Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Co-ultramicronized palmitoylethanolamide/luteolin normalizes GABA<sub>B</sub>-ergic activity and cortical plasticity in long COVID-19 syndrome

Viviana Versace<sup>a,⇑</sup>, Paola Ortellia<sup>1</sup>, Sabrina Dezi<sup>a</sup>, Davide Ferrazzoli<sup>a</sup>, Alessia Alibardia<sup>a</sup>, Ilenia Boninia<sup>a</sup>, Michael Englb<sup>b</sup>, Roberto Maestric<sup>c</sup>, Martina Assogna<sup>d</sup>, Valentina Ajello<sup>e</sup>, Elke Pucks-Faesf<sup>f</sup>, Leopold Saltuaria<sup>f</sup>, Luca Sebastianellia<sup>g</sup>, Markus Kofler<sup>f</sup>, Giacomo Koch<sup>g</sup><sup>⇑</sup><sup>2</sup>

<sup>a</sup>Department of Neurorehabilitation, Hospital of Vipiteno (SABES-ASDAA), Vipiteno-Sterzing, Lehrkrankenhaus der Paracelsus Medizinischen Privatuniversität, Italy
<sup>b</sup>Department of Biomedical Engineering, Scientific Institute of Montescano - IRCCS, Istituti Clinici Scientifici Maugeri, Pavia, Italy
<sup>c</sup>Department of Neurology, Hochzirl Hospital, Zirl, Austria
<sup>d</sup>Experimental Neuropsychophysiology Lab, Santa Lucia Foundation IRCCS, Rome, Italy
<sup>e</sup>Department of Neurology, Tor Vergata University Hospital, Rome, Italy
<sup>f</sup>Department of Neurorehabilitation, Hospital of Vipiteno (SABES-ASDAA), Vipiteno-Sterzing, Lehrkrankenhaus der Paracelsus Medizinischen Privatuniversität, Italy
<sup>g</sup>Department of Neuroscience and Rehabilitation, University of Ferrara, Ferrara, Italy

**Highlights**
- Long Covid patients with fatigue and cognitive problems show impairment of cortical GABA<sub>B</sub> activity and reduced plasticity in primary motor cortex.
- Co-ultramicronized palmitoylethanolamide with luteolin (PEA-LUT) 700 + 70 mg bid for 8 weeks restores GABA<sub>B</sub> neurotransmission and cortical plasticity.
- PEA-LUT is a candidate for the treatment of long Covid patients.

**Article info**
Article history:
Accepted 31 October 2022
Available online 11 November 2022

**Keywords:**
Palmitoylethanolamide
Long Covid
Transcranial magnetic stimulation
Long-interval intracortical inhibition
LTP-like cortical plasticity

**Abstract**
Objective: Transcranial magnetic stimulation (TMS) studies showed that patients with cognitive dysfunction and fatigue after COVID-19 exhibit impaired cortical GABA<sub>B</sub>-ergic activity, as revealed by reduced long-interval intracortical inhibition (LICI).

Aim of this study was to test the effects of co-ultramicronized palmitoylethanolamide/luteolin (PEA-LUT), an endocannabinoid-like mediator able to enhance GABA-ergic transmission and to reduce neuroinflammation, on LICI.

Methods: Thirty-nine patients (26 females, mean age 49.9 ± 11.4 years, mean time from infection 296.7 ± 112.3 days) suffering from persistent cognitive difficulties and fatigue after mild COVID-19 were randomly assigned to receive either PEA-LUT 700 mg + 70 mg or PLACEBO, administered orally bid for eight weeks. The day before (PRE) and at the end of the treatment (POST), they underwent TMS protocols to assess LICI. We further evaluate short-latency afferent inhibition (SAI) and long-term potentiation (LTP)-like cortical plasticity.

Results: Patients treated with PEA-LUT but not with PLACEBO showed a significant increase of LICI and LTP-like cortical plasticity. SAI remained unaffected.

Conclusions: Eight weeks of treatment with PEA-LUT restore GABA<sub>B</sub> activity and cortical plasticity in long Covid patients.

**Abbreviations:** TMS, transcranial magnetic stimulation; GABA, gamma-aminobutyric acid; PEA, palmitoylethanolamide; LUT, luteolin; PEA-LUT, palmitoylethanolamide co-ultramicronized with luteolin; UCI, long-interval intracortical inhibition; bid, bis in die; SAI, short-latency afferent inhibition; LTP, long-term potentiation; 2-AG, 2-arachidonoylglycerol; PCR, polymerase chain reaction; FSS, Fatigue Severity Scale; PCDS, Perceived Cognitive Difficulties Scale; MoCA, Montreal Cognitive Assessment; FAB, Frontal Assessment Battery; MEP, motor evoked potential; FDI, first dorsal interosseous; RMT, resting motor threshold; iTBS, intermittent theta burst stimulation.

* Corresponding author at: Department of Neurorehabilitation, Hospital of Vipiteno (SABES-ASDAA), Vipiteno-Sterzing, Lehrkrankenhaus der Paracelsus Medizinischen Privatuniversität, Margarethenstrasse 24, 39049 Vipiteno-Sterzing (BZ), Italy.

E-mail address: viviana.versace@sabes.it (V. Versace).

1 co-first authors.
2 co-senior authors.

https://doi.org/10.1016/j.clinph.2022.10.017
1388-2457/© 2022 International Federation of Clinical Neurophysiology. Published by Elsevier B.V. All rights reserved.
1. Introduction

Approximately 30% of individuals affected by COVID-19 including asymptomatic cases (Tenforde et al., 2020), and approximately 80% of hospitalized patients (Huang et al., 2021) may experience post-COVID sequelae including fatigue and cognitive impairment, along with other ongoing neuropsychiatric (e.g. depression) (Renaud-Charest et al., 2021) and physical (e.g. dyspnea) manifestations.

Symptoms may persist following the acute illness or may first appear after recovery from the initial disease and may fluctuate or relapse over time (Soriano et al., 2022).

Persons struggling with the effects of the so-called “post-acute COVID-19 syndrome” or “long Covid” may have noticeable troubles with attention, memory, and executive function, with a high impact on quality of life (Nalbandian et al., 2021). Cognitive symptoms are likely due to the dysfunction of frontotriastal and/or frontoparietal brain networks. An MRI study showed a reduction in grey matter thickness in the orbitofrontal cortex and parahippocampal gyrus in a large sample of patients presenting cognitive decline after mild COVID-19 (Douaud et al., 2022). 18FDG-PET/CT studies revealed hypometabolism of frontonal regions, including the olfactory gyrus (Guedj et al., 2021; Sollini et al., 2021). The neurophysiological alterations at cortical level are still partially obscure.

We have recently demonstrated by using paired-pulse transcranial magnetic stimulation (TMS) protocols in patients who developed fatigue and dysexecutive syndrome after severe COVID-19 a remarkable impairment of intracortical GABAergic activity, as shown by altered long-interval intracortical inhibition (LICI) (Ortelli et al., 2022; Versace et al., 2021). Subsequently, we were able to demonstrate similar alterations in patients with persistent fatigue and cognitive complaints after mild symptomatic COVID-19 (Ortelli et al., 2022).

Hypothesized mechanisms for such neurological alterations include direct viral damage, microvascular injury, persistent immune activation, and especially misguided host immunologic responses (Apple et al., 2022) leading to persistent neuroinflammation. Several animal and clinical studies have previously highlighted the therapeutic potential of ultramicronized palmitoylethanolamide (PEA) in various neurological diseases (Andresen et al., 2016; Beggiato et al., 2019; Caltagirone et al., 2016; Cordaro et al., 2020; Lunardelli et al., 2019; Onesti et al., 2019; Palma et al., 2016; Petrosino and Di Marzo, 2017). Interestingly, ultramicronized PEA reduced inflammatory, oxidative and coagulative alterations in the acute stage of COVID-19 (Albanese et al., 2022) and improved COVID-19-related olfactory dysfunction (Di Stadio et al., 2022). Moreover, a recently recognized PEA function is the enhancement of GABA neurotransmission through modulation of the release of the endocannabinoid 2-arachidonoylglycerol (2-AG) (Musella et al., 2017).

Hence, we conducted a double-blind, placebo-controlled, randomized clinical trial (RCT) to investigate the effects of an 8-week oral therapy cycle with palmitoylethanolamide (PEA) co-ultramiconized with the flavonoid luteolin (PEA-LUT) in patients with cognitive complaints and fatigue after mild COVID-19. We hypothesized that PEA-LUT could restore intracortical GABAergic neurotransmission measured by LICI.

2. Methods

2.1. Participants

The study was conducted at the ‘long Covid’ outpatient clinic of the Department of Neurorehabilitation (Hospital of Vipiteno, SABES-ASDAA) between September 2021 and March 2022.

Inclusion criteria were (a) previous diagnosis of SARS-CoV-2 infection confirmed through detection of virus RNA by polymerase chain reaction (PCR) testing of a nasopharyngeal swab; (b) subsequent recovery from infection as defined by two consecutive negative PCR tests separated by at least one day; (c) mild form of COVID-19 (symptoms might include fever, cough, sore throat, malaise, myalgia, anorexia, nausuea, diarrhea, anosmia and ageusia) without necessitating hospital admission; (d) complaints of sense of fatigue and/or cognitive difficulties persisting after SARS-CoV-2 infection documented through following self-administered questionnaires: Fatigue Severity Scale (FSS) and Perceived Cognitive Difficulties Scale (PCDS). FSS is a self-administered 9-item questionnaire that investigates the severity of fatigue in different situations during the previous week and ranks perceived severity on a 7-point Likert scale (1 = “strongly disagree”; 7 = “strongly agree”); FSS sum score ranges from 7 to 63, the cut-off for pathology and for inclusion in the study was FSS > 36) (Krupp et al., 1989). The PCDS scale assesses perceived cognitive difficulties (referring to one or more of the following: forgetfulness, cloudiness, difficulty in focusing, thinking and communicating) on a 4-point Likert-scale: 0 = “I have no cognitive difficulties”; 1 = “I have slightly more cognitive difficulties than before COVID”; 2 = “I have moderate cognitive difficulties most of the time; 3 = “I have persistent cognitive difficulties” (Ortelli et al., 2022). PCDS score ≥ 1 were considered for inclusion in the study.

No restrictions were considered regarding the interval between disease onset and study participation.

Exclusion criteria were (a) prior or concurrent diagnosis of neurological, psychiatric, endocrine, metabolic or cardiopulmonary conditions; (b) clinical and/or radiological evidence of COVID-19 related pneumonia during the active phase of the disease; (c) anemia; (d) pharmacological treatment with corticosteroids, antihistamines, antihypertensives, diuretics, antidepressants, anxiolytic or hypnotic drugs during the time of study.

Thirty-nine patients (mean age 49.9 ± 11.4 years, 26 females, mean education 13.4 ± 2.9 years, mean time from onset 296.7 ± 123.3 days) fulfilling the inclusion criteria were enrolled. Thirty-four patients were studied (five patients withdrew from the study after pre-intervention assessment). Their demographic and clinical characteristics are shown in Table 1. All patients were right-handed.

2.2. Study design

This randomized controlled trial (RCT) investigated the neurophysiological and cognitive impact of PEA-LUT administration in patients complaining of cognitive difficulties and fatigue after mild COVID-19 (henceforth “long Covid patients”).

The study was registered with clinicaltrials.gov (NCT05311852) on April 5, 2022 with "Actual Study Start Date" August 16, 2021 and “Actual Study Completion Date” March 15, 2022.
Patients were assigned to one of two study groups (n = 17 each), receiving either PEA-LUT (Glialia®, 700 mg + 70 mg, sublingual microgranule formulation, Epitech Group SpA, Saccolongo, Italy) or PLACEBO (sublingual inert microgranules), administered orally bid for eight weeks. Glialia® is licensed in Italy as an oral food product for special medical purposes, with anti-inflammatory and neuroprotective properties.

Group allocation was centralized and occurred in a pseudo-randomized manner taking into account a balance of age, gender, education, and duration of illness (Table 1).

All participants underwent neurophysiological and neuropsychological assessment the day before beginning treatment (pretreatment evaluation, PRE) and at the end of eight weeks PEA-LUT or PLACEBO administration (post-treatment evaluation, POST).

The study was approved by the local ethics committee (Comitato Etico dell’Azienda Sanitaria dell’Alto Adige, n. 99-2021) and was in accordance with the code of ethics of the World Medical Association. Written informed consent was obtained from all participants for the use of their clinical data for scientific purposes.

Primary outcome measure for PEA-LUT effects was the intracortical GABAergic neurotransmission indexed with LICI. Secondary neurophysiological outcome measures were short-lateness afferent inhibition (SAI) and long-term potentiation (LTP)–like cortical plasticity. As further exploratory outcomes, we also searched for modification in cognitive performance.

### 2.3. Neurophysiological and cognitive assessment

We recorded motor evoked potentials (MEPs) from first dorsal interosseous (FDI) muscle of the dominant side. TMS of the dominant primary motor cortex (M1) was performed with a high-power Magstim 200 (Magstim Co., Whitland, UK), through a 7 cm figure-of-eight coil, held over the optimum scalp position to elicit maximal MEPs for a given intensity in contralateral FDI, with a posterior-to-anterior current flow (Rossini et al., 1994; Rossini et al., 2015). Stimulation intensities were expressed as a percentage of maximum stimulator output (%) MSO. Surface electromyography signals were band-pass filtered (3–3000 Hz) and amplified with a Digitimer D440-4 amplifier (Digitimer Ltd., Welwyn Garden City, UK). Single sweeps were digitized (sampling rate 10 kHz) and recorded on a computer for post-hoc analysis using a CED 1401 A/D converter and Signal 6 software (Cambridge Electronic Design, Cambridge, UK). Resting motor threshold (RMT) was defined as previously described (Rossini et al., 2015).

Paired-pulse TMS was used to investigate LICI at interstimulus interval (ISI) 100 ms with a stimulation intensity of 130% RMT for both conditioning and test stimulus (Valls-Solé et al., 1992). The chosen ISI was the most effective in highlighting altered LICI in previous studies on long Covid patients (Ortelli et al., 2022; Versace et al., 2021). LICI is considered to be a phenomenon dependent on slow inhibitory postsynaptic potentials mediated through GABA<sub>A</sub>-receptors (Ziemann et al., 2015).

SAI was used to evaluate M1 inhibition induced by sensory afferents. SAI is a marker of inhibitory sensorimotor integration that depends mainly on the excitatory effect of cholinergic thalamo-cortical projections onto the inhibitory GABAergic cortical network (Tokimura et al., 2000). The conditioning stimulus was delivered to the ulnar nerve at the wrist (at an intensity just above the motor threshold for evoking a visible twitch in FDI) and preceded the TMS by an ISI corresponding to the latency of the N20 component of the ulnar nerve somatosensory evoked potential (Di Lazzaro et al., 2007). The intensity of the TMS test pulse over M1 was adjusted to elicit stable MEPs of approximately 1 mV peak-to-peak amplitude in the relaxed FDI.

For both LICI and SAI, twenty stimuli were delivered both to elicit test and conditioned MEPs in a pseudo-randomized sequence.
SAS/STAT statistical package, release 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

A total of 34 post COVID-19 patients completed the evaluation sessions. Demographic and clinical data are depicted in Table 1. An equal proportion of the female population (64.7%) was present in both groups. Patients did not differ significantly with respect to age, education, or time since onset of disease, FSS and PCDS. PEA-LUT and PLACEBO treatment was well-tolerated by all patients, and no side effects were reported.

3.1. Neurophysiological and cognitive assessment

All neurophysiological findings with repeated measures ANOVA results are reported in Table 2 (SAS/STAT statistical package, release 9.4 (SAS Institute Inc., Cary, NC, USA)).

In the LICI protocol performed at PRE, mean test MEP amplitude for the PEA-LUT group and for the PLACEBO group were 0.93 ± 0.42 mV and 0.86 ± 0.43 mV, respectively.

In the LICI protocol performed at PRE, mean test MEP amplitude for the PEA-LUT group and for the PLACEBO group were 0.90 ± 0.43 and 0.82 ± 0.43, respectively.

A significant interaction (treatment × time) was observed in the percent change of conditioned MEP amplitude in the LICI test. This finding indicates a different trend of this variable in PEA-LUT patients as compared to patients in the PLACEBO group. Indeed, post-hoc analysis revealed a significant increase in the amount of inhibition of the conditioned MEP from PRE to POST in the PEA-LUT group (P = 0.009) but not in the PLACEBO group (P = 0.72).

In the SAI protocol performed at PRE, mean test MEP amplitude for the PEA-LUT group and for the PLACEBO group were 0.98 ± 0.36 mV and 0.84 ± 0.35 mV, respectively.

In the SAI protocol performed at POST, mean test MEP amplitude for the PEA-LUT group and for the PLACEBO group were 1.01 ± 0.32 mV and 0.81 ± 0.40 mV, respectively.

No significant treatment or time effect and no interaction were found, indicating no differences in this variable in PEA-LUT and PLACEBO patients.

Repeated measures ANOVA for MEP amplitude modulation in the LTP-like cortical plasticity test revealed a significant interaction (treatment × time) 1 and 10 minutes following iTBS. Post-hoc testing revealed a significant increase of the MEP amplitude from PRE to POST in the PEA-LUT group but not in the PLACEBO group (P = 0.0009 and P = 0.01, for 1 and 10 minutes, respectively, in the PEA-LUT group; P = 0.38 and P = 0.56 for 1 and 10 minutes, respectively, in the PLACEBO group).

Results of cognitive tests are shown in Table 2. No significant interaction (treatment × time) was found in MoCA and FAB cognitive screening tests.

4. Discussion

In the present RCT oral PEA-LUT 700 + 70 mg administered bid for eight weeks increased the GABAergic activity of M1 measured with the LICI protocol in patients complaining of long-term fatigue and cognitive difficulties after mild COVID-19 (long Covid patients). In parallel, we also observed an improvement of LTP-like cortical plasticity.

The pathogenesis of long Covid has not yet been elucidated; potential contributors include persistent consequences of SARS-CoV-2 interactions with host microbiome/virome, clotting/coagulation issues, dysfunctional brainstem/vagus nerve signaling, neuro-inflammation, ongoing activity of primed immune cells, autoimmunity, dysregulation of the renin–angiotensin–aldoster one system, and endothelial cell damage (Nalbandian et al., 2021; Proal and VanElzakker, 2021).

Animal and organoid model studies have shown that SARS-CoV-2 is able to reach and infect cells of the central nervous system (CNS) and to produce neuro-inflammation (Song et al., 2020; Song et al., 2021).

In severe COVID-19 patients, encephalopathy is associated with systemic hyper-inflammation mainly provoked by an aberrantly excessive innate immune response (Gustine and Jones, 2021). Entry of pro-inflammatory cytokines (mostly IL-1β, IL-6, TNFα, and IL-17) into the CNS via disrupted blood–brain barrier may alter glial cell function, leading to microglial activation and proliferation (Najar et al., 2020). Similar pathophysiological mechanisms could also underlie long-term neurological symptoms after mild COVID-19 (Phetsouphanh et al., 2022).

PEA is a saturated N-acyl ethanolamide belonging to the family of endocannabinoids, naturally produced in the body, and largely found in several food sources which can exert anti-inflammatory and neuroprotective effects (Petrosino and Di Marzo, 2017).

In recent years, several experimental pre-clinical and clinical studies have indicated that ultramicronized PEA (a formulation

Table 2

| TEST | PEA-LUT | PLACEBO | ANOVA interactiontime × treatment | F-value | P-value |
|------|---------|---------|----------------------------------|---------|---------|
| Neurophysiological results | | | | | |
| Intracortical circuits | | | | | |
| LICI (% of test amplitude) | 66.6 (6.1) | 32.4 (5.8) | 66.8 (9.2) | 71.2 (15.8) | 4.9216 | 0.034 |
| SAI (% of test amplitude) | 70.9 (7.8) | 65.8 (6.0) | 66.1 (5.5) | 70.7 (6.2) | 0.0990 | 0.41 |
| LTP-like synaptic plasticity | | | | | |
| MEP amplitude (mV) - T2 | 0.85 (0.08) | 0.99 (0.10) | 0.91 (0.12) | 0.79 (0.11) | 2.9595 | 0.10 |
| MEP amplitude (mV) - T1 | 0.89 (0.08) | 1.30 (0.10) | 0.94 (0.12) | 0.84 (0.13) | 10.2947 | 0.003 |
| MEP amplitude (mV) - T10 | 0.79 (0.09) | 1.11 (0.06) | 0.87 (0.14) | 0.80 (0.14) | 5.5227 | 0.025 |
| MEP amplitude (mV) - T20 | 0.79 (0.09) | 0.87 (0.06) | 0.93 (0.17) | 0.78 (0.16) | 1.7757 | 0.19 |
| Neuropsychological evaluation - Behavioral and cognitive screening | | | | | |
| MoCA | 24.2 (3.2) | 25.9 (2.9) | 25.1 (2.8) | 26.1 (2.6) | 0.5222 | 0.48 |
| FAB | 15.9 (1.5) | 16.5 (1.3) | 16.5 (1.3) | 17.4 (0.9) | 0.978 | 0.330 |

PEA-LUT, co-ultramicronized palmitoylethanolamide/luteolin; LICI, long-interval intracortical inhibition; SAI, short-latency afferent inhibition; LTP, long-term potentiation; MEP, motor evoked potential; MoCA, Montreal Cognitive Assessment score; FAB, Frontal Assessment Battery.
Based on this evidence, we decided to investigate the impact of the oral administration of PEA-LUT on cortical GABA_ergic activity of long Covid patients with fatigue and cognitive difficulties. In fact, we have already demonstrated an impairment of GABA_ergic neurotransmission within M1, indexed by LICI, and to a lesser extent, of central cholinergic circuits, assessed by SAI, after both severe (Versace et al., 2021) and mild COVID-19 (Ortelli et al., 2022).

LICI is a well-known marker of GABA_B mediated intracortical inhibition within M1 (Ziemann et al., 2015). As demonstrated in studies of LICI, GABA_B-mediated inhibition is altered in various neuropsychiatric conditions such as psychotic mood disorders, epilepsy, Parkinson’s disease, traumatic brain injury, and dementia (Fatih et al., 2021). GABAergic interneurons, especially those expressing the Ca^{2+}-binding protein parvalbumin, inhibit M1 pyramidal cells through a negative feedback system (Sohal et al., 2009) and play a fundamental role in almost all neuronal coding and processing in the CNS.

Different cognitive abilities, mainly executive functions, are sensitive to cerebral GABA concentrations in the frontal cortex (Porges et al., 2017; Sumner et al., 2010). In particular, reduced LICI is now a recognized biomarker of fronto-temporal dementia (FTD) (Benussi et al., 2020) where it correlates with executive function deficit.

Interestingly, a 4-week treatment with PEA-LUT induced an improvement in frontal cognitive functions and a restoration of LICI in FTD patients (Assogna et al., 2020). Long Covid shares with FTD the impairment of executive functions (although to a different extent) and a comparable reduction of LICI (Benussi et al., 2020; Ortelli et al., 2022).

Moreover, degeneration of intracortical inhibitory GABAergic circuits within M1 has been reported in various affections of the central nervous system inducing fatigue (Lieber et al., 2005; Ridding et al., 1995; Vucin et al., 2011). In light of these studies, a downregulation of GABA activity and the consequent enhancement of cortical excitability could be seen as a compensatory mechanism for overcoming premature motor fatigue. On the other hand, one cannot exclude the possibility that cortical disinhibition is the cause of fatigue itself, as a system with upregulated excitability may have a lower range to further increase excitability.

SAI reflects M1 inhibition induced by sensory afferents and depends on the excitatory effect of cholinergic thalamocortical projections on inhibitory GABAergic cortical networks (Tokimura et al., 2000). Consolidated evidence points to a disrupted SAI mechanism in both Alzheimer’s and Lewy-body disease patients, where SAI correlate with memory function (Di Lazzaro et al., 2002; Di Lorenzo et al., 2013; Nardone et al., 2006).

Important indications on the relevance of LICI and SAI paradigms to investigate neurophysiological processes in the human cortex as well as their relationship to pathology come from TMS-electroencephalography (EEG) measurements. GABA_B-mediated inhibition of cortical activity in M1 and dorsolateral prefrontal cortex (DLPFC) can be obtained with LICI protocols (Daskalakis et al., 2008; Farzan et al., 2010; Premoli et al., 2014). Prefrontal LICI deficits are specific to patients with schizophrenia and other neuropsychiatric disorders (Tremblay et al., 2019). The attenuation of cortical excitability induced by SAI protocols identifies cholinergic changes in M1 and DLPFC and correlates with executive functions (Bikmullina et al., 2009; Noda et al., 2017).

We tested LICI at the ISI of 100 ms, which usually yields maximum MEP inhibition (Valls-Solé et al., 1992) and which was effective in highlighting altered LICI in long Covid patients (Ortelli et al., 2022; Versace et al., 2021). We found markedly reduced LICI in a similar range to our previous studies, in which we compared long Covid patients to a control population matched for age, sex and...
education (Ortelli et al., 2022; Versace et al., 2021). LICI increased significantly (i.e., percentage ratio of mean conditioned to mean test MEP amplitude decreased) after intervention in the PEA-LUT group, but not in the PLACEBO group.

We assessed SAI at the most effective ISI between cortical and peripheral stimulation, i.e., coinciding with the cortical somatosensory evoked potential component N20 following ulnar nerve stimulation at the wrist (Di Lazzaro et al., 2007), and found reduced SAI in line with our previous observations (Ortelli et al., 2022; Versace et al., 2021). Unlike LICI, SAI was not significantly improved by PEA-LUT therapy in the present study.

Furthermore, we investigated LTP-like cortical plasticity in M1 with the technique of iTBS given as iTBS, i.e., a stimulation with high-frequency bursts (5 Hz) at theta frequencies (50 Hz) which is able to induce homotopic plasticity (Huang et al., 2005). In iTBS studies indeed, cortical LTP-like plasticity is evidenced by the transient increase in MEP amplitude outlasting repetitive brain stimulation by seconds or minutes and reflecting activity-dependent changes in the effectiveness of synaptic transmission (Ziemann et al., 2008).

In the studied cohort of long Covid patients, iTBS failed to induce the expected potentiation of MEP amplitudes, thus indicating altered LTP-like cortical plasticity. The transient physiological MEP facilitation, however, was restored in the post-treatment evaluation only in the PEA-LUT group.

The pathophysiological cascade in patients with severe COVID-19 is related to over-stimulation of T cells and macrophages with a subsequent release of an enormous quantity of pro-inflammatory cytokines such as interleukins and chemokines that can result in multi-organ dysfunction (Yuki et al., 2020). Moreover, GABAergic neurons have a higher expression of ACE2 receptors (Chen et al., 2020; Mukerjee et al., 2019). If SARS-CoV-2 enters the brain it has the potential to access GABAergic neurons, leading to functional impairment until apoptosis and causing excitatory-inhibitory imbalance (Rami et al., 2020). Cytokine release from infected neurons and other activated microglia and astrocytes may also cause a decrease in GABA (Galic et al., 2012). GABAergic transmission is also impaired in hypoxic conditions (Oliviero et al., 2002), as also seen in severe COVID-19 patients with pneumonia (Versace et al., 2021).

The different pathways mentioned above through which PEA-LUT is able to enhance GABAergic transmission in the central nervous system may explain its effect on the GABAergic (LICI) circuits found in the present study.

The molecular mechanism by which PEA-LUT improves LTP-like cortical plasticity is very likely related to the involvement of the cannabinoid system. PEA as an endocannabinoid anandamide congener, is known to modulate glutamatergic transmission mainly through cannabinoid CB1 receptor and the transient receptor potential vanilloid 1 and to restore LTP mechanisms (al-Ghoul et al., 1993; Basavarajappa et al., 2014; Boccella et al., 2019; Guida et al., 2015; Lutz et al., 2015; Zimmermann et al., 2019). Hippocampal PEA modulates reward memory in mesolimbic areas through GPR55 receptors with the implication of glutamatergic projections emerging from ventral hippocampus (Kramar et al., 2017). Endocannabinoid signaling via anandamide or PEA is implicated in several neuronal functions and considered a potential therapeutic target for disorders associated with altered plasticity (Maccarrone, 2017; Zimmermann et al., 2019).

While we found that PEA-LUT had a beneficial impact on altered motor cortex physiology, we did not observe significant changes in the chosen cognitive measures.

MoCA and FAB, exploring global cognition and executive function respectively, exhibited a ceiling effect (Table 2) i.e. insufficient sensitivity for patients’ cognitive disturbances, thus preventing the possibility of observing cognitive improvement after treatment.

Future RCTs on selected groups of patients with more pronounced cognitive impairment or using more sensitive cognitive / behavioural outcome measures will be better able to assess the clinical impact of this or other treatments. Furthermore, studies with a longer observation period could evaluate the duration of the treatment effect.

In conclusion, the present RCT demonstrates that PEA-LUT is able to enhance GABAergic neurotransmission and LTP-like cortical plasticity in long Covid patients.

The mechanisms of action with which PEA-LUT exerted these effects are not deducible only from the current results and can be hypothesized on the basis of previous evidence, possibly depending on the reduction of central neuroinflammation or on the direct modulation of GABAergic and glutamatergic activity.
Conflict of Interest
The authors have no conflicts of interest to declare.

Data Availability Statement
The data that support the finding of this study are available upon request from the corresponding author.

Acknowledgements
The authors thank the pharmaceutical company Epitech Group SpA, Saccolongo, Italy, for the free supply of the active and placebo product.

The authors also thank Annelies Gruber for her excellent administrative support and Ellen Quirbach for language editing.

Funding Statement
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References
al-Ghoul WM, Li Volgi G, Weinberg RJ, Rustioni A. Glutamate immunocytochemistry in the dorsal horn after injury or stimulation of the sciatic nerve of rats. Brain Res Bull 1993;30(3–4):453–9.
Albanese M, Marrone G, Paulino A, Di Lauro M, Di Daniele F, Chiaramonte C, et al. Effects of Ultramircronized Palmitoylethanolamidole (um-PEA) in COVID-19 Early Stages: A Case-Control Study. Pharmaceuticals (Basel) 2022;15(2).
Andersen SR, Bing J, Hansen BM, Birnsic-Sorensen F, Johansens IL, Hagen EM, et al. Ulramicronized palmitoylethanolamide in spinal cord injury neuropathic pain: a randomized, double-blind, placebo-controlled trial. Pain 2016;157(9):2097–103.
Apple AC, Oddi A, Peluso MJ, Asken BM, Henrich TJ, Kelly JD, et al. Risk factors and abnormal cerebrospinal fluid associate with cognitive symptoms after mild COVID-19. Ann Clin Transl Neuro 2022;9(2):221–6.
Appollonio I, Leone M, Istella V, Piamarta F, Consoli T, Villa ML, et al. The Frontal Assessment Battery (FAB): normative values in an Italian population sample. Neurol Sci 2005;26(2):108–16.
Assogna M, Casula EP, Borghi I, Bonnì S, Samà D, Motta C, et al. Effects of Palmitoylethanolamide Combined with Luteolin on Frontal Lobe Functions, High Frequency Oscillations, and GABAergic Transmission in Patients with Frontotemporal Dementia. J Alzheimers Dis 2020;76(4):1297–308.
Assogna M, Di Lorenzo F, Martorana A, Koch G. Synaptic Effects of Palmitoylethanolamide in Neurodegenerative Disorders. Biomolecules 2022;12(8).
Basavarajappa BS, Nagre NN, Xie S, Subbanna S. Elevation of endogenous anandamide impairs LTP, learning, and memory through CB1 receptor signaling in mice. Hippocampus 2014;24(7):308–18.
Beggiato S, Tomasi MC, Ferraro L. Palmitoylethanolamidole (PEA) as a Potential Therapeutic Agent in Alzheimer's Disease. Front Pharmacol 2019;10:821.
Benussi A, Grassi M, Palluzzi F, Koch G, Di Lazzaro V, Nardone R, et al. Classification Accuracy of Transcranial Magnetic Stimulation for the Diagnosis of Neurodegenerative Dementias. Ann Neurol 2020;87(3):394–404.
Bikmullina R, Kicic D, Carlson S, Nikulin VV. Electrophysiological correlates of short-latency afferent inhibition: a combined EEG and TMS study. Exp Brain Res 2009;194(4):517–26.
Boccara S, Marabese I, Iannotta M, Belardo C, Neugebauer V, Mazzitelli M, et al. Metabotropic Glutamate Receptor 5 and B Modulate the Ameliorative Effect of Ultramricronized Palmitoylethanolamide on Cognitive Decline Associated with Neuropathic Pain. Int J Mol Sci 2019;20(7).
Caltagirone C, Cisari C, Schievan C, Di Paola R, Cordinaro M, Bruschetta G, et al. Ultramricronized Palmitoylethanolamide/Luteolin in the Treatment of Cerebral Ischemia: from Rodent to Man. Trans Stroke Res 2016;7(1):54–69.
Chen R, Wang K, Yu J, Howard D, French L, Chen Z, et al. The Spatial and Cell-Type Distribution of SARS-CoV-2 Receptor ACE2 in the Human and Mouse Brains. Front Neurol 2020;11:570395.
Cordinaro M, Cuzzocrea S, Crupi R. An Update of Palmitoylethanolamide and Luteolin Effects in Preclinical and Clinical Studies of Neuroinflammatory Events. Antioxidants (Basel) 2020;9(3).
Daskalakis ZJ, Farzan F, Barr MS, Maller JJ, Chen R, Fitzgerald PB. Long-interval cortical inhibition from the dorsolateral prefrontal cortex: A TMS-EEG study. Neuropsychopharmacology 2008;33(12):2869–5.
Di Lazzaro V, Olivierio A, Tonali PA, Marra C, Daniele A, Profice P, et al. Noninvasive in vivo assessment of cholinergic cortical circuits in AD using transcranial magnetic stimulation. Neurology 2002;59(3):392–7.
Palma E, Reyes-Ruiz JM, Lopergolo D, Roseti C, Bertolini C, Rufolo G, et al. Acetylcholine receptors from human muscle as pharmacological targets for ALS therapy. Proc Natl Acad Sci USA 2016;113(3):3060–5.

Petrosino S, Di Marzo V. The pharmacology of palmitoylethanolamide and first data on the therapeutic efficacy of some of its new formulations. Br J Pharmacol 2017;174(11):1349–65.

Pethsouphanh C, Darley DR, Wilson DR, Howe A, Munier CML, Patel SK, et al. Immunological dysfunction persists for 8 months following initial mild-to-moderate SARS-CoV-2 infection. Nat Immunol 2022;23(2):210–6.

Porges EC, Woods AJ, Edden RA, Puts NA, Chen H, et al. Frontal Gamma-Aminobutyric Acid Concentrations Are Associated With Cognitive Performance in Older Adults. Biol Psychiatry Cogn Neurosci Neuroimaging 2017;2(1):38–44.

Premoli I, Rivolta D, Eschenhahn S, Castellanos N, Belardinelli P, Ziemann U, et al. Characterization of GABAB-receptor mediated neurotransmission in the human cortex by paired-pulse TMS-EEG. Neuroimage 2014;103:152–62.

Proal AD, VanElzakker MB. Long COVID or Post-acute Sequelae of COVID-19 (PASC): An Overview of Biological Factors That May Contribute to Persistent Symptoms. Front Microbiol 2021;12:698169.

Ramani A, Müller L, Ostermann PN, Gabriel E, Abida-Islam P, Müller-Schiffmann A, et al. SARS-CoV-2 targets neurons of 3D human brain organoids. EMBO J 2020;39(20):e106230.

Renaud-Charest O, Lui LMW, Eskander S, Ceban F, Ho R, Di Vincenzo JD, et al. Onset and frequency of depression in post-COVID-19 syndrome: A systematic review. J Psychiatr Res 2021;144:129–37.

Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Di Iorio R, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: Basic principles and procedures for routine clinical application. Report of an IFCC committee. Electroencephalogr Clin Neurophysiol 1994;91(2):79–92.

Song E, Zhang C, Israelow B, Lu-Culligan A, Prado AV, Skriabine S, et al. Neuroinvasion of SARS-CoV-2 in human and mouse brain. bioRxiv 2020;2020.06.25.169946.

Song E, Zhang C, Israelow B, Lu-Culligan A, Prado AV, Skriabine S, et al. Neuroinvasion of SARS-CoV-2 in human and mouse brain. J Exp Med 2021;218(3).

Soriano JB, Marthy S, Marshall JC, Relan P, Diaz JV. A clinical case definition of post-COVID-19 condition by a Delphi consensus. Lancet Infect Dis 2022;22(4):e102–7.

Sumner P, Edden RA, Bompas A, Evans CJ, Singh KD. More GABA, less distraction: a neurochemical predictor of motor decision speed. Nat Neurosci 2010;13(7):825–7.

Tenforde MW, Kim SS, Lindsell CJ, Billig Rose E, Shapiro NI, Files DC, et al. Symptom Duration and Risk Factors for Delayed Return to Usual Health Among Outpatients with COVID-19 in a Multistate Health Care Systems Network – United States, March-June 2020. MMWR Morb Mortal Wkly Rep 2020;69(30):993–8.

Tokimura H, Di Lazzaro V, Tokimura Y, Oliviero A, Profice P, Insola A, et al. Short latency inhibition of human hand motor cortex by somatosensory input from the hand. J Physiol 2000;523 Pt 2(2):503–13.

Tremblay S, Rogasch NC, Premoli I, Blumberger DM, Casarotto S, Chen R, et al. Clinical utility and prospective of TMS-EEG. Clin Neurophysiol 2019;130(5):802–44.

Valls-Solé J, Pascual-Leone A, Wassermann EM, Hallett M. Human motor evoked responses to paired transcranial magnetic stimuli. Electroencephalogr Clin Neurophysiol 1992;85(6):355–64.

Versace V, Sebastianelli L, Ferrazzoli D, Romanello R, Ortelli P, Saltuari L, et al. Intracortical GABAergic dysfunction in patients with fatigue and dysexecutive syndrome after COVID-19. Clin Neurophysiol 2021;132(5):1138–43.

Vucic S, Cheah BC, Kiernan MC. Maladaptation of cortical circuits underlies fatigue and weakness in ALS. Amyotroph Lateral Scler 2011;12(6):414–20.

Xue X, Burns AM, Hallett M. Somatosensory-evoked potentials in patients with long-COVID. Eur J Neurol 2021;28(3):498–508.

Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 pathophysiology: A review. Clin Immunol 2020;215.108427.

Ziemia U, Paulus W, Nitsche MA, Berardelli A, et al. Consensus: Motor cortex plasticity protocols. Brain Stimul 2008;1(3):164–82.

Ziemia U, Reis J, Schwienke P, Rosanova M, Strafella A, Badawy R, et al. TMS and drugs revisited 2014. Clin Neurophysiol 2015;126(10):1847–68.

Zimmermann T, Bartisch JC, Beer A, Lomazzo E, Guggenhuber S, Lange MD, et al. Impaired anandamide/palmitoylethanolamide signaling in hippocampal glutamatergic neurons alters synaptic plasticity, learning, and emotional responses. Neuropsychopharmacology 2019;44(8):1377–88.