UNIVERSITÀ
DEGLI STUDI
DI PADOVA

DIPARTIMENTO
DI INGEGNERIA
DELL'INFORMAZIONE

DEPARTMENT OF INFORMATION ENGINEERING

MASTER DEGREE IN BIOENGINEERING

"HIGH-DENSITY EEG AND COGNITIVE ASSESSMENT IN COVID-19 SURVIVORS"

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Academic Year 2021 – 2022

April 14, 2022

To Marina and Andrea, mum and dad, who have always believed in me.
I would never have won alone.

Abstract

SARS-CoV-2 survivors experience new or persistent symptoms up to weeks or months after recovery. These manifestations are of different nature and can arise in both hospitalized and non-hospitalized individuals. Fatigue, memory and attentive disorders, sleep troubles, anxiety and depression are those shared by most of the subjects.

The aim of the thesis is to disentangle the physiopathology of long-term cognitive / affective impairment in COVID-19 survivors.

We recruited 33 participants: 16 and 17 patients respectively discharged from intensive care unit (ICU) and sub-intensive ward (noICU) of the Padova Teaching Hospital between March and May 2020 and 12 subjects who have never been infected by the virus (CTRL). Starting from June 2021, the controls and the COVID-19 survivors underwent a neuropsychological and sleep assessment. Our experimental protocol included a battery of cognitive and psychological tests, lasting approximately one hour, and a nap high-density (256 channels) electroencephalography (EEG) recording.

After the initial preprocessing step, the EEG patterns were segmented in 30-s epochs, then classified according to the patient's current sleep stage. Slow (i.e., [9-12] Hz) and fast (i.e., [12-16] Hz) sleep spindles were automatically detected and used as input of the EEG source analysis (ESI), aimed at localizing the spindle cortical generators. Some important parameters for the spindle analysis, such as the slow and fast spindle density, the peak frequency, the corresponding amplitude, and the spindle power, were then calculated.

COVID-19 survivors showed depressive and post-traumatic symptoms, scored the lowest on the physical quality of life survey and exhibited a memory and attentive functions' impairment. This evidence is more marked in the noICU population. The same group presented a different organization of the spindles' cortical generators and changes in the spindle parameters.

A correlation analysis, computed between the neuropsychological scores and spindle features, highlighted a relationship between the psychological evaluations with both the cognitive performances and the sleep spindle characteristics and the predictive potential of the Beck's Depression Inventory (BDI) and post-traumatic stress disorder (PTSD) scores with respect to the other variables.

Sleep neurophysiology turned out to be a valid tool to investigate the nature of the cognitive and executive functions' impairment manifested by the COVID-19 survivors, i.e., the ICU and noICU groups. According to spindle and correlation analysis' results, we hypothesized that the depressive state and the post-traumatic stress disorder, shown by COVID-19 recovered patients

and probably caused by the traumatic experience lived, involved both cognitive impairment and changes in sleep spindle features and distribution.

Sommario

A distanza di settimane o mesi dalla guarigione, in diversi contagiati dal virus SARS-CoV-2 persistono o insorgono alcuni sintomi. Queste manifestazioni sono di diversa natura. Stanchezza, deficit di memoria e attenzione, disturbi del sonno, ansia e depressione sono i sintomi condivisi dalla maggior parte dei soggetti.

Questa tesi ha l'obiettivo di valutare la fisiopatologia del deterioramento cognitivo/affettivo a lungo termine nei sopravvissuti al COVID-19.

Sono stati reclutati 33 partecipanti: 16 dimessi dal reparto di terapia intensiva (ICU) e 17 dal reparto di terapia sub-intensiva (noICU) del Policlinico Universitario di Padova tra marzo e maggio 2020. A questi sono stati poi aggiunti 12 soggetti che non sono mai stati contagiati dal virus (CTRL). A partire da giugno 2021, i controlli e i due gruppi di pazienti, ICU e noICU, sono stati sottoposti ad un'esaustiva valutazione neuropsicologica e ad una registrazione elettroencefalografica (EEG) ad alta densità (256 canali) effettuata durante il sonno pomeridiano.

Dopo una prima fase di pre-elaborazione, i tracciati EEG sono stati segmentati in epoche da 30 secondi, quindi classificati in base allo stadio del sonno . I fusi del sonno (sleep spindle) lenti ([9-12] Hz) e veloci ([12-16] Hz) sono stati rilevati automaticamente e utilizzati come input nell'analisi in sorgente (Electrical Source Imaging (ESI)), la quale mira a localizzare i generatori corticali di ciascuno spindle. Successivamente, sono stati calcolati alcuni parametri importanti per l'analisi dei fusi, come la densità, la frequenza di picco, l'ampiezza massima e la potenza.

I sopravvissuti al COVID-19 hanno mostrato sintomi depressivi e post-traumatici, hanno ottenuto il punteggio più basso nell'indagine riguardante la qualità di vita percepita e hanno mostrato una parziale compromissione della memoria e delle funzioni attentive. Questa evidenza è più marcata nel gruppo di pazienti che durante la loro degenza ospedaliera sono stati curati esclusivamente con ossigenoterapia, ovvero i noICU. Lo stesso gruppo ha presentato anche una diversa organizzazione dei generatori corticali dei fusi e variazioni nei valori dei parametri calcolati.

La correlazione tra i punteggi dei test neuropsicologici e le caratteristiche dei fusi ha evidenziato una relazione tra i punteggi delle valutazioni psicologiche sia con le prestazioni cognitive sia con le caratteristiche dei fusi e il potenziale predittivo dei punteggi delle valutazioni psicologiche rispetto alle altre variabili.

La neurofisiologia si è rivelata un valido strumento per indagare la natura dell'indebolimento delle funzioni cognitive ed esecutive manifestato nei sopravvissuti al COVID-19, sia nei pazienti ricoverati in terapia intensiva sia in quelli ricoverati in terapia sub-intensiva. In base ai

risultati emersi dalle analisi, si è ipotizzato che lo stato depressivo e i sintomi da disturbo da stress post-traumatico, mostrati dai soggetti guariti da COVID-19, fossero causati dall'esperienza traumatica vissuta, principale causa sia del parziale deterioramento delle funzioni cognitive ed esecutive sia delle variazioni delle caratteristiche dei fusi del sonno evidenziati in questi soggetti.

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Chapter 1

State of Art

1.1 Neurological assessment in the acute phase of COVID-19

1.1.1 Evidence from the literature

The SARS-CoV-2 is a respiratory virus that belongs to the large family of coronaviruses (CoVs) [1] and is responsible for the ongoing global pandemic. Almost 400 million cases of infection have been reported world-wide, with a global death toll of approximately 5.5 million [2].

COVID-19 patients can transmit the infection through the emission of small liquid particles, for example by coughing, sneezing, talking, singing, or breathing. These particles can be of various sizes, from the largest respiratory droplets to the smallest aerosols.

Most infected people develop mild to moderate symptoms (fever, cough, sore throat, headache, runny nose, fatigue, and muscle pain) and recover without needing special treatments [3]. However, some COVID-19 patients were observed to develop encephalopathy, slow cognitive activity, impaired concentration and memory, sleep disorders, personality changes and a reduced ability to sense taste and smell. In addition, an increase of the number of epileptic seizure events has been observed in the pandemic period.

One of the main purposes of the first studies focused on COVID-19 disease was assessing if behind these neurological manifestations was hidden or not a permanent damage to the central/peripheral nervous system caused by the virus. The electroencephalography (EEG) is one of the most important neurological diagnostic tests and it could be used as a tool to investigate COVID-19 patient brain patterns.

There is growing evidence in the literature that these symptoms are the direct consequence of the virus' noxious activity on the central nervous system (CNS) or on the peripheral nerves (Table 1.1).

Researchers	Materials and Methods	Results
Roberto et al. (September 2020) (Systematic review paper) [4]	• Sources: published articles in PUBMED by Medline, EMBASE and CENTRAL by the Cochrane Library • 177 patients affected by COVID-19 with descriptive EEG reports • Most of the studies employed continuous EEG recording, few studies employed a limited montage with a minimum of nine electrodes	• Disturbances of background activity such as generalized and focal slowing were seen in 113 and 14 cases respectively as well as epileptiform abnormalities, in 34 patients

	• The most common indications for EEG were mental status altered, encephalopathy, poor responsiveness after the withdrawal of sedation and seizure-like activity	• No consistent EEG findings specific to COVID-19 infection • COVID-19 associated encephalopathy and epileptiform patterns may be expected in critically ill patients
Pasini E et al. (2020) (experimental research paper) [5]	• EEG from 15 COVID-19 patients in the age range from 47 to 79 with suspected encephalopathy	• In all the cases the EEGs were abnormal • Slowing activity in the 4-8 Hz range with focal delta or theta waves was observed over the frontal or central region • In 66.67% of the cases no opening and closing of the eyes and no reactivity to external stimuli was documented
Vellieux G et al. (2020) (experimental research paper) [5]	• EEG test in 2 cases of COVID-19 patients (men aged 37 and 42)	• Continuous, slightly, asymmetric, monomorphic, two-phase, slow delta waves with a greater amplitude in both the frontal regions • These waves showed no reactivity to auditory of nociceptive stimuli
Koutroumanidis M et al. (2020) (experimental research paper) [5]	• EEG of 19 patients with COVID-19 at the age of 37 to 69, 13 of whom diagnosed with severe encephalopathy	• In all the cases non-specific markers of encephalopathy, diffuse slowing down and rhythmic delta activity were documented
Flamand M et al. (2020) (experimental research paper) [5]	• Several EEG tests were performed in an 80-year-old female patient suffering from COVID-19 • Additional symptoms: restless mental state with altered consciousness and focal epileptic seizures	• EEGs indicate a slowdown in basic activities and an epileptic status manifested in the frontal area • A three-phase wave activity was found, evidence of the neurological deterioration
Antony AR et al. (2020) (systematic review paper) [5]	• Data from 617 subjects with EEG scores reported in 84 studies • The age range was 45-69, including SARS-CoV-2 infected patients	• EEG abnormalities in the frontal area: focal background slowing, intermittent discharges and rhythmic delta activity. • Epilepsy-like discharges were observed
Karadas et al. (December 2021) (Experimental research paper) [6]	• Continuous EEG recordings (24h, 21 electrodes) of 87 intensive care patients (age > 18 y/o), who were diagnosed with COVID-19 and had shown a change in consciousness	• The most commonly EEG abnormality observed: focal and generalized slow waves • Specific epileptiform abnormalities featured sharp, spike or multi-spike

	before they needed mechanical ventilation • Investigations of demographic and clinical features and comorbid conditions • Thoracic computed tomography (CT) and routine laboratory tests were performed • Exclusion criteria: malignancy, severe metabolic syndrome, uncontrolled hypertension or diabetes, chronic liver or renal failure, decompensated congestive heart failure, current alcohol/drug use and psychiatric disorders	were detected in the 37.9% of the recordings • Non-convulsive epilepticus status (NCSE) was found in 5.7% of the patients

Table 1.1: *General review from the literature*

EEG technique is an important diagnostic tool to assess the negative effects of the virus on CNS in patients exhibiting neurological symptoms. Statistically significant EEG changes were observed in the continuous EEG of the COVID-19 patients followed up in the intensive care unit (ICU).

Karadas et al., reported that abnormal EEG findings were detected in 93.1% of the patients and were found to increase with the age and in patients with comorbidities [6].

The preliminary Long COVID-19 reports highlighted the presence of a general or central-frontal slowing of the arrhythmic background activity in most of the recorded EEG paths.

One of the most common abnormal EEG patterns observed was a rhythmic activity in the delta frequency range ([1, 4] Hz) mainly detected on the frontal lobe. Vellieux G et al. described these abnormal delta waves as continuous, slightly, asymmetric, monomorphic, two-phase and with a great amplitude. Several studies reported that some EEG signals did not vary when the patients underwent external stimuli, demonstrating that patients' reactivity was impaired.

In agreement with the onset of a growing number of epileptic seizures in the pandemic period, almost all studies, describing COVID-19 effects detectable by EEG, cite among the results the appearance of specific epileptiform abnormalities that featured sharp, spike or multi-spike.

Roberto et al. systematic review involved the EEG reports of 177 Covid-19 patients. Such a large cohort allowed the authors to investigate the effects of COVID-19 on brain activity also discriminating on the basis of disease severity. They found that encephalopathy and epilepsy-like discharges are recurrent in critically ill patients.

1.1.2 Open issues

Further studies are needed to be sure that the EEG findings described above are a direct consequence of SARS CoV-2 virus activity.

Among the several limits in the analyzed reports, the composition of the study sample is one of the most critical issues. Instead of a large and heterogeneous sample, the populations under investigation were characterized by:

a) homogeneity in illness severity: only patients hospitalized in the intensive care units and manifesting a change in consciousness were recruited;

b) absence of a control group: most of the studies did not include COVID- 19 negative patients;

c) limited number of subjects;

d) no specification of sedatives or antiepileptic drug received at the time of the EEG;

e) limited follow-up period.

Another important obstacle is the heterogeneity of the EEG techniques used and certain subjectivity of their interpretation. For example, the number of EEG electrodes varies from a minimum of 9 to a maximum of 256 in the different analyses.

Furthermore, the risk of the overinterpretation of changes in brain function as virus-specific is quite high. Brain hypoxia, microemboli or just prolonged ICU treatment with long term sedation represent much more common causes for the impaired function of the brain.

Several studies report the use of Magnetic resonance (MR) images for the interpretation of their EEG results. Interestingly, some authors declared they observed a normal MRI finding in most of their COVID-19 patients, while others reported cases in which EEG changes were associated with dramatic damage to the brain, presumably caused by the COVID-19 infection. So, we must be careful in validating the EEG finding with the results reported in the Computer Tomography (CT)/MRI scans.

Finally, to confirm the relationship of COVID-19 with the occurrence of neurological disorders might be promising the use of more sophisticated diagnostic methods such as the quantitative EEG, which can be used both as an assessment tool and as a neurofeedback therapy instrument for patients who have not fully recovered.

1.2 The long COVID-19

The “long COVID”, “Long Haulers” or “Post COVID syndrome” are some of the terms used to identify the persistence or the development of new symptoms in people who have recovered from SARS-CoV-2 infection. These manifestations can last weeks or months (Figure 1.1) and can be experienced both by hospitalized and non-hospitalized individuals.

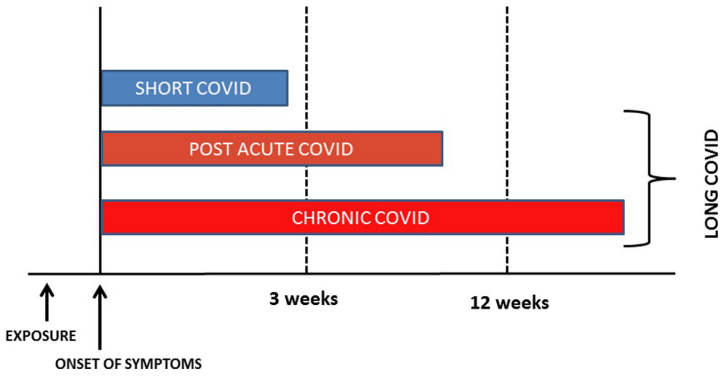


Figure 1.1: *Time-based COVID-19 disease classification. How the syndrome can be subdivided based upon symptoms duration in short, post-acute and chronic COVID-19 [7].*

Several cohorts of COVID-19 affected patients with persistent symptoms have been followed up after the first infection. This allows better investigation of the nature of these protracted disease manifestations and to identify the factors which are commonly associated with the development of long COVID.

The most common problems were fatigue, worsened quality of life, dyspnea, joint pain, chest pain, cough, skin rashes, palpitations, headache, diarrhea, and 'pins and needles' sensation. Patients also reported inability to do routine daily activities, in addition to mental health issues such as anxiety, depression and post-traumatic stress disorder.

A report from Italy found that 87% of people recovered during the acute phase of the disease showed persistence of at least one symptom even at 60 days. Of these, 32% had one or two symptoms, whereas 55% had three or more. Fever or features of acute illness were not seen in these patients. [7]

Another assessment has been conducted on a sample of 165 hospitalized patients without neurological implications six months after their discharge. The symptoms experienced by the patients vary in a wide range (Figure 1.2): fatigue (34%), memory/attention disorders (31%) and sleep troubles (30%) are the most common. The subjects have also declared they have dealt with depression/anxiety and visual disturbances. In addition, at the neurological examination, 40% of patients exhibited neurological abnormalities, such as hyposmia (18.0%), cognitive deficits (17.5%), postural tremor (13.8%) and subtle motor/sensory deficits (7.6%) [8]

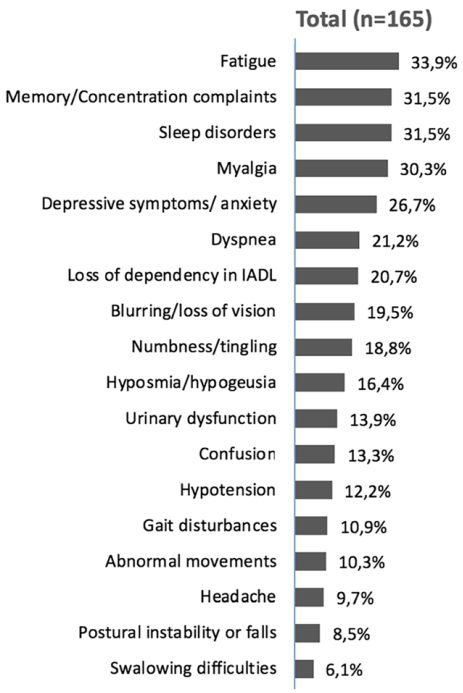


Figure 1.2: *Symptoms reported at 6-months [8].*

Later, the studies were focused on the possible influence of some variable in the belated development of the manifestations mentioned above.

It has been highlighted that the individual gender can intensify the risk of long COVID occurrence; indeed, the probability of symptoms appearance is twice greater in women rather than in men.

Then, individual age is a further variable that has an impact on the tardive COVID-19 related problems appearance; it has been reported the patients with long COVID are around four years older than those without.

The severity of SARS-CoV-2 infection has a key role on the long COVID effects; indeed, if the acute symptoms are more than five, the probability of developing one or more of the long COVID associated problems in the following months is greater. This is a crucial factor for neurological long COVID manifestations, including memory complaints and attention deficit, and it is further evidence of the central nervous system sensibility to prolonged inflammatory response and sustained intensive care.

	Total (*n*=165)	Mild (*n*=57)	Moderate (*n*=77)	Severe (*n*=31)	*p* value
Days of hospitalisation	11.6 ± 8.8	7.8 ± 4.2	12.3 ± 9.4	17.1 ± 10.4	**0.001#¶***
Oxygen therapy, *n* (%)	128 (77.6%)	20 (35.1%)	77 (100%)	31 (100%)	**0.001¶**
Non-invasive ventilation, *n* (%)	18 (11.0%)	0 (0%)	0 (0%)	18 (58.3%)	**0.001#¶**
Intubation, *n* (%)	2 (1.2%)	0 (0%)	0 (0%)	2 (6.4%)	**0.001#¶**
qSOFA	0.44 ± 0.53	0.0 ± 0.0	0.48 ± 0.50	1.10 ± 0.29	**0.001#¶***

*Significant for the comparison between mild vs moderate groups
#Significant for the comparison between moderate vs severe groups
¶Significant for the comparison between mild vs severe groups

Figure 1.3: *Clinical characteristics of the sample according to COVID-19 severity. How clinical assistance grows abreast the increasing disease sternness [8].*

Finally, many patients, who have faced such COVID-19 related syndrome, were also affected by other pathologies, therefore the presence of comorbidities is another factor that must be included in this list.

1.3 Neurological signals, brain functions and psychological disorders intercorrelation

The clinical entity of the long COVID-19 is not yet clear. Especially, the nature of the bad cognitive performances of the COVID-19 survivors has to be further investigated.
Are the memory and attention dysfunctions experienced by the recovered patients due to the noxious action of the virus on the CNS or a consequence of the psychological distress manifested by the individuals affected by the most severe form of the disease?
We look for a biomarker of cognitive disorders to elucidate their origin. Neurophysiology, a branch of biology and in particular of human physiology that studies the functioning of neurons and neural networks, may help us to answer this open issue. Specific cognitive and executive functions are codified by neurophysiological brain patterns detectable through electrodes homogeneously distributed on the scalp. In detail, through the analysis of nap EEG recordings we can investigate some sleep characteristic waveforms strictly linked to memory and attention activities.

1.3.1 Sleep stages

During sleep, we move through five different stages: N1, N2, N3, N4, all under the name of non-rapid eye movement (NREM) sleep, and rapid-eye movement (REM) sleep stage. The human body typically switches from one state to another during the night making on average from 4 to 6 full turns; it remains for about 90 minutes in each sleep stage.
NREM stages mostly occur during the early hours of the night, while they are replaced by longer REM episodes as the night progresses (Figure 1.4), [9].

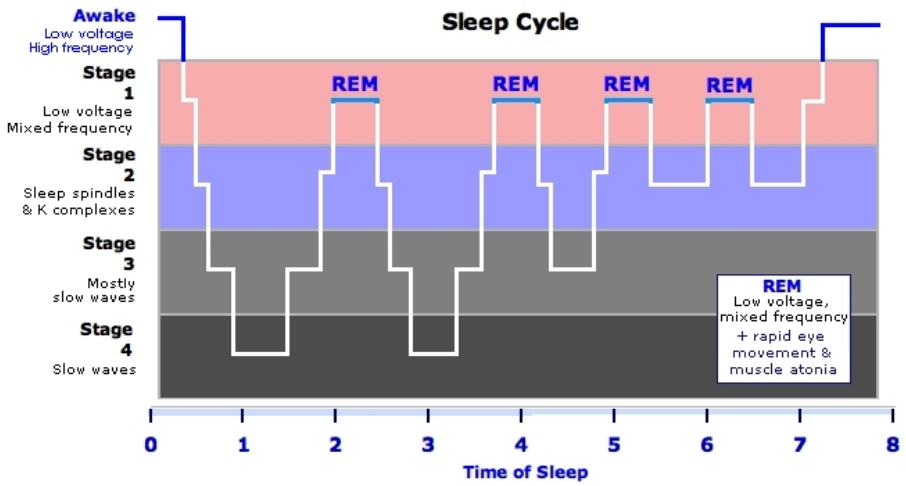


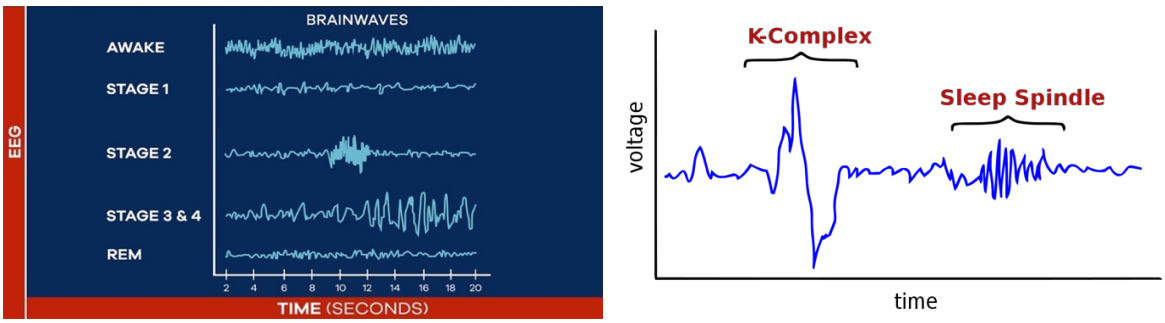
Figure 1.4: *The typical alternation of the different stages during a sleep of 8 hours: the repetition of a cycle that starts from the waking state, through the deeper sleep phases towards the REM stage [10].*

N1 sleep stage is the intermediate phase between waking state and sleep. Lower-frequency alpha and theta waves typically appear in the brain recorded path (Figure 1.5, a – second row). We may come across muscle spasm episodes, and we are easily awakened. Generally, an individual spends about five minutes in this first stage.

N2 is the sleep stage in which the subject typically spends more time overnight. The body temperature decreases, and the heart rate slows down. Characteristic waveforms are detectable by recording the brain signal during this sleep stage: the K-complexes, which denote a transition toward deeper sleep stages, and the sleep spindles (Figure 1.5, b).

Human mental and physical restoration takes place during N3 and N4 sleep stages, which are known as deep sleep. In N3, small and fast waves are intermixed with slower ones, known as delta waves (Figure 1.5, a -fourth row). Their presence increases during stage 4. In the deeper sleep stages, no eye movement or muscle activity are detectable.

Finally, REM sleep takes the name from the oscillatory and continuous eye movements that occur during this stage. The blood pressure and the heart rate increase and brain waves appear fast, desynchronized and with a low amplitude (Figure 1.5, a – last row). REM sleep stage is crucial for learning activity: during this phase daily information are processed to be stored in long-term memory [11].



(a) (b)

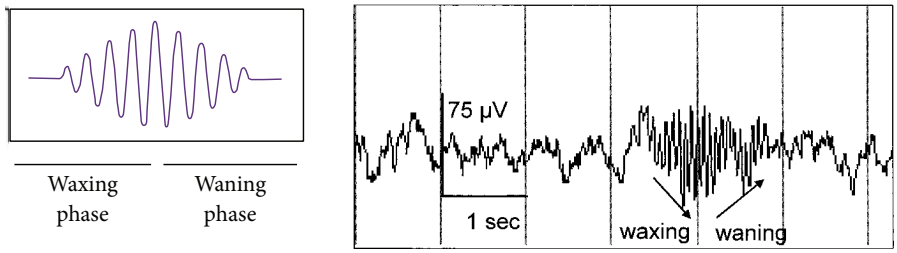
Figure 1.5: *Typical waveforms of the EEG signals recorded during each sleep stage (a), [11] and a detailed view of K-complexes and sleep spindles shapes (b), [12].*

1.3.2 Sleep spindles

Sleep spindles consist of brief bursts of rhythmic activity. Their minimum duration has been set to 0.5 seconds, although some authors have detected sleep spindles shorter than 0.3 seconds. A maximal duration limit has not been suggested yet.

Sleep spindle duration and frequency seem to be not directly associated. In general, they remain stable overnight in the same subject but can vary considerably between individuals.

Sleep spindles have a typical waxing and waning shape. During the initial phase, more cortical neurons are synchronously recruited, and the oscillation amplitude rapidly grows, then different neuronal mechanisms intervene by reducing the number of recruited neurons leading to a consequent amplitude decrease (Figure 1.6, a and b) [13].

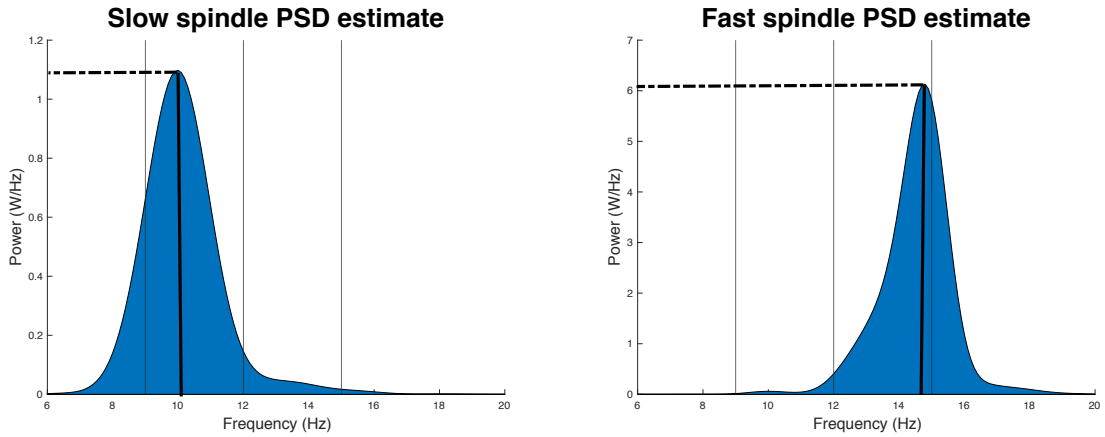


(a) (b)

Figure 1.6: *Depicted (a), [13] and realistic (b), [14] spindle waxing and waning shape.*

Spectral power analysis is an important tool that can be applied to nap EEG recorded signals. It allows for a detailed frequency-based investigation of the neuronal activity and the calculation of several important parameters, such as the spindle power, the peak frequency, and the spindle maximum amplitude. [15]

Sleep spindles frequency range typically spans between 11 and 16 Hz. Based on the peak frequency, spindles are distinguishable into two categories: the slow sleep spindles (9-12 Hz) and the fast sleep spindles (13-15 Hz) (Figure 1.7).



(a) (b)

Figure 1.7*: Example of slow (a) and fast (b) spindle power spectral density (PSD). Graphical representation of characteristic spindle parameters: spindle power (blue), peak frequency (continuous black line) and spindle maximum amplitude (black dash-dots line).*

It has been demonstrated that spindles are markers of neuronal plasticity and play a role in memory and cognition.

In 2013, the Canadian Lafortune and his collaborators investigated the possible correlation between sleep spindles and cognitive performances. Fifty-eight healthy middle-aged and older adults underwent nap EEG recording. Then, a spindle analysis was conducted on each brain recorded signal. Pearson's correlations were applied between the derived spindle features, such as the spindle density, the mean frequency and the mean spindle slope, and the cognitive test scores. The results showed that a higher spindle density was a predictor of better performances in tests investigating verbal learning, visual attention, and verbal fluency, suggesting that this parameter is an important biomarker of the cognitive functioning and of underlying neural networks integrity. [16]

Further investigations are needed to clarify the existing intercorrelation between stage 2 sleep spindles and cognitive functions, like memory and attention.

1.3.3 EEG source imaging tool

The electroencephalogram (EEG) is one of the oldest technologies to measure neuronal activity of the human brain and it is sufficient to conduct spindle analysis. However, EEG recordings

alone are not sufficient to deduce the cortical localization of the areas from which the measured neuronal activity originates. Adding detailed anatomical brain information to high-density EEG (> 64 channels) patterns and applying a sophisticated source localization algorithm, the cortical origins of the brain signal can be calculated and visually represented (Figure 1.8). EEG source imaging (ESI) is endowed with EEG advantageous properties, such as versatility, ease of use, affordability, and portability, and improves its functioning by deriving from the multichannel scalp recording the distribution of the underlying neuronal sources. Nowadays ESI has become the standard to localize human brain sensory, motor and cognitive functions in healthy or pathological subjects. [17]

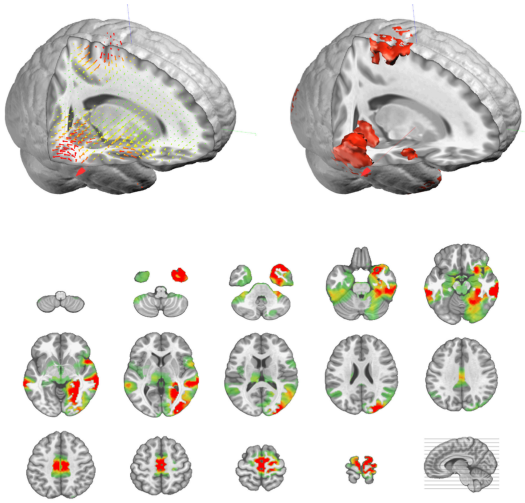


Figure 1.8: *Vectorial results from EEG source imaging (top left), tridimensional (top right) and bidimensional (bottom, through transverse slices) representation of source amplitudes (μA/mm^3), [17].*

Several studies aimed at identifying and evaluating slow and fast spindles cortical generators in healthy and pathological individuals. They started from magnetoencephalography (MEG) and electroencephalography (EEG) patterns and implemented the ESI neuroimaging technique. Differences were found in slow and fast spindle cortical source distributions in a cohort of healthy subjects. Slow spindles originated mainly in the frontal lobe, but some sources were also localized in the parietal lobe and in the limbic system. While fast spindle sources are largely distributed around the temporo-parietal regions and moderately in the frontal lobe**.** [18]

The use of neuroimaging technologies to investigate human brain functions, known as "functional neuroimaging", allowed us to identify the anatomical location of the neuronal networks codifying for the executive functions. A cohort of healthy patients underwent a functional MRI exam. While they were performing executive tasks employing working memory, response inhibition, verbal fluency, and planning, a neuronal activation was detected

over the prefrontal and parietal cortex, basal ganglia, thalamus, and cerebellum (Figure 1.9), [19].

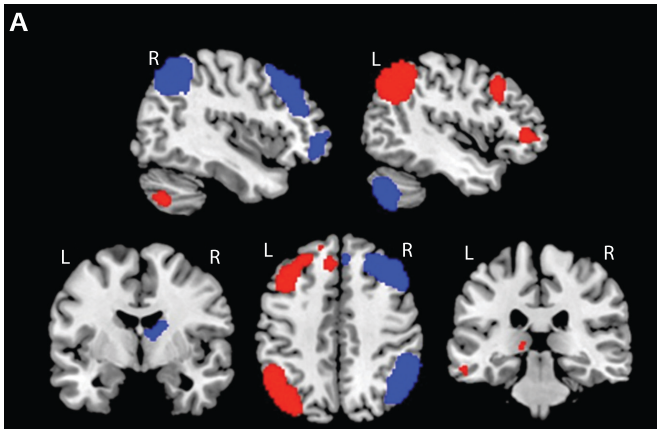


Figure 1.9: *Neuronal networks activated during the executive tasks. The brain template is neurologically oriented. In red are highlighted the regions activated in the left hemisphere, in blue the areas triggered in the right one [19].*

Therefore, executive and memory functions are processed in neural networks localized in anatomical regions of the cortico-thalamic-cortical circuit, areas in which sleep spindles are generated.

1.3.4 Neurophysiological biomarkers of psychological disorders

So far, we have reported literature evidence suggesting a likely correlation between cognitive and executive bad performances and sleep spindle parameter alterations; whose root cause could be a damage to the CNS or to the peripheral nerves.

However, an impairment of slow and fast spindle metrics, such as the spindle density, the peak frequency, the spindle power, and the cortical generators distribution, is also reported in psycho-affective disturbances.

An Italian American study, conducted in 2013, aimed at describing sleep spindle features and cortical distributions in people affected by major depressive disease (MDD). A sleep high-density electroencephalography (hdEEG) was performed on all recruited patients and on a group of healthy subjects. Four spindle parameters were investigated: spindle density, calculated by dividing the number of detected spindles by the total time the patient spent in NREM sleep stage (t_{NREM}), the spindle duration, the maximal amplitude, and the integrated spindle activity (ISA), determined by integrating the absolute amplitude values of each spindle divided by the t_{NREM}.

The comparison was performed before on spindle parameters calculated in the whole spindle frequency range [(11-15 Hz)], then a distinction was made between slow ([11-13 Hz]) and fast

([13-15 Hz]) sleep spindles. Patient's age and sex were considered as possible influencing factors.

In young to middle aged women with MDD was highlighted an increase in several sleep spindle parameters including density, duration, amplitude, and ISA, compared to the control group; whereas in men with MDD a decrease (or no changes) in spindle parameter values was detected. A two tailed unpaired t-test was performed on spindle density, amplitude and duration calculated in the pathological and healthy groups (Figure 1.10).[20]

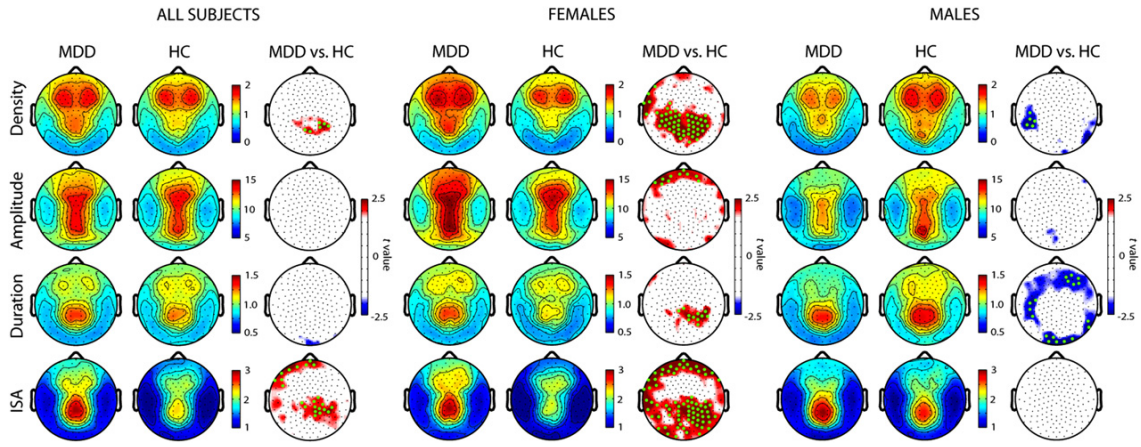


Figure 1.10: *Topographical maps: sleep spindle parameters (density, amplitude, duration, and ISA) differences between MDD population and healthy patients. Red and blue correspond to respective increase or decrease, green dots highlight the channel in which a statistically significant difference was found, [20].*

Alongside this, a recent study, published in 2021, investigated sleep microarchitectural differences (including spectral power and spindle activity) in trauma-exposed individuals that met or did not meet criteria for Post-Traumatic Stress Disorder (PTSD). The analysis of nap EEG recordings revealed a decreased beta spectral power during NREM sleep and an increased fast spindle frequency in subjects affected by PTSD. It was found that symptoms severity and variety are not associated with individual fast spindle frequencies that may be a possible biomarker of PTSD. [15]

1.3.5 Aim of the thesis

In our study, both people with and without previous COVID-19 infection underwent a cognitive and psychoaffective detailed assessment. The neuropsychological tests aimed at detecting an expected cognitive and executive functions impairment and a relevant depressive state in COVID-19 survivors compared to controls. A subsequent nap high-density EEG was recorded for each subject and a spindle analysis was performed to investigate the physiopathology of the long COVID-19 cognitive/affective symptoms. The thesis aims at shedding light on the

possible origin of long-term cognitive impairment in SARS-COV-2 infected patients at 12 months after their hospital admission for acute COVID-19.

Chapter 2

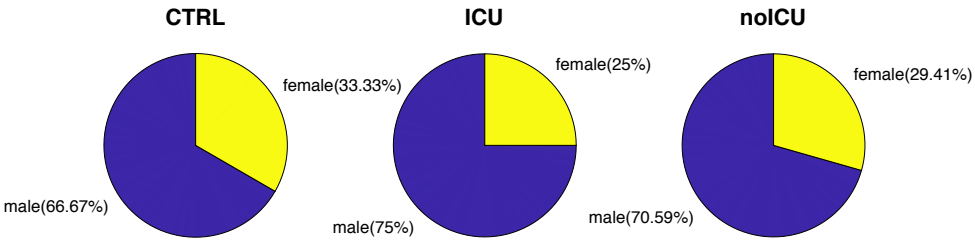
Data Acquisition

2.1 Participants

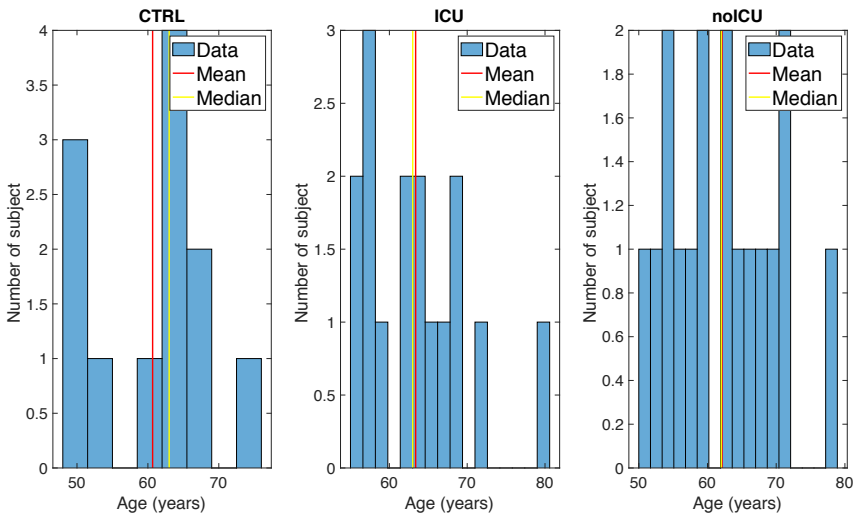
2.1.1 COVID-19 survivors

Thirty-three participants were affected by COVID-19 infection and, due to the strong respiratory symptoms, they were admitted to the Padua Teaching hospital between March and November 2020:

- 17 were treated in the sub-intensive care units where they received just oxygen assistance (noICU) (see Figure 2.1, c, f, i);
- 16, for whom oxygen treatment was not sufficient, spent part of their hospitalization in the intensive care units (ICU) (see Figure 2.1, b, e, h).



(a) (b) (c)



(d) (e) (f)

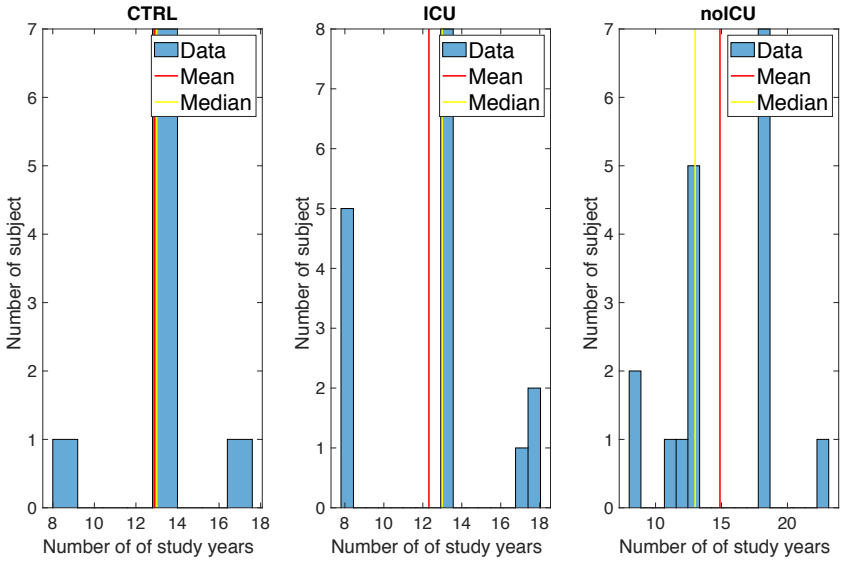


Figure 2.1. *The gender disparity across the different groups (a, b, c), the age range of individuals recruited (d, e, f) and the distribution of the education years among the three populations (g, h, i).*

91 COVID-19 survivors were, at first, identified to be recruited for this study, but 64% did not adhere for:

a) unreachability

b) health related or family issues

c) fear of contracting Covid-19 during the activity we propose or simply by leaving the house

d) sleep problems or the assumption of benzodiazepines or antidepressant drugs that can alter the architecture of the EEG signal recorded during the nap

e) waiver of participation after a first subscription (see Figure 2.2).

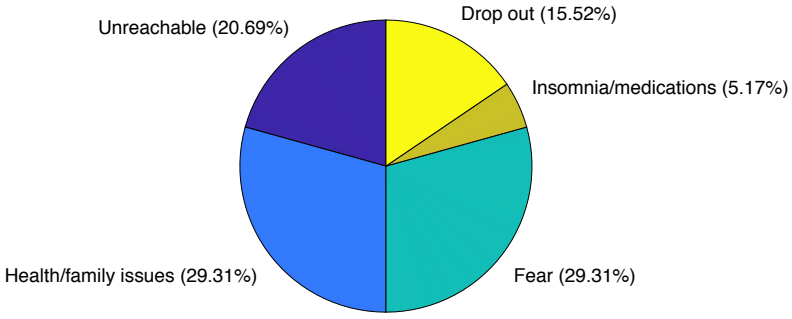


Figure 2.2. *The causes of non-adhesion: fifty-eight patients did not want to join the project and the main reasons of their refusal are summed up here.*

Additional exclusion criteria were: previous diagnosis of cognitive impairment, previous stroke or other central nervous system lesion.

2.1.2 Controls

12 subjects, never infected by COVID-19 were recruited (CTRL). 9 came from the Padova Teaching Hospital and 3 subjects from a previous dataset (see Figure 2.1, a, d, g).

2.2 Laboratory instrumentation and organization

EEG recordings were acquired at Padova Neuroscience Center.

The recording laboratory is organized in two rooms. The first consists of a workstation from where the operator can start, check, and stop EEG recordings and contains all the necessary equipment to carry out the examination (Figure 2.3).

The second is a dimly lit sound-attenuated and electrically shielded room with a swivel and folding chair, which allows the patient to assume a comfortable position during the EEG recording, and with the EEG amplifier (Figure 2.4).

Cognitive tests were performed inside this room before EEG acquisition.

Figure 2.3. *Experimental set-up: operator workstation.*

Figure 2.4. *Experimental set-up: high-density EEG recording setting.*

A 256-electrodes cap (Electrical Geodesic, Inc, Eugene, OR) was used to acquire both nap and resting state high-density EEG signals (Figure 2.5).

The cap was immersed in a solution composed of water, a measuring cup of shampoo and one of potassium at least ten minutes before the first recording (Figure 2.6). This step is crucial for decreasing the impedance value of most of the electrodes.

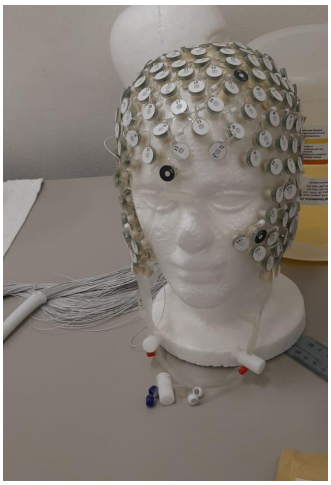


Figure 2.5. *256-electrode cap wearability.*

Figure 2.6. *Soaking 256-electrode cap.*

2.3 Procedure

All the subjects, partially sleep deprived (awakened at 5 AM), underwent a series of neuropsychological tests. The time required to perform this first step was approximately one hour.

Then, the operator measured the distance between the patient's nason and ineon and the one between the ear pressures. The cap reference electrode Cz should be located at the point of coordinates given by the midpoints of both the distances. Once the cap was on, by using some pipettes filled with the same solution in which the cap had been immersed, the electrodes with a higher impedance value were wetted again (electrode impedances must be kept below 50 kΩ). The patient lay down, and the recording started (see Figure 2.7 a).

The sampling rate used was 250 Hz and each recording lasted between one hour and one and a half hours. After ten minutes from awakening, three minutes of resting-state EEG were acquired, before with open and then with closed eyes. The time between patient awakening and the eyes-closed and eyes-open resting-state EEG was approximately ten minutes. A sitting posture and a thoughtless state were suggested for the resting-state tests (see Figure 2.7 b).

(a) (b)

Figure 2.7. *Control subject attitude during nap high-density EEG (a) and throughout the resting-state recordings (b).*

2.4 Neuropsychological assessment

A neuropsychological assessment was performed both on COVID-19 survivors and controls.

2.4.1 Cognitive evaluation

All the subjects underwent a series of tests aimed at evaluating different functions belonging to the cognitive domain, such as for example the complex attention, the ability to perform a task (executive function), the learning ability and the short- or long-term memory (Figure 2.8).

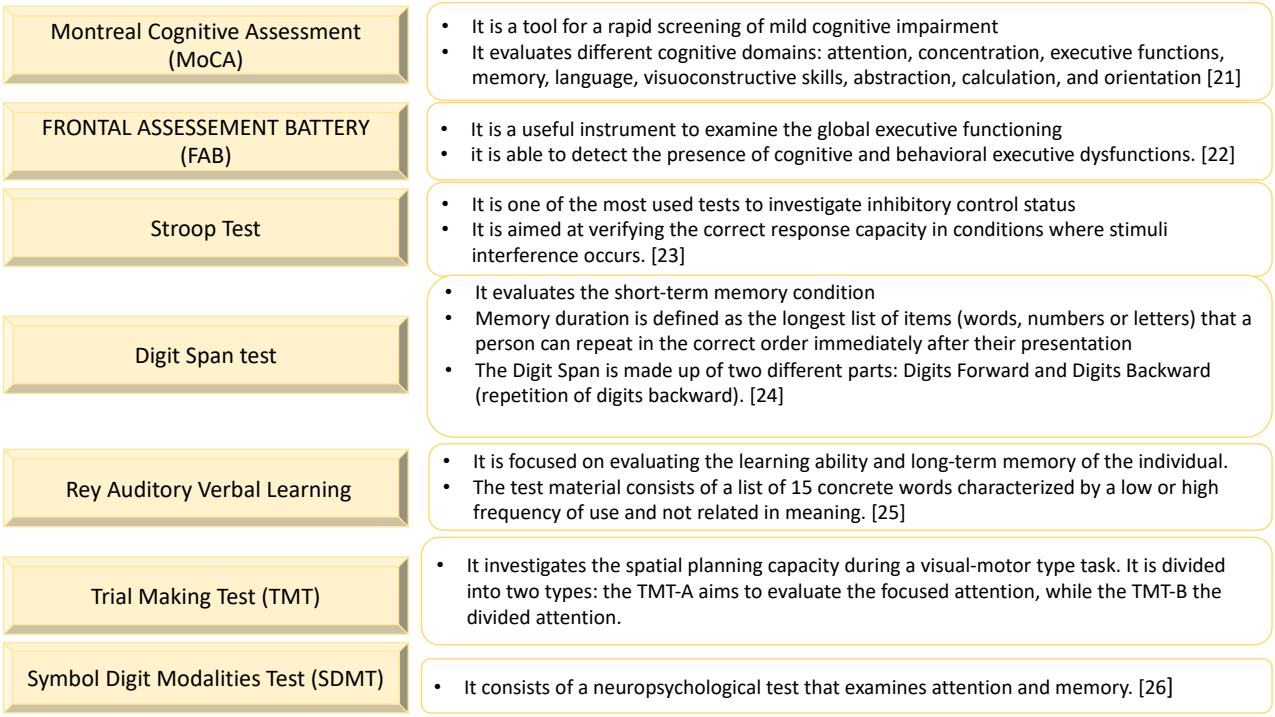


Figure 2.8: *Brief description of the neuropsychological test performed.*

2.4.2 Psychological evaluation

Other tests were also performed to assess the extent of depressive symptoms, the gravity of the disorders caused by the Covid-19 disease and how the patients perceive the quality of their own life (Figure 2.9).

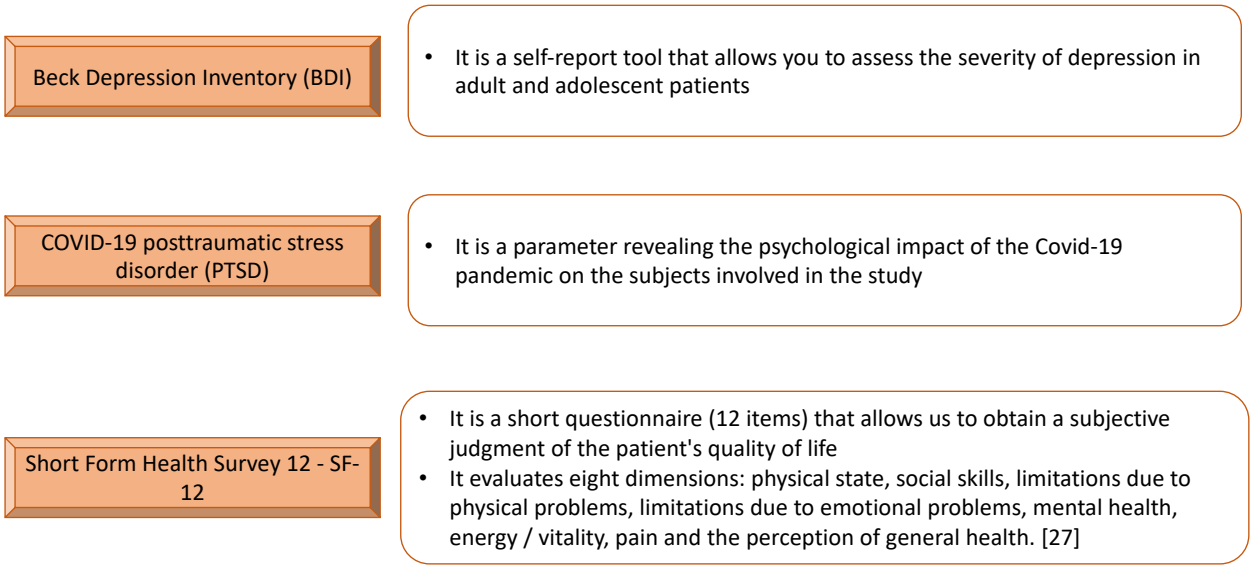


Figure 2.9: *Brief description of the psychological assessment tests.*

Chapter 3

Methods

3.1 Nap high-density EEG analysis

3.1.1 EEG pre-processing

The EEG patterns passed through two Butterworth filters: 1) a zero-phase-high-pass-filtered above 1 Hz and 2) zero- phase-low-pass-filtered below 40 Hz, avoiding phase distortion.
Channels in the cheeks and in the neck were discarded (204 channels left).
Through visual inspection the noisy channels were highlighted and, on average, four channels per individual were interpolated using the nearest-neighbor spline method.
Furthermore, the epochs including non-stereotyped artifacts, peri-stimulus eye blinks and eye movements were visually detected and discarded.
Nap signals were divided in 30-sec epochs and classified according to the current individual sleep stage: Wake, N1, N2, N3 and REM (statistics here – see Figure 3.1).
American Academy of Sleep Medicine (AASM) guidelines were followed in this step [28].

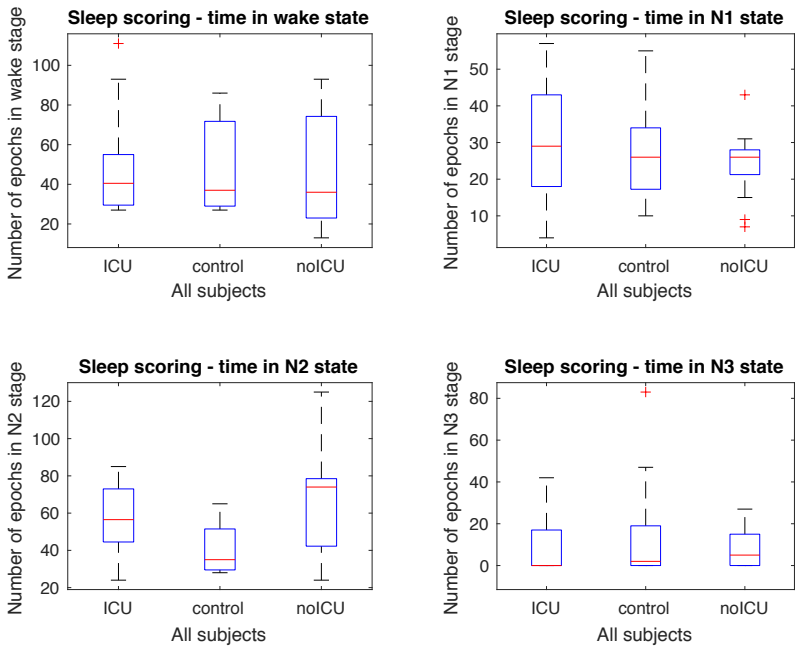


Figure 3.1: *Distribution of the time spent in the Wake, N1, N2 and N3 stage for each group.*

3.1.2. Spindle analysis

An algorithm implemented by Wonambi 6.12 [29] allowed us to automatically detect the sleep spindles in the pre-processed EEG paths.

Looking at each spindle peak frequency, a subdivision into slow ([9-12] Hz) and fast (]12-16 Hz]) spindles was accomplished. A Fast Fourier Transform analysis was conducted to check the spindle categorization.

Slow and fast spindle density is considered an important benchmark. It was computed for each subject simply by dividing the number of spindles automatically detected by the time spent in stage 2 (N2) in minutes. The spindle power, the spindle peak frequency and the corresponding amplitude were calculated for each slow and fast spindle detected. These parameters were then averaged for each subject. Finally, for each subject a topographical map at each fast and slow spindle onset has been generated. Fast and slow maps have been mediated separately at individual and group levels. Spindles containing unconventional, or muscle artifacts were discarded.

Subsequently, we focused on understanding in what position of the cortex the spindle potentials originated; this step is called spindle source reconstruction. The spindle starting point was the only segment needed for its source localization and it was defined as the initial deflection of the EEG signal from the baseline. The EEG was segmented 500 ms before and after each spindle onset.

Then, we applied the LORETA algorithm implemented in Cartool. It receives in input the averaged individual slow and fast spindle onsets and returns their cortex localization. [18]

The solution space is limited to the brain gray matter and the participant's age was considered to calibrate the conductivity of his/her skull. The Desikan– Killiany (DK) atlas [30] was taken as a reference to divide the individual gray matter into 83 parcels (Region Of Interest – ROI).

To represent the time and frequency content of the several diverging dipoles in each ROI, we computed a singular-value decomposition of all dipoles, and we recorded the resulting first singular vector.

In this way we provided a unique time-series, representative of the ROI's dipoles, which expressed most of the variability of the sources (~ 80%). [31]

Together with spindle source localization also onset spindle amplitudes were calculated. A visual comparison between the three groups (i.e., CTRL, nonICU participants and ICU participants) was made considering both the previous results.

3.2 Statistical analyses

The neuropsychological tests scores were adjusted for patient's age, sex and education (see Figure 2.1).

Two-sided Wilcoxon rank sum tests (WRST) were performed on the cognitive and psychological tests' outcomes. The WRST is used to compare two independent samples. It is a non-parametric test because it does not suppose any known distributions of the compared samples and, consequently, it has not to deal with any parameter. The statistical test hypotheses that the two samples under investigation belong to the same population or, equivalently, that their median value is approximately the same.

No corrections for multiple comparisons were applied because of the limited number of participants and the exploratory nature of the study.

Two-sided WRST were also implemented on the slow and fast spindle densities, on the spindle maximum amplitude values, on the spindle peak frequencies and on the spindle powers between the three different populations. The most meaningful differences were recorded and graphically represented below. Also in this case, no corrections for multiple comparisons were applied.

Then, we looked for a possible relationship between the neuropsychological test scores and the slow and fast spindle parameters (density, power, maximum frequency, and amplitude) through Pearson correlation coefficient (r). It reveals if the variables under investigation are linearly correlated or not. The r value varies between -1 and +1. A correlation coefficient of -1 indicates a strong negative relationship between the compared data: for every possible increase in one variable, it follows a possible decrease in the other parameter. Vice versa, r equal to +1 suggests a positive relationship: if the value of one variable grows, we expect a probable increase in the other as well. [32]

Then, Multiple Linear Regression (MLR) method supports us in defining the relationship among the psychological scores, the cognitive test performances, and the spindle characteristics. They have been used alternately as dependent / response and independent / explanatory variables of the model, which fits a linear equation to the experimental dataset.

3.3 Resting-state

The third database component, the resting-state high density EEG, could not have been included in the analysis for the following reasons:

1) The impedances of some electrodes certainly exceeded the 50 kΩ threshold.
   Indeed, before the resting-state recordings, the patient had been wearing the 256-electrodes cap for at least one hour, during which most of the electrodes dried out.

2) As reported in the literature, the fact of having slept shortly before the recording can vary the architecture of the resting-state signal pattern.

   Interestingly, a detailed analysis of topographical modifications in the delta ([1–4 Hz]), theta ([5–7 Hz]), alpha ([8–12 Hz]), beta-1 ([13–16 Hz]) and beta-2 ([17–24 Hz]) bands has highlighted that the post sleep EEG recording is characterized by a generalized reduction in beta activity and an increased delta activity in the posterior area of the scalp. [33]

   Therefore, in order to have a uniform sleep influence in the resting-state traces we should have selected for each group only those who woke up at the same stage (see Figure 3.2).

3) The presence of troubled and unusable paths

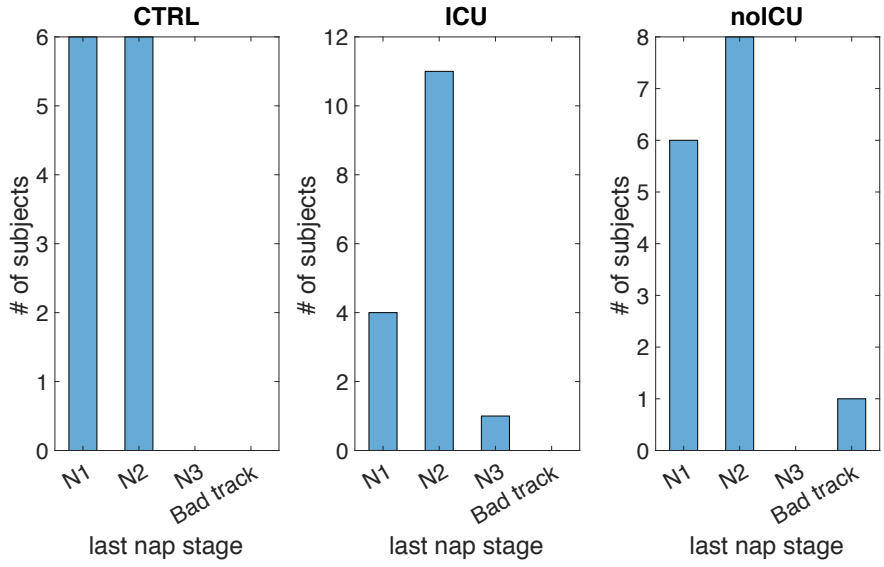


Figure 3.2: *Nap last stage. By adding selection criterion (2) to the presence of troubled and unusable paths and the limited number of subjects recruited, the available EEG tracks were few to guarantee the validity of the results that would have been obtained.*

Chapter 4

Results

Our experimental protocol aimed at assessing the correlation between executive deficits, memory impairment and neurophysiological changes and defining a clear neurological biomarker of the cognitive-psychological Long COVID-19 symptoms.

Not all subjects fell asleep during the high-density EEG recording and their patterns were not used in the analyzes (see Figure 4.1).

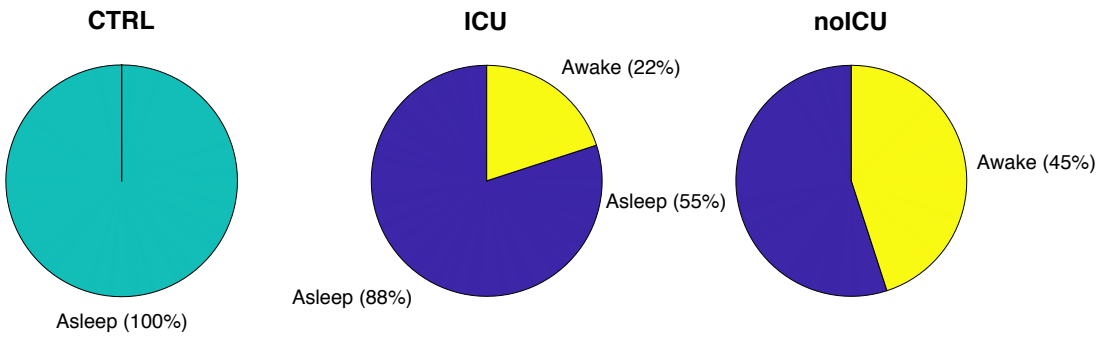


Figure 4.1: *Nap high-density EEG recordings available for each group.*

4.1 Spindle analysis results

4.1.1 Statistical results

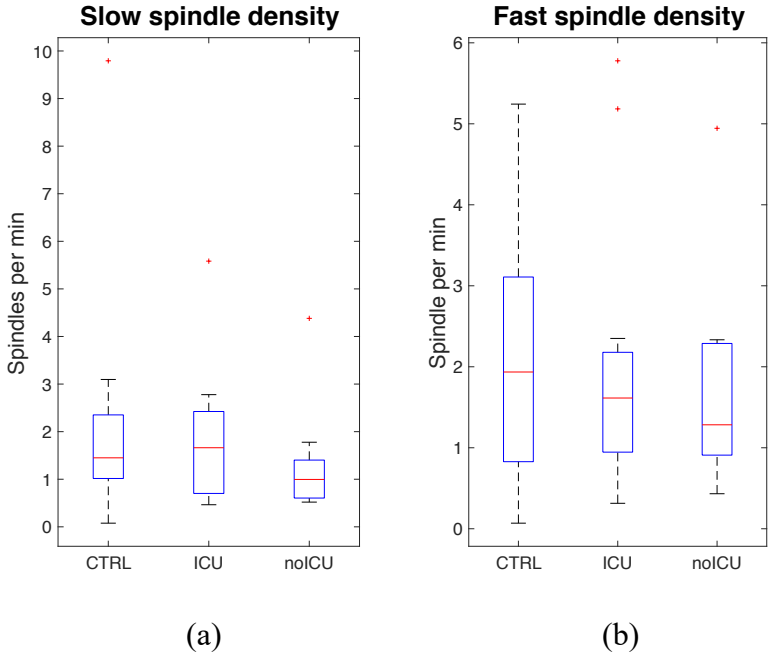
A higher spindle density implicates better performance on verbal learning, visual attention, and verbal fluency. The spindle density is considered a useful biomarker of cognitive functioning in older adults and may be an indicator of neuroanatomic integrity. [16]

Spindle density changes are also seen in patients affected by Mild Cognitive Impairment (MCI) and Parkinson Disease (PD), even at the first stages of the disorders, and their entity is linked to the cognitive decline severity. MCI and PD tend to reduce fast spindle density rather than slow one and to shift fast spindles, usually distributed in the frontal area, towards the parietal lobe [34].

In our study, fast and slow spindle densities did not considerably vary among the three different groups (see Figure 4.2).

We investigated other fast and slow spindle parameters, including the maximum spindle amplitudes, the corresponding frequencies, and the spindle powers.

Slow spindle frequencies calculated at the spindle peak significantly decreased ($p_{value=}$ 0.0477) in ICU patients compared to control population (see Figure 4.3, a).



(a) (b)

Figure 4.2: *Slow (a) and fast (b) spindle densities in the three groups.*

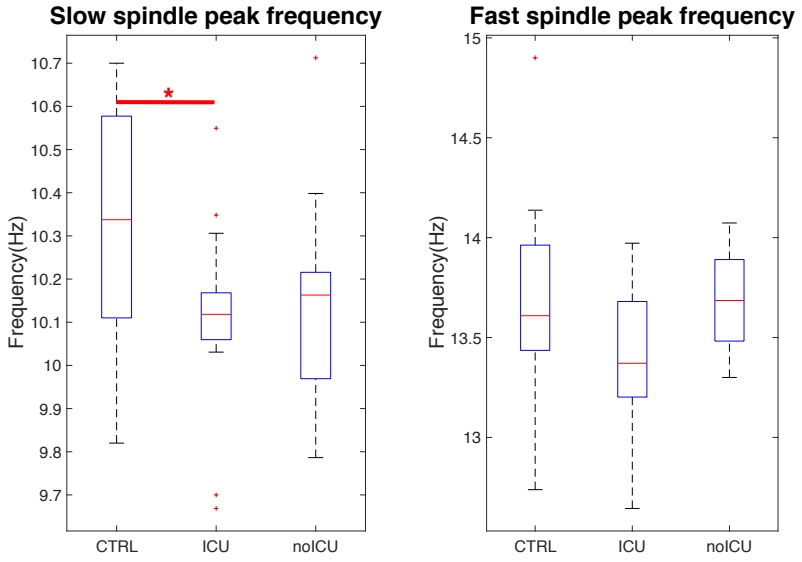


Figure 4.3: *Slow (a) and fast (b) spindle frequency among the three different populations. Significative differences notation: * if p value <0.05, ** if p value<0.01 and *** if p value <0.001.*

4.1.2 Spindle onset topographical maps

In the resulting slow spindle maps for the control group, we identify a frontal activation (see Figure 4.4, a), whereas the averaged fast spindles are more focused on the center-parietal area (see Figure 4.4, d). This distribution is in line with what we expected for people never affected by the disease.

In the Covid-19 survivors topographical results this distinction is less obvious. The slow spindles shifted towards the posterior area; they are focused on the central lobe for the ICU group (see Figure 4.4, b) and on the temporal lobe for the noICU population (see Figure 4.4, c). Meanwhile, in the ICU resulting maps the fast spindles seemed to spread out in the anterior area (see Figure 4.4, f).

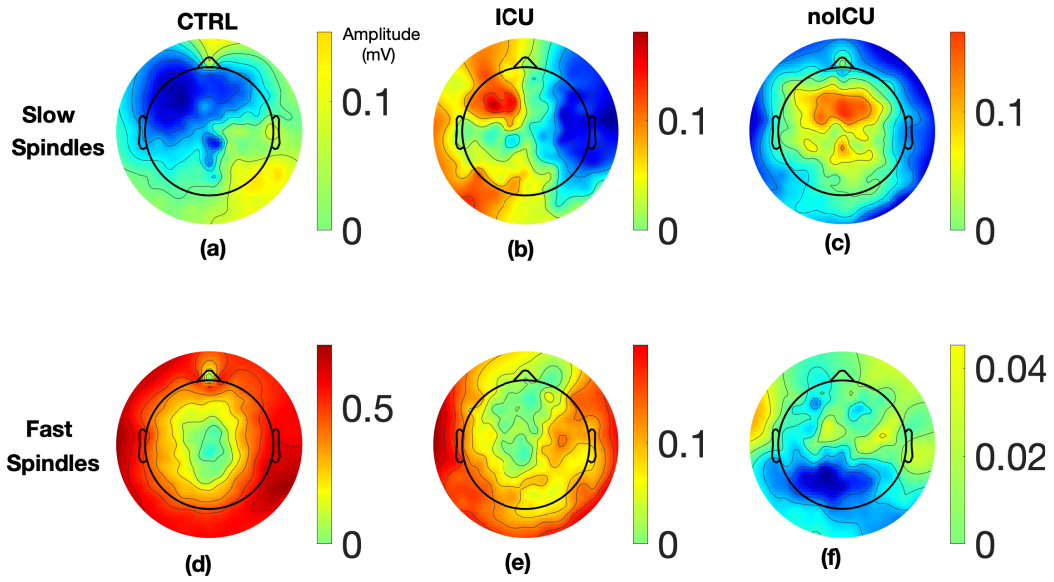


Figure 4.4: *Averaged fast and slow spindle topographical maps.*

4.1.3 Source analysis

The spindle source reconstruction revealed dissimilarities in fast and slow spindle source locating among the three populations.

Slow spindle sources in the control group are averagely distributed in the frontal area (see Figure 4.5, a), while the fast ones are mainly concentrated in the central region (see Figure 4.5, d).

In Covid-19 survivors the cortical starting points of the slow spindle onsets are mostly focused on the central-temporal area (see Figure 4.5, b, and c).

The ICU fast spindle sources are largely diffused in the frontal areas (see Figure 4.5, f), while the noICU ones are chiefly located in the tempo-parietal lobes (see Figure 4.5, e).

Moreover, a statistically significant difference between CTRL and noICU emerged from the fast spindle onset amplitudes. It was found that the fast spindle amplitudes measured at the spindle onset in the frontal and tempo-parietal lobes are higher in noICU rather than in CTRL (see Figure 4.6, e). Other remarkable statistically significant results did not arise.

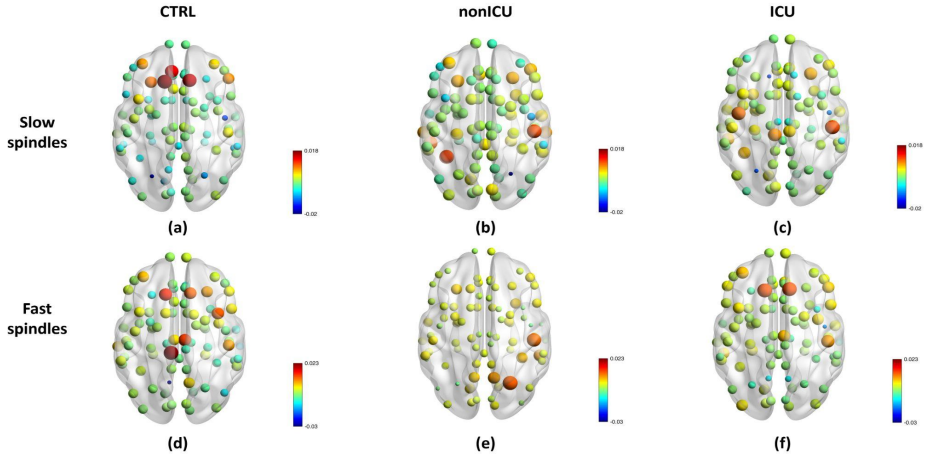


Figure 4.5: *Fast and slow spindle sources and amplitudes at their onset. Individual results were mediated within each population and node dimension and color were used to suggest averaged onset spindles'* amplitudes ($\mu A/mm^3$).

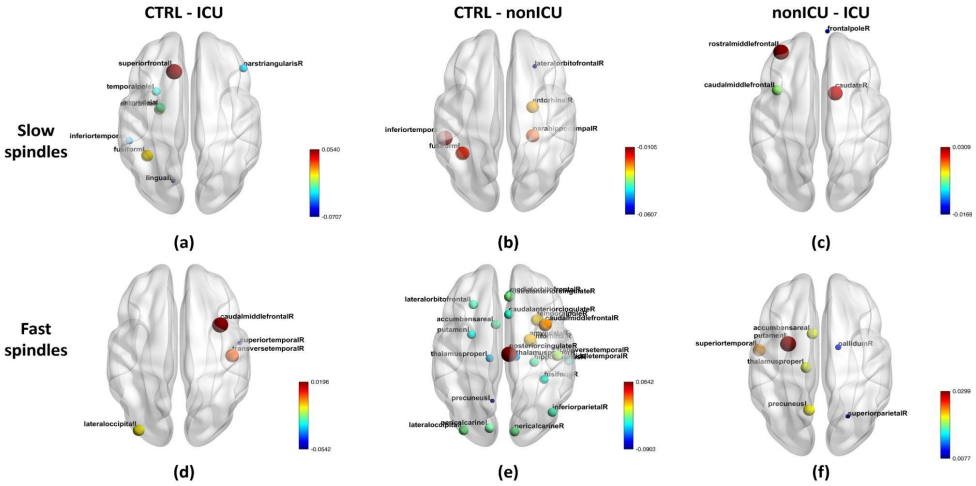


Figure 4.6: *A comparison of fast and slow spindle onset sources and amplitudes among the three groups. More intense color and greater node dimension mean that a significant difference has been found. Only ROIs in which the differences between the two groups were statistically significant (p value < 0.05) are reported.*

4.2 Neuropsychological results

4.2.1 Cognitive assessment

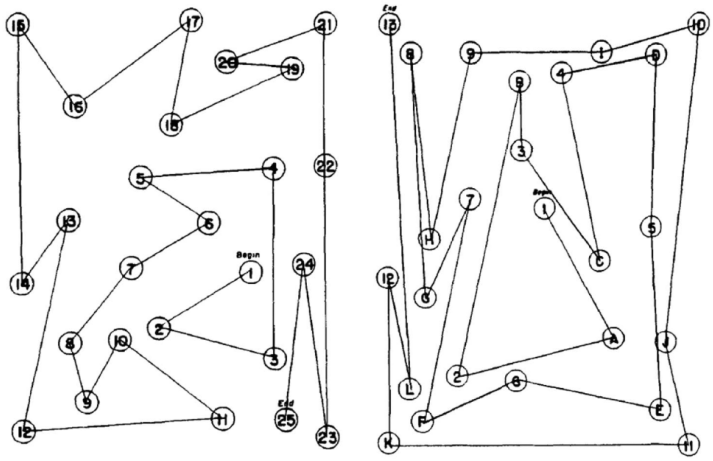
A worsening of executive performance in COVID-19 survivors was found through cognitive assessment tests. Particularly, the Trail Making Test (TMT) showed a deterioration of the attentive functions in patients who were admitted to intensive and sub-intensive care.

The TMT is made up of two subparts:

1) The TMT - A is focused on evaluating the selective attention; the person undergoing the test has to connect 25 consecutive numerical targets randomly painted on a sheet of paper (see Figure 4.7, a).

2) The TMT - B fixes the divided attention level of the patient; the association has to be made among 25 alternating alphanumerical targets (see Figure 4.7, b).

The subtraction between the two scores (TMT B - A) can be considered as an indicator of the subject frontal efficiency.



(a) (b)

Figure 4.7: *Example of a completed Trail Making Test: Part A (a) and B (b) [35].*

The two-sided Wilcoxon rank sum test revealed a statistical difference between CTRL and noICU in TMT – B (pvalue = 0.0556) and in TMT B-A (pvalue = 0.00459), (see Figure 4.8).

Because of the explorative nature of the study and the limited number of subjects recruited the p values were not adjusted.

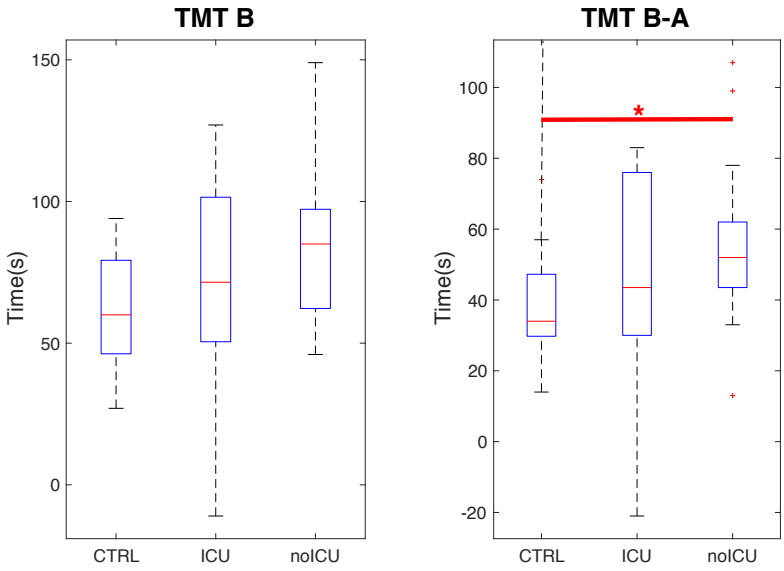


Figure 4.8: *Trail Making Test B and B-A score distributions for each population. Significative differences notation: * if p value <0.05, ** if p value<0.01 and *** if p value <0.001.*

4.2.2 Psychological assessment

A psychological evaluation was performed through the Beck Depression Inventory (BDI) to assess depressive symptoms entity, a Post-Traumatic Stress Disorder (PTSD) investigation to have an idea of how the COVID-19 disorder had an influence on patients' health mental state and a 12 -item short-form health survey to evaluate how subjects perceive the quality of their life (PCS-12).

A common trend emerged from psychological score statistics. Depression and posttraumatic stress increase in ICU and noICU populations compared to controls. Congruently, CTRL perceive to live qualitatively better than the ICUs and noICUs (see Figure 4.9).

The statistical test of Wilcoxon highlighted a substantial difference between CTRL vs ICU scores (pvalue = 0.0361) and CTRL vs noICU results (pvalue = 0.0236) collected from the survey about the physical quality of life.

CTRL and noICU statistically differ also in the PTSD outcomes (pvalue = 0.0624).

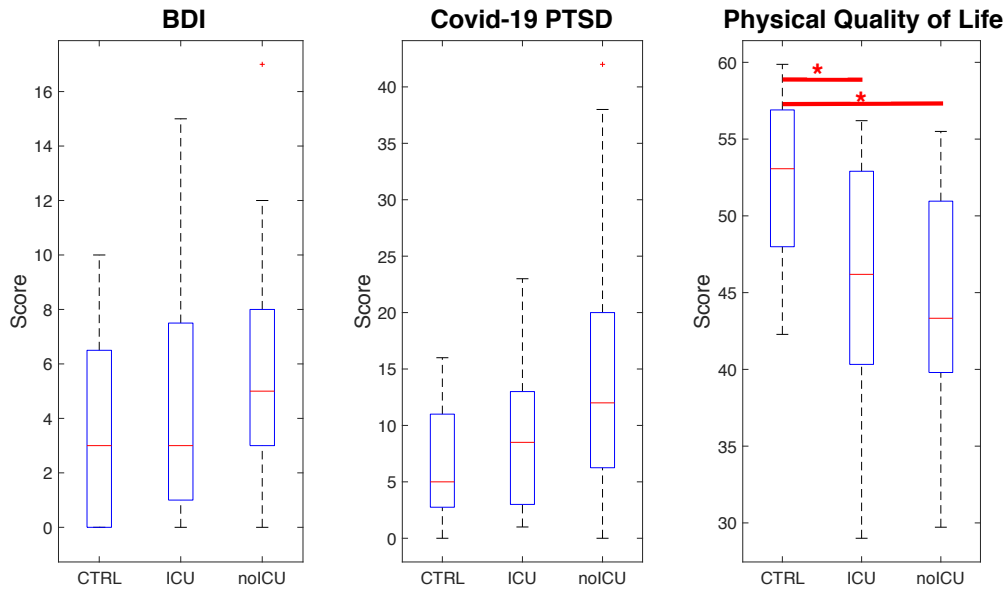


Figure 4.9: *Psychological assessment scores: Beck Depression Inventory (a), COVID-19 Post-Traumatic Stress Disorder (b) and, Physical Quality of life (c). Significative differences notation: * if p value <0.05, ** if p value<0.01 and *** if p value <0.001.*

4.3 Correlation analysis

4.3.1 Pearson correlation coefficients

Pearson Correlation was calculated between psychological test scores (Beck Depression Inventory: BDI; Post-traumatic stress disorder scale: PTSD; Physical and mental component summary scores: PCS-12 and MCS-12); cognitive performance (Montreal Cognitive Assessment: MoCA; Frontal Assessment Battery: FAB; Stroop task: Stroop_t; Stroop_e; Digit Span forward and backward: Digit FW, Digit BW; Rey Auditory Verbal Learning test: Rey_i, Rey_d; Trail Making Task: TMT A; TMT B, TMT B-A; Digit Symbol Modalities Test: Symbol), subjects' handedness dominance (Edinburgh Handedness Inventory: EHI) and participants' spindle features (slow and fast spindle density: D-slow, D-fast; peak frequency: fmax-slow, fmax-fast; maximum amplitude: Amplitude-slow, Amplitude-fast and sleep spindle power: Power-slow, Power-fast) .

Pearson correlation coefficient (r) highlighted a statistically significant linear correlation in the nonICU participants between:

- Psychological and cognitive test scores:
    - PTSD and: Digit BW (r=-0.6, pvalue= 0.069) and FAB (r=0.75, pvalue=0.0126)
    - BDI and: MoCA (r= -0.62, pvalue=0.057), Digit BW (r=-0.72, pvalue=0.0177) and FAB (r=0.69, pvalue=0.0258)
    - MCS-12 and Symbol Digit (r=-0.6, pvalue=0.0628)

- Psychological test scores and spindle parameters:
  - PTSD and: Fast spindle density (r= 0.6, pvalue= 0.069), Slow spindle maximum amplitude (r= 0.62, pvalue= 0.055) and Fast spindle maximum amplitude (r= 0.6, pvalue=0.062)
  - MCS-12 and: Fast spindle maximum amplitude (r= -0.57, pvalue=0.083)
  - PCS-12 and: Slow spindle peak frequency (r=0.63, pvalue=0.0503), Slow spindle maximum amplitude (r= -0.8, pvalue=0.0043) and Slow spindle power (r=-0.68, pvalue= 0.0306)

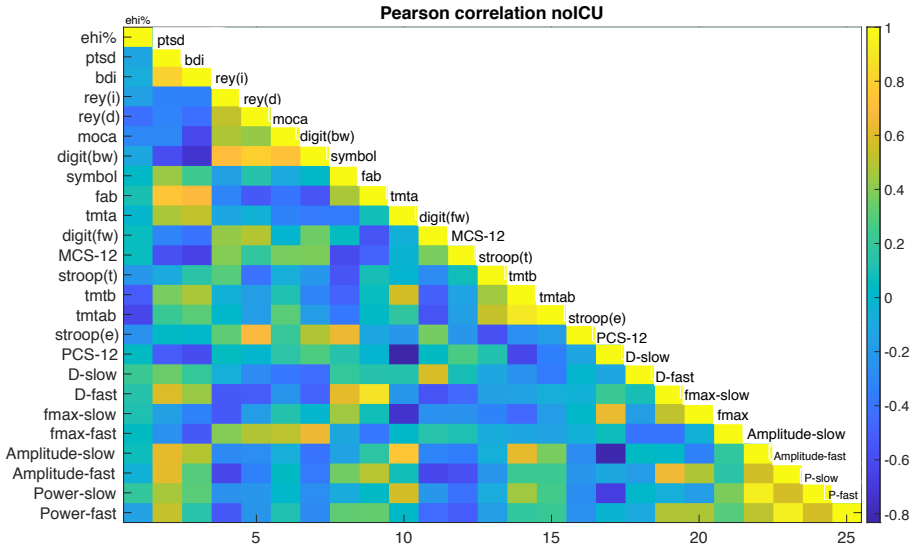
- Cognitive test scores and spindle parameters:
  - REY (i) and Fast spindle maximum amplitude (r=-0.63, pvalue= 0.051)
  - Symbol Digit and Fast spindle density (r= 0.61, pvalue=0.0584)
  - FAB and Fast spindle density (r= 0.88, pvalue=0.0007)
  - TMTA and: Slow spindle maximum amplitude (r= 0.76, pvalue= 0.0113) and Slow spindle power (r=0.57, pvalue=0.085)
  - Digit FW and: Slow spindle density (r= 0.58, pvalue= 0.0769), Fast spindle density (r= -0.56, pvalue= 0.092) and Fast spindle maximum amplitude (r= -0.63, pvalue= 0.052)

(see Figure 4.10, a).

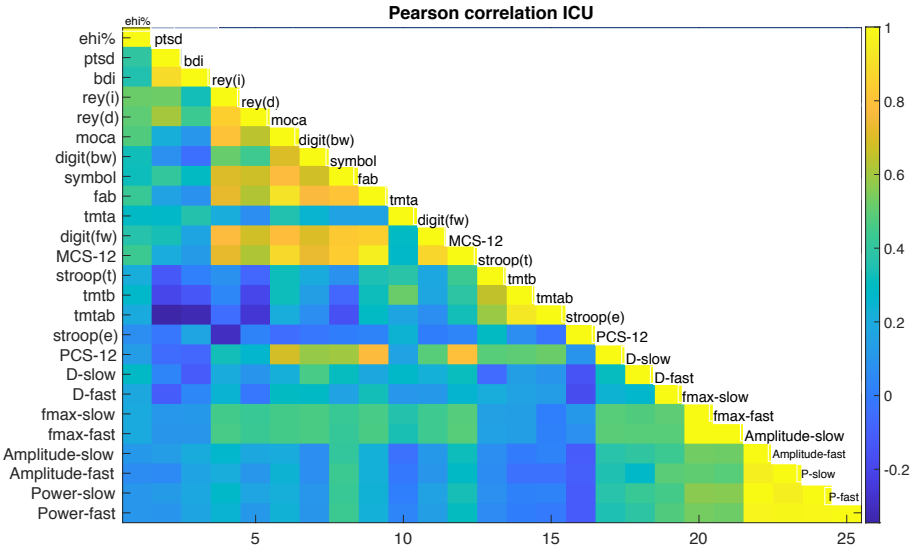
In ICU participants, a linear correlation emerged between psychological and cognitive test scores:

- PTSD and REY (d): (r= 0.6035, pvalue= 0.0103)
- MCS-12 and: REY (i) (r= 0.7248, pvalue= 0.001), REY (d) (r= 0.6177, pvalue= 0.0082), MoCA (r= 0.8839, pvalue< 0.0001), FAB (r= 0.9533, pvalue <0.0001), Symbol digit (r= 0.8403, pvalue<0.0001), Digit foreward (r= 0.8708, pvalue<0.0001) and backward (r= 0.7276, pvalue= 0.0009)
- PCS-12 and: MoCA (r= 0.6834, pvalue= 0.0025), Symbol digit (r= 0.5962, pvalue= 0.0115), FAB (r= 0.7759, pvalue= 0.0002)

(see Figure 4.10, b).



(a)



(b)

Figure 4.10: *Pearson correlation coefficients in noICU (a) and ICU (b) population. Yellow squares imply a strong positive relationship between two variables, while the blue ones suggest a negative correlation.*

4.3.2 Multiple linear regression

Multiple linear regression (MLR) analyses aimed at investigating the possible mutual predictability of the study variables. The psychological test scores, the cognitive performance and the spindle features have alternatively constituted the predictors and the predicted parameters of a linear model.

To determine how well the model fits the data and the predictive potential of the several variables we examine three model-derived measures: the adjusted R-squared, the F value and

the related pvalue. The R-squared value describes the percentage of variability of the predicted variable explained by the explanatory variable (or predictor). Typically, the greater the number of predictors, the better the description of the predicted variables and the higher the value of R-squared.

The F value in regression is the result of a test that compares the model with and without predictors, determining if the added variables improve or not the model's fit. The null hypothesis is that all the regression coefficients have no predictive capability, while a significant result (pvalue < 0.05) suggests that the parameters included in the model enhances the description of the predicted variable.

Multiple linear regression in noICU revealed that psychological tests scores can predict negative performance in attentive and memory tasks and changes in spindle features. PTSD, BDI and MCS-12 scores appeared to be quite good predictors of the subjects' outcomes in Digit Backward, FAB, MoCA, Symbol Digit and Trail Making Tests. While PCS-12 seems to be related to slow spindle maximum amplitude and power (Table 4.1).

Similarly, in ICU population, multiple linear regression analysis highlighted that executive and cognitive test scores (REY, Trail Making Test, FAB and Digit Forward) can be predicted by the psychological ones: PTSD, MCS -12 and PCS-12 (Table 4.2).

Predictor	Predicted variable	Adjusted R-square	F-statistics	pvalue
PTSD	DIGIT_BW	0.41	12.13	0.0033
PTSD	FAB	0.21	5.34	0.0355
PTSD	TMTB	0.31	8.19	0.0119
PTSD	TMTAB	0.27	6.93	0.0188
BDI	MoCA	0.27	6.77	0.0200
BDI	FAB	0.40	11.54	0.0040
MCS-12	SYMBOL	0.20	4.99	0.0412
PCS-12	Amplitude_SLOW	0.62	15.50	0.0043
PCS-12	Power_SLOW	0.40	6.87	0.0306

Table 4.1: *Evidence from multiple linear regression in noICU population.*

Predictor	Predicted variable	Adjusted R-square	F-statistics	pvalue
PTSD	REY(d)	0.24	5.79	0.0350
PTSD	TMTAB	0.19	4.61	0.0497
MCS-12	FAB	0.24	5.64	0.0324
MCS-12	TMTA	0.25	6.05	0.0275
PCS-12	REY(i)	0.36	8.56	0.0110
PCS-12	DIGIT_FW	0.29	7.03	0.0190

Table 4.2: *Evidence from multiple linear regression in ICU population.*

Chapter 5

Discussion

Our cohort underwent neuropsychological tests aimed at investigating the functioning of their attentional skills and working memory. In addition to these, we have made use of neurophysiology as a tool to help us to disentangle the pathophysiology of the possible negative executive and cognitive performance.

Sleep spindles are robust biomarker of cognition. It has been reported that the spindle-mediated information processing is a central mechanism for offline memory consolidation [38]. For example, taking a nap after a session of motor imagery (MI) practice might play a crucial role in the consolidation of the motor memory. It is also assumed that sleep spindle features, such as the spindle density, the topography at spindle onset and the distribution of their cortical generators, are related to the visuo-motor and verbal memory formation. Further analyses are suggested to assess probable sleep spindle parameter changes following dynamic mental activities, such as the MI practice [39].

Because of the fundamental function played by stage 2 sleep spindles in learning processes and memory consolidation, spindle analysis has become one of the most valuable clinical tools used in the field of neurodegenerative disorders. Sleep spindle alterations were detected in EEG patterns recorded from people affected by Mild Cognitive Impairment (MCI) and Alzheimer disease (AD). Both spindles and K-complexes appear significantly reduced and their decrease seems to be related to the severity of the cognitive decline [40].

Another evidence of the close correlation between these bursts of neural oscillatory activity and cognition come from investigation in the field of temporal lobe epilepsy. Epileptic patients showing memory impairment underwent nap EEG exam and a spindle analysis was conducted on the N2 sleep stage segments of the recorded patterns. From EEG source imaging (ESI) emerged that cortical spindle generators shift with respect to their physiological location [18].

The spindle activity may also be an important biomarker for studying Post Traumatic Stress Disorder (PTSD) and Major depression disorder (MDD) pathophysiology. Sleep spindle density, spindle peak frequency and maximum amplitude and cortical generators distribution seem to vary in subjects suffering from PTSD and / or MDD [15], [20].

EEG source analysis on COVID-19 survivors brain patterns revealed changes in the position of spindle cortical generators compared to the physiological one. Slow and fast spindle sources shifted to more posterior and anterior regions, respectively. This evidence seems to be partially

in accordance with sleep spindle generator modifications reported in patients affected by MDD and PTSD [41].
Spindle properties comparison highlighted differences between healthy controls and discharged patients (ICU and noICU). The increase of fast spindle peak frequency and the decrease of slow and fast spindle densities in COVID-19 survivors are in line with higher depression scores and may be a consequence of it. In background studies investigating sleep spindle features in individuals showing depressive symptoms, the fast spindle frequency and the spindle density were found respectively higher [42] and lower [43] in MDD patients with respect to healthy control subjects.
The correlation analysis results are in line with what we have found so far. Pearson Correlation outcomes revealed a significant relationship between psychological and cognitive test scores in COVID-19 survivors (both ICU and noICU) and between psychological performance and spindle features in the subjects discharged from the sub-intensive ward (noICU). From Multiple linear regression emerged the predictive potential of psychological test outcomes of the negative performance in attentive and memory tasks and alterations in spindle parameters.

Twelve months after discharge, the Covid-19 survivors exhibited executive and cognitive functions' impairments, perceived a worsening of their life quality, and showed depressive and PTSD symptoms. These are just some of the long-term manifestations that can arise after SARS-CoV-2 infection and last for weeks or even months. This phenomenon goes by the name of long-term COVID, which identifies the persistence of at least one symptom for more than twelve weeks after recovery.
There aren't many long-term follow up datasets, but all suggest that the clinical picture of long-COVID is more complex than the one described so far and that the long-term symptoms may persist for much longer than the time interval specified in the definition.

The nature of the memory and attentive function impairments is not yet clear.
On the one hand, there is strong evidence about the correlation between psychological disorders and the manifestation of neurocognitive deficits. PTSD can alter the neurocognitive functioning in several specific domains: the processing speed, the cognitive flexibility, the controlled and sustained attention are strictly linked to the psychological disease severity. This interconnection is also demonstrated by the convenient use of the measures of neurocognitive functioning, alongside demographic information, and clinical symptoms, to predict PTSD status months after onset [36].

Cognitive and functional impairment can also emerge in individuals suffering from Major depressive disorder (MDD). Processing speed, verbal learning and working memory appear to be the most damaged domains. There is strong evidence that MDD affects cognitive and executive functions both in individuals with a single or recurrent episodes [37].
On the other hand, there is growing evidence that the nervous system is frequently compromised in hospitalized patients with COVID-19.
Further research needs to be conducted to define whether the bad performances of patients recovered from COVID-19 in the tasks involving executive and cognitive functions are due to the harmful action of the virus on the CNS during the acute phase of the disease or if they are a consequence of the stress, anxiety and depression caused by the traumatic experience lived.

To sum up, both the neurological sleep signatures and the evident dependence of memory and attentive test performances from psychological scores suggest a more likely psychoaffective nature of the cognitive and executive functions' impairments. Depressive and Post Traumatic Stress Disorder symptoms experienced by COVID-19 survivors seem to have an impact on their cognitive performance and to be the origin underlying the sleep spindle feature alterations.

All the outcomes resulting from the neurocognitive tests, spindle and correlation analysis have differentiated the noICU subjects not only from the controls but also from the ICU patients.
Curiously, PTSD and depressive symptoms appear to be more marked in the subjects recovered in the sub-intensive ward than those admitted to the intensive care units and, in line with our hypothesis, the noICU group performed worse in the executive and cognitive tests and their sleep spindle alterations are more evident. The results emerging from the correlation analysis also confirm this trend: noICU cognitive / executive and psychological test scores appear to be strictly correlated and only in this subpopulation a correlation between the psychological performance and the spindle features was found.
Why did the noICU participants appear to have experienced higher levels of anxiety and stress? Severe COVID-19 illness is considered a risk factor in developing PTSD. Survivors of past coronavirus diseases, such as Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS), were followed up months or even years after their recovery and high PTSD rates emerged in hospitalized patients. A recent study, including 381 COVID-19 survivors with the most severe forms of the disease, found that 30.2% of the patients experienced PTSD symptoms in the weeks and months following hospital discharge [44]. Hence, we are not surprised by the recurrence of PTSD manifestations in COVID-19 survivors, but rather by their prevalence in the noICU population. Usually, cognitive disabilities

frequently occur in patients discharged from intensive care units rather than in those treated in medical wards. Commonly, intensive care survivors show impairments in cognition, psychological health, and physical function which may persist for months and years and are collectively called Post-intensive care syndrome (PICS) [45], [46].

Most likely, the reason for the clearer PTSD and depressive symptoms and for the worse perceived quality of life in noICU lies in the fact that during non-invasive high flow ventilation the patients remain conscious and receptive to external events. The critical health state of their bed neighbors, the lack of any human contact for weeks or even months, the terror in the eyes of healthcare workers and the chaotic and uncontrollable situation in the hospital are just some of those factors that have traumatized the patients, especially those who remained conscious during their hospital stay.

5.1 Limitations

A major limitation of our study is the lack of inclusion of patients showing more severe symptoms twelve months after the infection. By involving patients with symptoms of different magnitudes, from subclinical cognitive impairment to more critical neurological symptoms, we could arrive at different conclusions from those that emerged by examining our cohort of subjects.

The limited number of subjects recruited is another factor that may have biased the project results. Many patients recovered from SARS-CoV-2 infection refused to participate because they were afraid of being infected again by undergoing our experimental protocol or simply leaving the house. This evidence may indirectly reflect the anxiety and stress they still feel months after their recovery, and, therefore, it is in line with the participants' psychological performance.

Furthermore, it would have been useful to collect further information about the social and economic conditions of COVID-19 survivors. The worsened quality of life and depressive symptoms that emerged from psychological tests may be partially reconducted to a cause different from the COVID-19 due traumatic experience, such as economic difficulties or family troubles.

5.2 Conclusion

Our results show altered neurological sleep signatures, cognitive and executive function impairments, and depressive and PTSD symptoms in COVID-19 survivors, especially in participants admitted to medical wards, twelve months after their discharge. According to the results generated from the spindle and correlation analysis, we suppose that the anxiety and

stress experienced by hospitalized patients have an impact on both memory and attentive abilities and sleep spindle features. These findings may suggest that psychological disorders such as PTSD and depression may affect various domains of brain function and should not be underestimated.

Certainly, increasing the number of recruited individuals and including subjects with more severe long-term symptoms may be the following steps to strengthen our hypothesis or to gain new awareness in the long-term COVID-19 field. The use of fMRI, PET or other neuroimaging techniques could be a further helpful strategy to consolidate and improve our knowledge about the long-term neurological effects caused by COVID-19.

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