research-article

Stroke

ORIGINAL ARTICLE

Stroke Recovery–Related Changes in Cortical Reactivity Based on Modulation of Intracortical Inhibition

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BACKGROUND: Cortical excitation/inhibition dynamics have been suggested as a key mechanism occurring after stroke. Their supportive or maladaptive role in the course of recovery is still not completely understood. Here, we used transcranial magnetic stimulation (TMS)-electroencephalography coupling to study cortical reactivity and intracortical GABAergic inhibition, as well as their relationship to residual motor function and recovery longitudinally in patients with stroke.

METHODS: Electroencephalography responses evoked by TMS applied to the ipsilesional motor cortex were acquired in patients with stroke with upper limb motor deficit in the acute (1 week), early (3 weeks), and late subacute (3 months) stages. Readouts of cortical reactivity, intracortical inhibition, and complexity of the evoked dynamics were drawn from TMS-evoked potentials induced by single-pulse and paired-pulse TMS (short-interval intracortical inhibition). Residual motor function was quantified through a detailed motor evaluation.

RESULTS: From 76 patients enrolled, 66 were included (68.2±13.2 years old, 18 females), with a Fugl-Meyer score of the upper extremity of 46.8±19. The comparison with TMS-evoked potentials of healthy older revealed that most affected patients exhibited larger and simpler brain reactivity patterns ($P_{cluster}$<0.05). Bayesian ANCOVA statistical evidence for a link between abnormally high motor cortical excitability and impairment level. A decrease in excitability in the following months was significantly correlated with better motor recovery in the whole cohort and the subgroup of recovering patients. Investigation of the intracortical GABAergic inhibitory system revealed the presence of beneficial disinhibition in the acute stage, followed by a normalization of inhibitory activity. This was supported by significant correlations between motor scores and the contrast of local mean field power and readouts of signal dynamics.

CONCLUSIONS: The present results revealed an abnormal motor cortical reactivity in patients with stroke, which was driven by perturbations and longitudinal changes within the intracortical inhibition system. They support the view that disinhibition in the ipsilesional motor cortex during the first-week poststroke is beneficial and promotes neuronal plasticity and recovery.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: cortical excitability ■ electroencephalography ■ evoked potential ■ motor cortex ■ transcranial magnetic stimulation ■ upper extremity

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Nonstandard Abbreviations and Acronyms

EEG	electroencephalography
FM	Fugl-Meyer
HOA	healthy older adult
LMFP	local mean field power
RQS	regression quality score
SICI	short-interval intracortical inhibition
SP	single-pulse
TEP	transcranial magnetic stimulation-evoked potential
TMS	transcranial magnetic stimulation

Although more knowledge is continuously gained on the neurobiological processes occurring in the first weeks and months after a stroke, the mechanisms sustaining motor improvement are still not fully understood. There is substantial evidence that stroke induces functional plasticity partly driven by alterations in neuronal excitability.[1] Indeed, in the first phase after a stroke, the strong release of glutamate is excitotoxic and contributes to cell death, which is counteracted by the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) through cell hyperpolarization.[2] In mice, this phase in which inhibition in the perilesional area is beneficial lasts ≈3 days,[3] while its duration in humans remains unknown.[4] In the longer term, the effects are eventually reversed, so that a shift in the cortical excitatory/inhibitory balance toward excitation becomes beneficial for plasticity.[5] The resulting increase in excitability has been associated with the induction of structural plasticity and functional reorganization in motor regions.[6]

Collectively, this evidence suggests that changes in this balance could be 1 pivotal mechanism at the origin of neural plasticity after injury.[4,7] However, these mechanisms still need to be validated in longitudinal investigations in vivo in humans, to better understand the factors sustaining stroke recovery and to unveil potential targets for therapy tailored to the specific phase of the recovery process. Combining transcranial magnetic stimulation (TMS) and scalp electroencephalography (EEG) offers the possibility to directly assess the neuronal properties of the lesioned motor regions by studying the amplitude and dynamics of the TMS-evoked potentials (TEPs).[8] Such properties include cortical excitability[9] and neurotransmitter concentrations such as GABA.[10]

TMS-EEG has been successfully applied in stroke. Ipsilesional motor cortical excitability was reported higher in chronic stroke than in controls[11] and was related to poorer motor function.[12] Moreover, an abnormal brain reactivity to TMS, defined by a large and simple monophasic evoked activity, was observed in the most affected patients in all stroke stages ranging from acute to chronic.[13–16] This activity showed a similar profile as responses evoked in sleep and unresponsive wakefulness syndrome patients.[17] In addition, GABA receptors were suggested to be the main actors involved in this atypical brain response. Bai et al[15] recently showed a reduced intracortical GABA-B inhibition in the ipsilesional motor cortex of chronic stroke patients, by analyzing 1 specific component of such responses (N100). Crucially, TMS also offers the benefit of directly investigating inhibitory mechanisms (GABAergic) by applying paired-pulse short-interval intracortical inhibition (SICI) TMS protocols.[7]

Here, we longitudinally evaluated (acute to late subacute) a cohort of patients with stroke with TMS-EEG, in the framework of the TiMeS project.[18] The present study specifically focused on the analysis of the TMS-evoked responses and complements the study of TMS-induced oscillations,[19] the results of which will be put into perspective with those of the present study. Complementary TMS-EEG readouts enabled the study of cortical excitability and evoked dynamics from the individuals' brain reactivity, and their association with motor function at each stage and during the process of motor recovery. Additionally, by using for the first time SICI protocols in TMS-EEG coupling in patients with stroke, we monitored the changes in intracortical inhibitory activity, and their relationship with residual motor function, impairment, and recovery.

METHODS

For more details on the protocol and analysis, the reader might refer to the Supplemental Material and Fleury et al.[18]

Patient Population

Patients were recruited during the first-week poststroke, inclusion criteria consisted of being older than 18 years old, motor deficits of the upper limb (any degree, objectified by a clinical assessment), and absence of contraindications for magnetic resonance imaging or TMS. Fifteen healthy older adults (HOAs) aged-matched with patients (HOAs) were additionally recruited and underwent a single TMS-EEG recording session. The study was conducted in accordance with the Declaration of Helsinki and approved by Cantonal Ethics Committee Vaud, Switzerland (2018 to 01355), written informed consent was obtained.

Protocol Design

Patients underwent 3 sessions of assessments, at 1 (acute stage: A) and 3 weeks (early subacute stage), and 3 months (late subacute stage) poststroke (Figure 1), according to the SRRR consensus statement.[20] Each session comprised structural magnetic resonance imaging, TMS-EEG as well as a comprehensive battery of motor evaluations. This study is reported

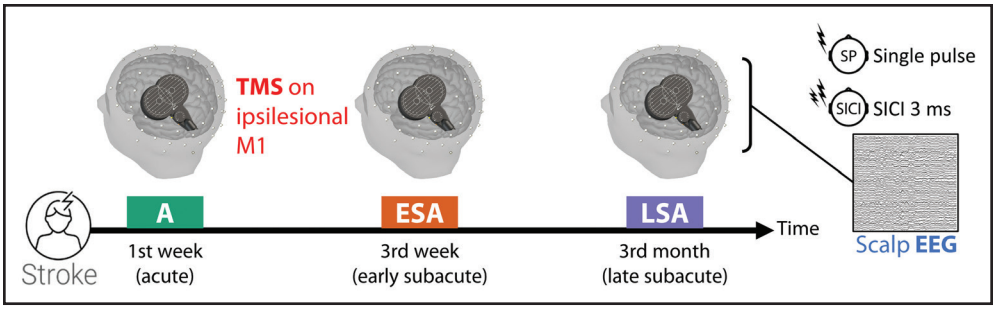

Figure 1. Protocol design.
Patients underwent 3 transcranial magnetic stimulation (TMS)- electroencephalography (EEG) recording sessions in the acute (A), early subacute stage (ESA), and late subacute stage (LSA). EEG was recorded while stimulating the ipsilesional motor cortex using supratreshold single pulses (SP condition) and paired-pulse short-interval intracortical inhibition protocol (SICI condition).

in compliance with STROBE guidelines (Strengthening the Reporting of Observational Studies in Epidemiology). The data related to this article are available upon reasonable request to the corresponding author.

Behavioral Assessment

The motor evaluation battery comprised of the (1) Fugl-Meyer of the upper extremity (FM-UE total, referred to thereafter as FM-total, max 60 points without reflexes) and each of its subscores: the upper extremity (FM-UE, max 30 points), the hand, and the wrist. For each hand, the following was assessed: (2) the maximum fist, key, and pinch force assessed in 3 trials and performed using a JAMAR hydraulic hand dynamometer; (3) the Box and Blocks; and (4) the 9-hole peg. For every motor score, with the exception of the FM, a ratio between the performance of the affected and nonaffected hand (affected/unaffected) was used for the analyses.

TMS-EEG Data Acquisition and Analysis

All the recommendations from international guidelines on TMS-EEG acquisition were followed, with the exception of a sham stimulation.[21] Two types of stimulation were applied on the ipsilesional (or left, for HOAs) motor cortex: suprathreshold single-pulse (SP) and SICI, comprised of an infrathreshold conditioning pulse followed by a suprathreshold SP, with an interstimulus interval of 3 ms. For each patient and stroke stage, a maximum of 180 SP and 180 SICI trials were applied (final mean number 169, minimum 80).

EEG data were preprocessed using the TESA (TMS-EEG Signal Analyser) toolbox.[22] For the purpose of visualization and cluster-based permutation statistics (see Statistics), topographies were flipped as necessary, ensuring that the left hemisphere was designated as the ipsilesional hemisphere for all patients. As a means to assess (1) the cortical excitability and (2) the complexity of the TMS-evoked dynamics, we computed for each stroke stage (1) the local mean field power (LMFP) of the early response (<80 ms), and (2) the number of evoked deflections (N_{def}) and the regression quality score (RQS) between stroke stages (see Supplemental Methods).

Statistics

Early TEPs from HOAs and patients in the acute stage were compared using cluster-based permutations (see Supplemental Methods). All remaining statistical analyses were performed using the JASP software (JASP Team [2022], Version 0.17.2.1). Bayes factors (BF_{incl} and BF_{10}) were used to quantify statistical evidence, and default values for priors were kept. BF_{incl} corresponds to the statistical evidence for including the factor or covariate in the model, across matched models. LMFP was evaluated using Bayesian 1-way ANOVA, for comparing patients to HOAs, and ANCOVA. This latter was focused on patients and comprised the stroke stage as a fixed factor, and the FM-total and the suprathreshold TMS intensity as covariates within a single model. RQS was analyzed using 2-way ANOVA, with stroke stages corresponding to reference TEPs and trials taken as factors. Additionally, at each stroke stage, we performed a comprehensive exploratory analysis comprised of Bayesian correlations between each pair of TMS-EEG readouts (3 readouts: LMFP, N_{def}, and RQS) and scores from the motor evaluation battery (9 scores, see Behavioral assessment). As the distributions of the motor scores in our patient cohort were not normal, Kendall nonparametric correlations were performed, and the 95% credible interval for τ_b (better adjusted for ties than τ_a) was reported, referred to hereafter as τ. Bayesian Kendall correlations between TMS-EEG readouts and the motor evaluation battery were also drawn for every evolution between stroke stages, which was measured as a percentage of change (eg, A versus early subacute stage: $(xESA-xA)/xA$) for each behavioral score and electrophysiological readout. To evaluate the influence of the conditioning pulse in the SICI paradigm over early LMFP, we calculated the arithmetic difference between SP and SICI (LMFP SP–LMFP SICI). Finally, to investigate the relationship between all the different TMS-EEG readouts (LMFP, N_{def}, and RQS) and lesion sites, a voxel-based lesion symptom mapping[23] (VLSM, see Supplemental Methods) and Bayesian Kendall correlations with lesion volume were performed, on the N=54 patients having an magnetic resonance imaging scan in the acute (N=51) or early subacute (N=3, if the acute scan was skipped) stages.

RESULTS

Seventy-six patients with stroke were enrolled in the study after being admitted to the cantonal hospital in Sion, Switzerland. Among them, 66 patients (age: 68.2±13.2 years old, 18 females) were included in this study, that is, patients with TMS-EEG recordings at

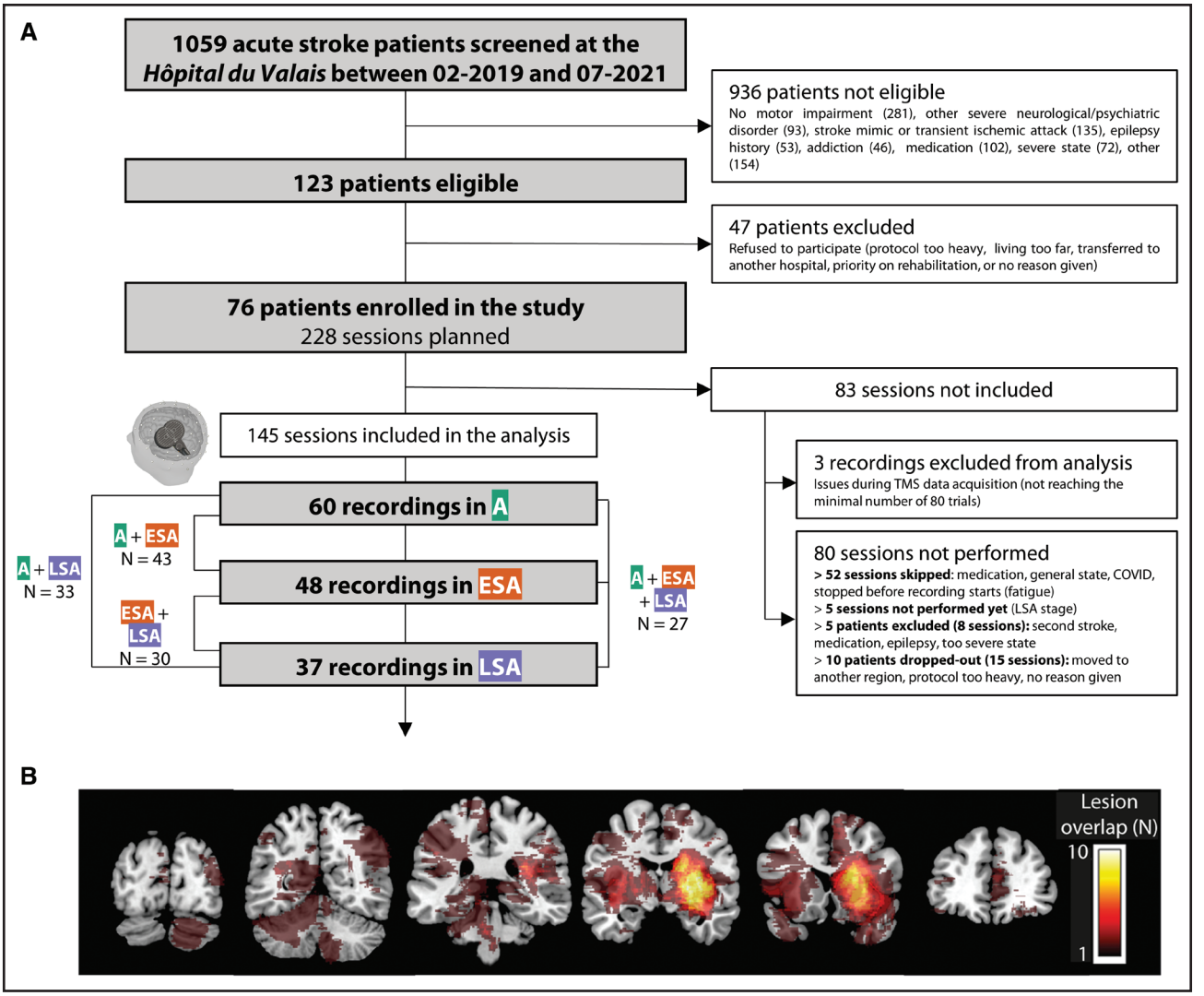

Figure 2. Patients flow chart and lesion heat map.
A, Patients inclusion flow chart. **B**, Lesion heat map of the patients, N=54, of whom 51 and 3 were taken from the acute and early subacute stages respectively, based on the first available magnetic resonance imaging (MRI) scan. Please note that 12 patients of the cohort did not undergo MRI in the acute or early subacute stages. ESA indicates early subacute; LSA, late subacute; and TMS, transcranial magnetic stimulation.

least in one of the recording sessions (see Figure 2A for the screening flow chart, and Table and Figure 2B for patients' characteristics). With the aim of determining factors specific to recovery, the subset including patients showing motor improvement was defined as the recovering group (N=40 in total). The latter was quantified by an increase in the Fugl-Meyer of the upper extremity, from the acute to either of the following stages (1 point minimum).

Brain Reactivity of Patients With Acute Stroke

Patients with acute stroke presented an abnormal brain reactivity when probing the ipsilesional motor cortex, compared with HOAs. Grand average TEPs revealed a larger and simpler pattern within the early part of the response (<100 ms) in the patient cohort (Figure 3A). This difference was significant at the group level, as shown by cluster-based permutation statistics, for both SP and SICI conditions ($P_{cluster}$<0.05). When exploring the data at the individual level, this abnormal reactivity seemed to be more pronounced in severely affected patients (Figure 3B). This interpatient variability was also evident when investigating the local response of the ipsilesional motor cortex longitudinally (Figure 4A; Figure S1A).

Motor Cortical Excitability Across Stroke Stages

LMFP of the early response was higher in patients with stroke than healthy controls for each stroke stage (Figure 4.B.1), as shown with moderate evidence by the Bayesian 1-way ANOVA (BF_{incl}=7.0 for the group effect) and with moderate to strong

Table. Patients' (Top) and Healthy Older Adults' (Bottom) Characteristics (Mean±SD)

Sex	Age, y	Handedness	Stroke type	Lesion location*	Lesion volume (voxels)†	Thrombolysis	Hemisphere affected	MEP negative A	rMT A (% MSO)	FM-total A (/60)	FM-total ESA (/60)	FM-total ELSA (/60)	Days post-stroke A	Days post-stroke ESA	Days post-stroke LSA
18 F/48 M	68.2±13.2	55 right-handed/8 ambidextrous/3 left-handed	63 ischemic/3 hemorrhagic; 57 first-ever/9 recurrent	33 subcortical/4 cortical/27 mixed/2 cerebellar/	16 k±29 k	N=20	31 left/33 right 2 bilateral	N=8	43±9.8	46.8±19	50.6±17	55.1±12	6.6±2.3	26.9±4.9	98.1±8.6
HOA: 11 F/4 M	HOA: 67.0±4.9								HOA: 43±11.0						

FM-total indicates Fugl-Meyer of the upper extremity; HOA, healthy older adult; MEP, motor-evoked potential; MRI, magnetic resonance imaging; and rMT, resting motor threshold.

*Lesion location from the whole cohort (extracted from clinical report, or T1 MRI of later stages for N=12 patients without MRI in acute and early subacute stages).

†Lesion volume computed from T1 MRI acquired in the acute or early subacute stage, for N=54 patients.

evidence by the subsequent post hoc comparisons (BF_{10}=13, 12, and 9.4 when comparing controls to acute, early and late subacute stages, respectively). However, despite a visual trend of decreased LMFP with time, post hoc comparison did not reveal any significant difference between stroke stages (0.1<all BF_{10}<1). This was further confirmed by the Bayesian ANCOVA focused on patients with stroke: the level of statistical evidence was inconclusive regarding stroke stages at the group level (BF_{incl}=0.7). However, it revealed strong evidence for a link between the LMFP and its covariate FM-total (BF_{incl}=16; using FM-UE: BF_{incl}=33), with larger signal power being associated with reduced upper limb scores. There was also extreme evidence for an effect of the TMS intensity used (BF_{incl}>1.4.10⁴), with higher LMFP linked with higher intensity. However, we found moderate evidence that the 2 covariates (TMS intensity and FM scores) were not correlated (BF_{10}=0.12 and 0.21 for FM-total and FM-UE, respectively). Moreover, abnormally high LMFP in the acute stage and its decrease with time were positively correlated with motor recovery towards the late subacute stage (Figure 4B2; see Tables S1 and S2 for detailed associations with scores from the motor evaluation battery).

TMS-Evoked Dynamics of the Motor Cortex Across Stroke Stages

The study of TMS-evoked dynamics revealed that their characteristics were singular in the acute stage. First, ANOVA investigating the evoked dynamics in different stroke stages, captured by RQS, revealed extreme evidence for an interaction effect between the factors reference TEP and single trial activity (BF_{incl} >1.10⁸, Figure 4C1). Post hoc tests showed strong evidence for a difference between stroke stages only when using the TEP from the acute stage as a reference (A>SA, BF_{10}=44; A>EC, BF_{10}=23). No evidence was found when using TEPs from the other stroke stages (all BF_{10}€[0.2–1.3]). Regarding the number of deflections, no effect was found for the factors stroke stages, FM-total score, or TMS intensity. Similarly, no evidence of relationships between the number of deflections and any of the motor scores was found. Among the tested TMS-EEG readouts, VLSM with the number of deflections in the acute stage revealed a significant association with the internal capsule (Figure 4C2). Thus, simpler responses, that is, fewer deflections, were predominantly related to lesions in the corticospinal tract. No correlation was found between TMS-EEG readouts and lesion volume. There was no evidence for the presence or absence of any correlations between these readouts and motor scores.

Unmasked Complementary Intracortical Inhibition Mechanisms

The association between the intracortical inhibition effect on excitability, that is, by contrasting LMFP from SP and SICI conditions, and motor improvement was only found when removing the abnormally large component (Figure 5A). First, moderate evidence was found for an absence of difference between HOAs and patients in any stroke stages (BF_{incl}=0.2, Figure 5B1). Second, the Bayesian ANCOVA focused on patients failed to find any evidence for an effect of stroke stage, FM-total, or TMS intensity (0.26<all BF_{incl}<0.89). However, moderate to very strong evidence was found for a negative link between the contrast in LMFP in the acute stage and motor recovery: patients who recovered the most were those presenting disinhibition, that is, higher level of LMFP in SICI than in SP (Figure 5B2; Tables S1 and S2). Longitudinal changes within the dynamics of the SICI-evoked response were also revealed in the recovering group. When comparing the dynamics in the acute and early subacute stages using RQS, moderate evidence was found with long-term motor improvement

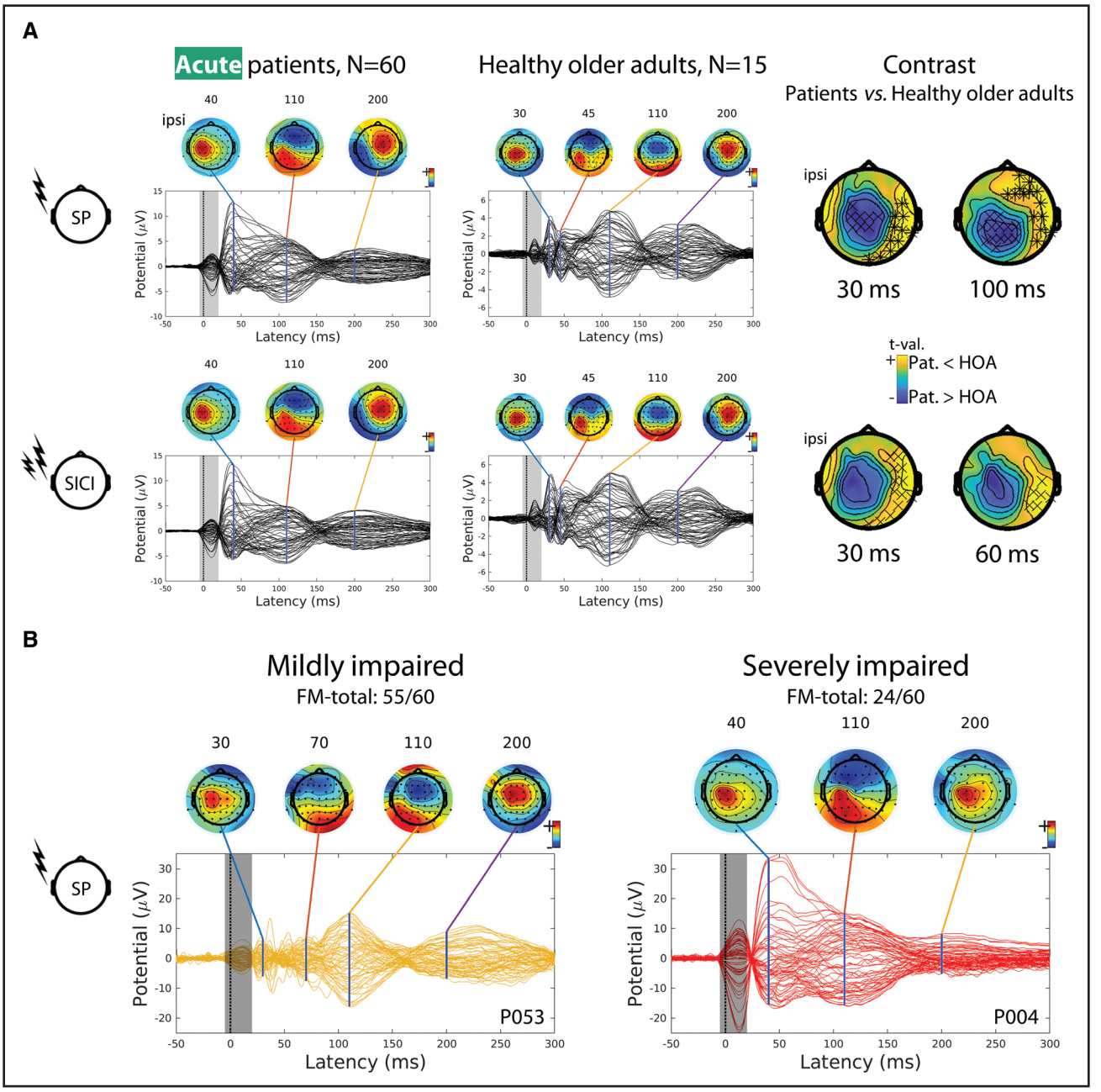

Figure 3. Abnormal brain reactivity in patients with acute stroke after the stimulation of the ipsilesional motor cortex. **A**, Grand average transcranial magnetic stimulation (TMS)-evoked potentials (TEPs) of the patient cohort in the acute stage (**left** column), and of the healthy older adults (**right** column), for each stimulation condition, that is, suprathreshold single pulse (SP, **top** row) and short-interval intracortical inhibition (SICI, **bottom** row). Left hemisphere was designated as the ipsilesional hemisphere for all patients (see text). Electrodes' time series are overlayed in a butterfly view from −50 to +300 ms relative to the stimulation onset, and topographies are plotted for the main activity peaks. The gray shaded area represents the time window interpolated around the TMS pulse (from −5 to +20 ms) not taken in the analysis. Topographies in the right column depict the beginning (**left**) and end (**right**) of the significant clusters found when contrasting TEPs between patients and healthy controls within the first 100 ms. Colormap codes for the local *t*-value, and black crosses and stars indicate electrodes belonging to a significant cluster (see Statistics section). **B**, Examples of TEPs in 2 representative patients in the acute stage with different initial motor deficit, as indicated by the Fugl-Meyer (FM) score of the affected upper extremity (FM-total). The 2 patients are labelled as mildly and severely impaired using the cutoffs proposed in Woytowicz et al.[24] Comparing TEPs reveals the difference in both maximum amplitude and spatial distribution of the signals, with the most affected patient (**right**) exhibiting a simpler, larger, and more spatially restricted activity, especially during the first 100 ms. LSA indicates late subacute.

in the late subacute stage (FM hand, τ € [−0.66 to 0.07], BF_{10}=5.8; 9-hole peg, τ € [0.09–0.67], BF_{10}=7.3; Figure 5C). Stronger motor improvements between the acute and the following stroke stages were linked with greater differences in the pattern of the SICI-evoked activity over time.

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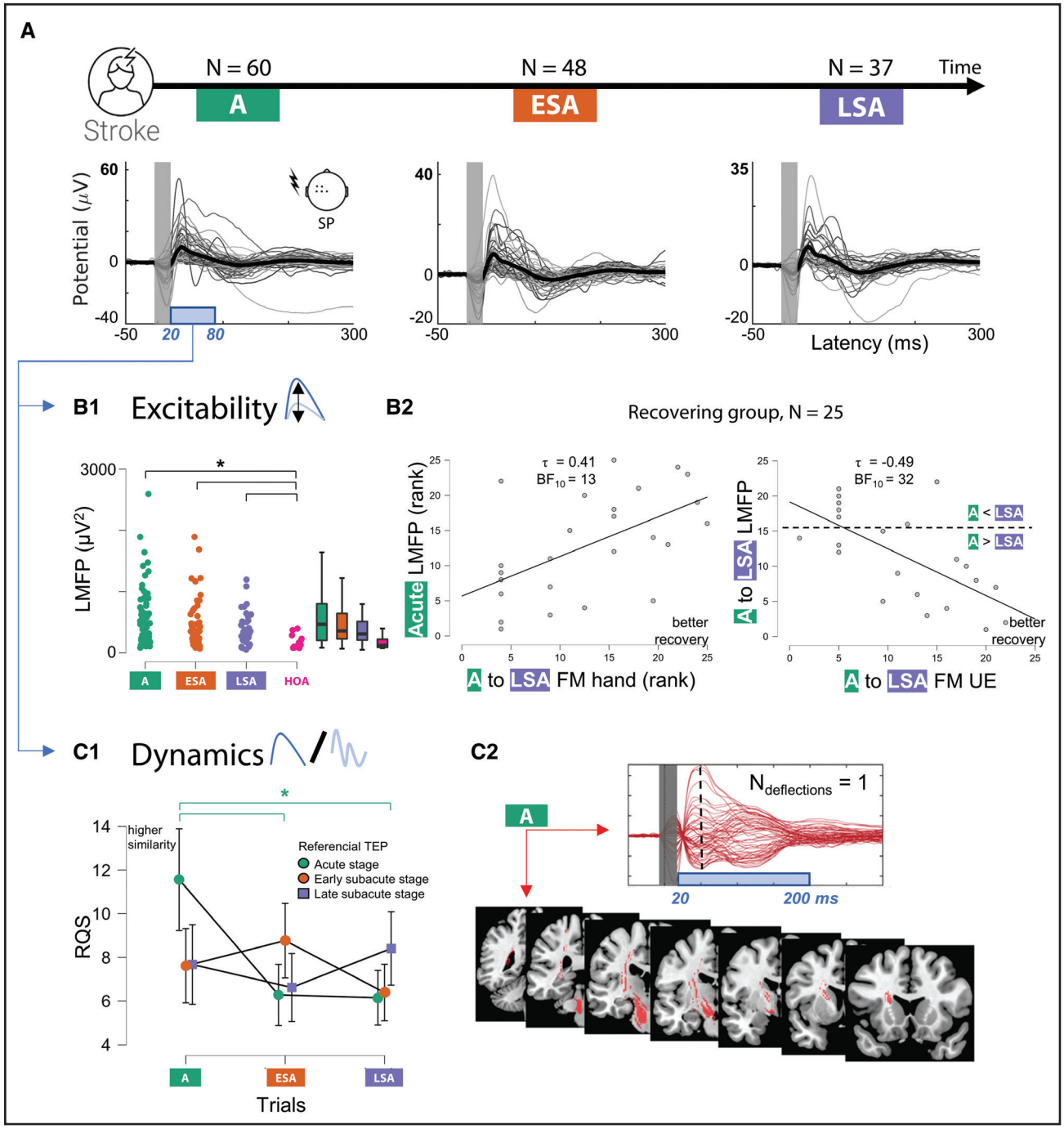

Figure 4. Longitudinal evolution of motor cortical excitability and transcranial magnetic stimulation (TMS)-evoked dynamics, and their association with motor recovery.

A, Local (TMS)-evoked potentials (TEPs) from the ipsilesional electrodes close to the stimulation site, across the 3 time points, for each patient (1 line represents 1 patient). Please note the decrease of max amplitude through stroke stages, and the inter-patients' variability in the evoked response amplitude and dynamics, respectively assessed using local mean field power (LMFP) and regression quality score (RQS) on the early part of the response (20–80 ms). **B1**, Distribution of LMFP across stroke stages, each being significantly higher than the one from healthy older adults (HOAs), as indicated by asterisks (see text). **B2**, Significant associations between high LMFP in the acute stage (**left**), and strong LMFP decrease toward the late subacute stage (**right**) on one hand, and better motor recovery on the other hand in the recovering group. Such correlations were also found within the whole cohort of patients, with weaker level of statistical evidence (see Tables S1 and S2). Correlations were performed using Kendall τ_b; values displayed on both axes corresponding to ranks. **C1**, Distribution of RQS through stroke stages (*y* axis), when using the local TEP of each stroke stage as a reference (represented by a different line). Higher RQS highlight higher similarity between the evoked dynamics of the reference TEP and the trials from other stroke stages. Stars indicate significant post hoc tests revealing strong evidence for a difference in evoked dynamics between the acute and both early and late subacute stages. **C2**, Association between response features and lesions maps were assessed using a voxel-based lesion symptoms mapping (VLSM). In the acute stage, the number of deflections within the first 200 ms was found negatively correlated with lesions in the internal capsule (depicted by red voxels, N=54).

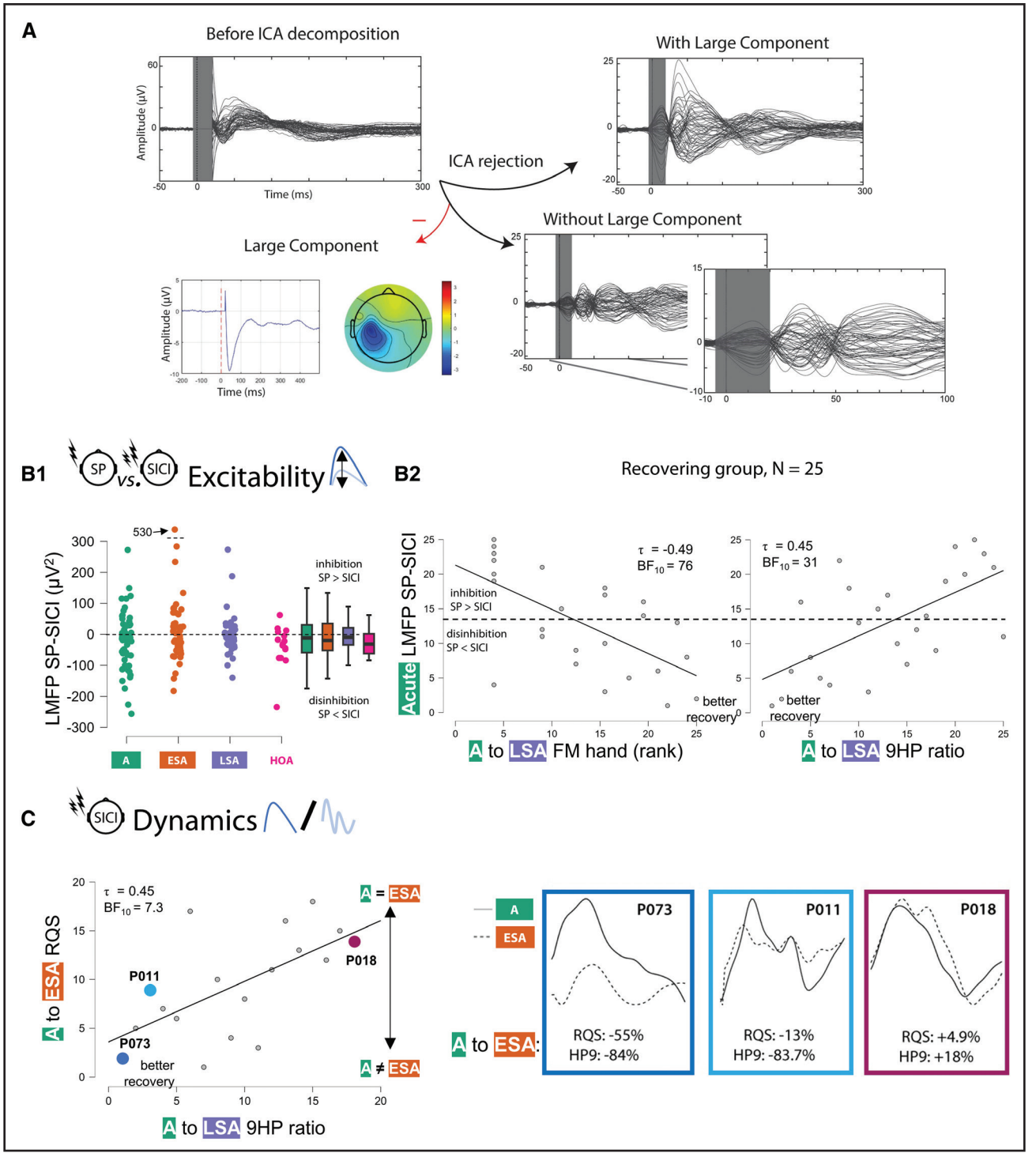

Figure 5. Complementary intracortical inhibition mechanisms revealed after removal of the abnormal large component. **A**, The large and monophasic evoked component, when visually detected, was removed during the second round of ICA decomposition before these complementary analyses. The removal of the large component (**bottom left**) unmasked weaker and more complex evoked signals (**bottom right**, without large component). **B1**, Distribution of the contrast between local mean field power (LMFP) of single-pulse (SP) and short-interval intracortical inhibition (SICI) protocol across stroke stages and for healthy older adults (HOAs). On average, negative values indicated a tendency toward disinhibition.[9,25–28] **B2**, Significant associations between high disinhibition in the acute stage (**left**), and better motor recovery in the recovering group. Such correlations were also found within the whole cohort of patients (see Tables S1 and S2). **C**, Significant association between change in regression quality score (RQS) for SICI condition from the acute to the early subacute stages (*y* axis) and motor recovery toward the late subacute stage (**left**). Plots of individual local TMS-evoked potentials (TEPs) after SICI stimulation in the acute (solid line) and early subacute (dotted line) stages for 3 representative patients. Respective evolution in RQS and 9-hole peg (9HP) are expressed below each signal. Note that a low value of RQS change between stroke stages correspond to a high similarity in the evoked signals dynamics, that is, no evolution in evoked dynamics, between these stages, which is associated with worse recovery (P018, **right**).

DISCUSSION

Abnormal Brain Reactivity in Patients With Stroke

Our results suggest that patients with stroke present atypical brain reactivity patterns, when the ipsilesional motor cortex is stimulated by means of TMS. In the acute stage, the pathological response was characterized by a large monophasic response, which contrasted with the weaker and more complex response observed in healthy young[29] and older adults (Figure 3). This result extends previous findings that spotted this abnormal reactivity in severely affected patients with stroke.[13,15,16] Results centered on early LMFP indicated that stroke presented a hyperexcitable ipsilesional motor cortex compared with age-matched controls, or with what is generally found in healthy young[8] and older[30] populations (Figure 4B1). Interestingly, the high interpatient variability regarding excitability explained the level of motor impairment: this hyperexcitability was mostly found in the most affected patients and was positively linked with the level of motor impairment (Figure 4B2).

Analysis of the evoked response's dynamics indicated that part of this abnormal reactivity was specific to the acute stage and evolved through stroke stages (Figure 4C1). Signal dynamics being sensitive to both local, for example, cytoarchitectonics and neurotransmitter concentrations,[10,31] and global properties, for example, structural and functional connectivity,[32] such results could highlight the poststroke reorganization processes occurring at these various levels. Regarding the global level, the VLSM revealed that acute signal dynamics were associated with the lesion load in the internal capsule (Figure 4C2), hosting the main outflow from motor cortical areas containing fibers from the corticospinal, corticorubral, and corticopontine tracts,[33] in line with previous work.[13] Such disruption of fibers connecting the cortex to subcortical structures prevents propagation and integration of the evoked activity to distant brain areas, leading to simpler responses reflected by fewer deflections. Recent work has succeeded in modeling this effect, showing that lesioned structural connectomes tended to produce simple and local TMS-evoked responses.[34] However, the longitudinal changes observed thereafter might rather underline reorganization processes occurring at the level of local intracortical inhibitory systems.

Disinhibition as a Key Mechanism for Successful Recovery

Recent animal work suggested that hyperexcitability and disinhibition states occur between the first week and 1-month poststroke and play an essential role for neuronal plasticity and recovery.[35] Indeed, in the acute stage, GABA-mediated ipsilesional intracortical inhibition is reduced compared with the unaffected hemisphere and with healthy controls.[36] Animal models[3,37] and human studies[38,39] suggest that this acute disinhibition is adaptive by enhancing ipsilesional neuronal excitability through reduction of cortical inhibition. This decrease in cortical inhibition is thought to promote plastic changes and reorganization to sustain the recovery of the lost functions.[7] If indirect proof of such disinhibition lies in the abnormally strong response observed here, the use of the SICI stimulation protocol enabled the direct probing of the intracortical GABAergic inhibitory system. In healthy young adults, SICI is known to induce an inhibition of the early TMS-evoked cortical activity.[9,25] In opposite, our results showed that SICI-induced inhibition was perturbated in the patient cohort, which showed strong inter-individual variability with a tendency toward disinhibition at the group level (Figure 5B1).

Whether the demonstrated disinhibition is for all patients, patient groups (eg, mild-moderate versus severely impaired) of adaptive or maladaptive nature remains not completely clear. In the present study, the acute disinhibition was associated with better recovery of distal impairment and fine motor skills, especially in the recovering group (Figure 5B2). These findings point to the fact that fine-tuned inhibitory activity is especially critical for more skilled hand functions, for example, as assessed by the 9-hole peg test. Although Tscherpel et al[13] also showed a relation between a large and simple reactivity in the acute stage and motor recovery, the direction of the effect was opposite to the present results. However, the link between abnormal reactivity and worse recovery reported in the previous study could be explained by a greater proportion of severely affected patients with limited improvement. The present cohort, which contained a higher proportion of mildly to moderately affected patients, might have exhibited the same physiological response to TMS but showed better recovery due to their overall less structurally damaged initial status. Severely impaired patients may also exhibit acute hyperexcitability and disinhibition to promote neuronal plasticity, but the presence of greater damage to the connectome, especially in key hubs,[40] would nevertheless hinder successful recovery over time. Finally, a disinhibition state was also present in our HOAs' group, which did not significantly differ from the patients (Figure 5B1). The perturbation of the GABAergic inhibitory system with age is known[30] and has been linked to a deficit in inhibitory control.[41] Further investigation would be needed to determine whether the acute beneficial disinhibition observed here is a direct result of poststroke mechanisms, or whether it highlights the anterior presence of a natural age-induced disinhibition that proved beneficial after stroke.

Interestingly, such beneficial disinhibition was also found in the present cohort of patients when focusing on late TMS-induced alpha oscillations,[19] which is also a marker of GABAergic system activity. The time course

of this disinhibition, that is, between the early and late subacute stages, and spatial localization, that is, more diffused across the brain, differed from the acute and local phenomenon found here. Taken all together, our results suggest that motor recovery is supported by a disinhibition phase occurring first in the acute stage to promote plastic changes and reorganization of the localized impacted areas, which then spread on a larger scale to allow remote plasticity and network reorganization towards the late subacute stage[5] (see Figure 6 of Harquel et al[19]).

Changes of Intracortical Inhibitory Activity Within Ipsilesional Motor Cortex and Motor Recovery

Previous work has hypothesized different roles for persistent disinhibition in the early or late chronic stage. Persistent disinhibition was notably observed at the cortical level in chronic patients with mild impairment.[15] While Ding et al[42] speculated that disinhibition could be detrimental for motor recovery, other studies showed that persistent disinhibition in the chronic stage might support recovery through enhanced plasticity in patients with residual deficits.[7,43] The functional role of persistent disinhibition in the chronic stage is thus unclear and the present longitudinal data contributed to addressing this question. Several readouts showed an evolution associated with motor recovery, and these results rather pointed toward a beneficial decrease of the disinhibition, that is, a restoration of a typical intracortical inhibitory activity within the ipsilesional motor cortex.

Although the association between the decrease in response power and motor recovery constitutes indirect evidence for a restoration of local inhibitory activity reducing cortical excitability (Figure 4B2), more direct evidence was found from the analysis of the SICI-evoked dynamics. First, the rapid change in evoked dynamics between the acute and early subacute stages was correlated with future motor recovery (Figure 5C). Second, the number of signal deflections increased with respect to motor recovery towards the late subacute stage, indicating a return to more complex response patterns for the recovering patients (Figure S1C2). Such changes in signal dynamics, that were absent when the motor cortex was probed using single pulses, might highlight the functional reorganization that is at stake within the ipsilesional and intracortical GABAergic inhibitory system.

Unmasking Complementary Intracortical Inhibition Mechanisms by Removing Atypical Large-Evoked Activity

We hypothesized that the large observed responses might mask further underlying intracortical inhibition mechanisms supported by neuronal activity of weaker power. In fact, the results related to excitability and early dynamics readouts were of the same nature for SICI as for single pulse (Figures 4 and 5). The removal of the large component (Figure 5A) unveiled complementary information on the intracortical inhibitory system. We propose that such large activity might predominantly originate from large layer V (L5) pyramidal cells present in the motor cortex, since both recording and stimulation techniques used here are biased toward this neuronal population.[44,45] On the one hand, these cells are known to generate the strongest electrical activity, at both microscale and mesoscale,[46] while being the most excitable neurons.[47] On the other hand, their specific shape and spatial orientation within the gyrus makes them more sensitive to the electrical field induced by TMS.[45] Therefore, TMS-EEG coupling might be oversensitive to deep L5 pyramidal neurons population and removing this large activity might allow to reveal activity from neurons within superficial layers eliciting weaker electrical potentials, such as inhibitory interneurons.

Limitations

Although this study followed the most recent guidelines on TMS-EEG coupling acquisition and data processing,[21] the absence of a realistic sham stimulation condition prevented the assessment of the influence of peripheral evoked potentials, resulting from the multisensory nature of TMS.[48] However, since this study mostly focused on early components, which are known to be much less sensitive to peripheral influence,[25,29,49,50] and employed a longitudinal analysis approach with the comparison with a proper age-matched control group, the absence of a sham stimulation condition does not affect the final interpretation of the results. Another limitation pertains to the distribution of the patient cohort concerning sex (73% of males), the severity of motor impairment in the acute stage and the extent of recovery. The majority of patients exhibited moderate to mild impairment. Conducting additional analyses on more heterogeneous groups in future studies will help refine the current conclusions regarding the relationship between changes in brain responsivity and motor recovery.

Conclusions

In conclusion, this work offers new insights into the longitudinal changes of cortical reactivity and local intracortical inhibition in the affected motor cortex after a stroke. The present results strongly support the critical impact of intracortical disinhibition evolving in the acute stage on residual motor function and recovery, especially skilled distal functions, and the importance of reorganization within the intracortical inhibition system of the lesioned motor cortex to sustain long-term motor

recovery. Furthermore, this study demonstrates that TMS-EEG provides an excellent opportunity to determine the reactivity of the affected motor cortex after a stroke, even in patients with impacted corticospinal tract preventing the monitoring of peripheral motor activity with EMG. This knowledge provides a strong basis for developing TMS-EEG toward a clinical tool to phenotype patients and to develop biomarkers related to recovery and treatment response.

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Supplemental Material

Supplemental Methods
Supplemental Results
Tables S1–S2
Figure S1

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