Long-COVID mechanisms: Hypothesis for long COVID based on AI and modelling (D1.8 – SGA3)

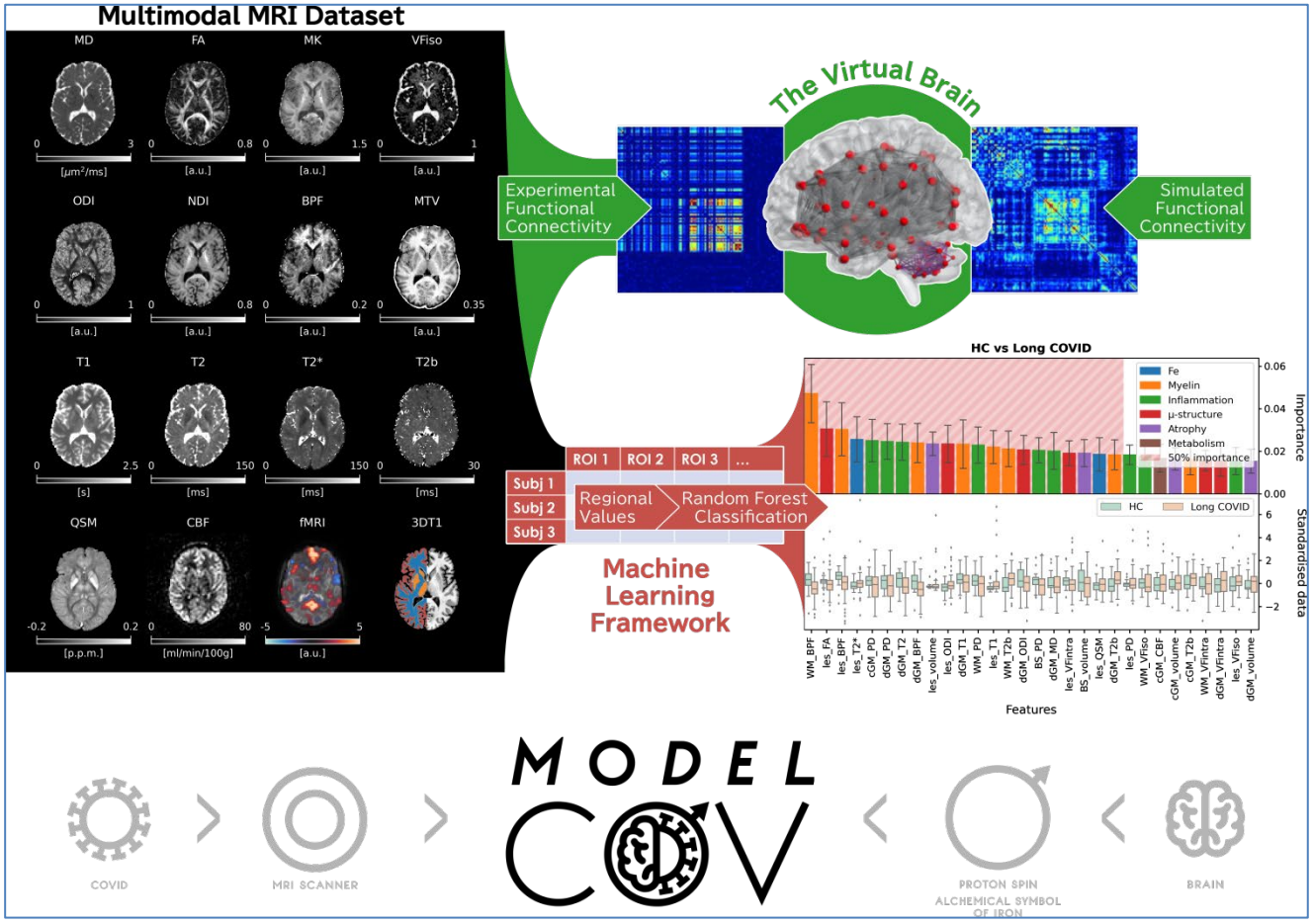

Figure 1: Multi-modal Magnetic Resonance imaging (MRI) data

Multi-modal Magnetic Resonance imaging (MRI) data was acquired on a cohort of healthy controls, COVID-19 and long COVID participants. Biophysically meaningful feature maps were generated to contribute to a voxel-based of this cohort. Featured extracted from macro-areas can be used for classical statistical models to determine mechanisms of long COVID and to explain clinical scores. Random Forest machine learning pipelines can be then used on small sample data to determine the most significant features classifying long COVID subjects compared to COVID and healthy controls. The virtual brain model (TVB) can then be run on functional series and diffusion connectomic for identifying patterns of excitatory/inhibitory balance for each participant.

Project Number:	945539	Project Title:	HBP SGA3

Document Title:	Long-COVID mechanisms: Hypothesis for long COVID based on AI and modelling
Document Filename:	D1.8 (D108) SGA3 M42 SUBMITTED 230929.docx
Deliverable Number:	SGA3 D1.8 (D108)
Deliverable Type:	Other
Dissemination Level:	PU = Public
Planned Delivery Date:	SGA3 M42 / 30 SEP 2023
Actual Delivery Date:	SGA3 M42 / 29 SEP 2023
Author(s):	Claudia WHEELER-KINGSHOTT, UCL (P82) Fulvia PALESI, UNIPV (P70) Bhavana SOLANKY, UCL (P70) Madiha SHATILA, UCL (P70) Elena GROSSO, UNIPV (P82) Antonio RICCIARDI, UCL (P70) Jed WINGROVE, UCL (P70) Marta GAVIRAGHI, UNIPV (P82)
Compiled by:	Claudia WHEELER-KINGSHOTT, UCL (P82) Fulvia PALESI, UNIPV (P70)
Contributor(s):	Claudia WHEELER-KINGSHOTT, UCL (P82) and Fulvia PALESI, UNIPV (P70) contributed to all sections Bhavana SOLANKY, UCL (P70) contributed to section 2.2 Madiha SHATILA, UCL (P70) contributed to section 2.3 Elena GROSSO, UNIPV (P82) contributed to section 3.2 Antonio RICCIARDI, UCL (P70) contributed to section 3.2 Jed WINGROVE, UCL (P70) contributed to section 3.4 Marta GAVIRAGHI, UNIPV (P82) contributed to section 3.5
WP QC Review:	Giovanna RAMOS QUEDA, AMU (P78), Pilar F. ROMERO, UPM (P68)
WP Leader / Deputy Leader Sign Off:	Viktor JIRSA, AMU (P78)
T7.4 QC Review:	N/A
Description in GA:	MRI data and meta-data from MRI analysis will be available on up to 144 subjects, 40 controls and 104 COVID. Models of brain dynamics from TVB will be demonstrated and compared between COVID and control subjects. BMFs extracted from qMRI and TVB modelling will be identified to explain long-COVID symptoms. Possible long-COVID scenarios will be proposed through MIP algorithms run on the entire cohort of subjects.

Abstract:	In this study we acquired a rich magnetic resonance imaging (MRI) dataset that provided voxel-based metrics of inflammation, microstructural integrity, myelin, and metabolic alterations in people who were infected with the SARS-CoV-2 virus (COVID cohort), developed long COVID or were never exposed to it (control cohort). A cohort of 127 subjects was recruited and 124 subjects underwent MRI. Maps sensitive to iron accumulation were also extracted to be entered in statistical analysis and assess whether this could be a mechanism of long COVID. Raw data went through automatic pipelines to generate the maps and macro-areas were averaged to provide a table of biophysically meaningful features (BMF) to be entered in statistical analysis. Linear regression models and random forest algorithms were run to identify the most significant features distinguishing control subjects from COVID and long COVID subjects. Resting state functional imaging data were analysed to search for signatures of anosmia in an initial cohort of subjects, and the virtual brain (TVB) analysis was performed to extract alterations in excitatory/inhibitory balance. TVB metrics were included in the table of features. Long COVID subjects have alterations of several metrics, suggestive of inflammation, iron accumulation and myelin damage that should be followed up longitudinally to understand the evolution of brain tissue property alterations and suggest potential interventions.
Keywords:	Magnetic Resonance Imaging, MRI, quantitative imaging, microstructure, COVID-19, long COVID, the virtual brain modelling, myelin, iron, functional, biophysically meaningful features (BMF)
Target Users/Readers:	Neuroscientists, clinicians, neurologists, MR Physicists, computer scientists, biomedical engineers, radiographers, MRI manufacturers, the general public affected by COVID, pharma companies

Table of Contents

Table of Tables

Table of Figures

1. Introduction

Long-COVID has been defined clinically. Subjects show persistent neurological symptoms, e.g. anosmia, fatigue, brain fog (memory) and depression. Mechanisms are still unclear. We have applied a rich advanced quantitative Magnetic Resonance Imaging (qMRI) protocol to a cohort of people with long COVID and compare objective measures of tissue features between long COVID and those who had COVID, but recovered from it, as well as with people who never contracted the SARS-CoV-2 virus (control cohort). From the qMRI data we extracted quantitative maps of biophysically meaningful features (BMFs) of the brain that can then be used to model possible mechanisms of long COVID. Our initial results show that inflammation is present in people with long COVID symptoms. We also found that there are functional alterations in people with persistent anosmia (Wingrove et al, 2023), with possible iron overload, prominent damage to the brainstem and an altered excitatory/inhibitory balance. In people with long COVID, metrics of myelin damage are the most altered of all in long COVID subjects.

As long COVID is still elusive, we have applied brain modelling methods such as the virtual brain (TVB) developed in HBP and part of the EBRAINS tools. We have also contributed to expand the atlas database available on EBRAINS by sharing the features and maps for a cohort of a total of 124 subjects, once published. We have explored machine learning algorithms available on the Medical Informatics Platform (MIP) of EBRAINS and wrote a wrapping routine for applying Random Forest (RF) machine learning (ML) algorithm as a classifier of long COVID patients, using BMFs calculated in macro-areas of the brain to reach a ranking of importance in the classification task. TVB metrics and iron specific BMFs will be added to the database and RF run to see whether the classification changes and COVID mechanisms are identified. The RF code is shared on GitHub.

Overall, we have contributed to acquire and share data, apply brain modelling and ML tools for classification and shown how advanced qMRI BMFs that can help us understand mechanisms of long COVID, as summarised in Figure 1. This work will interest several communities of scientists, clinicians, physicists and biomedical engineers, neuroscientists and the population in general who is eager to know the neurological consequences of the SARS-CoV-2 pandemic.

2. Rich database creation

During this project a rich database of biophysically meaningful features (BMFs) was created for uploading to EBRAINS. Description of the database includes the quantitative magnetic resonance imaging (qMRI) protocol, clinical and paraclinical scores as well as blood samples.

2.1 Subjects

The cohort of the study includes participants of an initial COVID-19 study and of a prospective cohort of long COVID and healthy control subjects. Overall, the cohort consists of 124 participants (see Table 1).

We recruited people who either had recovered from a SARS-CoV-2 infection (COVID) or believed that they had not been infected (Control), or who were diagnosed with long COVID (long COVID). Subjects were either self-referred to us, recruited from other COVID-19 studies at UCL/UCLH (COVID-19 Staff Testing of Antibody Responses Study (Co-Stars, ClinicalTrials.gov: NCT04380896) and Finding Out if COVID-19 Infection Can be pREdicted by ChAnges in Smell and/or Taste (FORECAST, ClinicalTrials.gov: NCT04377815) or referred from the National Hospital for Neurology and Neurosurgery (NHNN) or UCLH long COVID clinics.

The final inclusion criteria for the COVID group were:

- History of a positive Polymerase Chain Reaction (PCR) or a positive lateral flow antigen test (LFT), followed by a negative test shortly before the MRI appointment;
- Serum antibody positive test for SARS-CoV-2 infection using the EDI™ Coronavirus COVID-19 IgM and IgG enzyme-linked immunosorbent assay (ELISA) kit (sensitivity and specificity of 92% and 96.5%, respectively, at the time of scanning.

Similarly, the final inclusion criteria for the Control group were:

- A self-declaration of never having contracted the virus because they had not tested positive to either PCR or LFT since the beginning of the pandemic and had not suffered from symptoms suggestive of COVID-19 (in 2020) (e.g., fever, persistent cough and anosmia);
- An EDI™ ELISA serum antibody negative test for SARS-CoV-2 at the time of scanning.

The inclusion criteria for the long COVID group were:

- Subjects with at least one persistent neurological symptom of anosmia, brain fog (memory), fatigue or depression, persistent after >12 weeks since SARS-CoV-2 infection and not explained by the subject' pre-COVID-19 clinical history.

Table 1: Demographics and description of the cohort assembled for this project

GROUPS	Number of subjects	Mean Age (Std Dev)	Female/Male	Description
Healthy Controls	25	38(12)	13/12	Participants who resulted negative to antibodies (pre-vaccine) or N-protein blood tests.
COVID-19	41	38(11)	32/9	Participants who had a history of positive SARS-CoV-2 tests results or had a positive antibody blood test.
Long COVID	58	50(19)	42/16	People clinically defined as suffering from long COVID syndrome by neurological clinics in the UK.

Demographics and description of the cohort assembled for this project, comprising people who never contracted the SARS-CoV-2 virus (Healthy Controls) or who had COVID-19 or long COVID.

2.2 Quantitative Magnetic Resonance Imaging & Spectroscopy

The qMRI protocol was developed previously at the NMR Research Unit, Queen Square Multiple Sclerosis Centre, to be ultra-fast and efficient for acquiring a large number of datasets and extract several BMFs sensitive to inflammation, microstructure changes, myelin, brain function (see Figure 2 for an example of BMF maps) and metabolism (see Figure 3 for an example of magnetic resonance spectroscopy of the brainstem). The MRI acquisition protocol is described in Table 2 and included: Clinical fluid-attenuated inversion recovery (FLAIR) sequences for detecting brain abnormalities, 3DT1 for brain volume measurements, quantitative PD for MTV calculations sensitive to the solid fraction, quantitative T2* for susceptibility weighted and quantitative susceptibility mapping (QSM) for microbleeds and tissue composition, multi-shell diffusion-weighted imaging (DWI) for microstructure assessment, resting state functional MRI (fMRI) for brain function, quantitative magnetization Transfer (qMT) and bound pool fraction (BPF) for myelin integrity, MR spectroscopy (MRS) for metabolic profiling and arterial spin labelling (ASL) for blood perfusion. The protocol has been submitted for publication, while the functional MRI (fMRI) data was analysed and published, demonstrating alterations of functional connectivity in people with persistent anosmia (Wingrove, 2023).

Table 2: Quantitative Magnetic Resonance Imaging (qMRI) acquisition protocol

	Voxel [mm]	FOV [mm]	# Slices	Sequence details	TE [ms]	TR [ms]	FA [°]
FLAIR 3'26"	FH=1 AP=1 RL=1	FH=256 AP=256 RL=176	176 sag	3D-IR, TSE-factor=182 SENSE: AP=3, RL=2 TI=1650 ms	266	4800	40
3DT1 1'55"	FH=1 AP=1 RL=1	FH=256 AP=256 RL=176	176 sag	3D-FFE, TFE-factor=225 SENSE: no	3.1	6.9	8
fMRI 6'47"	FH=3 AP=3 RL=3	FH=230 AP=230 RL=146	42 axial	FFE, EPI-factor=25, SENSE=3 dynamic scans=100	25	4000	90
pCASL 7'15"	FH=3 AP=3 RL=3	FH=240 AP=240 RL=90	30 axial	3D-SE, TSE-factor=27 SENSE: AP=1.9, RL=1 dynamic scans=12, delay=2000 ms	12.1	4266	90
b0 31"	FH=2 AP=2 RL=2	FH=224 AP=224 RL=144	72 axial	SE, EPI-factor=55, SENSE=2 fold-over direction: PA	96	6287	90
b0 (AP) 31"	FH=2 AP=2 RL=2	FH=224 AP=224 RL=144	72 axial	SE, EPI-factor=55, SENSE=2 fold-over direction: AP	96	6287	90
DWI 8'41"	FH=2 AP=2 RL=2	FH=224 AP=224 RL=144	72 axial	SE, EPI-factor=55, SENSE=2 b-values={0, 1000, 2800, 2000} # directions={4, 20, 36, 20}	96	6279	90
IR 4'28"	FH=2 AP=2 RL=2	FH=224 AP=224 RL=144	72 axial	SE, EPI-factor=55, SENSE=2 TI=[50-1910] ms, 12 TIs, dTI=120 ms	59	6885	90
qMT 4'57"	FH=2 AP=2 RL=2	FH=224 AP=224 RL=144	72 axial	SE, EPI-factor=55, SENSE=2 offset: {96(x2), 13.7(x5), 3(x5)} MHz FAs: {100(x2), 890(x5), 593(x5)}°	59	7626	90
B1	FH=2	FH=224	72	SE, EPI-factor=55, SENSE=2	59	15	120/

(DAM) 30”/30”	AP=2 RL=2	AP=224 RL=144	axial			x10^3	60
B1 (AFI) 1’57”	FH=4 AP=4 RL=4	FH=256 AP=256 RL=176	44 sag	3D-FFE, SENSE: no	2.2	30/ 180	60
SPGR (multi-TE) 4’6”	FH=1 AP=1 RL=1	FH=256 AP=256 RL=176	256 sag	3D-FFE, SENSE: no	2.3-25.4 8 TEs dTE=3.3	29	24
SPGR 4’6”	FH=1 AP=1 RL=1	FH=256 AP=256 RL=176	256 sag	3D-FFE, SENSE: no	2.3	29	4
MRS 4’52”	~FH=20 ~AP=20 ~RL=20			SV-PRESS, NEX = 128, SW = 2000Hz, NP = 1024	35	2000	90

FH/AP/RL = feet-head/anterior-posterior/right-left; FOV = field of view; TE/TR/TI = echo/repetition/inversion time; FA = flip angle; FFE/TFE = fast/turbo field echo; (T)SE m= (turbo) spin-echo; (FLA)IR = (fluid-attenuated) inversion recovery; EPI = echo planar imaging; fMRI = functional MRI; pCASL = pseudo-continuous arterial spin labelling; DWI = diffusion-weighted imaging; qMT = quantitative magnetisation transfer; DAM/AFI = dual angle method/actual flip angle; SPGR = spoiled gradient echo; MRS = magnetic resonance spectroscopy; SV-PRESS = (single voxel) point-resolved spectroscopy; NEX = number of excitations; SW = Spectral width; NP = number of spectral points. All EPI-based acquisitions (shaded cells) were set with a unified readout.

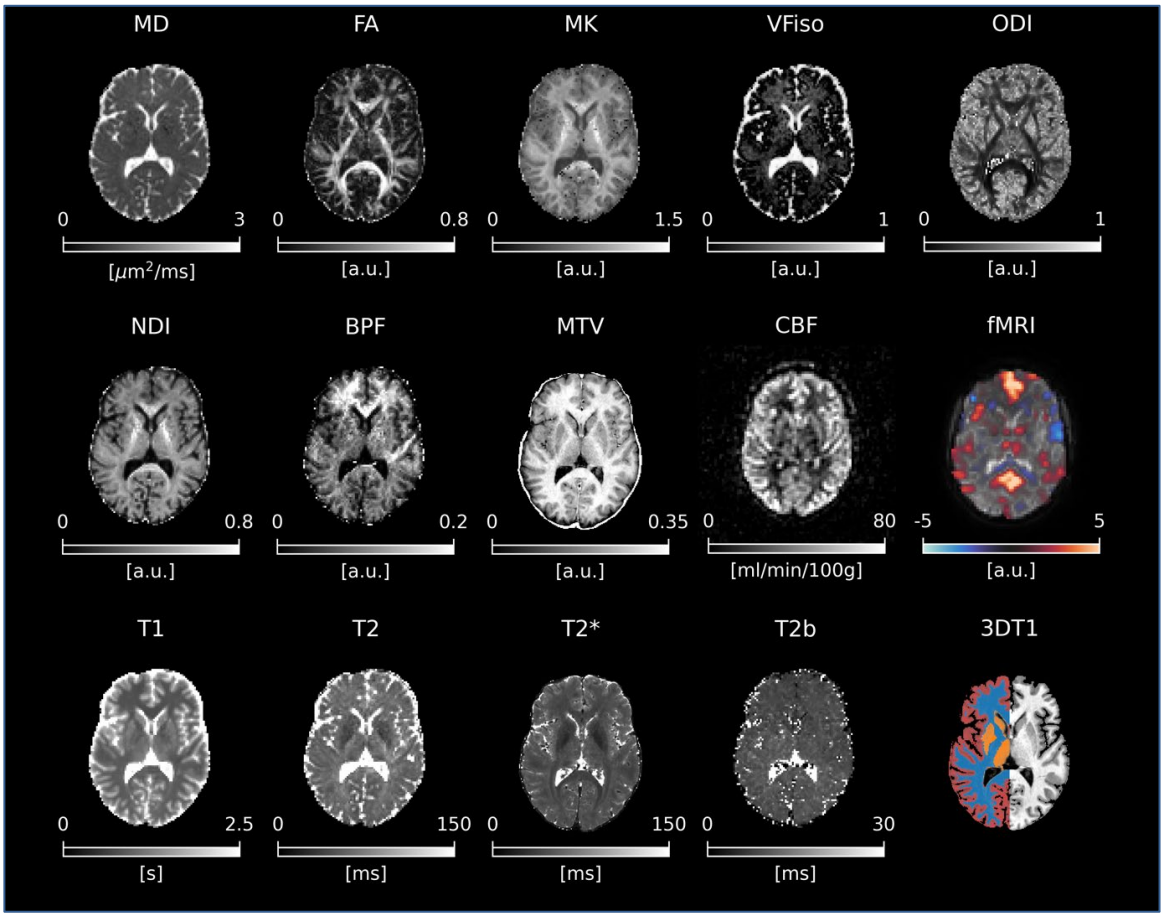

Figure 2: Maps of quantitative metrics calculated from the MRI protocol acquired in this study

MD = Mean Diffusivity; FA = Fractional Anisotropy; MK = Mean Kurtosis; VFiso = volume fraction of the isotropic component; ODI = Orientation Dispersion Index; NDI = Neurite Density Index; T1 = longitudinal relaxation time; T2 = transverse relaxation time; T2* = transverse relaxation time affected by local magnetic susceptibility; T2b = transverse relaxation time of bound protons; BPF = Bound Pool Fraction; MTV = macromolecular tissue volume; CBF = Cerebral Blood Flow; fMRI = resting state functional MRI; 3DT1 = high structural resolution anatomical T1-weighted scan.

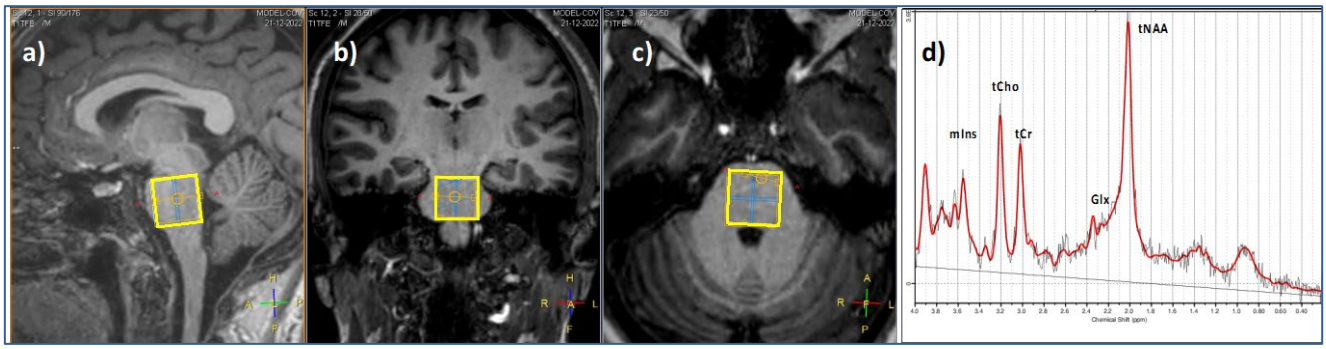

Figure 3: Magnetic resonance spectroscopy (MRS) of the brainstem acquired for this study

a, b, c) Sagittal, coronal and axial view of the cubic MRS voxel positioning on the brainstem - yellow box, 1cm dimension; d) Example of fitted spectrum with main metabolite labelled: tNAA = total N-Acetyl Aspartate; Glx =. Glutamine + Glutamate; tCr = total Creatine; tCho = total Choline; mIns = myo-Inositol.

2.3 Clinical and para-clinical scores

Participants of the prospective cohort were asked to undergo a neurological assessment comprising tests for muscle strength (MRC Muscle Strength Scale for upper and lower limb), cognition (Symbol Digit Modality Test, SDMT) and the University of Pennsylvania Smell Identification Test (UPSIT). In addition, patient-reported outcome measures (PROMs) and cardiovascular risks factors were also collected. The PROMs included the Quality of Life questionnaire (EQ-5D-5L), the Modified Fatigue Impact Scale (MFIS) and the Patient Health Questionnaire-9 (PHQ-9) depression test. The participant responses were inputted into a tablet computer using the REDCap mobile app and the data was uploaded to the MODEL-COV database on REDCap, hosted on the UCL server. Participants were also able to complete the questionnaires in paper form, if preferred.

All data, i.e. BMFs from qMRI analysis, clinical and paraclinical scores as well as blood sample results were compiled in a database for classical statistics and RF analysis, which will be available on EBRAINS after publication.

2.4 Blood samples

We acquired blood samples from all subjects to confirm their COVID status. We analysed the stored serum and extracted antibodies for CoV-2 N, CoV-2 RBD and CoV-2 S.

CoV-2 S was the major indicator of COVID-19 in pre-vaccination time.

However, post vaccination time, it is almost impossible to find COV-2 RBD and COV-2 S antigens negative due to vaccination. However, CoV-2 N stays negative during vaccination and indicates whether the person was infected by the SARS-CoV-2 virus and therefore was ill with COVID-19 if CoV-2 N is positive. We used these results to validate the inclusion of subjects in the Ctrl cohort.

3. Data analysis

3.1 EBRAINS contribution

We have exploited, benchmarked and enhanced the EBRAINS infrastructure by:

- Contributing to the EBRAINS Medical Data Analytics, by developing a flexible code to simplify the use of the Random Forest (RF) algorithm for classification and feature ranking (https://github.com/hex808080/MODEL-COV).
- Sharing acquisition protocols with other sites who would like to acquire consistent data, after publication of the protocol.

- Involving healthcare companies. We are open to liaise with manufacturers to share a simplified version of our protocol with other users through industry-driven networks.
- Uploading on EBRAINS the atlas of features calculated for the MODEL-COV cohort and the csv file with all the BMFs entered in the statistical analysis (currently stored on the following Directories: https://drive.ebrains.eu/d/73dd614abae84beeb6c3/ and https://drive.ebrains.eu/d/46787f216a504ef4a529/ and available on EBRAINS at this link https://search.kg.ebrains.eu/instances/1a8c1e14-5c81-4d61-af2c-154beb771b97 at the end of the curation process).
- Exploiting TVB tools already available from EBRAINS (https://drive.ebrains.eu/f/c0e09710330744588270/).
- Identifying the brain network of anosmia and sharing the regions of interest (ROIs) through GitHub (https://github.com/marta-gaviraghi/olfactory_atlas.git).

3.2 Statistical analysis

3.2.1 Classical statistic

An initial analysis of BMFs from macro-areas (white matter, grey matter, deep grey matter and brainstem) of 59 subjects acquired in 2020, pre-vaccination, seems to indicate that iron accumulates in tissue in the presence also of inflammation, especially in people with anosmia. This was obtained using linear regression models with age and gender as covariates (paper in preparation).

On the overall cohort of 124 subjects, once the robust means of all the BMFs metrics have been computed using the interquartile range (IQR) rule, a descriptive analysis of individual variables will be conducted to identify potential erroneous values. Basic statistical moments will be explored within the R Studio framework, which also enables to obtain histograms for visualizing the distribution of each metric.

Subsequently, differences between patients (COVID and long COVID) and controls will be assessed with a linear regression, with age and gender as covariates. The same approach will be employed to determine which set of BMFs will better explain the clinical scores in this cohort of patients, including SDMT, UPSIT, MSIF and PHQ-9 scores.

Furthermore, an advanced latent variable model will be explored to identify which BMFs are related to neurodegeneration, myelin integrity and inflammation and how they may contribute to the explanation of the clinical scores. It will be interesting to understand the role of iron accumulation in the context of long COVID, where people have suffered symptoms for several months.

Finally, a refinement of the linear model will be done performing a LASSO (least absolute shrinkage and selection operator) analysis. This process will help select the best set of predictors and improve the accuracy and interpretability of the resulting statistical model.

3.2.2 Random Forest Machine Learning

Given the relatively small number of subjects and the large number of variables, we have explored possible ML algorithms including those proposed as part of the Medical Informatics Platform (MIP). We chose to use a Random Forest (RF) approach to test differences between groups and extract the most significant BMFs that explain group belonging. Interestingly, metrics revealing myelin damage are those that most significantly explain group differences, together with BMFs that are descriptive of inflammation. This work is ongoing as we are extracting features of iron accumulation and we are running TVB analysis on the entire cohort (see 3.3.), which will provide extra BMFs to be included in the database that is fed to the RF algorithm. The RF code is available on GitHub.

3.3 Whole-brain simulation with TVB

We exploited EBRAINS computational models, included in TVB framework, to create personalized virtual brains of control subjects and people affected by COVID and long COVID for investigating their whole-brain dynamics. In particular, the Wong-Wang model was used to extract subject-specific parameters relevant to the global coupling between different brain regions, and their overall excitatory-inhibitory balance.

We started from diffusion and resting-state functional MRI data of each single subject to build personalized whole-brain structural and functional connectomes, respectively. The subject-specific structural connectome was reconstructed by combining advanced probabilistic tractography results (using the Anatomically-Constrained Tractography framework with a high number of streamlines) with an ad-hoc parcellation atlas, including both cerebral and cerebellar structures. The subject-specific functional connectome was instead created by computing the Fisher-z transformed coefficient resulting from the correlation of the time-course of BOLD signals between pairs of nodes. These connectomes were directly imported and used in the FAST TVB Neuroinformatics Platform, downloaded from EBRAINS, to simulate brain dynamics. The simulation step required tuning of the model to identify the optimal combination of global and local parameters at network-level, providing an evaluation of important physiological features such as the balance between the excitatory and inhibitory synaptic strengths. In detail, these parameters are G, which is the global coupling, Ji, which represents the strength of inhibitory synapses, and JNMDA and w+, which represent the strength of excitatory synapses.

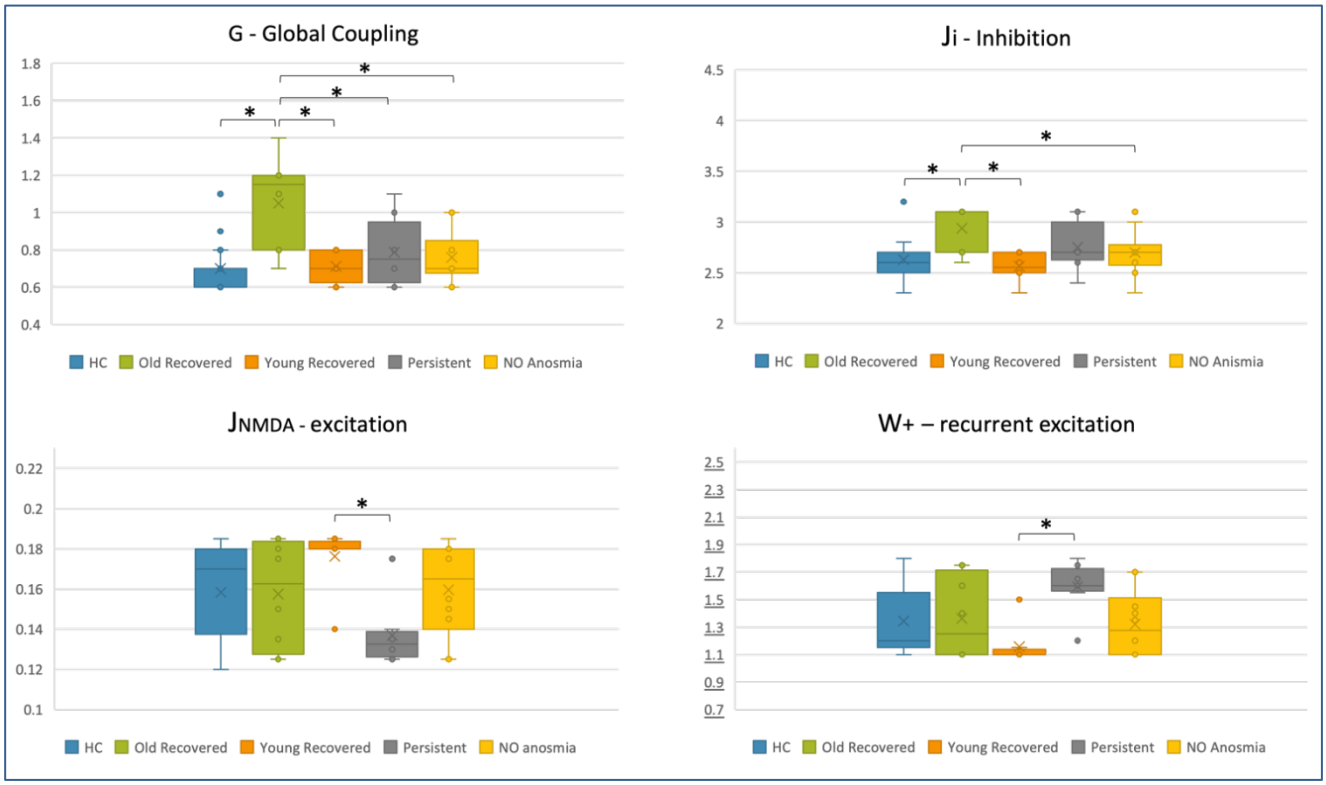

Figure 4: Boxplot of model parameters from simulating brain dynamics

Parameters of Global coupling (G), inhibition (Ji), excitation (JNMDA) and recurrent excitation (w+) are extracted for each subject from the virtual brain simulations using the Wong-Wang model. Here we show the boxplot of these parameters for subset of participants divided into 5 groups of healthy controls (HC), persistent anosmia subjects (Persistent), young participants who recovered from anosmia (Young Recovered), subjects who recovered from anosmia and were age matched to the persistent group (Old Recovered) and participants who did not have anosmia but had contracted the SARS-CoV-2 virus (NO anosmia). *indicates significant differences ($p<0.05$).

This pipeline was applied to 55 subjects divided as follows: 21 healthy controls, 8 COVID participants that recovered from anosmia, 8 young COVID participants who recovered from anosmia, 8 COVID participants with persistent anosmia, and 10 COVID participants without confirmed anosmia. A multivariate general linear model with age and gender as covariates demonstrated that all

parameters were altered in participants with COVID (Figure 4). The analysis will continue until we include the entire cohort of subjects.

Interestingly, participants who recovered from anosmia showed significantly higher value for G and Ji with respect to the other groups, meaning that distant nodes were more strongly connected and showed higher synchronicity. This finding suggests that people recovering from anosmia may be characterized by hypersynchrony, which is typical of impaired brain networks attempting to create compensation mechanisms. Furthermore, young participants who recovered form anosmia showed significantly higher JNMDA values and lower w+ values with respect to persistent anosmia subjects, suggesting that acute or chronic phases are characterized by decrease excitation strength while an increment is related to the ability of NMDA receptors to perform neuroplasticity and to recover from injury.

3.4 fMRI of anosmia

Forty-six subjects underwent a resting state fMRI protocol to understand possible changes in resting state functional connectivity due to anosmia symptoms post COVID-19. The study found significant resting state connectivity difference between individuals who had persistent anosmia after contracting COVID-19 and those who recovered from it or never contracted the SARS-CoV-2 virus. In particular, there was hypo-connectivity between the left orbito-frontal cortex and a region in the left dorsal anterior cingulate cortex, known to be involved in odour processing. Interestingly there was hyper connectivity between the right anterior insula and the left crus I region of the cerebellum. Full results are to be found in Wingrove et al, 2023. We are in the process of repeating this analysis across the whole cohort of 124 participants.

3.5 Olfactory circuit evaluation

We were further interested in analysing how specific brain networks are affected by COVID. Since it is known that a significant percentage of people infected by SARS-CoV-2 present with anosmia, we investigated how the olfactory circuit was altered. To detect acute or chronic impairment, both participants with persistent anosmia and those who recovered were included in the analysis.

An overall number of 35 grey matter nodes were identified based on structural and functional brain networks linked to anosmia, as reported in literature. The regions included the olfactory bulb, cortical areas (i.e. anterior olfactory nucleus, olfactory tubercle, piriform cortex, entorhinal cortex and orbitofrontal cortex), subcortical areas (i.e. amygdala, thalamus, hippocampus, hypothalamus, insula), brain stem, cerebellar area (i.e. crus I, crus II, lobule VI, lobule VIII, lobule IX and dentate nucleus). Most of them were extracted from standard parcellation atlases, such as Brodmann, Harvard Oxford, Juelich atlas, while the olfactory bulbs were manually drawn by three different operators and the final ROI segmentation was obtained considering the voxel segmented by at least two operators.

To generate the white matter bundles of the olfactory circuit, high resolution whole-brain tractography of 10 healthy subjects of the Human Connectome Project were used. Starting from the identified 35 ROIs, for each subject, all the combinations of tracts connecting two ROIs were extracted. Only tracts existing in all subjects were then considered as realistically belonging to the network. Each resulting tract was registered into the common MNI space and the average tract of the 10 subjects was calculated. Our goal was to create an atlas of tracts, so each voxel was associated with one tract only, therefore if the voxel belonged to two or more tracts, the tract with the highest number of streamlines in that specific voxel was considered (Figure 5).

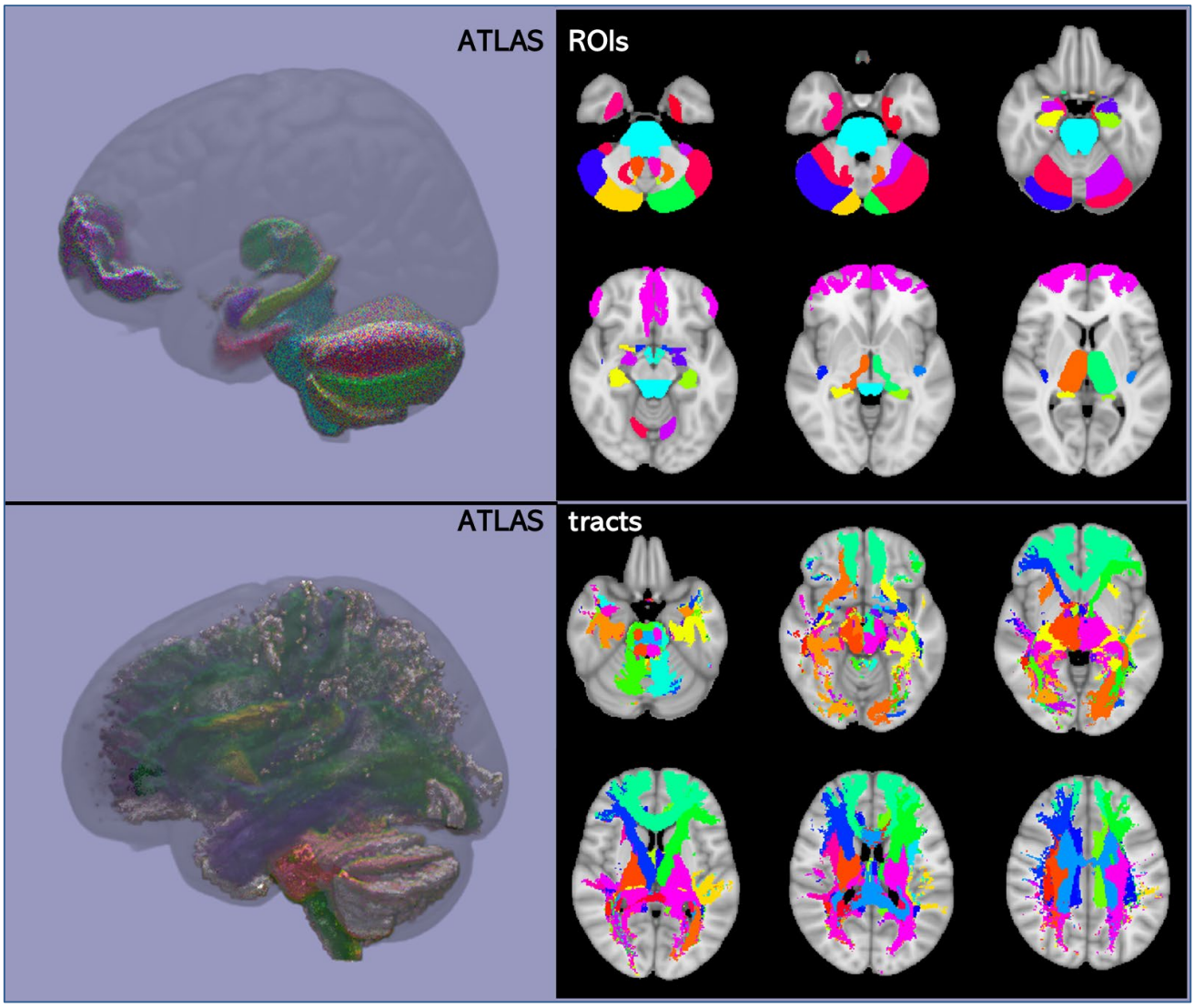

Figure 5: Atlas of the olfactory network white matter tracts

Different colours show ROIs corresponding to different white matter axonal bundles.

The atlas of ROIs and tracts obtained with this method has been subsequently applied to a subset of 48 subjects of the COVID database (17 Controls, 14 COVID, 8 COVID anosmia, 9 COVID recovered anosmia) to investigate whether there are statistically significant variations, between the different groups, in the microstructural features and in the volume of the regions and tracts involved in the olfactory circuit. Results will be presented at scientific conferences as well as written up for peer reviewed publication. ROI files are available on GitHub and upon request after publication (https://github.com/marta-gaviraghi/olfactory_atlas.git).

4. Looking Forward

The database gathered during these last 18 months of HBP, thanks to the open call on COVID-19, allowed us to put together an extensive database of 124 subjects that will be further analysed for the months to come in order to gather as much information as possible on long COVID.

The database will contribute to a rich atlas-based set of features of the human brain, obtained with MRI that will be available to others for research questions that may also be beyond long COVID.

The research outcomes from this project will leverage publications that will have scientific and clinical impact. It will also potentially lead to further grant funding for longitudinal studies of long COVID cohorts.

The richness of the protocol and the advanced statistical analysis, including ML algorithms and TVB brain modelling, will drive future technical innovations in terms of clinical impact of these tools in understanding new diseases such as the neurological consequences of SARS-CoV-2 infection.

5. References

5.1 Publications and events

- **P4182:** Wingrove et al, Aberrant olfactory network functional connectivity in people with olfactory dysfunction following COVID-19 infection: an exploratory, observational study, eClinicalMedicine 58, Elsevier 2589-5370, doi: 10.1016/j.eclinm.2023.101883
- Gandini Wheeler-Kingshott et al., Advanced magnetic resonance imaging to study brain tissue alterations in people infected with SARS-COV-2, ISMRM Annual Meeting 2021
- Wingrove et al., Persistent anosmia following COVID-19 results in significant connectivity differences within olfactory regions: a resting state fMRI analysis, ISMRM Annual Meeting 2022
- Grosso et al., Subject-specific -separation method: the effect of introducing a personalised relaxometric constant, ISMRM Annual meeting 2023
- Grosso et al., Quantitative Susceptibility Mapping and χ - separation method: how MRI can help us understand COVID-19, Human Brain Project Summit 2023
- Ricciardi et al., Advanced magnetic resonance imaging to study brain alterations in people infected with SARS-COV2, Human Brain Project Summit 2023

5.2 Data

- Ransom Forest wrapper code: https://github.com/hex808080/MODEL-COV
- Olfactory network atlas: https://github.com/marta-gaviraghi/olfactory_atlas.git
- NIFTI MAPS for the entire cohort: https://drive.ebrains.eu/d/73dd614abae84beeb6c3/
- METADATA for the entire cohort: https://drive.ebrains.eu/d/46787f216a504ef4a529/
- Link EBRAINS per database: https://search.kg.ebrains.eu/instances/1a8c1e14-5c81-4d61-af2c-154beb771b97