Appendix 1: Supplementary material

Cerebral Blood Flow maps

Raw Arterial Spin Labeling MRI data are transformed to cerebral blood flow (CBF) weighted images. First, difference images are calculated by a control minus tag subtraction. Next, a proton density ASL reference is used to create a fractional difference image. The CBF-weighted images are subsequently scaled to absolute units of volume of blood flow per 100 gram of tissue per minute (mL/100g/min). Established values for this conversion to absolute CBF are based on the best-practise equation published previously in the ASL white paper (1). The CBF maps are calculated in the native orientation of the raw data, based on manual landmarking of each participant, as performed by a research MRI technologist. Group analysis is then facilitated by transforming the maps to a standard brain atlas template: the average adult brain provided by the Montreal Neurological Institute (MNI) (2). Altogether, the steps listed here are part of one software package that is available through the functional MRI of the brain (FMRIB) Software Library (FSL) and is based on previous work (3). Regional CBF values are extracted by overlaying anatomical reference locations onto individual CBF maps. Group average CBF values are calculated in the regions described below.

Resting State Brain Connectivity (fALFF) maps

The resting-state functional MRI data are collected as an image time series of 250 brain volumes, with the signal time-course at each voxel location reflecting blood oxygenation level-dependent (BOLD) contrast (4). The amplitude of low-frequency fluctuation (ALFF) metric of the resting-state fMRI data thus reflects the intensity of regional spontaneous brain activity as indexed by

the BOLD signal, with functional brain maps derived using a power spectrum decomposition. Frequency content between 0.01 and 0.08 Hz is selected as the BOLD signals of interest. To better generalize the ALFF maps and reduce confounds from physiological noise sources, the maps are normalized by dividing the ALFF image intensity values by the power content over the entire frequency range (per voxel), thus yielding fractional ALFF (fALFF) maps (5). The fALFF maps are registered to the MNI standard space in the same manner as the CBF maps. Group average fALFF values are calculated in the regions described below.

Regions of Interest for hypothesis testing of CBF and fALFF maps

A total of 10 brain regions of interest (ROI) were selected a priori as brain locations of interest. These ROIs are masks that delineate anatomical subregions on the basis of an established Harvard-Oxford brain atlas. The ROIs are overlaid on the CBF and fALFF maps to allow regional estimates, defined as the mean value of each metric in the ROI. A total of 10 CBF and 10 fALFF values are thus extracted per participant / session to facilitate group analyses. These regions have been implicated in COVID-19 neuroimaging findings, according to a literature search that was conducted in June 2021. In the table below there are two or more references for each ROI. It should be noted that this list is not exhaustive and it should not be considered a systematic review of the literature.

Region of Interest (ROI)	**PubMed links with embedded PMID provided**
Thalamus	https://pubmed.ncbi.nlm.nih.gov/?term=33501506+32728799+33398411
Caudate	https://pubmed.ncbi.nlm.nih.gov/?term=33452633+34149394
Hippocampus	https://pubmed.ncbi.nlm.nih.gov/?term=33501506+32728799+32838240
Anterior parahippocampal gyrus	https://pubmed.ncbi.nlm.nih.gov/?term=34189535+32728799+34182098

Appendix 1, as supplied by the authors. Appendix to: MacIntosh BJ, Ji X, Chen JJ, et al. Brain structure and function in people recovering from COVID-19 after hospital discharge or self-isolation: a longitudinal observational study protocol. *CMAJ Open* 2021. DOI:10.9778/cmajo.20210023.

Amygdala	https://pubmed.ncbi.nlm.nih.gov/?term=33501506+32728799+34182098
Orbitofrontal Cortex	https://pubmed.ncbi.nlm.nih.gov/?term=34189535+33501506+33398411+32444492
Anterior Cingulate Gyrus	https://pubmed.ncbi.nlm.nih.gov/?term=34189535+32728799+33398411+32838240
Insula	https://pubmed.ncbi.nlm.nih.gov/?term=34189535+33452633+32838240
Frontal Medial Cortex	https://pubmed.ncbi.nlm.nih.gov/?term=33720371+32294339
Posterior Cingulate Gyrus	https://pubmed.ncbi.nlm.nih.gov/?term=32728799+33452633+32838240

Electroencephalography analysis

EEG signals are acquired with a 256 Hz sampling rate using the Muse headset (RRID: SCR_014418), which contains two frontal channels (AF7 and AF8 positions) and two temporoparietal channels (TP9 and TP10 positions), referenced to a fifth channel located at Fpz. Raw EEG signals are processed first by applying a 2 - 36Hz bandpass filter and then rejecting data containing large noise (> 100 microvolts) using tools available in EEGLAB (6). Data from eyes closed and eyes open recordings are merged into one recording and independent component analysis is used to identify and reject at most one component that reflects eye blinks (7). The data are then split into 2 second epochs. The power spectral density (PSD) is obtained for each epoch and averaged across epochs for eyes closed and eyes open conditions, separately. The two frontal signals (left and right, AF7 and AF8) are averaged, and the two temporoparietal signals (left and right, TP9 and TP10) are averaged. The mean power is calculated for the following frequency bands: delta (2-4Hz), theta (4-7Hz), alpha (7-14Hz), and beta (14-30Hz) bands. Thus, there are 4 frequency bands, two brain locations, and two conditions for a total of 16 EEG outcomes to consider for hypothesis testing.

Statistical model for primary hypothesis testing

The primary hypothesis relies on the initial, cross-sectional data and is tested using R (www.r-project.org) . The statistical model uses a one-way ANOVA to test for an effect of group (e.g. testing whether at least one of the four groups is not like the others) with age and sex as covariates. In a sensitivity analysis related to this hypothesis, "days since COVID-19 infection" is added as an additional covariate to characterize the influence of this variable on the primary effect of group.

Longitudinal data (i.e. initial and follow-up visits) are assessed with the secondary hypothesis, which tests for differences in the brain measures over time between groups. The statistical analysis involves linear mixed-effects modeling, with group and session considered as fixed effects, and days since COVID-19 infection considered a random effect.

There is a need to account for the risk of false discovery for each of the outcome measures listed above. Namely, there are 10 ROIs from the CBF maps, 10 ROIs from the fALFF maps, and 16 EEG outcomes measures, which will be corrected for multiple comparisons at a false discovery rate of $q = 0.05$.

Data Sources

The table below provides a list of the data sources for the assessments described in this protocol. All items are accessible on the web. References and details are provided. Web links were accessed on 13 July 2021.

Assessment	Source for the assessment	Detail and references
On-site		
Anatomical MRI	http://sabre.brainlab.ca/docs/processing/stage7.html	Lesion analysis is one component to the suite of Lesion Explorer tools (8).
ASL Cerebral Blood Flow	https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BASIL	A graphical user interface is available (9).
fMRI fALFF	https://www.nitrc.org/frs/?group_id=296#	A series of shell scripts are available for download (5).
EEG	https://choosemuse.com/	Wearable EEG systems offer portable data collection (10).
Olfaction	https://sensonics.com/product/smell-identification-test/	The UPSIT is available for purchase and includes instructions on how to administer the test (11).
Vision	https://michaelbach.de/fract/	A visual test battery that is free for use as a computer program (12).
Vision	https://www.precision-vision.com/products/contrast-sensitivity-tests/peak-contrast-sensitivity/pelli-robson/pelli-robson-contrast-sensitivity-chart/	A visual test to see how well participants can see faint objects (13).
NIH Cognitive Toolbox	https://www.healthmeasures.net/explore-measurement-systems/nih-toolbox/intro-to-nih-toolbox/cognition	The NIH Toolbox Cognition Battery was developed as part of a NIH blueprint for neuroscience research (14).
Cognitive Mnemonic Similarity Task	https://faculty.sites.uci.edu/starklab/mnemonic-similarity-task-mst/	Download and implementation instructions for this memory test are provided from this lab's website (15).
Remote		
NIH Emotion	https://www.healthmeasures.net/explore-measurement-systems/nih-toolbox/intro-to-nih-toolbox/emotion	The NIH Toolbox Emotion Battery was developed as part of a NIH blueprint for neuroscience research (16).
Mild Behavioral Impairment Checklist (MBI-C)	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5652315/	A modified version of the MBI-C questionnaire has been adopted (17).

Appendix 1, as supplied by the authors. Appendix to: MacIntosh BJ, Ji X, Chen JJ, et al. Brain structure and function in people recovering from COVID-19 after hospital discharge or self-isolation: a longitudinal observational study protocol. *CMAJ Open* 2021. DOI:10.9778/cmajo.20210023.

NIH PROMIS	https://commonfund.nih.gov/promis/index	This NIH toolbox was a consensus-based framework and we have opted to use the dyspnea, sleep disturbance, cognitive complaints, and fatigue scales (18).
Functional status post-COVID-19	https://erj.ersjournals.com/content/erj/56/1/2001494/F1.large.jpg	This test is advocated as a tool to track functional status over time after COVID-19 (19).
SF-36	https://www.rand.org/health-care/surveys_tools/mos/36-item-short-form/survey-instrument.html	This short survey was designed to assess health-related quality of life (20).

References

1. Alsop DC, Detre JA, Golay X, Günther M, Hendrikse J, Hernandez-Garcia L, et al. Recommended implementation of arterial spin-labeled perfusion MRI for clinical applications: A consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia: Recommended Implementation of ASL for Clinical Applications. Magn Reson Med. 2015 Jan;73(1):102–16.

2. Mazziotta J, Toga A, Evans A, Fox P, Lancaster J, Zilles K, et al. A probabilistic atlas and reference system for the human brain: International Consortium for Brain Mapping (ICBM). Philos Trans R Soc Lond B Biol Sci. 2001 Aug 29;356(1412):1293–322.

3. Chappell MA, MacIntosh BJ, Donahue MJ, Günther M, Jezzard P, Woolrich MW. Separation of macrovascular signal in multi-inversion time arterial spin labelling MRI. Magn Reson Med. 2010 May;63(5):1357–65.

4. Ogawa S, Menon RS, Tank DW, Kim SG, Merkle H, Ellermann JM, et al. Functional brain mapping by blood oxygenation level-dependent contrast magnetic resonance imaging. A comparison of signal characteristics with a biophysical model. Biophys J. 1993 Mar;64(3):803–12.

5. Zou Q-H, Zhu C-Z, Yang Y, Zuo X-N, Long X-Y, Cao Q-J, et al. An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: fractional ALFF. J Neurosci Methods. 2008 Jul 15;172(1):137–41.

6. Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods. 2004 Mar 15;134(1):9–21.

7. Delorme A, Sejnowski T, Makeig S. Enhanced detection of artifacts in EEG data using higher-order statistics and independent component analysis. NeuroImage. 2007 Feb 15;34(4):1443–9.

Appendix 1, as supplied by the authors. Appendix to: MacIntosh BJ, Ji X, Chen JJ, et al. Brain structure and function in people recovering from COVID-19 after hospital discharge or self-isolation: a longitudinal observational study protocol. *CMAJ Open* 2021. DOI:10.9778/cmajo.20210023.

8. Ramirez J, Gibson E, Quddus A, Lobaugh NJ, Feinstein A, Levine B, et al. Lesion Explorer: a comprehensive segmentation and parcellation package to obtain regional volumetrics for subcortical hyperintensities and intracranial tissue. NeuroImage. 2011 Jan 15;54(2):963–73.

9. Groves AR, Chappell MA, Woolrich MW. Combined spatial and non-spatial prior for inference on MRI time-series. NeuroImage. 2009 Apr 15;45(3):795–809.

10. Krigolson OE, Williams CC, Norton A, Hassall CD, Colino FL. Choosing MUSE: Validation of a Low-Cost, Portable EEG System for ERP Research. Front Neurosci [Internet]. 2017 Mar 10 [cited 2021 Jul 19];11. Available from: http://journal.frontiersin.org/article/10.3389/fnins.2017.00109/full

11. Wolfensberger M, Schnieper I, Welge-Lüssen A. Sniffin'Sticks: a new olfactory test battery. Acta Otolaryngol (Stockh). 2000 Mar;120(2):303–6.

12. Bach M. The Freiburg Visual Acuity test--automatic measurement of visual acuity. Optom Vis Sci Off Publ Am Acad Optom. 1996 Jan;73(1):49–53.

13. Elliott DB, Sanderson K, Conkey A. The reliability of the Pelli-Robson contrast sensitivity chart. Ophthalmic Physiol Opt J Br Coll Ophthalmic Opt Optom. 1990 Jan;10(1):21–4.

14. Weintraub S, Dikmen SS, Heaton RK, Tulsky DS, Zelazo PD, Bauer PJ, et al. Cognition assessment using the NIH Toolbox. Neurology. 2013 Mar 12;80(11 Suppl 3):S54-64.

15. Stark SM, Kirwan CB, Stark CEL. Mnemonic Similarity Task: A Tool for Assessing Hippocampal Integrity. Trends Cogn Sci. 2019 Nov;23(11):938–51.

16. Salsman JM, Butt Z, Pilkonis PA, Cyranowski JM, Zill N, Hendrie HC, et al. Emotion assessment using the NIH Toolbox. Neurology. 2013 Mar 12;80(11 Suppl 3):S76-86.

17. Ismail Z, Agüera-Ortiz L, Brodaty H, Cieslak A, Cummings J, Fischer CE, et al. The Mild Behavioral Impairment Checklist (MBI-C): A Rating Scale for Neuropsychiatric Symptoms in Pre-Dementia Populations. J Alzheimers Dis JAD. 2017;56(3):929–38.

18. Cella D, Yount S, Rothrock N, Gershon R, Cook K, Reeve B, et al. The Patient-Reported Outcomes Measurement Information System (PROMIS): progress of an NIH Roadmap cooperative group during its first two years. Med Care. 2007 May;45(5 Suppl 1):S3–11.

19. Klok FA, Boon GJAM, Barco S, Endres M, Geelhoed JJM, Knauss S, et al. The Post-COVID-19 Functional Status scale: a tool to measure functional status over time after COVID-19. Eur Respir J. 2020 Jul;56(1).

20. Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care. 1992 Jun;30(6):473–83.

Appendix 1, as supplied by the authors. Appendix to: MacIntosh BJ, Ji X, Chen JJ, et al. Brain structure and function in people recovering from COVID-19 after hospital discharge or self-isolation: a longitudinal observational study protocol. *CMAJ Open* 2021. DOI:10.9778/cmajo.20210023.