Changes in Brain Activation Pattern During Working Memory Tasks in People With Post-
COVID Condition and Persistent Neuropsychiatric Symptoms

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*Neurology®* Published Ahead of Print articles have been peer reviewed and accepted for publication. This manuscript will be published in its final form after copyediting, page composition, and review of proofs. Errors that could affect the content may be corrected during these processes.
Contributions:
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Thomas M Ernst: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data

Figure Count:
6

Table Count:
1

Search Terms:

Acknowledgment:
We thank our research participants for their participation. We also thank Dr. Andrea Levine for referring some of the post-COVID participants to the study.

Study Funding:
This work was supported by a grant from the National Institute of Neurological Disorders and Stroke (R21-NS121615).

Disclosures:
S. Kottilil is a member of the Scientific Advisory Board at Merck, Regeneron, Silverback therapeutics, and Zhuhai Yufan Biotechnologies and The Liver Company, and has received grants paid to the institution from Gilead Sciences and Arbutus Pharmaceuticals; L. Chang reports no disclosures relevant to the manuscript; M.C. Ryan reports no disclosures relevant to the manuscript; H. Liang reports no disclosures relevant to the manuscript; X. Zhang reports no disclosures relevant to the manuscript; E. Cunningham reports no disclosures relevant to the manuscript; J. Wang reports no disclosures relevant to the manuscript; E. Wilson reports no disclosures relevant to the manuscript; E.H. Herskovits reports no disclosures relevant to the manuscript; T. Ernst reports no disclosures relevant to the manuscript.
Abstract

**Background and Objectives:** Post-COVID condition (PCC) is common and often involves neuropsychiatric symptoms. This study aimed to use blood-oxygenation-level-dependent functional MRI (BOLD-fMRI) to assess whether participants with PCC had abnormal brain activation during working memory (WM), and whether the abnormal brain activation could predict cognitive performance, motor function or psychiatric symptoms.

**Methods:** The PCC participants had documented COVID-19 at least 6 weeks prior to enrollment. Healthy control participants had no prior history of COVID-19 and negative tests for SARS-CoV-2. Participants were assessed using three NIH-Toolbox (NIHTB) batteries for Cognition (NIHTB-CB), Emotion (NIHTB-EB) and Motor function (NIHTB-MB), as well as selected tests from the Patient-Reported Outcomes Measurement Information System (PROMIS). Each had BOLD-fMRI at 3 Tesla, during WM (N-back) tasks with increasing attentional/WM load.

**Results:** 169 participants were screened; 50 fulfilled the study criteria and had complete and usable datasets for this cross-sectional cohort study. 29 PCC participants were diagnosed with COVID-19 242±156 days earlier, had similar ages (42±12 vs. 41±12 years), gender proportion (65% vs. 57%), racial/ethnic distribution, handedness, education, and socioeconomic status, as the 21 uninfected healthy controls. Despite the high prevalence of memory (79%) and concentration (93%) complaints, the PCC group had similar and normal performance on the NIHTB-CB as the controls. However, PCC participants had greater brain activation than the controls across the network (p-FDR-corrected=0.003, T-max=4.17), with greater activation in the right superior frontal gyrus (p=0.009, Cohen’s-d=0.81, 95%CI [0.15-1.46]) but lesser deactivation in the default mode regions (p=0.001, d=1.03, 95%CI [0.61-1.99]). Compared to controls, PCC participants also had poorer dexterity and endurance on the NIHTB-MB, higher T-scores for negative affect and perceived stress, but lower T-scores for psychological well-being on the NIHTB-EB, as well as more pain symptoms and poorer mental and physical health on measures from PROMIS. Greater brain activation also predicted poorer scores on measures that were abnormal on the NIHTB-EB.

**Discussion:** PCC participants with neuropsychiatric symptoms demonstrated compensatory neural processes with greater usage of alternate brain regions, and reorganized networks, to
maintain normal performance during WM tasks. BOLD-fMRI was sensitive for detecting brain abnormalities that correlated with various quantitative neuropsychiatric symptoms.

Introduction

Post-COVID condition (PCC) or long-COVID syndrome are highly prevalent (~42% based on a recent meta-analysis\(^1\)), and the symptoms may persist for two years or longer.\(^2\text{-}^4\) Neuropsychiatric symptoms are particularly common, including fatigue, inability to concentrate or “brain fog”, headaches, hyposmia/dysgeusia, sleep disorders, anxiety, and depression.\(^3\text{-}^5\) Although the pathophysiology or mechanisms underlying these persistent symptoms remain unclear, neuropathology of COVID-19 patients who died within 2 months of illness found microvascular injury with leakage of fibrinogen, along with activated microglia and hypertrophic astrocytes within the olfactory bulb and brain stem.\(^6\) Patients who died on average 28 days after hospitalization showed neuronal loss in the cerebellum, axonal swelling and neuronal degeneration in the pons, and widespread activation of glia and immune cells.\(^7\) Non-human primates infected with SARS-CoV-2 also show similar neuroinflammatory changes and Purkinje cell death in the cerebellum on necropsy.\(^8\) However, without post-mortem brain tissue, whether evidence of brain injury persists in PCC with ongoing neuropsychiatric symptoms after acute COVID-19 illness remains unknown.

Several neuroimaging studies evaluated brain abnormalities in post-acute COVID-19 patients. PET studies showed decreased glucose metabolism in cortical and subcortical regions that correlated with poorer cognitive function and other functional complaints within the first few months of recovery from COVID-19.\(^9\text{-}^11\) However, six-months after acute COVID-19, glucose metabolism and cognition were found to be normal despite memory and attention complaints.\(^12\) On MRI, three-months after acute COVID-19, recovered participants showed enlarged gray matter volumes in bilateral olfactory cortices, hippocampi, insula, Heschl’s gyrus, Rolandic operculum, and cingulate gyri.\(^13\) In contrast, a larger longitudinal MRI study found that recovered COVID-19 participants had greater decreases in cortical thickness than controls in multiple brain regions, including the orbitofrontal cortex and the parahippocampal gyrus, two brain regions related to olfaction.\(^14\) Several diffusion tensor imaging (DTI) studies found variable abnormalities in brain diffusivity and fractional anisotropy,\(^13\text{-}^15\text{-}^17\) indicating disruption of microstructural integrity, which persisted in hospitalized patients at 1-year follow-ups.\(^18\) However, task-activated blood-oxygenation-level-dependent functional MRI (BOLD-fMRI) has not been used to evaluate how brain function is impacted in individuals with persistent neuropsychiatric symptoms after recovery from acute COVID-19.

We aimed to evaluate whether participants with long-COVID symptoms for at least 6 weeks after their acute illness have abnormal brain function on quantitative neurobehavioral measures and abnormal brain activation on task-activated BOLD-fMRI. We hypothesized that compared to controls, these recovered COVID-19 patients with long-COVID syndrome would show persistent cognitive deficits and neuropsychiatric symptoms on three NIH toolbox® (NIHTB) batteries, including the Cognitive Battery (NIHTB-CB), Emotional
Battery (NIHTB-EB), and Motor Battery (NIHTB-MB), and more symptoms for pain and poorer physical and mental health, on the Patient-Reported Outcomes Measurement Information System® (PROMIS). We also expected that post-COVID-19 participants would require greater brain activation than healthy controls, indicating greater usage of alternate brain regions to compensate for the residual brain injury or ongoing neuroinflammation. In addition, we expected that the greater brain activation in post-COVID-19 participants would be related to deficits on measures that were abnormal on the NIHTB batteries and PROMIS.

**Methods**

All participants were recruited through referrals from our medical center and flyers distributed locally. To minimize potential sources of bias or confounds, healthy controls were recruited to match the age range, self-reported gender and racial/ethnic proportions of the Post-COVID group. Self-reported race and ethnicity classifications were in accordance with U.S. Census data.

Participant inclusion criteria for both groups included men or women of any race or ethnicity, 18-75 years of age, able and willing to provide informed consent. Post-COVID participants were required to have confirmed COVID-19 diagnosis from medical records and a documented positive PCR test for SARS-CoV2 at least 6 weeks prior to enrollment (to minimize risk for ongoing infection and ensure that they no longer require hospitalization) and had at least one persistent post-COVID symptom. Healthy controls were required to have no history of COVID-19 symptoms or illness, and a negative PCR test within 7 days or a negative SARS-CoV-2 antigen test on study day. Exclusion criteria for both groups included: 1) significant co-morbid psychiatric illness that may confound study measures; 2) any confounding neurological disorders, including significant prior head trauma with loss of consciousness >60 minutes; 3) taking medications that could significantly alter functional brain imaging studies; 4) any current or history (within the past two years) of severe substance use disorder, according to the Diagnostic and Statistical Manual of Mental Disorders-5); tobacco use was allowed; 5) positive urine toxicology screen on day of assessments; 6) pregnancy or breast-feeding (self-report); 7) contraindications for MR studies (e.g., metallic objects, electronic implants, or severe claustrophobia).

Each participant was assessed using a standardized neuropsychiatric evaluation, including substance use history, electrocardiogram, urine toxicology, and screening blood tests (complete blood count and comprehensive metabolic panel) from medical records within one year or collected at the screening visit. The post-COVID-19 participants also completed a survey regarding their acute COVID-19 symptoms and treatments, any premorbid conditions, and current long-COVID symptoms and severities (Table 1).

**Standard Protocol Approvals, Registrations, and Patient Consents**

Written informed consent was obtained from all participants according to the Declaration of Helsinki using a protocol approved by the University of Maryland, Baltimore Institutional Review Board (Human Research Protections Office at the University of Maryland, Baltimore; protocol # HP-00092062).
Quantitative Neurocognitive Tests and Psychiatric Symptom Assessments
Each participant was evaluated with three NIHTB batteries and selected PROMIS measures. The Cognition Battery (NIHTB-CB) assessed seven cognitive domains: 1) Attention/Executive Functioning, 2) Episodic Memory, 3) Working Memory, 4) Language, 5) Executive Function, 6) Processing Speed, 7) Immediate Recall. The Emotional Battery (NIHTB-EB) assessed four domains: Negative Affect, Psychological Well-Being, Stress and Self-Efficacy, and Social Relationships. The Motor Battery (NIH-MB) assessed five domains: 1) Dexterity, 2) Strength, 3) Balance, 4) Endurance, 5) Locomotion. The PROMIS measures were selected from four domains to assess symptoms related to Depression, Anxiety, Fatigue, and Pain, which derived scores for Global Physical Health and Global Mental Health. Findings regarding these measures were reported previously in a larger cohort, but the current subsets of the participants’ data are presented here.

Activation Paradigm, Image acquisition and processing
Each participant performed three N-back WM tasks (0-back, 1-back, 2-back) during BOLD-fMRI using a block design (30s task period alternating with 30s rest period, four repeats over 4 minutes for each task, with five targets presented at random times per 30s task block) as described. Briefly, during the 0-back task, participants pressed a button with their dominant hand index finger whenever a number flashed on the screen, and during the 1-back and 2-back tasks, the participant responded whenever the target letter was the same as the previous screen (1-back) or two screens previously (2-back). The task periods alternating with passively viewing various symbols during the rest period.

All scans were acquired on a 3T MR Scanner (Siemens Prisma). Structural MRI included a sagittal 3D magnetization prepared rapid gradient echo (repetition time/echo time [TR/TE]/inversion time [TI] 2,200/4.47/1,000 ms, 1-average, 256x256x160 matrix) and an axial fluid attenuated inversion recovery FLAIR sequence (TR/TE/TI59,100/84/2,500 ms, 1-average, 204x256x44 matrix). A board-certified neuroradiologist (EH) and an experienced board-certified neurologist (LC) both reviewed all structural MRIs, blinded to the COVID-status of the participants, to exclude those with major structural abnormalities. BOLD fMRI used a single-shot gradient-echo echo-planar sequence (TE/TR=30/3000ms, ~42 axial 3-mm slices, 3-mm resolution, 80 excitations) with real-time motion correction. The MRI trigger pulse was synchronized to a custom-stimulus software written in Matlab (The MathWorks, Inc., Natick, MA). Participants responded with a button push during the task periods, providing reaction time and accuracy. Only fMRI data with <1.5mm translations and <1.5º rotations during each scan, and ≥70% performance accuracy were included in the final analyses.

fMRI scans were acquired from 53 subjects, but scans from the 2-back task were excluded for five subjects in the Post-COVID group due to excess motion (n=1) or <70% accuracy (n=4), while scans from six controls were removed, due to significant medical issues discovered after the scans (n= 2) or <70% accuracy (n=4); see details in eFigure1. Data were processed using Statistical Parametric Mapping (SPM)12. To avoid potential bias, all fMRI data were
preprocessed by an engineer blinded to the participants’ COVID status. For each task, a mask was created by combining activation maps (1-sample t-test) for each group at p ≤ 0.05.

**Sample Size Estimate and Statistical analyses**

We estimated that with 20 subjects per group, assuming α = 0.05 and variability with a standard deviation of 50% in each group, we expected 89% power to detect a 1.5-fold change in outcome variables between groups. Of note, similar sample sizes per group yielded significant group effects in our prior fMRI studies.\(^{27-29}\)

Statistical analyses were performed in R (version 4.1.2). Demographic data were compared between groups using t-tests, Chi Square tests, and Fischer’s Exact Test. Group differences on NIHTB & PROMIS measures were assessed using unpaired t-tests for fully-corrected T-scores (adjusted for age, gender, education, and race/ethnicity) or analyses of covariance (ANCOVA) for raw scores (with adjustments for age, gender, and Index of Social Position (ISP) calculated from the Hollingshead Four Factor Index of Socioeconomic Status).\(^{30}\)

Cohen’s d effect sizes with 95% Confidence Internals were calculated using the R package ‘effsize’.

Group differences in BOLD activation throughout the brain were compared in SPM12, using two-sample t-tests (with the appropriate mask), thresholded at ≥100 voxel clusters and T ≥ 1.7. Only corrected p-values at the cluster level, with a false discovery rate (FDR) ≤ 0.05, were considered significant. Since portions of the WM network may also be involved in emotional regulation,\(^{31}\) linear regression was used to explore whether the abnormal brain activation predicted neurobehavioral outcome measures that were abnormal on the NIHTB or PROMIS. BOLD signals were also extracted at the 3 cluster maxima (6x6x6 mm = 216 mm\(^3\)) for each task to perform post-hoc analyses.

**Data Availability**

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

**Results**

**Participant Characteristics (Table 1)**

Between February 2021-February 2022, 169 potential participants were pre-screened by telephone, 61/169 (36%) were enrolled for additional on-site screening, and 57/61 (93%) fulfilled study criteria and completed the behavioral studies and 53/61 (87%) completed the fMRI scans. 50 participants (29 post-COVID and 21 controls) with usable fMRI scans are reported (see Participant Flow diagram, eFigure 1).

The post-COVID-19 participants were diagnosed 219±137 days (~7 months) earlier, and had similar age, gender proportion, racial and ethnic distribution, handedness, education, and socioeconomic status (from the ISP), as the healthy controls. Although the PCC group tended to have higher body mass indices than the controls, the proportions of participants in the overweight/obese categories were not different between groups. The two groups also had
similar proportions that used tobacco, marijuana, or alcohol; similar prevalence of co-morbid illnesses, including hypertension, diabetes, overweight/obese, and chronic obstructive pulmonary disease. Their vaccination status for SARS-CoV-2 was also not different between groups.

Amongst the post-COVID-19 participants, 9/29 were hospitalized, and 19/29 required one or more treatment(s) during their acute illness, which included supplemental oxygen \(n=11\), nasal cannula \(O_2\), Hi-Flow/BiPAP, ventilation, or extracorporeal membrane oxygenation (ECMO), steroids \(n=15\), dexamethasone, prednisone, methylprednisolone, or hydrocortisone), remdesivir \(n=5\), monoclonal antibodies \(n=3\, bamlanivimab, etesevamab\), antibiotics for secondary infections \(n=5\, azithromycin, ceftriazone\), and/or apixaban for deep vein thrombosis \(n=1\). See Table 1 for the number of participants who received one or more of these treatments.

Of the 50 usable fMRI datasets, nine participants (six post-COVID, three controls) had minor abnormalities on their structural MRIs that were not exclusionary. Five (3 post-COVID participants, 2 controls) had slightly more than age-related white matter lesions, two (one in each group) had lacunar infarcts, one control had microhemorrhages, and one control had both a small \(6\)-mm old infarct and a microhemorrhage.

**Neuropsychiatric Symptoms in Post-COVID-19 Participants (Table 1, Figure 1A)**

The Post-COVID-19 participants endorsed a high prevalence of cognitive complaints [concentration problems (92.9%), memory problems (78.6%), confusion (64.3%)] and neurological symptoms [headaches (57.1%), visual disturbances (50%), gait disturbance (50%), paresthesia (42.9%), and coordination problems (39.3%)]. The common acute symptoms of hyposmia and dysgeusia persisted in 28.6% of post-COVID-19 participants. They also had a high prevalence of new onset psychiatric and other symptoms, including fatigue (85.7%), depression/anxiety (67.9%), sleep disturbance (64.3%), myalgia (60.7%), light-headedness (46.4%) and urinary problems (27.6%, including frequency, dysautonomia and recurrent urinary tract infections).

**NIH Toolbox Batteries and PROMIS Measures**

Despite the high prevalence of neurocognitive complaints, the post-COVID participants had normal performance on all seven cognitive domains of the NIHTB-CB (data not shown, but similar to those reported in the larger cohort\(^23\)). However, on the NIHTB-MB, the post-COVID-19 participants performed poorer than controls on locomotion (4-Meter walk, Cohen’s \(d=-0.74, 95\%\text{CI}: [-0.14, -1.34], p=0.01\)), endurance (2-Minute Walk, \(d=-0.93, 95\%\text{CI}: [-0.31, -1.55], p=0.003\)), and dominant hand dexterity (9-Hole Pegboard Test, \(d=-0.79, 95\%\text{CI}: [-0.19, -0.38], p=0.007\)). Figure 1B. The two groups’ performance were similar on the Standing Balance Test and the non-dominant hand Pegboard.

In addition, on the NIHTB-EB (Figure 1C), the post-COVID participants showed markedly poorer psychological well-being than controls, including lower positive affect \(d=-1.02, 95\%\text{CI}: [-0.41, -1.63], p=9\times10^{-4}\) and general life satisfaction \(d=-1.20, 95\%\text{CI}: [-0.57, -
1.82], \( p=1.3 \times 10^{-4} \), and much higher perceived stress (\( d=0.95 \), 95%CI: [0.34, 1.56], \( p=0.002 \)) and negative affect measures, including anger (\( d=0.93 \), 95%CI: [0.33, 1.54], \( p=0.002 \)), sadness (\( d=0.85 \), 95%CI: [0.25, 1.56], \( p=0.004 \)), fear somatic arousal (\( d=2.23 \), 95% CI: [1.50, 2.96], \( p=5 \times 10^{-10} \)), and fear affect (\( d=1.04 \), 95%CI: [0.43, 1.65], \( p=7 \times 10^{-4} \)).

PROMIS measures corroborated with the NIHTB-EB. The post-COVID-19 participants had much higher T-scores than controls for mental health symptoms, including depression and anxiety, as well as for physical health symptoms, with more fatigue and pain measures, leading to poorer overall mental (\( d=-1.37 \), 95%CI: [-0.73, -2.00], \( p=2 \times 10^{-5} \)) and physical health (\( d=-2.59 \), 95%CI: [-1.81, -3.37], \( p=6 \times 10^{-12} \)) (Figure 1D).

**BOLD-fMRI during WM Tasks**

Across all participants, brain activation showed the typical load-dependent increases in the WM network with increasing task difficulty.\(^{24,25}\) Post-COVID-19 participants and controls showed no group differences in brain activation during the 0-back and 1-back tasks (Figure 2A). However, Post-COVID-19 participants had greater brain activation than controls (\( T=4.17, P_{FDR-corr}=0.003 \)) on the 2-back task for a large cluster in the WM network, with three subcluster maxima centered at the right posterior cingulate (PCG: 2, -50, 26 & 8, -56, 28) and right superior frontal gyrus (SFG: 36, 2, 28) (Figures 2B, 3, and 4).

Using extracted BOLD signals at the cluster maxima, we delineated that the greater BOLD signals in the post-COVID participants than the controls were due to greater activation in the SFG region, but lesser deactivation in the PCG region (Figure 3A). The extracted BOLD signals across the three tasks further demonstrate that the group differences increased parametrically (linear-mixed effects model-\( p \)-values=0.002-0.018), with only the 2-back task showing significant group differences on post-hoc analyses (post-hoc-\( p=0.001-0.009 \)) (Figure 3B). Despite these group differences in brain activation, the two groups had similar accuracy and reaction times for each task (Figure 3C). Furthermore, an anti-correlation between deactivation on the 2-back task in the PCG (the default mode region) and activation in the SFG was observed only for the control, but not the post-COVID group (Figure 3C, bottom).

Several brain regions showed lesser activation on the 2-back task at the uncorrected cluster-level in post-COVID participants than controls (Figure 4). The two large clusters with lesser activation included subcluster maxima in the left hemisphere for postcentral gyrus, insula, precentral gyrus, and inferior parietal lobule (red regions, Figure 4). By contrast, all brain regions with greater activation on the 2-back task in the post-COVID group than controls were in the right hemisphere (green regions, Figure 4).

For the 2-back task, when comparing COVID-19 severity during the acute phase, post-COVID subjects who were hospitalized with more severe illness showed lesser activation than the non-hospitalized individuals in several frontal regions [right and left subgyral and left SFG, \( P_{FDR-corrected}=0.001 \) (cluster level)]. The extracted BOLD signals from these cluster maxima are shown (Figure 5, A–C). We further evaluated for possible gender-
specific differences (COVID-19-by-gender, co-varied for age and ISP), but found no gender-specific effects on brain activation. Furthermore, BOLD signals in the WM network on the 2-back task predicted T-scores on the NIHTB-EB measures that showed significant group differences (eTable 1). Specifically, we found similar relationships for both groups in the right SFG and right parietal regions, where higher BOLD signals correlated with lower Positive Affect and more Perceived Stress (right SFG: \( r=0.49, p<0.001, \beta=-6, 95\% CI [-10, -2] \); right parietal: \( r=0.50, p<0.001, \beta=14, 95\% CI [6, 23] \). Figures 5, D and E). However, we also found differences (interactions) in the correlations between the two groups: only the COVID-19 group with higher BOLD signals at the right extranuclear region had greater Anger Affect \( (r=0.69, p<0.001, \beta=15, 95\% CI [6, 25]) \), higher BOLD signals at the right superior temporal gyrus correlated with more Sadness \( (r=0.68, \beta=15, 95\% CI [8, 23], p<0.001) \), and higher BOLD signals at the left frontal white matter correlated with lower Psychological Well-Being \( (r=0.68, \beta=-24, 95\% CI [-35, -14], p<0.001) \). (Figure 5, F–H).

Similarly, BOLD signals in the WM network on the 2-back task predicted the scores on PROMIS measures that showed significant group differences (eTable 1). Greater brain activation in the left frontal lobe on the 2-back task predicted more psychiatric symptoms and poorer mental health on PROMIS (eFigure 2). Specifically, across all participants, greater BOLD signals in the left anterior cingulate gyrus predicted higher levels of anxiety \( (r=0.51, p=0.001, \beta=18, 95\% CI [10, 27], eFigure 2A) \), greater BOLD signals in the left insular-subgyral region predicted more depressive symptoms \( (r=0.78, p<0.001, \beta=31, 95\% CI [20, 42], eFigure 2B) \), and greater BOLD signals in the left inferior frontal gyrus predicted poorer global mental health \( (r=0.51, p<0.001, \beta=-7, 95\% CI [-10, -3], eFigure 2C) \).

Lastly, on the NIHTB-MB, COVID-19 participants had poorer dexterity than controls across the age spectrum (Dominant Hand, \( d=-0.85, group-p=0.007, 95\% CI [-1.50, -0.20] \), Figure 6A) and poorer endurance (2-Minute Walk, \( d=-0.83, 95\% CI [1.48, -0.17], group-p=0.002 \), especially in the older participants, \( (\beta=0.8, 95\% CI [0.2,1.5], group-by-Age-p=0.02, Figure 6B) \). In brain regions that showed non-significant lesser activation in the post-COVID participants than controls, lower BOLD signals in the left postcentral gyrus predicted poorer dexterity on the Pegboard Dominant Hand T-scores only for the COVID subjects \( (r=0.58, p=0.003, \beta=11, 95\% CI [5,18], Figure 6C) \), while lower BOLD signals in another region in the left postcentral gyrus predicted poorer endurance on the 2-Minute Walk across all participants \( (r=0.51, p<0.001, \beta=16, 95\% CI [8,24], Figure 6D) \). Although COVID-19 participants also had slower locomotion on 4-Meter Walk than the controls, none of the brain regions showed a correlation with the performance on this test.

**Discussion**

Participants with Post-COVID condition, on average 7 months after their COVID-19 diagnosis, reported a high prevalence of neuropsychiatric symptoms, including “brain fog” and concentration or memory problems. Despite these subjective complaints, the post-COVID participants had relatively normal performance with objective cognitive testing on
the NIHTB-CB, including attention and WM, and during the fMRI tasks. However, they had poorer dexterity and endurance on the NIHTB-MB relative to both the healthy controls and the normative database, especially in the older post-COVID individuals. In addition, the greater emotional distress and negative affect endorsed on the NIHTB-EB and the mental health symptoms assessed on PROMIS concurred with the post-COVID participants’ complaints. Furthermore, our post-acute COVID-19 participants had poorer global physical health, attributed by significantly more fatigue and pain symptoms.

This is the first task-activated BOLD-fMRI study in recovered COVID-19 patients. Although the post-COVID participants had normal and similar accuracy and reaction times as the controls, they had greater brain activation, with lesser deactivation in right PCG and greater activation in right SFG within the WM network on the more difficult 2-back task. These findings indicated a reorganized WM network, with greater or compensatory usage of the non-dominant brain regions, but less usage of the parietal default mode resources, to maintain normal performance. Furthermore, greater brain activation predicted more severe neuropsychiatric symptoms in these recovered patients.

**Cognitive Performance and Neuropsychiatric Symptoms in Post-COVID Participants**

The normal performance in our post-COVID participants assessed objectively in seven cognitive domains on the NIHTB-CB is consistent with the normal Mini-Mental State examination in 443 mainly non-hospitalized individuals, ~9 months after their SARS-CoV-2 infection, relative to 1328 matched controls.\(^{32}\) Another study of 31 long-COVID participants 202±58 days after acute illness with neurocognitive complaints of impaired attention, memory, and multitasking abilities, also found normal neuropsychological tests scores and on the Montreal Cognitive Assessment.\(^{12}\) More sensitive tests that can assess fatigue and cognitive endurance are needed.

However, the post-COVID participants’ higher T-scores on psychological and emotional symptoms, perceived stress, and negative affect, as well as their lower T-scores on psychological well-being and general life satisfaction on the NIHTB-EB, are consistent with their subjective complaints, and were further corroborated by the higher levels of depressive and anxiety symptoms, and poorer mental health on PROMIS. These findings concur with a meta-analysis report that evaluated 10,530 patients and found a high prevalence of these symptoms in those with post-COVID syndrome at mid (3-6 months) to long-term (> 6 months) follow-ups which suggested that these symptoms might become even more prevalent over time.\(^{33}\)

In addition, the elevated T-scores on pain measures (behavior, quality, intensity, interference) in the post-COVID participants are also consistent with a comprehensive review of 54 reports that found a high prevalence of pain in 21,668 COVID-19 patients, including 33.9% reported headaches, 47.1% had a sore throat, 61.0% had myalgia or arthralgia, 17.7% had chest pain, and 14.5% had abdominal pain.\(^{34}\)
Relative to the controls, our post-COVID participants had poorer dexterity in their dominant hands across the age spectrum, and lower endurance on the 2-Minute Walk Test. The slower performance on the 2-Minute Walk Test is similar to that reported in 37/66 (56%) hospitalized COVID patients at 3-6 months follow-up, but the abnormality persisted in only 14/161 (8.7%) hospitalized patients one year later. Similarly, lower endurance on the 6-Minute Walking Test was reported in 79% of hospitalized COVID-patients at 6-week follow-up, and in only 29% of those at 6-month follow-up. Although two-third of our post-COVID participants were not hospitalized, they showed relatively lower endurance, especially the older participants.

**Reorganized brain activation patterns on BOLD-fMRI in post-COVID-19 participants**

During WM tasks, men typically activate bilaterally or predominantly the right hemisphere network, while women typically show greater activation lateralized to the left hemisphere. However, although two-third of our post-COVID participants were women, they had greater activation in the non-dominant right hemisphere, which indicates a reorganized neural network, with suboptimal activation in the normal network but greater activation in alternate brain regions, to maintain normal performance during the N-back tasks, and likely on the NIHTB-CB. Such a reorganized neural network was also observed in people with HIV, who showed similar adaptation of their neural network during task-activated BOLD-fMRI, even in cognitively asymptomatic individuals.

The cluster maxima in the PCG coincided with the well-described medial parietal node in the default mode network (DMN), showing that greater activation in this region resulted from lesser deactivation in the COVID-19 group than the control group. Such diminished or deficits in task-induced deactivation in the DMN were also found in individuals with mild Alzheimer’s disease (AD), those with only the APOE4 genetic risk for AD, and middle-aged individuals with subclinical cognitive decline. Other brain disorders with lower than normal deactivation in the DMN include schizophrenia, idiopathic generalized epilepsy, attention deficit/hyperactivity disorder, and obsessive-compulsive disorders. Less deactivation in the DMN suggest less brain reserve, since the deactivation might provide reallocation of neural resources for other brain regions or networks. The relatively greater SFG activation in our post-COVID participants is consistent with greater attentional modulation of the top-down dorsal attentional network. Greater activation in this region of the brain is often observed with brain injury in those with chronic neuroinflammation, such as that seen in persons with HIV infection or mild traumatic brain injury. Since the inverse correlation between greater deactivation in the DMN and the greater activation in SFG was seen only in the controls, the post-COVID participants might have deficits in the DMN due to COVID-19-induced brain injury, requiring compensatory usage of alternative brain regions in the contralateral right hemisphere, such as the greater activation in the SFG.

**Brain activation on the 2-back task predicted neuropsychiatric symptoms and motor performance**

Greater activation in the WM network predicted less positive affect and more perceived stress across all participants, but more symptoms of anger and sadness, and poorer psychological...
well-being only in the post-COVID participants. The associations across all participants might be due to the greater stress from the pandemic, while the associations between greater left frontal activation and the psychological symptoms might reflect the greater severity of these symptoms in the Post-COVID participants. Although the exact pathophysiology of PCC remains unclear, peripheral immune markers, such as C-reactive protein and peripheral inflammatory markers including lymphocytes and platelets, correlated with greater severity of depressive symptoms in post-COVID patients. Future studies evaluating both peripheral and CSF immune markers may provide further insights into these relationships. The altered brain activation was observed only with the higher attentional/WM load, which suggest that higher cognitive demand in their daily lives could further contribute to the negative psychological and psychiatric symptoms in post-COVID participants.

Lastly, lesser brain activation on the 2-back task within the left dominant postcentral sensorimotor gyrus predicted poorer dominant hand dexterity in the post-COVID participants, and lower endurance with the 2-Minute Walk across all participants. These findings further delineated the relationships between the sensorimotor cortex and motor function.

**Limitations**

Our study has several limitations. First, the moderate sample size precluded the evaluation of further subgroup analyses, such as gender-specific effects, group differences in age-dependent changes, or more detailed evaluation of disease severity, in relation to the symptoms associated with PCC. Second, this study was conducted primarily during the Delta variant phase in the United States; therefore, how the new variants might affect the brain could not be assessed. Third, without SARS-CoV2 antibody testing, we cannot exclude the remote possibility of prior asymptomatic infections in the controls. Furthermore, longitudinal follow-ups are needed since some long-COVID symptoms decreased at 2-year follow-up, while psychiatric or cognitive function worsened over time, especially in older COVID-19 survivors. Lastly, due to the study’s cross-sectional design, the abnormal brain activation in the Post-COVID group cannot be causally attributed to COVID-19.

In summary, the NIHTB and PROMIS measures quantified the post-COVID participants’ neuropsychiatric symptoms, including high levels of fatigue, pain and emotional symptoms, as well as motor deficits, but demonstrated normal cognitive function. On BOLD-fMRI, these post-COVID-19 participants had a reorganized network with less activation indicating suboptimal functioning in the normal network, but greater brain activation in the contralateral hemisphere, likely to maintain normal performance during the WM tasks and on the NIHTB-CB. The abnormal brain activation predicted poorer motor dexterity and endurance, as well as neuropsychiatric symptoms, which might support the WM network’s involvement in emotional regulation. Task-activated BOLD-fMRI is sensitive and objective for evaluating post-COVID brain abnormalities, especially with a parametric design using increasing cognitive load (i.e., a brain stress test). Future longitudinal follow-ups using these quantitative measures and task-activated BOLD-fMRI will allow us to delineate whether or when these altered brain activation patterns and the neuropsychiatric symptoms will
normalize. Finally, correlations between immune or neuronal injury markers, as well as other brain imaging biomarkers (e.g., MR spectroscopy) are needed to further delineate the possible relationship between immune activation and persistent brain injury in post-COVID participants.

Figure Legends

Figure 1. Prevalence of neuropsychiatric symptoms and the assessment scores from the NIHTB batteries and the PROMIS. (A) Self-reported neuropsychiatric symptoms by the COVID-19 participants. (B) NIH Toolbox Motor Battery (C) NIH Toolbox Emotion Battery (D) PROMIS.
Figure 2. Group fMRI activation maps and group comparison during the N-back tasks. 
(A) **Top panel:** The 0-Back and 1-Back task paradigms, as shown sequentially on the presentation computer during the fMRI, are illustrated on the left, and the typical activation maps for each group are shown in three orientations. No group differences were found on these tasks (hence the group comparison data are not shown). **(B) Bottom panel:** The 2-back task paradigm is illustrated on the left, and the group activation maps show the typical WM activation patterns, including activation in the bilateral dorsolateral or inferior prefrontal cortices, the anterior cingulate cortex, the precuneus and bilateral posterior parietal and occipital regions and the cerebellum. Note the higher T-max scores on the color scale for the 2-back task than the 0-back and 1-back tasks shown above. Cortical surface maps and axial an image below show the brain regions with greater activation in post-COVID-19 participants compared to healthy controls on the two-sample t-tests (co-varied for age, gender, and Index of Social Position). Clusters with corrected-p<0.05 and ≥100 voxels are shown, with cluster maxima shown in the posterior cingulate gyrus and the superior frontal gyrus (see also Figure 3).

Figure 3. Brain activation differences (and %BOLD signals) on the 2-back task, and performance during the N-back tasks. (A) On the 2-back task, COVID-19 participants had greater BOLD signals than uninfected controls, subcluster maxima are shown in two subregions of the posterior cingulate gyrus (PCG) and the superior frontal gyrus (SFG). Group comparisons were evaluated with analysis of covariance (ANCOVA, co-varied for age, gender, and ISP) at the cluster level p-corrected=0.003, 7,770 voxels and Tmax=4.17. The bar graphs below show the % BOLD signal extracted from a 27-voxel region of interest.
(red circles) center at each of the three cluster maxima shown on the map, showing less deactivation in the PCG regions but greater activation in the SFG. (B) In each of the cluster maxima shown in A, BOLD signals from 0-back, 1-back and 2-back tasks were extracted to illustrate that the two groups were different (on the linear mixed effects model), and the differences are maximal and significant only with the 2-back task (post hoc p-values are shown). (C) Performance with % accuracy and reaction times during the 3 N-back tasks show no group differences. Bottom graph: greater deactivation (more negative % BOLD signals) in the PCG predicted greater activation in the SFG only in the controls, while post-COVID participants showed only minimal deactivation and primarily a positive correlation between the PCG and SFG.
Figure 4. Regional group differences on activation during the 2-back task. The activation maps show both brain regions that are greater (green) or lesser (red) in the post-COVID group compared to the controls. The table on the right shows the Montreal Neurological Institute (MNI) coordinates of where the group differences are located both with the corrected $p$-values with false discovery rate (FDR) and the uncorrected $p$-values at the cluster level, as well as the maximum T-scores in each of the subclusters. The three significant clusters (at the FDR-corrected level) that are greater in post-COVID than controls are also shown in Figures 2 and 3 above.
Figure 5. Hospitalized effect on regional % BOLD signals and regional brain activation predicted emotional symptoms on the NIH-THB-Emotion Battery. (A-C) Comparison of COVID-19 severity on brain activation during the 2-Back Task. The scatterplots show the %BOLD signals extracted from the cluster maxima in brain regions showing significantly less activation (FDR-corrected-p=0.001, cluster level) in hospitalized post-COVID-19 participants (purple) than those who were not hospitalized (green), in the right and left sub-gyral region and the left superior frontal (SFG) across the age range. (D & E) Across all participants, those with higher % BOLD signals in the right SFG had lower scores for positive affect, and those with higher % BOLD signals in the right parietal region endorsed more perceived stress. (F-H) Lower regional activation predicted higher T-scores on Anger and Sadness and lower level of Psychological Well Being only in the post-COVID-19 group.
Figure 6. Poorer motor performance was related to lower % BOLD signals in the left postcentral gyrus (the sensorimotor cortex) (A & B) Post-COVID-19 participants (red dots) had slower performance on manual dexterity (9-Hole Pegboard dominant hand) and poorer endurance (2-Minute Walk) as shown by their lower T-scores on these tasks compared to the healthy controls (blue dots) across the age spectrum, especially for the older participants on the 2-Minute Walk task. (C) Only post-COVID-19 participants who had lesser brain activation in the left postcentral gyrus had poorer dexterity in their dominant hand; no such relationship was found in the healthy controls. (D) Across all participants, those with lesser brain activation in another region of the left postcentral gyrus had poorer endurance.

http://links.lww.com/WNL/C748

http://links.lww.com/WNL/C749

http://links.lww.com/WNL/C750
References


Table 1. Participant Characteristics (Mean ± S.D., number or %)

<table>
<thead>
<tr>
<th></th>
<th>Post-COVID (n=29)</th>
<th>Healthy Controls (n=21)</th>
<th>P-Value</th>
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<tr>
<td>Age (Years)</td>
<td>42.4 ± 12.3</td>
<td>41.5 ± 12.2</td>
<td>0.81†</td>
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<tr>
<td>Sex [%] Men / [%] Women</td>
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<td></td>
<td></td>
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<tr>
<td>10 (34.5%) / 19 (65.5%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Race/Ethnicity [% White / Hispanic / Asian / Black / Biracial]</td>
<td>19 / 1 / 0 / 8 / 1</td>
<td>8 / 3 / 2 / 8 / 0</td>
<td>0.12²</td>
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<tr>
<td>Handedness (Right / Left)</td>
<td>27 / 2</td>
<td>20 / 1</td>
<td>0.75⁵</td>
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<tr>
<td>Education [% Graduate / Undergraduate / Some College / High School]</td>
<td>8 / 8 / 8 / 5</td>
<td>11 / 7 / 1 / 2</td>
<td>0.10³</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>30.7 ± 8.0</td>
<td>26.5 ± 6.2</td>
<td>0.05²</td>
</tr>
<tr>
<td>Index of Social Position</td>
<td>30.4 ± 14.1</td>
<td>27.4 ± 14.8</td>
<td>0.46³</td>
</tr>
</tbody>
</table>

## Substance Use History

| # Lifetime / Past-month Tobacco Use | 10 / 1 | 6 / 1 | 0.89⁶ / 1¹ |
| # Lifetime / Past-month Marijuana Use | 14 / 3 | 6 / 2 | 0.27⁷ / 1³ |
| # Lifetime / Past-month alcohol use | 23 / 23 | 18 / 18 | 0.83⁸ / 0.83⁸ |

## Co-Morbid Medical Illnesses Prior to COVID-19 or Vaccination Status at Study

| Hypertension (%) | 6 | 1 | 0.09⁹ |
| Diabetes (%)     | 4 | 0 | 0.11¹¹ |
| Overweight / Obese (%) | 9 / 13 | 4 / 6 | 0.96¹² |
| Chronic Obstructive Pulmonary Disease (%) | 4 | 0 | 0.25¹⁰ |
| Vaccination status (yes / no / unknown) for SARS-CoV2 | 16 / 13 / 0 | 17 / 1 / 3 | 0.06⁸ |

## Post-COVID-19 Participant History and Symptoms

<table>
<thead>
<tr>
<th>Days since Diagnosis [range]</th>
<th>242 ± 156 [42-484]</th>
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<tbody>
<tr>
<td># Hospitalized / # Non-Hospitalized</td>
<td>9</td>
</tr>
</tbody>
</table>

## # of Participants That Received COVID-19 Treatments

| Hi-Flow/BiPAP, Ventilation, ECMO, Steroid¹³; Remdesivir + Apixaban | 1 |
| Hi-Flow/BiPAP, Steroid⁶ + Remdesivir | 2 |
| Ventilation + Steroid⁶ | 1 |
| Nasal Cannula O₂, Steroid⁴ + Antibiotics⁹ | 1 |
| Nasal Cannula O₂, Steroid⁶ + Remdesivir | 3 |
| Steroid⁴ + Antibiotic⁹ | 3 |
| Steroid⁴ + Monoclonal Antibody¹⁰ | 1 |
| Monoclonal Antibody¹⁰ / Steroid⁶ / Nasal Cannula O₂ | 1 / 4 / 2 |
| No treatment | 10 |

## Neurological Post-COVID Symptoms

| Concentration Problems | 92.9 [14.3 / 53.6 / 25.0] |
| Memory Problems        | 78.6 [17.9 / 39.3 / 21.4] |
| Confusion              | 64.3 [42.9 / 14.3 / 7.1]  |
| Headaches              | 57.1 [7.1 / 35.7 / 14.3]  |
| Dizziness              | 57.1 [28.6 / 14.3 / 14.3] |
| Gait Disturbance       | 50.0 [28.6 / 17.9 / 3.6]  |
| Visual Disturbances    | 50.0 [21.4 / 21.4 / 7.1]  |
| Paresthesia            | 42.9 [17.9 / 10.7 / 14.3] |
| Coordination Problems  | 39.3 [14.3 / 25.0 / 0.0]  |
| Hyposmia               | 28.6 [14.3 / 10.7 / 3.6]  |
| Dysgeusia              | 28.6 [14.3 / 7.1 / 7.1]   |
| Postural Instability   | 14.3 [3.6 / 7.1 / 3.6]    |
| Other Neurological     | 14.3 [10.7 / 3.6 / 0.0]   |

## Psychological/Other Post-COVID Symptoms

| Fatigue               | 85.7 [10.7 / 28.6 / 46.4] |
| Depression or Anxiety | 67.9 [21.4 / 32.1 / 14.3] |
| Sleep Disturbances    | 64.3 [17.9 / 21.4 / 25.0] |
| Myalgia               | 60.7 [21.4 / 32.1 / 7.1]  |
| Light-headedness      | 46.4 [17.9 / 17.9 / 10.7] |
| Urinary Problems      | 25.0 [17.9 / 3.6 / 3.6]   |
Index of Social Position was calculated from the Hollingshead Four Factor Index of Socioeconomic Status. T-test; Chi Square test; Fischer’s exact test; Includes two post-COVID using cannabidiol Dexamethasone, Prednisone, Methylprednisolone, or Hydrocortisone; Bamlanivimab or Etesevimab Azithromycin or Ceftriazone
Changes in Brain Activation Pattern During Working Memory Tasks in People With Post-COVID Condition and Persistent Neuropsychiatric Symptoms

Linda Chang, Meghann C Ryan, Huajun Liang, et al.

*Neurology* published online April 26, 2023
DOI 10.1212/WNL.0000000000207309

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