

Letter to the Editor

Response to Questions Regarding “Altered Resting-State Electroencephalogram Microstate Characteristics in Stroke Patients”

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Thank you for forwarding the thoughtful letter regarding our paper “Altered resting-state electroencephalogram microstate characteristics in stroke patients” published in the *Journal of Integrative Neuroscience*. We sincerely appreciate your interest in our work and the time you took to raise these important questions. We welcome the opportunity to address the concerns and questions raised, as they help clarify the study’s methods and also highlight areas where future research could be refined. We have carefully considered the comments and provide the following responses:

Point 1: “...the electrical activity of the cortex can be highly dependent on the location and volume of the stroke [1]. Subcortical strokes can produce a completely different resting electroencephalogram (EEG) pattern from strokes that involve the cortex. Brainstem strokes may present completely differently on the resting EEG from supratentorial strokes involving the cortex. Also, the extent of the stroke nucleus can strongly influence the cortical electrical activity [2].”

Author response to Point 1:

Research published in *The New England Journal of Medicine* has revealed that similar clinical symptoms can result from lesions in distinct brain locations that share the same brain network [3]. The correlation between the location of lesions and clinical symptoms is complex. Our study aimed to address the same clinical symptoms. Although the location of lesions may vary, it can also lead to similar changes in brain function within a single brain network (as shown in Table 1).

It is more important that the current understanding of how different lesion locations in stroke patients affect microstate dynamics remains limited. Previous research has revealed that the transition probabilities from microstate A and microstate D to microstate C significantly decreased

whereas the transition probabilities from microstate A to microstate D and from microstate D to microstate B significantly increased in patients with acute brainstem infarction relative to those in healthy individuals, suggesting an increased tendency to activate microstate B and a corresponding rise in the extent of neural activity over time [1]. The previous studies of simultaneous EEG-functional magnetic resonance imaging (fMRI) have shown a significant correlation between microstate B and the visual network (VN) [2,4,5]. The previous studies also revealed that microstate A is related to the sensorimotor network (SMN), microstate C is associated with the salience network (SN), and microstate D is connected with the dorsal attention network (DAN). However, there is still a lack of exploration into the different effects of lesion location (cortical, subcortical, brainstem) on brain dynamics in stroke patients.

This current study included 24 stroke patients, among whom 4 had cortical lesions, 10 had subcortical lesions, 1 had brainstem lesions, 5 had cortical and subcortical lesions, and 4 had subcortical and brainstem lesions. The study did not involve subgroup analyses based on lesion location, which is a limitation. Thus, we are currently unable to determine whether different lesion locations led to distinct EEG microstate patterns. This will be a key question that can be explored from a functional network perspective in our future research. We plan to expand sample size for more detailed stratified analyses. Additionally, if requested, we can perform subgroup analyses with the current data, although the small sample size may limit the statistical power of such analyses.

Point 2: “...whether only patients with an ischemic stroke or also with a hemorrhagic stroke were included, was not mentioned. If patients with hemorrhagic stroke were also included, whether the hemorrhage was accompanied by edema or not should be mentioned, because the degree of



Table 1. Detailed demographic characteristics and clinical evaluations of stroke patients.

Subjects	Gender	Age	Diagnosis	Lesion location	Time of onset (months)	Hemiplegic side	FMA	FMA-UE	FMA-LE	ARAT	MMSE
Sub01	male	65	ischemic	subcortical + brainstem	11	right	60	36	24	11	30
Sub02	female	46	hemorrhagic	subcortical	12	left	37	15	22	2	28
Sub03	male	67	ischemic	subcortical	1	left	62	40	22	27	26
Sub04	male	67	ischemic	subcortical	11	left	45	26	19	2	23
Sub05	male	65	ischemic	subcortical	3	left	21	9	12	0	26
Sub06	male	38	ischemic	subcortical	1	right	64	41	23	37	26
Sub07	male	37	hemorrhagic	cortical	11	left	41	18	23	2	30
Sub08	male	59	ischemic	subcortical + brainstem	3	right	54	31	23	19	27
Sub09	male	56	ischemic	subcortical	8	right	61	41	20	27	24
Sub10	female	53	ischemic	cortical + subcortical	5	left	25	10	15	0	24
Sub11	female	75	ischemic	subcortical	11	right	62	28	34	13	26
Sub12	female	62	hemorrhagic	subcortical	10	right	34	16	18	1	28
Sub13	male	44	ischemic	subcortical	10	left	56	40	16	10	30
Sub14	male	44	ischemic	subcortical + brainstem	7	right	16	8	8	0	23
Sub15	female	66	ischemic	cortical + subcortical	11	left	37	18	19	0	25
Sub16	female	67	ischemic	cortical + subcortical	5	right	19	10	9	0	23
Sub17	male	67	ischemic	cortical	10	left	42	26	16	6	25
Sub18	male	50	ischemic	subcortical + brainstem	11	right	73	47	26	13	29
Sub19	male	49	ischemic	cortical	1	left	81	52	29	33	28
Sub20	female	30	ischemic	cortical + subcortical	6	right	36	15	21	7	29
Sub21	male	67	ischemic	cortical	10	left	85	55	30	38	30
Sub22	male	61	hemorrhagic	subcortical	7	right	16	8	8	0	23
Sub23	female	49	hemorrhagic	brainstem	8	right	63	38	25	15	28
Sub24	male	34	ischemic	cortical + subcortical	7	right	46	22	24	5	28

FMA, Fugl-Meyer assessment; FMA-UE, Fugl-Meyer assessment of upper extremity; FMA-LE, Fugl-Meyer assessment of lower extremity; ARAT, action research arm test; MMSE, mini-mental state examination.

perifocal edema can strongly influence cortical electrical activity [4]. It is also important to know how many patients with hemorrhagic stroke had or did not have intraventricular intrusion.”

Author response to Point 2:

As explicitly stated in Section 2.1 Participants, our study enrolled patients with “first-ever ischemic or hemorrhagic stroke”. The study did not involve separate subgroup analyses for ischemic or hemorrhagic stroke, because our primary research objective was to elucidate the global patterns of cortical electrophysiological alterations induced by post-stroke motor dysfunction. Our research enrolled 5 patients with hemorrhage (sub 02, 07, 12, 22, and 23), with onset times of 12 mo, 11 mo, 10 mo, 7 mo, and 8 mo, respectively. We traced the medical history of the patients and confirmed that the most recent neuroimaging prior to study enrollment showed no evidence of perifocal edema or intraventricular intrusion, which suggested that their effect on electrophysiological measurements was likely minimal in our study. As noted by the reader, we acknowledge that perilesional edema may indeed influence electrophysiological activity. We agree that future investigations could incorporate stroke-phase classification, pathological subtypes, and multimodal neuroimaging data (e.g., magnetic

resonance imaging (MRI)/computed tomography (CT)) to facilitate more nuanced subgroup analyses.

Point 3: “...the latency between the acute stroke and the EEG recordings was not measured, or was not included in the analysis. The cortical electrical activity may strongly depend on the “age” of the stroke [5]. The “older” the stroke, the more likely it is that cortical activity, and thus EEG activity, will recover. Electrical activity may also depend on the response to stroke rehabilitation. Patients who have full functional and structural recovery may be associated with normal electrical activity, compared to patients who do not fully recover. Therefore, the final outcome of the 19 patients must be included in the analysis.”

Author response to Point 3:

This is a significant issue. As presented in Table 1 of our response, we have documented the latency between acute stroke and the EEG recordings, which ranged from 1 to 12 mo across participants. Table 1 also details motor function of the affected limb in stroke patients using the Fugl-Meyer assessment (FMA) and the action research arm test (ARAT) scales. We explored correlations between temporal characteristics (duration, occurrence, and coverage) and transition probabilities (TP) of four microstates and clinical outcomes, including the FMA and ARAT scores,

in stroke patients. We found that the TP from microstate A to microstate D had a significant positive correlation with the Fugl-Meyer assessment of lower extremity (FMA-LE) scores, which did not survive false discovery rate (FDR) adjustment. This indicated a trend where the higher the TP from microstate A to D was, the higher the patient's FMA-LE scores would be, revealing that these networks were continuously switched. Although all patients showed motor dysfunction based on the scoring criteria and none achieved full recovery, the varying degrees of dysfunction may still have been a source of EEG heterogeneity. Additionally, as Fox has highlighted, lesion network mapping focuses on the spatial component of lesion-induced symptoms, but the temporal component may be equally significant [3]. Therefore, future studies can employ subgroup analyses based on severity levels of dysfunction and longitudinal follow-up designs to further elucidate the relationship between latency, deficits and EEG activity.

Point 4: "...a previous stroke was an exclusion criterion, but the exclusion was based on history only, suggesting that patients with a previous subclinical stroke on imaging were included in the study. This issue should be clarified."

Author response to Point 4:

Although subclinical stroke (also termed silent cerebral infarction) presents no neurological symptoms, emerging evidence has suggested that it may still exert effects on electrophysiological activity of brain [6]. Yang *et al.* [6], found that patients with silent cerebral infarction had lower P300 amplitude and longer latency than did healthy individuals. Therefore, we fully concur with the reader's comment. A limitation of our research was the absence of neuroimaging (e.g., MRI) to exclude participants with subclinical stroke, which may have had a confounding effect on the observed outcomes. Thus, it is significant to incorporate neuroimaging techniques (e.g., MRI) to screen for and exclude individuals with a history of silent cerebral infarction in order to enhance methodological rigor in future investigations.

Point 5: "...stroke may be manifested not only by limb weakness but also by dysarthria, aphasia, or dysphagia. However, the FMA does not record and assess these features, which is why the severity of the deficits may have been misclassified. Furthermore, the ARTA test is inadequate to assess the bulbar symptoms of stroke patients."

Author response to Point 5:

The diversity of clinical manifestations of stroke, such as dysarthria, aphasia, or dysphagia, may indeed influence the comprehensive evaluation of global neurological function in patients. However, our study was specifically designed to investigate the underlying mechanism of brain motor reorganization, with a primary focus on the motor function of hemiplegic limbs and EEG activity. Thus, we selected two movement-specific assessment scale: FMA and ARAT. FMA is considered by many in the field of stroke rehabilitation to be one of the most comprehensive

quantitative measures of motor impairment after stroke [7]. ARAT is a reliable, valid measure of arm motor status after stroke and has established value for characterizing clinical states [8]. Since all enrolled patients in this study presented with limb-movement disorders as the primary clinical manifestation (as explicitly specified in the inclusion criteria), prioritizing motor function assessment was justified. However, we must acknowledge that the lack of assessment for other neurological symptoms (e.g., dysarthria, aphasia, or dysphagia) may have resulted in an incomplete characterization of patients' overall neurological deficits. Future studies could incorporate comprehensive scales or domain-specific assessment tools, such as the Western Aphasia Battery (WAB) and the Water-swallowing test (WST), to fully delineate the spectrum of neurological impairments in stroke patients [9,10].

Point 6: "...patients taking anti-seizure and antipsychotic drugs were excluded, but not patients taking sedatives or hypnotics. Since the latter can greatly reduce cortical activity at rest, we should know how many of the patients suffered from insomnia, anxiety, or depression, and required appropriate medication. Those patients also need to be excluded from the analysis."

Author response to Point 6:

It is indeed a methodological limitation of our study that we did not exclude participants using sedatives or hypnotics. Specifically, we neither systematically collected data on such medication use nor assessed related psychological symptoms (e.g., insomnia, anxiety, or depression). Previous studies examining EEG activity demonstrated that sedatives or hypnotics can affect EEG activity [11,12]. For example, in a report that evaluated the power spectral profiles of various sleep agents in healthy individuals, zolpidem reduced activity in the lower frequency bands and increased activity in the middle frequency bands, whereas suvorexant had no effects in any of the frequency bands [13]. To address these limitations, future studies should implement stricter screening protocols to exclude participants using sedative/hypnotic medications, while incorporating standardized psychometric assessments to comprehensively evaluate psychological status.

Author Contributions

HL: Writing original Draft, Formal analysis, Visualization; ZM: Resources, Investigation; JZ: Data Curation, Investigation; JW: Methodology, Funding acquisition; MZ: Conceptualization, Supervision, Funding acquisition; XH: Conceptualization, Writing review and Editing, Funding acquisition; JX: Conceptualization, Funding acquisition. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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