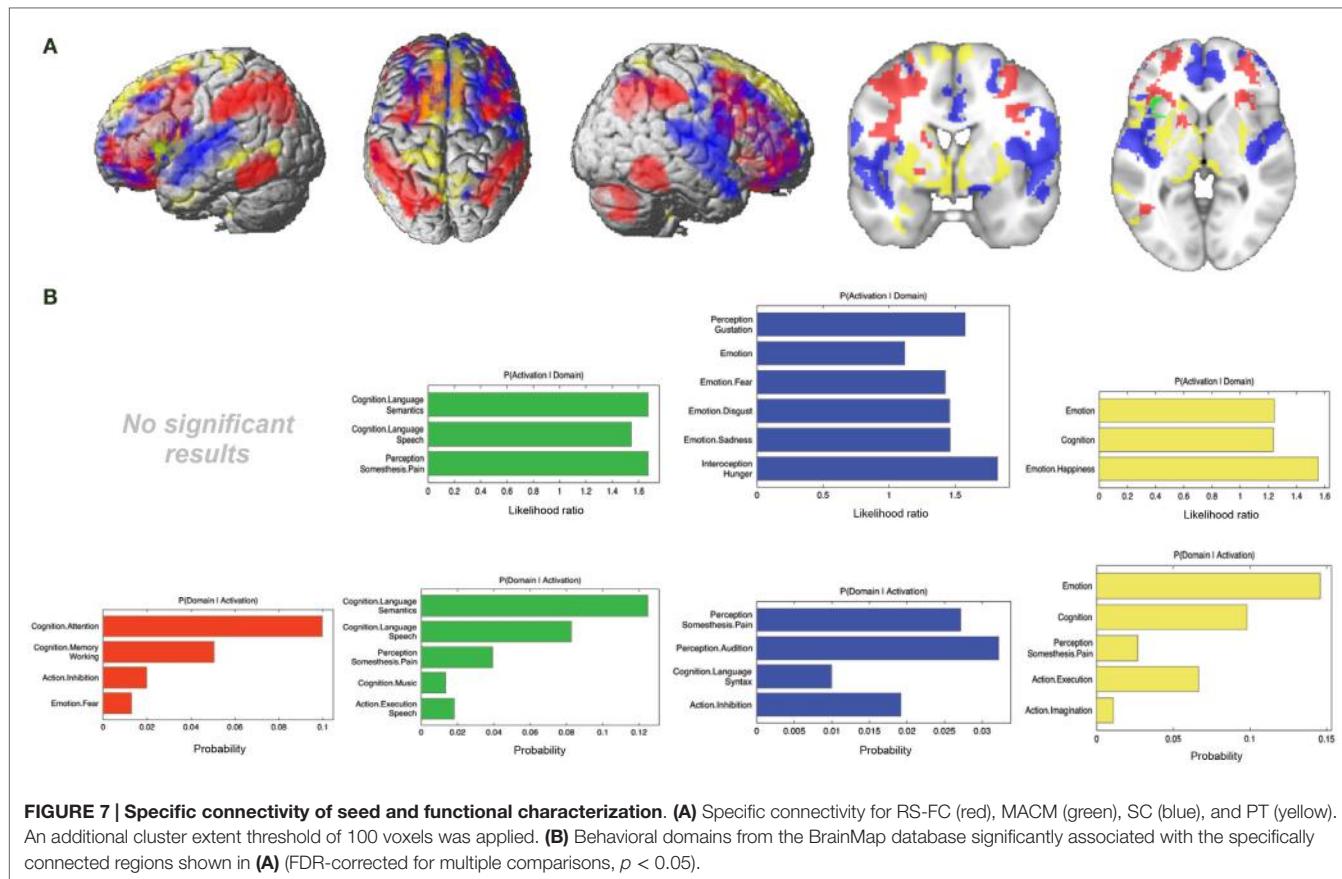


FIGURE 7 | Specific connectivity of seed and functional characterization. (A) Specific connectivity for RS-FC (red), MACM (green), SC (blue), and PT (yellow). An additional cluster extent threshold of 100 voxels was applied. **(B)** Behavioral domains from the BrainMap database significantly associated with the specifically connected regions shown in **(A)** (FDR-corrected for multiple comparisons, $p < 0.05$).

memory (54), and inhibitory control (55). Importantly, the IFS location identified in the current study was found to be part of this multi-demand network, indicating that TMT-MS performance is related to brain structure in a region involved in executive rather than motor functions. This association between certain aspects of motor performance and cognitive or executive functions has already been suggested in earlier studies (56, 57).

At first glance, these results contradict the intention of the TMT-MS to measure motor speed, and to serve as a baseline measure for higher, executive aspects of the test (2). However, one may argue that since subjects are given specific instructions to follow a dotted line while making sure that the line drawn touches every circle along the path, the accurate completion of this task should in fact draw heavily on executive control processes. It may hence not surprise that performance in a task requiring a relatively high degree of executive motor control and attention is related to a structure that is part of the multi-demand network involved in executive functions (51). In turn, there was no significant association between performance and GMV in cortical or subcortical motor structures as may have been expected. In this context, it must be noted that adequate hand motor abilities are a necessary prerequisite for performing the TMT-MS test successfully; i.e., subjects have to be able to use their hand to draw the required lines. Hence, the reliance of TMT-MS completion on an intact cortical and subcortical motor system is obvious. What we found, however, is that performance (i.e., the speed at which the task is completed)

may seem to primarily depend on executive rather than more basic motor control processes. Does this contradict the assumption that the TMT-MS test is a baseline measure of motor speed? Not necessarily, but rather, given our findings, we would argue that motor speed in a more naturalistic setting should be more strongly associated with executive rather than primary motor function.

In congruence with the present results, previous studies have linked longer reaction times and motor slowing with sustained attention (58). However, lesion studies have associated slowing in motor processes with lesions in the right lateral frontal lobe (59, 60). Consequently, these results contrast with the findings of the present study. Additionally, the present results differ from those obtained using tasks that are commonly employed to investigate changes to the motor system following stroke; for instance, in functional neuroimaging studies using fist opening/closure paradigms (61, 62). Here, activation and interactions of the primary motor cortex as well as the lateral and medial pre-motor cortices are of essential importance. Similar regions were found in another functional neuroimaging study which used a finger tapping paradigm and focused on healthy subjects (63). In turn, activations involving the inferior frontal cortex and other regions of the executive, multi-demand network are not prominently seen. This implicates a potentially important distinction between neuroimaging assessments of stroke patients, in which more fundamental aspects of motor performance are usually tested, and paper-and-pencil tests that apparently, even when aimed at

TABLE 3 | Specifically present connectivity of IFS seed.

Region	x	y	z	Cytoarchitectonic assignment
RS-FC				
Cluster 1 (5322 voxels)				
L rectal gyrus	-4	24	-26	
Cluster 2 (4183 voxels)				
	-30	-72	20	
Cluster 3 (3958 voxels)				
	14	18	-28	
Cluster 4 (2318 voxels)				
	36	-64	24	
Cluster 5 (1630 voxels)				
R Cerebellum (Crus 2)	44	-66	-50	
Cluster 6 (1357 voxels)				
L inferior temporal gyrus	-52	-50	-26	
Cluster 7 (817 voxels)				
R inferior temporal gyrus	54	-50	-26	
MACM				
Cluster 1 (279 voxels)				
L insula lobe	-30	22	-10	
SC				
Cluster 1 (26511 voxels)				
R medial temporal pole	32	6	-33	
Cluster 2 (7299 voxels)				
	-39	3	-27	
Cluster 3 (2577 voxels)				
R superior frontal gyrus	21	33	30	
Cluster 4 (1710 voxels)				
L middle frontal gyrus	-40	51	10	
Cluster 5 (875 voxels)				
	-24	30	-23	
Cluster 6 (525 voxels)				
	28	-46	36	Area hIP1 (IPS)
Cluster 7 (341 voxels)				
L inferior frontal gyrus (p. Opercularis)	-57	15	7	Area 44
Cluster 8 (229 voxels)				
L SMA	-8	17	52	Area 6
Cluster 9 (153 voxels)				
L precentral gyrus	-33	-7	54	
Cluster 10 (122 voxels)				
L inferior frontal gyrus (p. Orbitalis)	-46	26	-5	
PT				
Cluster 1 (919 voxels)				
L superior medial gyrus	-8	54	28	
Cluster 2 (748 voxels)				
R superior medial gyrus	10	56	24	
Cluster 3 (387 voxels)				
L paracentral lobule	-10	-34	60	Area 4a
Cluster 4 (308 voxels)				
R precuneus	8	-66	40	Area 7A (SPL)
Cluster 5 (234 voxels)				
L inferior frontal gyrus (p. Orbitalis)	-48	22	-4	Area 45
Cluster 6 (232 voxels)				
L precuneus	-2	-72	36	Area 7P (SPL)
Cluster 7 (179 voxels)				
L middle temporal gyrus	-58	-28	-12	
Cluster 8 (111 voxels)				
	-4	-36	-48	
Cluster 9 (107 voxels)				
L middle occipital gyrus	-52	-70	-2	

x, y, and z coordinates refer to the peak voxel in MNI space. R, right; L, left.

testing basic motor speed, are more reflective of executive motor control. In summary, we would thus argue that the distinction between motor and “higher cognitive” tasks, which seems rather prevalent in (neuroimaging) stroke research, may be slightly misleading, as executive motor control functions may play a major role in the everyday impairments following stroke.

Core Network

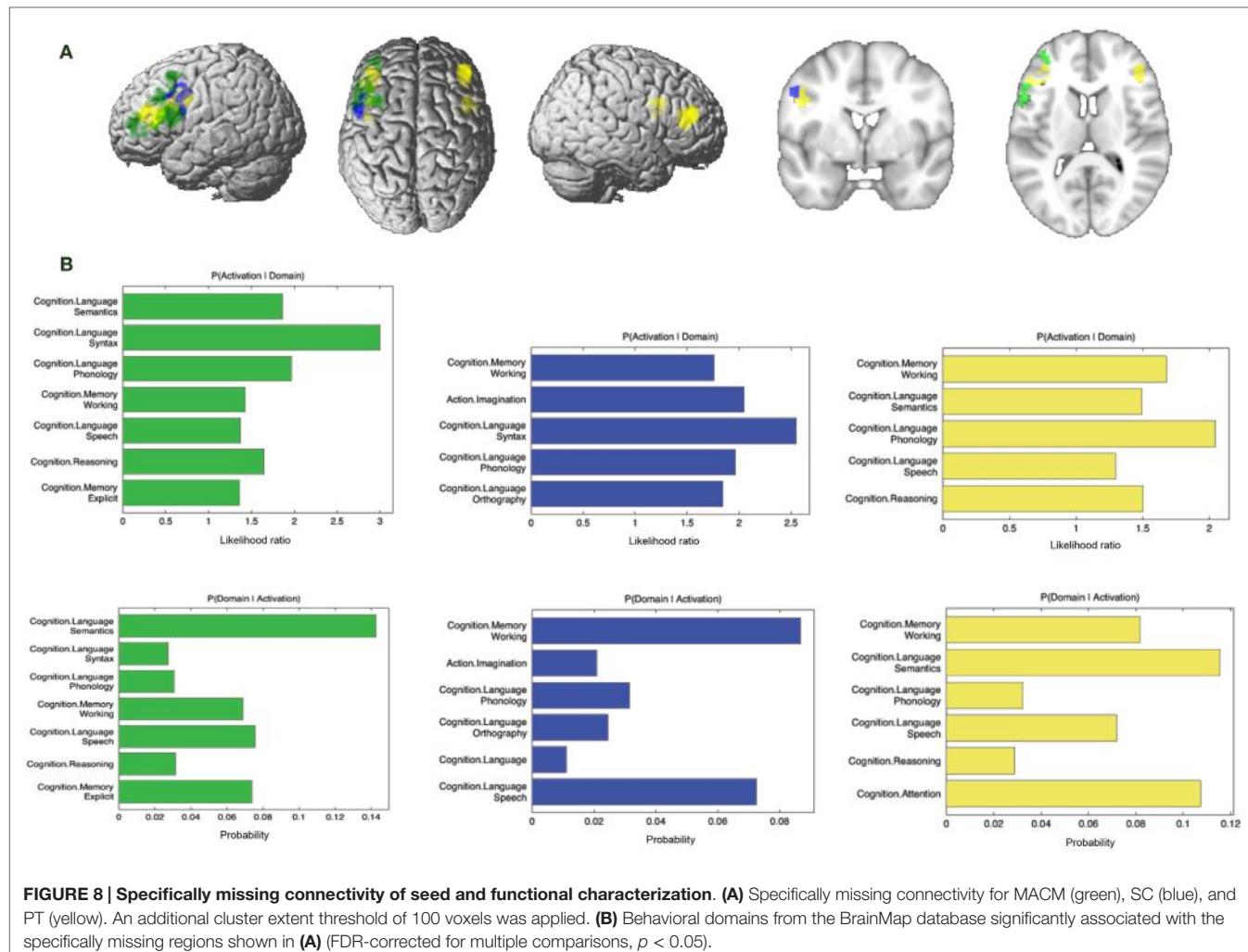
Notably, all three FC approaches (RS-FC, MACM, and SC), together with locations revealed as structurally connected by PT, converged on a network comprising of the left inferior gyrus extending into the left IFS and an additional cluster in the right Brodmann Area 45. In combination with the observation of a fairly restrictive region associated with TMT-MS performance, these results suggest a core network of mostly regional connectivity that is in line with the current view on the role of the inferior frontal cortex in executive functioning (51).

Additionally, the right IFG, bilateral adjacent pre-motor cortices, and anterior insula were additionally found to converge when looking only at the FC approaches, namely, RS-FC, MACM, and SC (but not PT). Similar as the IFS seed, most of these clusters overlap with regions previously described to be part of the multiple-demand network (51, 52). In particular, the bilateral IFG, and left anterior insula as well as the MCC were the regions that overlapped with the multiple-demand network. Thus, we here show that, across different (functional) connectivity approaches the IFS shows robust interactions with regions associated with multiple cognitive demands. This is additionally supported by the functional characterization of the network robustly connected with the IFS across the different FC approaches, which show an association with multiple cognitive tasks. These observations thus continue to emphasize the important role of cognitive functions in the TMT-MS and thus suggest that this test might be tapping into executive rather than primary motor function.

Convergence and Differences Between Connectivity Measures

Convergence Among Modalities

Functional interactions can be probed by using different approaches, each having their own methodological features, and potentially also different biases even though the same statistical analyses and thresholds were used for each of the modalities. The use of the different modalities in the current study provided an opportunity to systematically compare all the different approaches. Despite the conceptual differences between the different modalities, a common network was revealed. When comparing the modalities RS-FC, MACM, and SC networks through a minimum statistic conjunction analysis, all three approaches converged on a core network that included adjacent parts of left IFG, its right-hemispheric homolog, right precentral gyrus, left middle cingulate cortex, middle orbital gyrus, and insular cortex. These results are in line with previous studies that used different seeds and therefore different networks, and also showed convergence between RS and MACM (18–20), between RS and SC (21, 22, 28), between RS and fiber tracking (23–26), and between RS, MACM, and SC (27, 64). As a result, it can be suggested that future studies could benefit from a multi-modal approach and

**TABLE 4 | Specifically absent connectivity of IFS seed.**

Region	x	y	z	Cytoarchitectonic assignment
MACM				
Cluster 1 (735 voxels)				
L inferior frontal gyrus (p. triangularis)	-42	40	-2	
L inferior frontal gyrus (p. triangularis)	-50	38	6	
L inferior frontal gyrus (p. triangularis)	-52	20	30	Area 45
Cluster 2 (166 voxels)				
L middle frontal gyrus	-44	12	38	Area 44
SC				
Cluster 1 (205 voxels)				
L precentral gyrus	-50	4	16	
PT				
Cluster 1 (629 voxels)				
L inferior frontal gyrus (p. triangularis)	-42	32	6	
Cluster 2 (339 voxels)				
R inferior frontal gyrus (p. triangularis)	46	34	6	Area 45
Cluster 3 (119 voxels)				
R precentral gyrus	54	6	18	Area 44

x, y, and z coordinates refer to the peak voxel in MNI space. R, right; L, left.

the consequent use and interpretation of the convergent network rather than focusing on a unimodal approach.

Furthermore, our resulting similarity between the SC and PT networks and the networks obtained from the other two modalities supports the idea that FC can be used to reflect structural connectivity and that SC of GMV can reflect functional networks in the brain (21, 22, 27). Consequently, our results together with previous findings provide evidence for the fact that SC is functional in nature.

Differences Among Modalities

Despite the convergence observed across all approaches, divergent connectivity patterns were also found when looking at contrasts of the different modalities. This is not surprising, given that the approaches use different data and methods in order to determine connectivity between a seed region and the rest of the brain. Previous studies have similarly reported striking differences between RS-FC and MACM connectivity approaches (20, 27). Clos et al. (27) and Jakobs et al. (20) have already argued that the differences that result from these two approaches may be the

result of the conceptual differences between the methods. While RS-FC is based on correlation of fMRI time-series measured in the absence of an external stimulus (5, 65), MACM delineates networks that are conjointly recruited by a broad range of tasks (3). That is, RS and MACM derive FC from different mental states, in the absence and presence of a task, respectively. As a result, spontaneous networks related to self-initiated behavior and thought processes that can be captured in the task-free state may be largely missed in MACM analyses (3).

In particular, RS-FC of our seed was specifically found in a number of regions that have been predominantly associated with executive functions, such as working memory, attention, action inhibition, and spatial cognition. Importantly, there were no regions that were present in SC, PT, and MACM, but absent in RS-FC as revealed by the specifically absent RS-FC. This indicates that RS-FC captures the broadest network. By contrast, specific connectivity observed for MACM was found to be mainly associated with language-related functions such as semantics and speech. In turn, specifically absent regions in MACM were found to be mainly associated with cognitive functions such as working memory and explicit memory as well as language-related functions. As already mentioned above, these diverging patterns, with RS-FC capturing a broader network than MACM is possibly due to the conceptual differences. Moreover, these two approaches also differ in the subject groups assessed. While a group of 109 subjects were recruited for the RS-FC analysis, the MACM analysis relied on a large amount of published neuroimaging studies from the BrainMap database (10), with the selection criteria being activation of our identified seed region. Thus, it is possible that this difference in subject groups may have also contributed to the difference in results obtained.

In contrast to the FC approaches mentioned above, specific SC connectivity was observed in regions found to be mainly associated with functions related to emotion (fear, disgust, and sadness) and perception (pain, gustation, audition, hunger, and somesthesia). Additional functions observed included action inhibition and cognition. On the other hand, functional characterization of areas that were found to be specifically absent for SC connectivity revealed an association with functions related to cognition and language such as working memory, phonology, orthography, syntax, and speech. Given these results, it can be noted that the specific SC network showed a prominent association with perception and emotional processing. The strong association with emotional processing in SC is particularly interesting since the functional characterization of the seed region and the conjunction network did not indicate such an involvement. Moreover, while the specific RS-FC network revealed regions that were predominately related to cognition and the MACM network revealed regions that were predominantly related to language, the SC network found such regions to be specifically missing. These differences may be largely due to the conceptual differences between the FC modalities described above and SC. The exact biological basis of SC is still rather unclear (27), but it has been hypothesized that SC networks arise from synchronized maturational change that could be mediated by axonal connections forming and reforming over the course of development (66). Therefore, early and reciprocal axonal connectivity between regions is expected to have a mutually trophic effect on regional growth in an individual brain

leading to covariance of regional volumes across subjects (14). That is, the correlation of anatomical structure between regions is the result of similarities in maturational trajectories (14). The specific connectivity pattern of the SC modality may thus be reflecting synchronized developmental patterns within a network of regions associated with perception and emotional processing. This could thus be the reason for particular regions to be present in the SC network and not in the MACM and RS-FC networks since the latter two modalities are more likely to highlight regions that are related to certain functions rather than long-term anatomical interactions. Additionally, SC is also likely to include other influences such as common genetic factors, developmental brain symmetry, neuromodulator distributions, and vascular territories (14, 15), which contribute to its more widespread distribution.

In congruence with the specific SC network, the PT network also showed a prominent association with perception and emotional processing while functional characterization of areas that were found to be specifically absent for PT connectivity revealed an association with functions related to cognition and language. These results further imply that the regions that were specifically associated with SC may reflect dominant long-term synchronized maturational patterns. However, despite the differences observed, it should be noted that the core network showed that the resulting SC network (also) revealed functional relations despite the fact that it was defined by anatomical covariance. SC may hence be regarded as a measure potentially bridging between structural and functional connectivity aspects. However, when comparing the PT to the other three networks, contrasting regions can be observed. This could be due to biases related to the use of conventional diffusion tensors. Such tensors can only capture the principal diffusion direction, and thus makes them prone to errors induced by crossing fibers (67). As a result, this could have limited the possible resulting convergence amongst the four modalities.

CONCLUSION

In summary, the present results demonstrate a significant correlation between TMT-MS performance and GMV in the lower bank of the IFS, which was functionally characterized as being involved in cognitive tasks. Additionally, all connectivity approaches used (RS-FC, MACM, SC, and PT) converged on a network comprising of regions that overlap with the multiple-demand network. Results therefore indicate that performance (i.e., the speed at which the task is completed) may primarily depend on executive function, thus suggesting that motor speed in a more naturalistic setting should be more strongly associated with executive rather than primary motor function. Moreover, the common connectivity resulting from the different modalities used verifies that common networks can be revealed across highly divergent methods.

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