Gray Matter Changes Following Mild COVID-19: An MR Morphometric Study in Healthy Young People

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Background: Although COVID-19 is primarily an acute respiratory infection, 5%–40% of patients develop late and prolonged symptoms with frequent neurological complaints, known as long COVID syndrome. The presentation of the disease suggests that COVID infection may cause functional and/or morphological central nervous system alterations, but studies published in the literature report contradictory findings.
Purpose: To investigate the chronic effects of COVID-19 on cerebral grey matter in a group of young patients without comorbidities, with mild course of COVID infection and no medical complaints at the time of examination.
Study Type: Prospective.
Population: Thirty-eight young (age = 26.6 ± 5.0 years; male/female = 14/24), adult participants who recovered from mild COVID infection without a history of clinical long COVID and 37 healthy control subjects (age = 25.9 ± 2.8 years; male/female = 14/23).
Field Strength/Sequence: Three Tesla, 3D T1-weighted magnetization-prepared rapid gradient-echo, 2D T2-weighted turbo spin-echo.
Assessment: MRI-based morphometry and volumetry along with neuropsychological testing and self-assessed questionnaire.
Statistical Tests: Fisher's exact test, Mann–Whitney *U*-test, and multiple linear regression analyses were used to assess differences between COVID and healthy control groups. $P < 0.05$ was used as cutoff for significance.
Results: In the COVID group, significantly lower bilateral mean cortical thickness (left/right-hemisphere: 2.51 ± 0.06 mm vs. 2.56 ± 0.07 mm, $\eta^2_p = 0.102$/2.50 ± 0.06 mm vs. 2.54 ± 0.07 mm, $\eta^2_p = 0.101$), lower subcortical gray matter (57881 ± 3998 mm^3 vs. 60470 ± 5211 mm^3, $\eta^2_p = 0.100$) and lower right olfactory bulb volume (52.28 ± 13.55 mm^3 vs. 60.98 ± 15.8 mm^3, $\eta^2_p = 0.078$) were found. In patients with moderate to severe anosmia, cortical thickness was significantly lower bilaterally, as compared to patients without olfactory function loss (left/right-hemisphere: 2.50 ± 0.06 mm vs. 2.56 ± 0.05 mm, $\eta^2 = 0.173$/2.49 ± 0.06 mm vs. 2.55 ± 0.05 mm, $\eta^2 = 0.189$). Using further exploratory analysis, significantly reduced cortical thickness was detected locally in the right lateral orbitofrontal cortex in the COVID group (2.53 ± 0.10 mm vs. 2.60 ± 0.09 mm, $\eta^2_p = 0.112$).
Data Conclusion: Even without any subjective or objective neurological complaints at the time of the MR scan, subjects in the COVID group showed gray matter alterations in cortical thickness and subcortical gray matter volume.
Level of Evidence: 2
Technical Efficacy: Stage 3

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Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus is typically an acute respiratory illness. Neurological symptoms and complications are quite rare in the acute/subacute phase, the only characteristic and apparently specific neurological symptom is the loss of smell and taste, which may be present in 40%–90% of cases.[1] After the acute illness has resolved, some patients may retain some of their symptoms for a longer time, or even develop new symptoms.[2] This is termed the long COVID syndrome and occurs in 5%–40% of patients after acute infection.[3–5] Long COVID can be defined as "the continuation or development of new symptoms 3 months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least 2 months with no other explanation" (WHO). It can develop after any severity of COVID-19 infection, but in most people with long COVID the acute infection was mild,[3] which is probably due to the fact that the vast majority of SARS-CoV-2 infections are mild.[6] The most characteristic symptoms of long COVID persisting beyond 6–12 months may suggest an involvement of the central nervous system: physical and mental fatigue, migrating/multiplex pain complaints, persistent olfactory disturbance, sleep disturbance, dyspnea, cognitive and concentration impairment ("brain fog").[4,5] The causes of these symptoms are unclear. Some hypotheses suggest that cardio-pulmonary damage, persistent viral infection, post-infectious immune response, post-infectious transient autonomic dysfunction, psychological, and psycho-social factors may be involved in the development of the symptoms.[7] These mechanisms can cause neurological symptoms even without direct central nervous system involvement: eg, cardiopulmonary involvement can cause fatigue or psychological disturbance can cause sleep and concentration problems. However, there is also a possibility that the direct or indirect consequences of viral infection may affect the nervous system, leading to long COVID. Therefore, it is particularly important to detect or refute the central nervous system effects of the SARS-CoV-2 virus in people who have experienced mild COVID-19.In patients with severe COVID-19 requiring intensive care unit treatment, conventional MRI techniques sometimes detect brain abnormalities during and after acute infection: microbleeds, ischemic lesions, and white matter abnormalities have been reported.[8] However, no clear central nervous system abnormalities underlying long COVID have yet been detected by conventional MRI.[9] The results of quantitative MRI studies looking for subtle differences—not visible by direct visual analysis—are surprisingly contradictory. Some have suggested that, in long COVID, the volumes of some gray matter areas are increased.[10,11] Others have found that after severe COVID, gray matter volume decreases.[8] After mild COVID infection, some authors found no gray matter abnormalities at all.[12] Most quantitative MRI studies investigating the period following COVID-19 infection have found abnormalities in structures associated with the olfactory system.[13–19] Using quantitative brain MRI methods, Douaud et al[19] studied a large group of middle-aged/elderly patients before and after COVID infection. They found that, following acute COVID, there is a decrease in global brain size and a regional decrease in gray matter thickness in olfactory system regions and other limbic structures, suggesting that COVID may result in a global effect on the entire gray matter mass of the brain and a local (specific) gray matter damage in olfactory-system related regions.[19]

The conflicting data regarding the effect of COVID-19 on the central nervous system may have several reasons, because these studies included patients who: 1) had different severities of acute COVID-19 disease, 2) had varying frequency rates and severities of long COVID 3) were of varying—typically older—age, 4) may have been affected by comorbidities to varying degrees. Older patients, who also have more severe symptoms of acute COVID-19, are more likely to have comorbidities that can affect the central nervous system independently of COVID-19 (eg, hypertension, atherosclerosis, malignancies, diabetes).[20]

The aim of this study was to investigate the chronic effects of COVID-19 on gray matter. To avoid the confounding effects of the previously described variables, a young homogeneous population was included with a history of mild acute COVID infection, no medical complaints (including long COVID symptoms) at the time of the study, and no comorbidities.

Materials and Methods

Participants

The procedures in this study were carried out in accordance with the Declaration of Helsinki. The study was approved by the National Public Health Center (registration number: 6843-5/2021/EÜIG). All subjects were informed about the study and provided written informed consent.

Young (age <40 years), right-handed, Caucasian adult participants who recovered from mild COVID infection (COVID group) and age-, sex-, and education-matched control participants (healthy control group—HC) were recruited through online social media platforms. They underwent an oral interview regarding their current health status on the day of MRI examination. The COVID infection-related symptoms and the date of the first COVID+ test were also recorded.

The full list of inclusion criteria for the COVID group was as follows:

1. Infected with SARS-CoV-2 based on polymerase chain reaction (PCR) or fast antigen test.
2. At least 60 days elapsed after the first positive COVID test.
3. Tested as positive during the first or second COVID-19 wave in Hungary (before the end of January 2021).[21]
4. No hospitalization, pneumonia, or dyspnea during the infection.
5. Not vaccinated prior to the COVID infection.
6. No acute or chronic health problems at the time of the MRI scan.

Date of infection was determined based on official COVID+ test results. According to self-reports, control subjects have not had COVID infection or showed COVID+ or flu-like symptoms since the beginning of the pandemic.

Psychological Tests and Questionnaires

Psychological tests and questionnaires were performed on the same day as the MRI.

Mental health was assessed using the Beck Depression Inventory[22] and Spielberger's State-Trait Anxiety Inventory.[23] Fatigue Impact Scale was used to assess the effect of fatigue on activities of daily living.[24] This instrument includes three subscales (cognitive, physical, and social). Edinburgh Handedness Inventory was also recorded.[25]

All participants underwent the following screening: Verbal memory and learning were assessed by Rey Auditory Verbal Learning Test.[26] The total learning scores and delayed recall scores were used in the evaluation. Attention and working memory skills were measured using the Digit Span (DS) test, Corsi Block Tapping (CBT) test, and Rey-Osterrieth Complex Figure test (RCFT).[26] Regarding the DS and CBT, the forward and backward scores were used. Only recall scores were analyzed in this study.

COVID infection-related symptoms were assessed retrospectively with the adapted version of a self-assessed questionnaire developed by Jeong and colleagues.[27] It consisted of the 23 most common symptoms in COVID infection, and the items could be answered on a 10-point Likert scale (from 1—no symptoms at all to 10—worst symptom possible).

MRI Acquisition

All images were acquired at 3 T (MAGNETOM Prisma^{Fit}, Siemens Healthcare, Erlangen, Germany) with a 64-channel Head/Neck coil. A sagittal 3D T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) sequence was obtained for each subject according to the morphometry protocol recommended for Freesurfer reconstruction: repetition time (TR)/inversion time/echo time (TE) = 2530/1100/3.37 msec; flip angle = 7°; 176 slices; slice thickness = 1 mm; FOV = 256 × 256 mm^2; matrix size = 256 × 256; receiver bandwidth = 200 Hz/pixel; available at https://surfer.nmr.mgh.harvard.edu/fswiki/. A 2D T2-weighted turbo spin-echo sequence was acquired in coronal plane for measuring olfactory bulb (OB) volumes: TR/TE = 4880/85 msec; flip angle = 120°; 29 slices; slice thickness = 1.6 mm; distance factor = 0% (i.e., no gap); FOV = 200 × 162.5 mm^2; matrix size = 512 × 416; phase oversampling = 25%; receiver bandwidth = 195 Hz/pixel; turbo factor = 15; averages = 2.

Image Analyses

Cortical thickness and subcortical volume measurements were performed on the 3D T1 images using Freesurfer 6.0 image analysis suite, documented and freely available online (https://surfer.nmr.mgh.harvard.edu). Each image was processed on exactly the same computer and operating system. Major processing steps are illustrated in Fig. 1. Talairach transformation, the removal of non-brain tissues (i.e., skull strip), the boundary between white matter and gray matter (i.e., white surface), the outer boundary of the cortex (i.e., pial surface) and final cortical and subcortical segmentations were visually verified for each subject and troubleshooting was performed when necessary according to the recommended workflow (https://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/TroubleshootingDataV6.0). Technical details are described on the FreeSurfer wiki page (https://surfer.nmr.mgh.harvard.edu/fswiki).

The volume of the OB was assessed by manual segmentation (G.O. with 15 years of experience in MRI processing) on thin slice 2D coronal T2-weighted images using 3D Slicer 4.10.2 r28257 (Fig. 2). Posterior borders were defined based on Rombaux et al.[28] The posterior border of manual segmentation was assessed as follows: The transition of the OB to the olfactory tract is either accompanied by an abrupt change in the measured coronal area or not. In case of an abrupt change in the area, the slice with the highly decreased area was considered as the posterior border of the OB. In cases where no abrupt change was observed (but rather a constant gradual decrease), the posterior border was defined by finding the first pair of slices with closely equal areas, in such case, the first (anterior) slice was considered as the posterior border of the OB.

For head-size correction in case of volumetric measures, the reciprocal of volumetric scaling factor calculated by FSL-SIENAX was used as a surrogate of intracranial volume (referred to as ICV hereafter), https://fsl.fmrib.ox.ac.uk/fsl/fslwiki.

Statistical Analyses

Statistical analyses were performed using SPSS, version 23.0 (IBM Corp., Armonk, NY, USA). Sex distribution and education level were compared by Fisher's exact test between COVID and HC groups, while age, cognitive test scores, and mental health-related questionnaire results were compared by Mann–Whitney *U*-test.

First, global differences in COVID vs. HC groups were analyzed, such as mean cortical thickness, total subcortical gray matter volume, and total brain volume. In a second round, local differences—that are not explained by global differences—were examined. Both steps involved the use of multiple linear regression models. Subjects with missing data were excluded from all subsequent analyses. The assumptions of multiple linear regression were satisfied as judged by checking for independence of errors, linearity, homoscedasticity, multicollinearity, outliers, and normality assumptions of the residues. $P < 0.05$ was used as cutoff for significance. The effect sizes were assessed by partial eta squared (η^2_p) for linear regression models and by eta squared (η^2) for Mann–Whitney tests.

CORTICAL THICKNESSES. Multiple linear regression analyses were performed to assess possible differences between COVID and HC groups in the left- and right mean cortical thicknesses. The cortical thickness served as dependent, while group membership, sex, and age as independent variables. Since normality of overall model residuals was slightly non-normal for the analysis of right mean cortical thickness ($P = 0.040$ by Shapiro–Wilk normality test), multiple linear regression was also repeated by applying reflect and logarithmic transformation on the right mean cortical thickness: $\log_{10}$(maximum value + 1 − actual value).

Since data transformation had no effect on the significance pattern and regression analysis is fairly robust to non-normality, the results will be presented based on the original untransformed data.

The effects of COVID-related anosmia on the left- and right mean cortical thicknesses were assessed using Mann–Whitney *U*-test by comparing patients exhibited no loss of smell during COVID

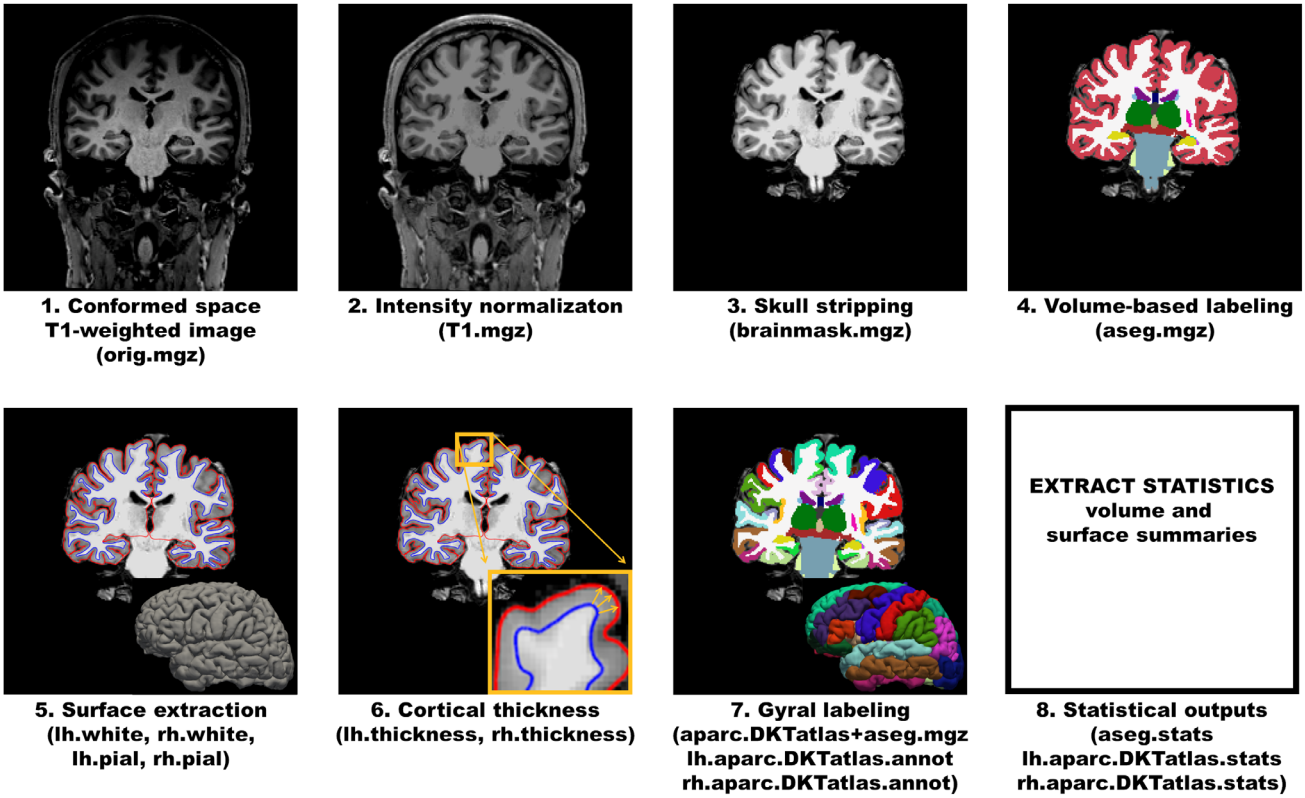

FIGURE 1: Graphical illustration of the major processing steps of standard Freesurfer pipeline. This pipeline was used to extract subcortical volumes and cortical thicknesses. Output file names, according to Freesurfer nomenclature, are shown in parentheses. Images were obtained from a 27-year-old male subject in the COVID group.

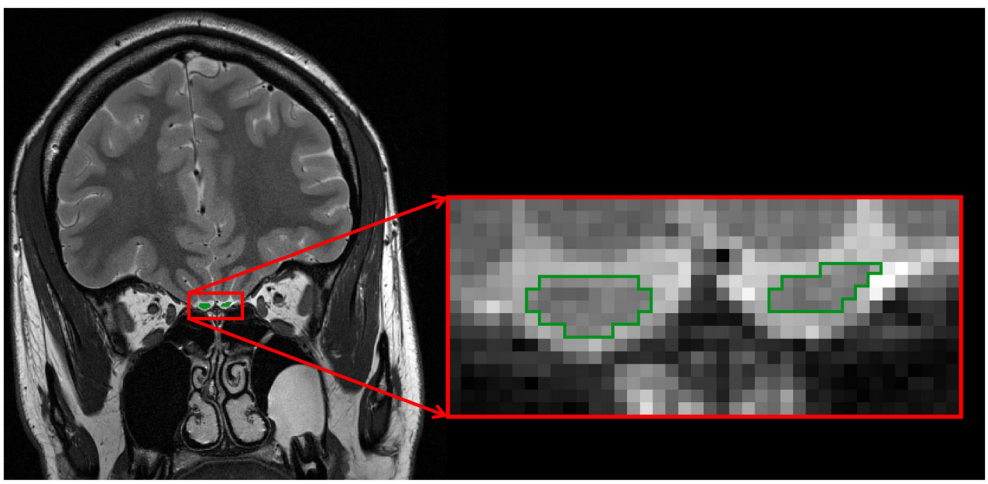

FIGURE 2: Manual segmentation of olfactory bulb. An example of the coronal T2-weighted image used to measure the volume of olfactory bulb is shown as background. Green indicates the outlines of manually delineated olfactory bulbs. Images were obtained from an 18-year-old female subject in the COVID group.

(scored 1 on the scale from 1 to 10; N = 6) and patients exhibited moderate to most severe anosmia during the infection (scored ≥5 on the scale from 1 to 10; N = 32). To control for the possible confounding effects of age and sex, multiple linear regression analyses were also performed including group membership (anosmia vs. normosmia), sex, and age as independent variables, while cortical thickness served as dependent variable. Since normality of overall model residuals was slightly non-normal for the analysis of right mean cortical thickness ($P = 0.042$ by Shapiro–Wilk normality test), multiple linear regression was also repeated by applying reciprocal transformation on the right mean cortical thickness: 1/right mean cortical thickness. Since data transformation had no effect on the significance pattern, results will be presented based on the original untransformed data.

The effects of COVID-related loss of taste (i.e., hypogeusia) on the left- and right mean cortical thicknesses were assessed using

Mann–Whitney *U*-test by comparing patients exhibited no loss of taste during COVID (normogeusia, scored 1 on the scale from 1 to 10; N = 10) and patients exhibited moderate to most severe hypogeusia during the infection (scored ≥5 on the scale from 1 to 10; N = 25). To control for the possible confounding effects of age and sex, multiple linear regression analyses were also performed.

Since no hypothesis was formulated on lateralization effects of COVID and because left- and right mean cortical thicknesses led to the same results, all of the above analyses were repeated using the overall mean cortical thickness (i.e., average of left- and right mean cortical thicknesses).

SUBCORTICAL GRAY MATTER VOLUME. Possible difference between COVID and HC groups in the total subcortical gray matter volume (i.e., SubCortGrayVol) was assessed by using a multiple linear regression model including SubCortGrayVol as dependent, while group membership, sex, age, and ICV as independent variables.

The effects of COVID-related anosmia and loss of taste on SubCortGrayVol were investigated in two separate multiple linear regression models including group membership (anosmia vs. normosmia/hypogeusia vs. normogeusia), age, sex and ICV as independent variables.

TOTAL BRAIN VOLUME. The volume of the segmented brain generated by FreeSurfer (i.e., BrainSegVol) was compared between HC and COVID groups using multiple linear regression analysis including group membership, sex, age, and ICV as independent variables.

OLFACTORY BULB VOLUME. The volumes of manually segmented left and right olfactory bulbs and total OB volume (i.e., sum of the left and right volumes) were compared between COVID and HC groups using multiple linear regression analyses. As birhinal olfactory tests usually reflect the function of the more sensitive nostril (i.e., no summation observed) and given the correlation between olfactory function and OB volume, the larger of the two measured OB volumes (i.e., Max_OB) was also tested as a dependent variable.[29] Age, gender, group membership, and ICV were included in the models as independent variables.

EXPLORATORY ANALYSES. As exploratory analyses, COVID and HC groups were also compared regarding the thickness of each cortical region segmented based on the Desikan–Killiany–Tourville (DKT) atlas included in Freesurfer (31 cortical regions per hemisphere), using multiple linear regression analyses corrected for age and sex. To assess whether any of the cortical structures show a difference between the two groups that cannot be explained by changes in cortical thickness in general, the analyses were repeated by including mean cortical thickness as an additional independent variable (i.e., left mean cortical thickness for the left hemispheric cortical structures and right mean cortical thickness for the right ones). Based on the results of the above exploratory analyses, the right lateral orbitofrontal cortex (OFC) was further investigated, and every analysis performed for the mean cortical thicknesses was repeated for the thickness of right OFC too.

Similar exploratory analyses were performed for the volumes of left and right subcortical structures, using multiple linear regression analyses corrected for age, ICV, and sex. To assess whether any of these subcortical structures (i.e., thalamus, caudate, putamen, pallidum, hippocampus, amygdala, and accumbens) show a group difference that cannot be explained by changes in subcortical volume in general, the analyses were repeated using the total subcortical gray matter volume segmented by Freesurfer (instead of ICV) as an independent variable in addition to age and sex.

Results

Forty-five subjects were recruited in both the COVID and HC groups. Seven participants from the COVID group and eight from the HC group showed uncertainty about the exact date or absence of infection or reported clinically relevant psychiatric symptoms. Therefore, the final sample included 38 COVID and 37 HC participants (mean age ± SD: 26.6 ± 5.0 and 25.9 ± 2.8 years, respectively).

Age or *sex* distribution, *education level*, cognitive performance, and mental health scores were not significantly different between COVID and the healthy control (HC) groups (Table 1). Table 2 shows the symptoms of patients during acute COVID-19 disease and the time elapsed between the positive COVID test and MR imaging.

Cortical Thicknesses

Based on multiple linear regression, the COVID group showed significantly smaller *left-*, *right-* and *overall mean cortical thicknesses* compared to the HC group (2.51 ± 0.06 vs. 2.56 ± 0.07 mm, $\eta^2_p = 0.102$; 2.50 ± 0.06 vs. 2.54 ± 0.07 mm, $\eta^2_p = 0.101$ and 2.50 ± 0.06 vs. 2.55 ± 0.07 mm, $\eta^2_p = 0.105$, respectively; Fig. 3a). *Age* was inversely related to the *left-*, *right-*, and *overall mean cortical thicknesses*. The association between *age* and a thinner cerebral cortex has been described previously.[30] *Sex* had no significant effect ($P = 0.102$, $P = 0.133$, and $P = 0.110$). There were no significant interactive effects of *age* × *group*, *age* × *sex*, and *sex* × *group* on the *mean cortical thicknesses*.

Patients with *anosmia* showed decreased *left-*, *right-*, and *overall mean cortical thicknesses* compared to patients without smell loss symptom (2.50 ± 0.06 vs. 2.56 ± 0.05 mm, $\eta^2 = 0.173$; 2.49 ± 0.06 vs. 2.55 ± 0.05 mm, $\eta^2 = 0.189$ and 2.49 ± 0.06 vs. 2.56 ± 0.05 mm, $\eta^2 = 0.195$, respectively). The significant effect of *anosmia* was unchanged when correcting for *age* and *sex* through a multiple linear regression model (Fig. 3b). The inverse relationship between *age* and *left-*, *right-*, and *overall mean cortical thicknesses* were indicated by these models as well, while *sex* had no effect ($P = 0.103$, $P = 0.086$, and $P = 0.087$). There were no significant interactions between any pairs of independent variables. Patients with *hypogeusia* showed no significantly different *left-*, *right-*, or *overall mean cortical thicknesses* compared to patients without taste loss (2.51 ± 0.05 vs. 2.52 ± 0.08 mm, $\eta^2 = 0.014$, 2.50 ± 0.04 vs. 2.50 ± 0.08 mm, $\eta^2 = 0.020$ and 2.51 ± 0.05 vs. 2.51 ± 0.08 mm, $\eta^2 = 0.017$; $P = 0.506$, $P = 0.418$ and $P = 0.460$, respectively). The group difference remained non-significant when correcting for age and

TABLE 1. Summary of Characteristics and Psychological Measures of COVID and Control Groups

Characteristics	COVID Group (N = 38)	Control Group (N = 37)	*P* Values
Age (years)[a]	26 (23–29.3)	25 (24–27.5)	0.662[c]
Sex[b]			
Female	24 (63%)	23 (62%)	1.000[d]
Male	14 (37%)	14 (38%)	
Education level[b]			
Secondary school	17 (45%)	15 (40.5%)	0.506[d]
Bachelor's or equivalent level	10 (26%)	7 (19%)	
Master's or equivalent level	11 (29%)	15 (40.5%)	
Mental health[a]			
BDI	6.5 (3–13.3)	4 (1–10)	0.077[c]
STAI-T	41.5 (36–45.3)	40 (34.5–46)	0.478[c]
STAI-S	37 (33–42.3)	35 (32.5–41.5)	0.158[c]
FIS cognitive	6.5 (3–14.3)	7 (3–12.5)	0.943[c]
FIS physical	4.5 (2–10.5)	6 (2–10)	0.905[c]
FIS social	8.5 (3–17.3)	9 (4.5–14.5)	0.989[c]
FIS total	18 (11–39.5)	23 (9.5–38)	0.968[c]
Cognitive performance[a]			
Rey AVLT total learning	58 (51–64)	59 (51–63.5)	0.518[c]
Rey AVLT delayed recall	13 (11–15)	13 (11–14)	0.743[c]
Digit span forward	7 (6–7.3)	7 (6–7)	0.687[c]
Digit span backward	5 (5–6)	5 (4–6)	0.261[c]
CBT forward	6 (5–7)	6 (5.5–7)	0.128[c]
CBT backward	6 (6–6.3)	6 (5–7)	0.619[c]
Rey CFT[e]	24.5 (21.3–29)	25 (21.6–29)	0.658[c]

[a]Values are presented as median (interquartile range).
[b]Values are presented as frequency (percent).
[c]Mann–Whitney *U*-test (2-sided exact *P* value).
[d]Fisher's exact test (2-sided exact *P* value).
[e]Due to the exclusion of subjects who were familiar with the test the reported numbers are based on N = 37 COVID and N = 36 control subjects.

sex through a multiple linear regression model ($P = 0.801$, $P = 0.787$, and $P = 0.790$, respectively).

Subcortical Gray Matter Volume

Based on multiple linear regression, the COVID group showed significantly smaller *subcortical gray matter volume* (*SubCortGrayVol*) than controls (57881 ± 3998 vs. 60470 - ± 5211 mm^3, $\eta^2_p = 0.100$; Fig. 3c). Intracranial volume (ICV) was positively related to *SubCortGrayVol*, and *SubCortGrayVol* was smaller in females (57038 ± 3621 vs. 62717 ± 4413 mm^3), while *age* had no significant effect ($P = 0.098$). There were no significant interactions between any pairs of independent variables.

Anosmia or *hypogeusia* was not related to significantly different *SubCortGrayVol* ($P = 0.823$, $\eta^2_p = 0.002$ and $P = 0.508$, $\eta^2_p = 0.015$, respectively). The positive relationship between ICV and *SubCortGrayVol* and smaller *SubCortGrayVol* in females (significant for the model with anosmia and $P = 0.059$ for the model with hypogeusia) were indicated by these models as well, while *age* had no significant effects ($P = 0.303$ and $P = 0.697$).

TABLE 2. COVID-Related Symptoms During the Infection and Elapsed Time Between the Positive COVID Test and MRI Scanning

COVID Symptoms (1–10 Scale), N = 38[a]	
Febrile sense	3.5 (1–6.3)
Chills	4 (1–7)
Cough	4 (2–6)
Sputum	2 (1–3)
Sore throat	2 (1–5)
Runny nose	2 (1–5.3)
Nasal stuffiness	3 (1–6)
Loss of smell (anosmia)	10 (7.5–10)
Chest pain	2 (1–5)
Chest discomfort	2 (1–4.3)
Shortness of breath	1 (1–3.3)
Loss of taste (hypogeusia)	8 (1–10)
Loss of appetite	4 (1–7.3)
Epigastric soreness	1 (1–2)
Nausea	1 (1–1.3)
Vomiting	1 (1–1)
Abdominal pain	1 (1–1)
Diarrhea	1 (1–2)
Constipation	1 (1–1)
Headache	6 (3.8–8)
Muscle ache	5 (2.5–7)
Arthralgia	3 (1–7)
General weakness	7 (4.8–10)
Elapsed time (days)[b]	178 (112–241.3)

Values are presented as median (interquartile range).
[a]The COVID questionnaire consisted of 23 symptoms, and the items could be answered on a 10-point Likert scale (from 1—no symptoms at all to 10—worst symptom possible).
[b]Elapsed time indicates time between the first positive COVID test and MRI measurement.

There were no significant interactions between any pairs of independent variables.

Total Brain Volume

The volume of the segmented brain (i.e., *BrainSegVol*) was not significantly different between the HC vs. COVID groups (1204051 ± 114416 vs. 1174879 ± 99044 mm^3, $\eta^2_p = 0.008$; $P = 0.451$). However, a significant interaction between *sex* and *age* (i.e., *sex* × *age*) was detected, thus the significant interaction term was also included in the model. The HC vs. COVID group difference was non-significant by this model either ($P = 0.168$).

Olfactory Bulb Volume

After visual inspection of the high-resolution T2-weighted images, 10 subjects (two COVID and eight HC subjects) with low image quality were excluded from the OB analysis. COVID patients had significantly lower *right OB volume*, as compared to the HC group (52.28 ± 13.55 vs. 60.98 ± 15.84 mm^3, $\eta^2_p = 0.078$; Fig. 4a), while *left OB volume* was not significantly different (52.13 ± 13.34 vs. 57.13 ± 13.12 mm^3, $\eta^2_p = 0.044$; $P = 0.101$). *Max_OB* and *total OB volumes* were also significantly lower in the COVID group (55.14 ± 13.06 vs. 62.95 ± 14.76 mm^3, $\eta^2_p = 0.074$ and 104.41 ± 25.83 vs. 118.11 ± 27.49 mm^3, $\eta^2_p = 0.066$, respectively; Fig. 4c). *Age*, *sex*, and ICV were not indicated as significant predictors of *OB volumes*. There were no significant interactions between any pairs of independent variables. Neither smell nor taste disturbances showed any significant association with *OB volumes*.

Exploratory Analyses

The results of the exploratory analyses suggest that all cortical regions of the DKT atlas were thinner in the COVID group than in the HC group, many of these differences reached the statistical significance ($P < 0.05$ uncorrected for multiple comparisons; Table S1, Supplemental Material). However, when the analyses were also corrected for the *hemispheric mean cortical thickness* none of the differences remained significant, even when no correction for multiple comparisons was applied ($P \geq 0.088$). The *right lateral orbitofrontal cortex* (*OFC*) was the only cortical structure that showed a trend-like thinning in the COVID group even after correcting for the *hemispheric mean cortical thickness* ($P = 0.088$, $\eta^2_p = 0.041$). If the significant interaction term between sex and age (i.e., *sex* × *age*) was also included in this model, the difference even became significant ($\eta^2_p = 0.081$). The group difference is demonstrated in Fig. 4b (2.53 ± 0.10 vs. 2.60 ± 0.09 mm, $\eta^2_p = 0.112$ uncorrected for *hemispheric mean cortical thickness*). Subjects of the COVID group with *anosmia* or *hypogeusia* showed decreased *right lateral OFC thickness* (2.51 ± 0.09 vs. 2.62 ± 0.10 mm, $\eta^2_p = 0.181$ and 2.51 ± 0.09 vs. 2.59 ± 0.09 mm, $\eta^2_p = 0.182$ uncorrected for *hemispheric mean cortical thickness*). If the significant interaction term between *sex* and *age* (i.e., *sex* × *age*) was also included in these models, the differences remained significant.

Based on the exploratory analyses for subcortical regions, all subcortical gray matter structures were smaller in the COVID group, as compared to the HC group, but only the difference for the *left and right putamen volume* reached the statistical significance during pairwise comparisons (uncorrected for multiple comparisons; Table S2, Supplemental Material). However, when controlling for the *total subcortical gray matter volume*

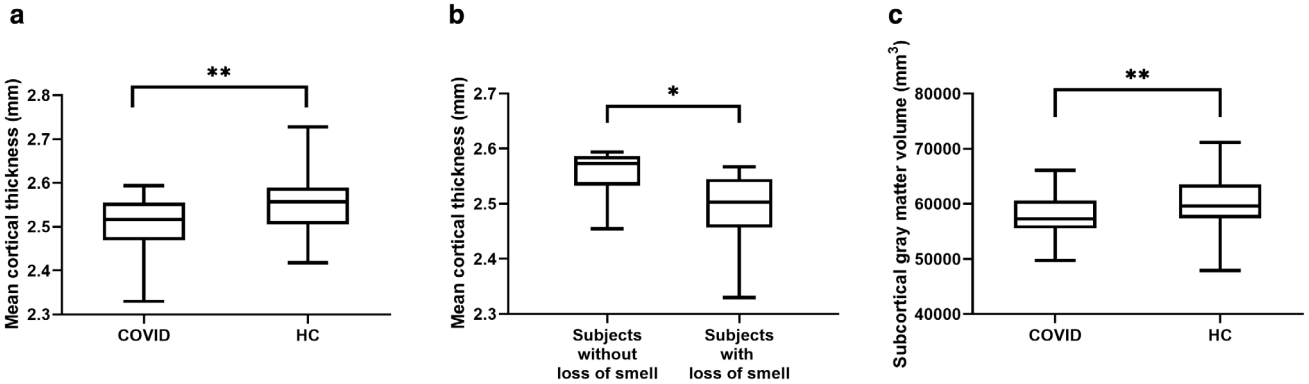

FIGURE 3: Summary of the main findings. (a) Group differences in overall mean cortical thickness between the COVID group and healthy controls (HC). (b) Group differences in overall mean cortical thickness between COVID subjects with and without smell loss. (c) Group differences in subcortical gray matter volume between the COVID group and healthy controls. Whiskers are set at minimum and maximum, the horizontal line marks the median, whereas box indicates the interquartile range (25%–75%) of the raw data. * and ** respectively indicate $P < 0.05$ and $P < 0.01$ based on multiple linear regression.

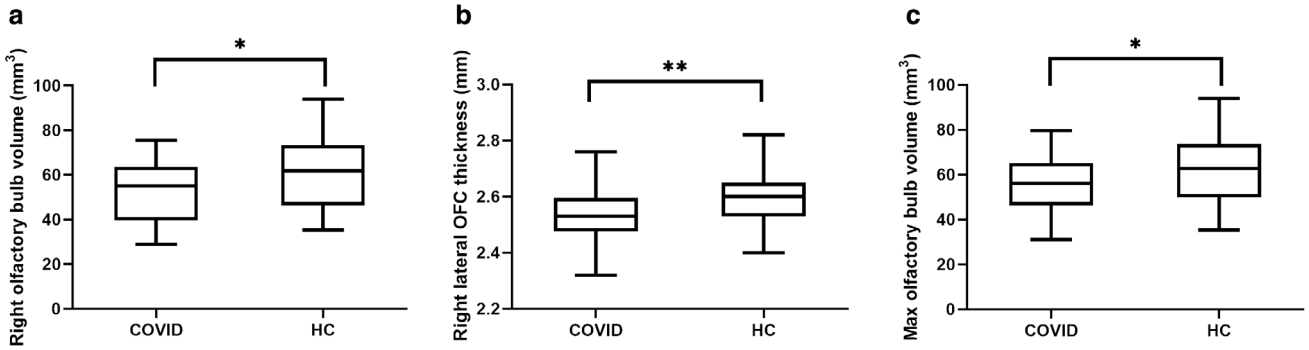

FIGURE 4: Volume differences between COVID and healthy control (HC) groups within the olfactory system. (a) Group differences in the right olfactory bulb (OB) volume between the COVID and healthy control (HC) groups. (b) Group differences in the right lateral orbitofrontal cortex (OFC) thickness between the COVID group and healthy controls. (c) Group differences in the maximal olfactory bulb (Max_OB) volume between the COVID and healthy control (HC) groups. Whiskers are set at minimum and maximum, the horizontal line marks the median, whereas box indicates the interquartile range (25%–75%) of the raw data. * and ** respectively indicate $P < 0.05$ and $P < 0.01$ based on multiple linear regression.

(which was significantly smaller in the COVID group) none of the subcortical subregions were significantly different between the two groups (uncorrected $P \geq 0.11$).

Discussion

This study examined young healthy adults by using MR morphometric methods. Two groups were compared: those who had COVID infection but were asymptomatic at the time of the examination (COVID group) and those who had not had COVID infection (HC group). The COVID group had the most typical course of COVID-19 disease: complete recovery after a mild acute episode without residual symptoms. Even without any subjective or objective neurological complaints at the time of the MR scan, as evidenced by psychometry, the subjects in the COVID group showed gray matter alterations in cortical thickness and subcortical gray matter volume.

Cortical thickness was used to estimate cortical gray matter because several previous studies have shown that cortical thickness appears to be a more sensitive indicator of regional cortical atrophy than regional cortical volume or regional cortical surface area.[31] The cortical thickness of each region was corrected for the average thickness of the whole cortex. This was done because the mean cortical thickness alone indicated a highly significant difference between the COVID and HC groups. The fact that all cortical regions were thinner in the COVID group further strengthened the suspicion that the whole cortex was affected, rather than individual brain regions. A previous study has also corrected the thickness of each region for the average thickness of the whole cortex.[32] Following the same rationale, since the volume of each subcortical gray matter region was smaller in the COVID group and the total subcortical gray matter volume also showed a significant difference between the two groups, correction for the total subcortical gray matter volume was performed when analyzing the volumes of subcortical gray matter regions.

In the COVID group, the mean cortical thickness and the total subcortical gray-matter volume were smaller compared to the control group. This supports the hypothesis that following COVID infection, gray matter may be globally

affected.[19] These findings, for the mean cortical thickness and the total subcortical gray-matter volume are partly supported by the longitudinal results from the exploratory approach by Douaud and colleagues in older COVID patients.[19] However, it is of note that due to the very different age ranges and statistical models, the two studies should be compared with caution. Moreover, there is no clear explanation for such global findings. It is conceivable that persistent viral infection (i.e., termination of the symptoms and disease is not accompanied by elimination of the virus itself), abnormal immune response, neuroinflammation-induced neurodegeneration, hypoxia/ischemia-induced damage, blood–brain barrier dysfunction, hypercoagulopathy may be involved: these pathological processes do not necessarily affect a single brain region but rather the whole gray matter.[19,33,34] In this study, however, a decrease in cortical thickness was associated with olfactory dysfunction during the acute phase of COVID-19. The olfactory system involvement in COVID-19, probably due to the high viral load in the nasal epithelium and in the olfactory bulb, has already been described.[33,35] Moreover, expression of SARS-CoV-2-specific entry proteins has been described in neural cells along the olfactory pathway.[33] The long-term damage to the olfactory system has also been confirmed by this study, a reduction in the size of the olfactory bulb in the COVID group was found. Abnormal volume and signal intensity of the olfactory bulb has been reported in previous MRI studies performed during acute and chronic phase of COVID-19 related anosmia.[13–16]

Furthermore, a decrease in right lateral orbitofrontal cortex (OFC) thickness was found in the COVID group compared to the control group, which may also indicate the involvement of the olfactory system. Moreover, subjects of the COVID group with anosmia or hypogeusia showed significantly decreased right lateral OFC thickness. Douaud et al also showed a reduction in OFC thickness after COVID-19.[19] Others have demonstrated OFC dysfunction by smell-evoked functional MRI following COVID-19.[17] Using arterial spin labeling MRI in post-COVID patients, a decreased cerebral blood flow was also shown in the OFC.[18,36] The OFC is a higher order brain structure responsible for processing smell and taste stimuli.[37] In addition, the OFC is a central component of the reward system, involved in pain processing, decision-making, and emotion processing.[37,38] The fact that the OFC may also be affected in asymptomatic patients after mild COVID-19 compared to controls suggests that more severe damage in OFC may lead to long COVID, as some of the most common central nervous symptoms of long COVID (persistent olfactory and taste disturbance, migrating/multiplex pain complaints, concentration disturbances, or mental fatigue) could theoretically be attributed to the dysfunction of this structure. Indeed, a positron emission tomography (PET) study in long COVID patients demonstrated hypometabolism in the OFC.[39]

It is important to emphasize that this study focused on young subjects, not only because this age group was underrepresented in previous neuroimaging studies, but also because they are affected by long COVID to the same extent as older age groups.[3] It is also important to focus on young people in neuroimaging studies because, although very rarely, they too can die from SARS-CoV-2 infection. In contrast to older age groups, it is mainly the central nervous system disorders (epilepsy, intellectual disability, Down syndrome) that increase COVID-related mortality in young people.[21] However, it cannot be determined whether this might be related to the gray matter disturbance found in the present study.

Limitations

The present study is not without limitations. First, although this study focused on relatively young (<40 years) non-hospitalized COVID-19 subjects with no known preexisting comorbidities, recruited during the first two pandemic waves to minimize confounding effects, sample sizes were modest and unequal between COVID subgroups. Second, the study was cross-sectional. Future longitudinal studies should clarify the causality and whether COVID-related morphometric changes are reversible or not. A previous longitudinal PET study found an improvement in brain metabolic dysfunction following COVID-19,[40] however, a previous longitudinal morphometric MR study found no reversibility of gray matter damage.[19] Third, COVID-related symptoms at the time of infection relied on self-reported questionnaire assessed retrospectively. Fourth, the possibility of OFC damage has been raised and discussed. This was done because out of all the cortical and subcortical regions examined, this was the only one where a statistical trend-like difference between the COVID and HC groups was found even after correcting for the global measure (i.e., hemispheric mean cortical thickness); furthermore, several previous neuroimaging studies have suggested damage to this structure as a consequence of COVID-19.[17–19,36,39]

Conclusion

This study showed decreased mean cortical thickness and subcortical gray matter volume in young adult subjects without medical complaints at the time of examination, who recovered from mild course of COVID infection. Moreover, local gray matter changes in olfactory-system related regions were observed.

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Conflict of Interest

The authors report no competing interests.

Data Availability Statement

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

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