Bluetooth Communication Interface for EEG Signal Recording in Hyperbaric Chambers

Lucio Pastena, Emanuela Formaggio, Fabio Faralli, Massimo Melucci, Marco Rossi, Riccardo Gagliardi, Lucio Ricciardi, and Silvia F. Storti

Abstract-Recording biological signals inside a hyperbaric chamber poses technical challenges (the steel walls enclosing it greatly attenuate or completely block the signals as in a Faraday cage), practical (lengthy cables creating eddy currents), and safety (sparks hazard from power supply to the electronic apparatus inside the chamber) which can be overcome with new wireless technologies. In this technical report we present the design and implementation of a Bluetooth system for electroencephalographic (EEG) recording inside a hyperbaric chamber and describe the feasibility of EEG signal transmission outside the chamber. Differently from older systems, this technology allows the online recording of amplified signals, without interference from eddy currents. In an application of this technology, we measured EEG activity in professional divers under three experimental conditions in a hyperbaric chamber to determine how oxygen, assumed at a constant hyperbaric pressure of 2.8 ATA, affects the bioelectrical activity. The EEG spectral power estimated by fast Fourier transform and the cortical sources of the EEG rhythms estimated by low-resolution brain electromagnetic analysis were analyzed in three different EEG acquisitions: breathing air at sea level; breathing oxygen at a simulated depth of 18 msw, and breathing air at sea level after decompression.

Index Terms—Bluetooth, electroencephalogram (EEG), hyperbaric chamber, oxygen toxicity, signal processing.

I. INTRODUCTION

S INCE the 1970s, signal recording in hyperbaric chambers has presented numerous practical challenges in the evaluation of biological parameters during training and in

Manuscript received June 17, 2014; revised October 27, 2014; accepted December 20, 2014. Date of publication January 16, 2015; date of current version July 03, 2015. This work was supported by Rilevazioni elettrofisiologiche in immersione Cap. 1322, Italian Ministry of Defense, Direzione Generale della Sanità Militare, 2010 under Grant SMD L-023. (Corresponding author: Emanuela Formaggio.)

L. Pastena is with the Department of Neurological Sciences, University of Rome, La Sapienza, 00192 Rome, Italy (e-mail: lucio.pastena@gmail.com).

E. Formaggio is with the Department of Neurophysiology, Foundation IRCCS San Camillo Hospital, 30126 Venice, Italy (e-mail: emanuela. formaggio@univr.it).

F. Faralli, M. Melucci, R. Gagliardi, and L. Ricciardi are with the Italian Navy Medical Service Comsubin Varignano, 19025 Le Grazie (La Spezia), Italy (e-mail: fabio.faralli1958@gmail.com, massimo.melucci@me.com, ricgal@inwind.it, lucio.ricciardi@uninsubria.it).

M. Rossi is with the Ates Medica Device – EbNeuro, 37030 Verona, Italy (e-mail: marco.rossi@ebneuro.com).

S. F. Storti is with the Department of Computer Science, University of Verona, 37134 Verona, Italy (e-mail: silviafrancesca.storti@univr.it).

Color versions of one or more of the figures in this paper are available online at http://ieeexplore.ieee.org.

Digital Object Identifier 10.1109/TNSRE.2015.2391672

operational situations of professional and student divers. The main limitation is that the chamber is enclosed in a steel casing, like a Faraday cage that shields against electromagnetic waves entering from the environment and blocks the electromagnetic waves generated inside from propagating outside. This means that an electromagnetic-wave generator placed inside the chamber cannot be externally detected. The second limitation is that the power supply of equipment inside the chamber cannot be powered by alternating current as it may generate sparks, creating a considerable safety hazard in the presence of a gas such as hydrogen or oxygen. Therefore, the equipment needs to be powered by low-voltage continuous current and special safety precautions taken when opening and closing the power supply circuit to prevent the occurrence of sparks.

Till now, the main problem with the steel casing shielding electromagnetic wave propagation has been solved by running wire connections from the biological signal sources, e.g., an electrocardiogram (ECG) or an electroencephalogram (EEG), to outside the chamber. A wire-to-wire connection embedded in a pressure-resistant resin material links the two sockets, one inside and the other outside the chamber. The apparatus is screwed to the wall of the chamber. Even with this setup, however, the recording of biological signals like EEG (order of μV) remains suboptimal. Steps to improve the EEG signal quality entail keeping the cable length to a minimum to prevent disturbance from eddy currents during EEG recording and placing the human subject as close as possible to the chamber wall. But because the EEG signals near the recording source cannot be amplified owing to the potential hazard of the occurrence of sparks from the device power supply, and because separate units for signal amplification and recording are not commercially available, the recording device needs to be located as close as practical to the outer wall of the chamber to reduce eddy currents, and the cables outside the chamber shielded to reduce electromagnetic interference [Fig. 1(a)].

Given this myriad of difficulties in quantifying the EEG signal, EEG studies in hyperbaric chambers are not so common. EEG was first recorded in animals in 1945 and in humans in 1969, and specifically for the study of the high-pressure nervous syndrome (HPNS) in 1968–1969 [1]–[3]. In 1974, Rostain and Naquet performed EEG studies in humans to identify the effects of helium and oxygen (He/O₂) gas mixtures [4]; Rostain continued this line of research from 1974 until 1988, experimenting with He/O₂ mixtures in particular [5]. Only recently has Fourier analysis been applied to human EEG recordings in a hyperbaric chamber to quantify the EEG signal [6]–[8].

538

1534-4320 © 2015 IEEE. Personal use is permitted, but republication/redistribution requires IEEE permission. See http://www.ieee.org/publications_standards/publications/rights/index.html for more information.





B) HOLTER APPARATUS - OFF-LINE RECORDING ® - ALL TYPES OF PRESSURE CHAMBERS ©



Fig. 1. Different modalities of biological signal recording inside a hyperbaric chamber. (A) Old apparatus. Wired connections from the EEG signal sources to the EEG acquisition unit are embedded in a pressure-resistant resin material. Since the device power supply inside the chamber is critical, EEG signals are amplified outside the Faraday cage. (B) Holter apparatus. The device amplifies signals close to the subject and records them on a removable storage memory. The device is powered by low-voltage direct current, which does not cause dangerous sparks during opening and closing of the power supply circuit. Signal recording is possible only in off-line mode. (C) Bluetooth technology. The EEG system is composed of a portable recorder connected via Bluetooth wireless transmission to a notebook that visualizes the EEG signal. The acquisition module acquires samples from the amplified signals coming from the channels on the cap. The hyperbaric chamber has pressure-resistant Plexiglas portholes made from transparent resins through which the Bluetooth radio waves are transmitted outside the chamber. The notebook, located outside the chamber, is placed next to the porthole to obtain more stable data acquisition. With this technology, the amplified online signals can be recorded without interference from eddy currents caused by connection cables.

With the development of the Holter monitor, i.e., an ambulatory data collection device that amplifies signals (heart rhythm) by means of sensors attached to the wearer's chest and records them on a removable storage memory, were many of these disadvantages resolved. The Holter monitor is powered by low-voltage direct current (dc) which does not generate sparks during opening and closing of the power supply circuit. Furthermore, because the signals are amplified near their source, extremely short cables can be used, thus minimizing interference from eddy currents; after am-

HYPERBARIC ROOM

el Wal

plification, the signals can be recorded with higher accuracy, again, without eddy current interference. The older "off line" recording method consisted of a mass memory recording that could be read outside of the chamber; however, the subject could not be monitored in an "online" mode, precluding prompt intervention if the EEG indicated a dangerous situation [Fig. 1(b)].

relative humidity

With the development of the Bluetooth communication protocol, signal transmission via low-length radio waves obviated the need for cables. Using the same radio communication



Fig. 2. (A) During the experiment, the subject reclines on a cot with his eyes closed. (B) The 32-channel EEG system is connected via Bluetooth wireless transmission to a notebook that visualizes the EEG signal outside the hyperbaric chamber. (C) The EEG recording system is located outside the hyperbaric chamber, near the porthole.



Fig. 3. (A) Thirty-two-channel HydroCel Geodesic Sensor Net Map. Each blue and red sensor corresponds to an amplifier channel, while the white ones are stabilizer electrodes. (B) Electrode positions displayed on a head model. (C) A navy diver wearing the EEG cap outside the hyperbaric chamber.

system, multiple devices can communicate and exchange data via cable-free connections over short distances.

Bluetooth applications are indoor environments, where attenuation by walls and signal fading due to signal reflections can both reduce the range. Applied to portable devices, Bluetooth technology allows to record online signals already amplified without the interference of eddy currents caused by cables [Fig. 1(c)].

In this technical report, we present a Bluetooth device that allows the recording of biological signals in a hyperbaric chamber despite the presence of a Faraday cage. Although such systems can be used for a variety of purposes, here we focus on their potential application in hyperbaric chambers. In detail, 32-channel EEG was recorded with a Bluetooth EEG system in 11 professional divers. A 20-min EEG recording was carried out under three different experimental conditions: air (baseline at sea level, 1 atmosphere absolute pressure [ATA]); oxygen breathing (at a simulated depth of 18 msw, 2.8 ATA); and air (after decompression, 1 ATA). The Bluetooth low-length radio waves can pass through the resin portholes of the hyperbaric chamber walls. The advantages of wireless technology for transferring data outside a hyperbaric chamber are also discussed.

II. DEVICE DESCRIPTION

The EEG equipment used in this study is a portable recorder connected via Bluetooth wireless transmission to a notebook that visualizes the EEG signal (EBNeuro – ATES Medica Device, Verona, Italy) (Fig. 2). The Bluetooth Neurotravel module is an embedded Class 2 module with an integrated antenna.

The Bluetooth EEG system includes a cap with 32 channels, an acquisition unit, and a data processing and storage unit. Although the study was conducted using this acquisition system, other Bluetooth systems can probably be used for the same purpose (e.g., B-Alert X24 EEG SystemEmotiv EPOC, g.Nautilus – g.tec's wireless EEG system, Enobio Wireless EEG, etc.).

The acquisition unit (Neurotravel GEM100/SMART) detects signals on the subject by means of an acquisition module connected to the notebook via Bluetooth. This module acquires samples from the amplified signals coming from the channels on the cap. An anti-aliasing filter precedes EEG signal digitalization at 250 Hz. Signals are recorded with 16-bit resolution.

The EEG cap (Electrical Geodesic Inc., OR, USA) (Fig. 3) is composed of Ag/AgCl electrodes, which are soaked in a physiological-potassium solution for about 5 min before beginning the recording. The solution creates a microclime between the subject's scalp and the electrodes that keeps the impedances low even during long EEG recordings. The net was adjusted so that 18 (placed on the nasion), Cz, Oz, and the pre-auricular points were correctly placed according to the international 10/20 system. By virtue of the net's geodesic tension structure, all electrodes were evenly distributed over the scalp. The data were recorded against a vertex electrode reference (Cz) at a sampling rate of 250 Hz using the software package Geodesic EEG System on Neurotravel technology (ATES Medica Device, and Electrical Geodesic, Inc.), and subsequently re-referenced using the average reference for the analysis. Electrode impedances were verified and showed mean resistances in the range of 20–80 k Ω .

III. APPLICATIONS

A. Oxygen Toxicity

The use of an oxygen mixture and closed-circuit breathing apparatus (CCBA) for diving at various depths augments the risk of central nervous system oxygen toxicity (CNS O2T). The EEG pattern is known to significantly change during saturation dives [6]. Oxygen poisoning can manifest itself with a range of symptoms and complications: from nausea, dizziness, hearing and visual disturbances, amnesia, vertigo, irritability, and localized muscular twitching to loss of consciousness, tonic-clonic seizures, as well as EEG alterations including an increase in theta waves, a decrease in alpha waves, microsleep, and a decline in psychometric performance. Prolonged exposure to hyperbaric oxygen (HBO) will result in permanent paralysis and death [9]. To prevent this syndrome, prompt recognition of the earliest symptoms, as described after dry or humidified hyperbaric oxygen exposure, is vital. Therefore, one of the most important applications of a Bluetooth EEG technology in a hyperbaric chamber is to measure the EEG activity and determine how oxygen and hyperbaric pressure variables affect bioelectrical brain activity in professional divers. As a first application, we determined how oxygen, breathed at a constant hyperbaric pressure of 2.8 ATA (simulated depth of 18 msw), affects the bioelectrical activity in human professional divers. This EEG activity was then compared with the pre- and post-oxygen conditions at 1 ATA pressure breathing air.

B. Subjects and Experimental Protocol

The EEG was recorded inside a hyperbaric chamber using the Bluetooth wireless technology as described above [Fig. 1(c)]. The study population was 11 healthy male navy divers (mean age, 46.2 ± 4.9 years). Exclusion criteria were a medical history of respiratory problems, sleep disturbances, smokers, and overweight. The internal ethics committee of the University approved the experimental protocol, and oral and written informed consent was obtained from the subjects before participating in the study.

A simulated depth of 18 msw (2.8 ATA) was selected for the study because exposure at this breathing depth is a routine part of navy diver training courses. The hyperbaric chamber used in the study complied with Italian Navy standards for safety equipment and emergency procedures. All subjects were studied individually. They were accompanied during the simulated dive

by a technician who assisted the subject if needed and were monitored by closed-circuit television. Arousal was maintained throughout the whole recording session by administering an external acoustic stimulation as soon as one 30-s epoch showed a reduction of more than 50% of the background alpha rhythm, according to the American Academy of Sleep Medicine scoring rules [10]. The procedure was carried out by an experienced neurophysiologist who was also responsible for detecting any epileptiform phenomena. Visual inspection of the EEG signals showed no abnormalities. The divers had complete saturation of blood haemoglobin; nonetheless, the true value of oxygen partial pressure (pO_2), which requires arterial sampling, cannot be performed inside a hyperbaric chamber.

Each recording session lasted 20 min, during which the subject was reclined on a cot with eyes closed. A baseline 20-min EEG recording was made at 1 ATA breathing air (AIRpre) in an open chamber. A 2-min compression profile (descent rate, 9 m min⁻¹) breathing air was used to reach the oxygen stage at a pressure of 2.8 ATA. At this pressure, the subject breathed pure oxygen via an oronasal mask (O₂) and a second 20-min EEG recording was acquired. The atmosphere within the hyperbaric chamber was controlled to maintain a total pressure of 2.8 ATA. After decompression, back on air breathing, the EEG of each subject was recorded for 20 min (AIRpost), discarding the first 2 min (ascent rate, 9 m min⁻¹). The overall time of EEG recording was about 1 h [Fig. 4(a)]. The chamber was compressed with air and a separate breathing circuit was used for the oxygen stage. As the chamber was equipped with a continuous ventilation system, eventual oxygen leaks from the mask were not thought to influence the atmosphere inside the hyperbaric chamber [8].

C. Scalp Maps of EEG Spectral Power

The data were preprocessed and processed as described in [8]. The FFT relative power (%) was then estimated for the delta (1-4 Hz), theta (5-7 Hz), alpha (8-12 Hz), beta1 (13-15 Hz), and beta2 (15-30 Hz) frequency ranges.

Comparison of brain activity between air breathing at sea level (1 ATA) and oxygen (2.8 ATA) conditions in the same subjects showed a rapid and significant decrease in delta relative power in the posterior regions starting already in the early minutes of the test, with a parallel and significant increase in the alpha rhythm in the same regions [Fig. 4(b)]. After decompression (AIRpost), the delta relative power significantly decreased from baseline (AIRpre) over all electrodes until minute 8. At 11'-12', this decrease was principally localized in the posterior regions. The increase in alpha rhythm was uniformly distributed over the cerebral cortex until minute 8; this increase was still significant in the posterior regions until the end of the 20 min. Because a decrease in delta activity during oxygen breathing may be a sign of reduced performance of cortical inhibitory mechanisms, an increase in alpha activity can be detected in the central regions where this activity is normally very low. During prolonged exposure to hyperbaric oxygen, EEG changes occurred in all subjects with good reproducibility. From this we would assume that during oxygen breathing vasoconstriction is followed by vasodilatation, with an increase in alpha activity in the posterior regions [11]. This is consistent with the observa-



Fig. 4. (A) Dive profile and recording sessions. A 20-min baseline EEG recording was made at 1 ATA breathing air (EEG1 – AIRpre) in the open hyperbaric chamber. In the closed chamber, a 2-min compression profile (descent rate, 9 m min⁻¹) breathing air was used to reach the next stage at a pressure of 2.8 ATA (compression). At this pressure, the subject breathed pure oxygen via the oronasal mask and a 20-min EEG was acquired (EEG2 – O₂). After decompression, back on air breathing, the EEG of each subject was recorded for 20 min (EEG3 – AIRpost), discarding the first 2 min (ascent rate, 9 m min⁻¹) (decompression). (B) Power spectra density $[\mu V^2/Hz]$ of the EEG signal average of 20-min EEG recording (only 19 channels visualized in the international 10–20 system positions) in one professional diver in the three different conditions: air (blue), oxygen breathing (green), and air after decompression (red).

tion that vascular response to hyperoxia leads to a reduction in cerebral blood flow.

D. Cortical Source Analysis of the EEG Rhythms

1) Loreta Imaging: The data were preprocessed as before. The EEG data for each condition (20 min for each) were divided into epochs of 2 s. Because EEG spectral frequency bands are known to reflect different functions and behave statistically independently of one another, the analysis was done separately in the following six bands: delta (1.5-6 Hz), theta (6-8 Hz), alpha (8-12 Hz), beta1 (12-18 Hz), beta2 (18-21 Hz), and beta3 (21-30 Hz) [12]. In order to localize the cortical sources of scalp EEG activity, standardized low-resolution brain electromagnetic tomography (sLORETA; http://www.unizh.ch/keyinst [13]) was used. The sLORETA method is a properly standardized, discrete, 3-D distributed, linear, minimum norm inverse solution. The particular form of standardization used in sLORETA endows the tomography with the property of exact localization to test point sources, yielding images of standardized current density with exact localization, albeit with low spatial resolution (i.e., neighboring neuronal sources will be highly correlated) [13].

In the frequency domain, LORETA [14] can be computed from EEG cross-spectra (see Appendix).

Under the three conditions, for each subject separately and all subjects together, the EEG cross-spectra and then the corresponding 3-D-cortical distribution of the electric neuronal generators were computed with sLORETA for each frequency band. The spectral current density was mapped into a 3-D representation: one map for each frequency band in the AIRpre condition (1-20 min) and seven maps for the O₂ and AIRpost conditions, with seven intervals of analysis (1', 2', 5', 8', 11'-12', 16'-17', and 19'-20'). Minutes 19-20 from the EEG of subject no. 3, minute 5 from subject no. 8, and minute 2 from subject no. 9 were discarded due to loss of the EEG signal via Bluetooth.

2) Statistical Analysis of the Loreta Solutions: Statistical analyses were performed using the sLORETA software package. The difference in source localization of cortical oscillations between the three experimental conditions (AIRpre, O_2 , AIRpost) in each frequency band was assessed by voxel-by-voxel independent sample t-tests based on sLORETA log-transformed current density power. Due to the non-Gaussianity nature of the data, in the resulting statistical 3-D images the cortical voxels showing significant differences were identified by a nonparametric approach (statistical nonparametric mapping [SnPM]) via randomizations [15], [16]. Using this randomization strategy, we determined the critical probability threshold values for the actually observed t-values with correction for multiple comparisons across all voxels and all frequencies. A total of 5000 permutations were used to determine the significance for each randomization test. Log of ratio of averages were used and considered with a 0.95 level of significance. The use of statistical nonparametric maps applied to LORETA images has been validated in previous studies [17]–[19]. As the LORETA method does not need any "distributional assumptions," it yields an adjusted t-critical value that is effective for controlling type I error [20]. The LORETA images were statistically compared between conditions (O2 versus AIRpre, AIRpost versus AIRpre). The t-values corresponding to p < 0.05 were plotted onto a magnetic resonance imaging (MRI) template with a scale bar indicating statistical power.

3) Results: Average of LORETA solutions (i.e., relative current density at cortical voxels) modelling the distributed EEG sources for delta, theta, alpha1, alpha2, beta1, beta2, and beta3



Fig. 5. Averaged sLORETA solutions (current density at cortical voxels) of EEG sources for each frequency band, delta (1.5–6 Hz), theta (6–8 Hz), alpha (8–12 Hz), beta1 (12–18 Hz), beta2 (18–21 Hz), and beta3 (21–30 Hz), and for each condition (AIRpre, O_2 , AIRpost). The left side of the maps (top view) corresponds to the left hemisphere.

bands presented specific spatial features during air breathing in resting state condition, during oxygen breathing, and during air breathing after the decompression stage.

As compared to baseline, during oxygen breathing (2.8 ATA), the brain activity showed a rapid and significant decrease in delta and theta sources in the posterior regions starting from the early minutes of oxygen breathing and a parallel significant increase in the alpha and beta1 sources in the same regions (Fig. 5). As shown on the t-maps (O₂ versus AIRpre), a significant decrease was detected for the theta sources in particular and persisted throughout the entire recording during oxygen breathing. The alpha sources markedly, albeit less significantly, localized in the same posterior regions. The beta2 and beta3 bands were less involved during oxygen breathing as compared to the baseline (Fig. 6).

After decompression (AIRpost), the delta and theta sources significantly decreased from baseline (AIRpre) principally over the posterior regions until minute 5. At 11'–12', the decrease was still present but less significant over the posterior regions. After 20 min, the power resembled that of AIRpre even if it did not completely return to the baseline (Fig. 5). This modification is shown on the t-maps (AIRpost versus AIRpre). The alpha and beta1 sources significantly increased in the same posterior regions during the first 2 min after decompression before returning almost completely to baseline at 19'–20'. The beta2 and beta3 bands were less involved during AIRpost as compared to the baseline (Fig. 6).

These results show that oxygen exposure can affect the cortical sources of resting state rhythms, i.e., the slow rhythms significantly decrease as a sign of vasoconstriction followed by vasodilatation, with an increase in alpha activity in the posterior regions. They also suggest that it may be possible to define and recognize landmarks of oxygen-induced brain activity, which could inform decisions in the medical treatment of subjects reporting "diving-related problems."

IV. CONCLUSIONS

Mobile EEG systems have been developed in biomedical contexts for monitoring medical conditions and have also found a particular place in neurological applications (i.e., epilepsy, sleep, brain–computer interface). Here, we focus on their potential application in hyperbaric chambers.

EEG studies in hyperbaric chambers are not common. The main technical limitations are the practical problems with recording the EEG signal inside a steel-enclosed chamber, i.e., a Faraday cage, and that the power supply of the equipment inside the chamber cannot be powered by alternating current because of the hazard of sparks.

The advantages of Bluetooth technology for recording biological parameters in a hyperbaric chamber are many: the EEG signal quality is better than with older technologies because the transmission of the amplified signals is less subject to environmental interference and because it enables wireless connection without cables which can generate eddy currents. Hyperbaric chambers are equipped with pressure-resistant Plexiglas portholes through which Bluetooth radio waves are transmitted outside the chamber. By placing the EEG system outside the chamber next to the porthole, we can obtain more stable data



Fig. 6. sLORETA statistical maps of delta, theta, alpha, beta1, beta2, and beta3 oscillations. Significant results are projected onto a cortical surface. Colored areas represent the spatial extent of voxels with a significant difference (yellow and light blue-coded for p < 0.05, corrected for multiple testing) in source current density in O₂ versus AIRpre and AIRpost versus AIRpre. The color scale represents log F-ratio values (p < 0.05).

acquisition. In addition, so-called hull penetrators (consisting of two sockets connected wire-to-wire and embedded in the pressure-resistant resin), are generally no longer provided on hyperbaric chambers.

Our preliminary experimental results indicate that this dedicated system can create an EEG platform with a signal acquisition quality comparable to current EEG systems. The principal results of EEG recording in professional divers during three different conditions (breathing air inside a hyperbaric chamber at sea level, breathing oxygen at a simulated depth of 18 msw, and breathing air at sea level after decompression) showed that cortical sources are affected by oxygen breathing for at least 20 min. The oxygen condition was characterized by a marked amplitude increase in the alpha and beta1 sources in the parietal and occipital areas and an amplitude decrease in the occipital delta and theta sources. These results are in line with those obtained from the scalp which showed a decrease in the relative power of slow rhythms and an increase in the relative power of fast rhythms. These findings support the hypothesis that long-term oxygen exposure may differently affect cortical sources of EEG.

There are many situations in which the human brain is exposed to hyperoxia, including medical therapy, diving, industrial health, and safety in toxic environments. The most widely used technique to study hyperoxia in a normobaric condition is perfusion-MRI. Several studies reported that steady-state cerebral blood flow (CBF) decreases and cerebral vascular resistance increases during acute exposure to normobaric hyperoxia in healthy subjects [21]–[23]. The CBF decreased independently of arterial partial pressure of carbon dioxide (pCO_2) indicating an independent cerebral vasoconstrictive effect of hyperoxia. Supporting these observations, Visser and colleagues monitored by transcranial Doppler (TDC) the blood flow velocity in the right middle cerebral artery and found that the mean TDC velocity decreased during hyperbaric oxygen (HBO) exposure (30 min exposure to 2.8 bar pure oxygen) probably due to vasoconstriction of the cerebral resistance vessels, whereas it increased in the subject who experienced a generalized seizure [24].

EEG has rarely been used for the evaluation of brain activity during hyperoxia. Visser *et al.* described the effects of HBO exposure on quantitative EEG and noted that the electrical changes were minor and not considered indicative of a HBO effect on the brain in the group of healthy subjects who showed no signs of toxicity. Preconvulsive EEG changes were detected in only one subject who experienced a seizure, but they were too insignificant and did not clearly herald clinical signs [25]. Kaskinoro and colleagues found that prolonged oxygen administered at high concentrations under normal atmospheric pressure and with normal ventilation does not cause significant EEG slowing or produce any epileptiform activity in the EEG in healthy subjects [26].

Differently, as observed by the significant changes in the cortical neuronal activity assessed by our EEG recordings, we would assume that the prolonged hyperoxia in these healthy subjects induced vasoconstriction followed by vasodilatation, with an increase in alpha activity in the posterior regions. These findings could support the oxygen tolerance test (OTT)

in military divers and the evaluation of subjects with susceptibility to CNS O_2T . In particular, the Italian Navy OTT procedure exposes divers to 2.8 ATA (the same pressure as in our experiment) for 15 min in a dry chamber while breathing 100% oxygen through a face mask. The EEG could be easily used as a biomarker to investigate possible alterations in electrical brain activity during the test, e.g., increased power in the low-frequency bands (i.e., delta, theta) with reductions in the higher frequency bands (i.e., alpha, beta) and abnormal source localization (e.g., LORETA) analyses.

This first application has produced encouraging results; however, two major limitations of the study merit discussion. First, we cannot completely exclude a slight increase in the alpha components as a marker of increased drowsiness over the duration of the recording. Nonetheless, in our results we observed an increase in alpha activity, especially during oxygen breathing, and an almost complete return to the baseline at the end of the AIRpost condition. From this transient modification we can assume that the results were not significantly altered. Moreover, drowsiness-alpha activity is typically characterized by decreased amplitude over the occipital areas, as compared with the wakefulness-alpha rhythm, simultaneous to the appearance of a slower alpha pattern localized over the anterior regions [27]. Differently, our results showed that the alpha increase is localized especially over the parietal and occipital regions.

The second limitation is that during oxygen breathing, the subjects were simultaneously exposed to high pressure and pure oxygen. These two variables were not independently manipulated and we were unable to quantify the effects of each variable on the results. Therefore, we are planning to include in the protocol of future studies an EEG evaluation during normobaric hyperoxia in order to determine how oxygen and hyperbaric pressure, respectively, affect bioelectrical activity in human professional divers.

APPENDIX

Loreta in Frequency Domain: LORETA images corresponding to the estimated neuronal generators of brain activity within a given frequency band are defined as follows. For a given subject, let $\Phi_{i,t}$ denote a vector comprised of the scalp electric potentials measured at each scalp electrode (any reference electrode is allowed), at time instant t ($t = 1, ..., N_{\tau}$), and for EEG epoch i ($i = 1, ..., N_{\epsilon}$). Let $\Phi_{i,t}^{\Omega}$ denote the band filtered EEG, where Ω denotes the frequency band of interest. The instantaneous current density estimate is computed as the linear transformation

$$\hat{J}_{i,t}^{\Omega} = T\Phi_{i,t}^{\Omega} \tag{1}$$

where T denotes the inverse of the transfer matrix or lead field matrix K [18], [28]. The LORETA image for the frequency band Ω is then defined as the spectral density of estimated current density signals. Based on Parseval's theorem on the equivalence of power expressed in the time and frequency domains [29], the LORETA image can be computed as:

$$\operatorname{diag}\left[\frac{1}{N_{\tau}N_{\epsilon}}\sum_{\substack{i=1,\ldots,N_{\epsilon}\\t=1,\ldots,N_{\tau}}} (\hat{J}_{i,t}^{\Omega})(\hat{J}_{i,t}^{\Omega})^{T}\right] = \operatorname{diag}(TS_{\Phi}^{\Omega}T^{T}) \quad (2)$$

where diag denotes the diagonal of a matrix, the superscript T denotes vector transposition, and S_{Φ}^{Ω} is, except for a scale factor, the Hermetian EEG cross-spectral matrix [30]

$$S_{\Phi}^{\Omega} = \frac{1}{N_{\epsilon}} \sum_{\substack{i=1,\dots,N_{\epsilon}\\\forall\omega\in\Omega}} (\Phi_{i,\omega}^{\Omega}) (\Phi_{i,\omega}^{\Omega})^*$$
(3)

where $\Phi_{i,\omega}^{\Omega}$ denotes the discrete Fourier transform at frequency ω and the superscript * denotes complex conjugate and vector transposition [31].

REFERENCES

- R. Cohn and I. Gersch, "Changes in brain potentials during convulsions induced by oxygen under pressure," *J. Neurophysiol.*, vol. 8, no. 4506, pp. 155–160, 1945.
- [2] R. W. Brauer, S. Dimov, X. Fructus, P. Fructus, A. Gosset, and R. Naquet, "Neurologic and encephalographic syndrome of hyperbarism," *Rev. Neurol. (Paris)*, vol. 121, pp. 264–265, Sep. 1969.
- [3] G. L. Zal'tsman, "Hyperbaric epilepsy and narcosis (neurophysiological studies) Leningrad: Sechenov Institute of Evolutionary Physiology and Biochmistry," *Moscow: Russian Acad. Sci.*, 1968.
- [4] J. C. Rostain and R. Naquet, "Le Syndrome Nerveux fonction de divers modes de compression," *Rev. EEG Neurophysiol.*, vol. 4, pp. 107–124, 1974.
- [5] J. C. Rostain, M. C. Gardette-Chauffour, J. P. Gourret, and R. Naquet, "Sleep disturbances in man during different compression profiles up to 62 bars in helium-oxygen mixture," *Electroencephalogr Clin. Neurophysiol.*, vol. 69, pp. 127–135, Feb. 1988.
- [6] L. Pastena, G. Mainardi, F. Faralli, and R. Gagliardi, "Analysis of cerebral bioelectrical activity during the compression phase of a saturation dive," *Aviat. Space Environ. Med.*, vol. 70, pp. 270–276, Mar. 1999.
- [7] L. Pastena, F. Faralli, G. Mainardi, and R. Gagliardi, "EEG patterns associated with nitrogen narcosis (breathing air at 9 ATA)," *Aviat. Space Environ. Med.*, vol. 76, pp. 1031–1036, Nov. 2005.
- [8] L. Pastena, E. Formaggio, S. F. Storti, F. Faralli, M. Melucci, R. Gagliardi, L. Ricciardi, and G. Ruffino, "Tracking EEG changes during the exposure to hyperbaric oxygen," *Clin. Neurophysiol.*, vol. 126, pp. 339–347, Feb. 2015.
- [9] K. W. Donald, "Oxygen poisoning in man," Br. Med. J., vol. 1, p. 667, May 1947.
- [10] C. Ibner, S. Ancoli-Israel, A. Chesson, and S. E. Quan, *The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications*, 1st ed. Westchester: Amer. Acad. Sleep Medicine, 2007, vol. III.
- [11] L. K. Weaver and S. Howe, "Normobaric measurement of arterial oxygen tension in subjects exposed to hyperbaric oxygen," *Chest*, vol. 102, pp. 1175–1181, Oct. 1992.
- [12] S. Kubicki, W. M. Herrmann, K. Fichte, and G. Freund, "Reflections on the topics: EEG frequency bands and regulation of vigilance," *Pharmakopsychiatr Neuropsychopharmakol*, vol. 12, pp. 237–245, Mar. 1979.
- [13] R. D. Pascual-Marqui, "Standardized low-resolution brain electromagnetic tomography (sLORETA): Technical details," *Methods Find Exp. Clin. Pharmacol.*, vol. 24 Suppl D, pp. 5–12, 2002.
- [14] R. D. Pascual-Marqui, C. M. Michel, and D. Lehmann, "Low resolution electromagnetic tomography: A new method for localizing electrical activity in the brain," *Int. J. Psychophysiol.*, vol. 18, pp. 49–65, Oct. 1994.
- [15] A. P. Holmes, R. C. Blair, J. D. Watson, and I. Ford, "Nonparametric analysis of statistic images from functional mapping experiments," *J. Cereb. Blood Flow Metab.*, vol. 16, pp. 7–22, Jan. 1996.
- [16] T. E. Nichols and A. P. Holmes, "Nonparametric permutation tests for functional neuroimaging: A primer with examples," *Hum. Brain Mapping*, vol. 15, pp. 1–25, Jan. 2002.
- [17] P. Anderer, R. D. Pascual-Marqui, H. V. Semlitsch, and B. Saletu, "Differential effects of normal aging on sources of standard N1, target N1 and target P300 auditory event-related brain potentials revealed by low resolution electromagnetic tomography (LORETA)," *Electroencephalogr Clin. Neurophysiol.*, vol. 108, pp. 160–174, Mar. 1998.

- [18] R. D. Pascual-Marqui, D. Lehmann, T. Koenig, K. Kochi, M. C. Merlo, D. Hell, and M. Koukkou, "Low resolution brain electromagnetic tomography (LORETA) functional imaging in acute, neuroleptic-naive, first-episode, productive schizophrenia," *Psychiatry Res.*, vol. 90, pp. 169–179, Jun. 1999.
- [19] D. Pizzagalli, R. D. Pascual-Marqui, J. B. Nitschke, T. R. Oakes, C. L. Larson, H. C. Abercrombie, S. M. Schaefer, J. V. Koger, R. M. Benca, and R. J. Davidson, "Anterior cingulate activity as a predictor of degree of treatment response in major depression: Evidence from brain electrical tomography analysis," *Amer. J. Psychiatry*, vol. 158, pp. 405–415, Mar. 2001.
- [20] P. Flor-Henry, J. C. Lind, and Z. J. Koles, "A source-imaging (low-resolution electromagnetic tomography) study of the EEGs from unmedicated males with depression," *Psychiatry Res.*, vol. 130, pp. 191–207, Feb. 2004.
- [21] C. J. Lambertsen, R. H. Dough, D. Y. Cooper, G. L. Emmel, H. H. Loeschcke, and C. F. Schmidt, "Oxygen toxicity; effects in man of oxygen inhalation at 1 and 3.5 atmospheres upon blood gas transport, cerebral circulation and cerebral metabolism," *J. Appl. Physiol.*, vol. 5, pp. 471–486, Mar. 1953.
- [22] N. A. Watson, S. C. Beards, N. Altaf, A. Kassner, and A. Jackson, "The effect of hyperoxia on cerebral blood flow: A study in healthy volunteers using magnetic resonance phase-contrast angiography," *Eur. J. Anaesthesiol.*, vol. 17, pp. 152–159, Mar. 2000.
- [23] T. F. Floyd, J. M. Clark, R. Gelfand, J. A. Detre, S. Ratcliffe, D. Guvakov, C. J. Lambertsen, and R. G. Eckenhoff, "Independent cerebral vasoconstrictive effects of hyperoxia and accompanying arterial hypocapnia at 1 ATA," *J. Appl. Physiol.*, vol. 95, pp. 2453–2461, Dec. 2003.
- [24] G. H. Visser, R. A. Van Hulst, G. H. Wieneke, and A. C. Van Huffelen, "Transcranial Doppler sonographic measurements of middle cerebral artery flow velocity during hyperbaric oxygen exposures," *Undersea Hyperb Med.*, vol. 23, pp. 157–165, Sep. 1996.
- [25] G. H. Visser, R. A. v. Hulst, G. H. Wieneke, and A. C. van Huffelen, "The contribution of conventional and quantitative electroencephalography during monitoring of exposure to hyperbaric oxygen," *Undersea Hyperb Med.*, vol. 23, pp. 91–98, Jun. 1996.
- [26] K. Kaskinoro, A. Maksimow, H. Scheinin, R. Laitio, R. Aantaa, T. Kärki, S. Hinkka-Yli-Salomäki, and S. Jääskeläinen, "Normobaric hyperoxia does not induce significant electroencephalogram changes in healthy male subjects," *Internet J. Neuromonitoring*, vol. 6, no. 1, 2008.
- [27] J. L. Cantero, M. Atienza, and R. M. Salas, "Human alpha oscillations in wakefulness, drowsiness period, and REM sleep: Different electroencephalographic phenomena within the alpha band," *Neurophysiol. Clin.*, vol. 32, pp. 54–71, Jan. 2002.
- [28] R. D. Pascual-Marqui, "Review of methods for solving the EEG inverse problem," *Int. J. Bioelectromagn.*, vol. 1, no. 1, pp. 75–86, 1999.
- [29] J. W. Cooley, P. A. W. Lewis, and P. D. Welch, "The Fast Fourier transform and its application to time series analysis," in *Statistical Methods for Digital Computers*, K. Enslein, A. Ralston, and H. S. Wilf, Eds. New York, NY, USA: Wiley, 1977, pp. 377–423.
- [30] D. R. Brillinger, *Time Series: Data Analysis and Theory*. New York, NY, USA: McGraw-Hill, 1981.
- [31] E. Frei, A. Gamma, R. Pascual-Marqui, D. Lehmann, D. Hell, and F. X. Vollenweider, "Localization of MDMA-induced brain activity in healthy volunteers using low resolution brain electromagnetic tomography (LORETA)," *Hum. Brain Mapping*, vol. 14, pp. 152–165, Nov. 2001.



Emanuela Formaggio was born in Sassari, Italy in 1980. She received the Master's degree in electronic engineering and the Ph.D. degree in bioengineering from the University of Padova, Padova, Italy, in 2005 and 2010, respectively.

From 2006 to 2010 she was a Research Fellow at the Department of Neurological and Movement Sciences, University of Verona, Verona, Italy. From 2010 she has a research fellowship with the Foundation IRCCS "San Camillo" Hospital, Venezia, Italy. Her research interest concerns fMRI analysis, coregistration of

EEG-fMRI signals, EEG-TMS coregistration, and EEG source localization for clinical applications (epilepsy, stroke, disorders of consciousness).



Fabio Faralli was born in Arezzo, Italy in 1958.

He is a Diving and Hyperbaric Medicine Specialist. He has been the Italian Navy delegate at the NATO Underwater Diving Working Groups from 2012 to 2013, and at the NATO Submarine Emergency and Rescue Working Group from 1998 to 2002. He was Former Chief of Underwater Medicine Research Unit of COMSUBIN – Italian Navy. From 1995 to 2014, he was a Teacher at the University of Chieti, at the Italian Naval Academy, at the Diving School of COMSUBIN and Professional Diving

Schools, at S. Anna School, and CNR in Pisa. He is the author of about 80 national and international papers on diving medicine.



Massimo Melucci received the B.S. degree in nursing from the University of Pisa, Pisa, Italy, in 2006.

Since 1996 he has been a Petty Officer in the Nurse officer Section of Pathophysiology Underwater COMSUBIN. He previously served on ships and submarines of the Italian Navy. From 1995 he worked in the trial of electroencephalographic (EEG) techniques applied to diving, and in particular the registration of EEG during hyperbaric diving. Since 2010 he has participated as a member of the research project of the Ministry of Defense L023,

experiencing the ability to record EEG to divers in the water through the use of a helmet which he is coinventor.



Marco Rossi received the Electronic Engineer Diploma in 1980, degree in industrial engineering from the University of Rome, Rome, Italy, Guglielmo Marconi in 2008, and is an Italian accredited engineer.

He has been the CEO and Manager of Strategic Marketing medical products line of EBNeuro S.p.A. Italy, Florence since 2012. His research interests include biomedical signal acquisition for EEG/EMG/ECG using advanced technology of Blue-tooth, WiFi/TCPIP, with real-time signal analysis by uP/DSP; Trancranial Magnetic Stimulation,

coils, and neuronavigation. He has coauthored about 15 thesis degree graduation in collaboration with Verona and Padua University, speaker at scientific conferences, and author of conference papers on EEG and TMS application.



Lucio Pastena received the Ph.D. degree in neurophysiology from Scuola Normale Superiore di Pisa (Medical College), Pisa, Italy.

He was qualified with honor as medical doctor in 1961 (University Roma "La Sapienza", Italy). He specialized in neurology and psychiatry. He is an Associate Professor in neurology at the Department of Neurological Sciences University Roma "La Sapienza." He is a Lieutenant Commander Director of the Italian Navy Research Program: Electrophysiological Signals in Diving (D.G.SM 7). His interests

are the arrest-mechanisms of a epileptic crisis, the automatic proceeding in medical data, and the recording of bioelectrical signals in underwater environments. At present he is a retired professor.



Riccardo Gagliardi was born in La Spezia, Italy in 1949.

From 1982 to 1983 he was Medical Officer at the Medical Service of COMSUBIN (Italy); from 1983 to 1996 Chief of the Medical Service and of the Underwater Medicine Research Unit of COMSUBIN. From 1996 to 1998 he was Director of the Medical Services of the Italian Navy Naval Base in La Maddalena. He was a Former teacher of Diving Medicine at the Italian Naval Academy and at the Specialization School in "Medicina del Nuoto e delle Attività

Subacquee" at the University of Chieti. He is the author of several national and international papers on diving medicine.



Lucio Ricciardi was born in Pavia, Italy in 1950. He qualified from the University of Pavia: M.D., in 1969–1975, Postgraduate School of Sports Medicine, 1979–1982.

He was a Research Fellow, Department of Physiology, University of Leiden, NL (1983–1986), a Visiting Scientist, Department of Medical Physiology, University of Calgary, CDN (1985), a Research Fellow (1975–1980), an Established Researcher Institute of Human Physiology, University of Pavia (1980–1992), an Associate Professor of

Applied Physiology, at the same school (1992–1994); of Human Physiology, University of Pavia 2nd School of Medicine (1994); of Human Physiology, University of Insubria School of Medicine (1998–2011). He is retired, and is now a Sports Physician. He is also an Environmental Physiologist and is a Former Italian Navy Surgeon Lt., Adviser to the Navy.



Silvia F. Storti was born in Arzignano (VI), Italy in 1980. She received the Master's degree in electronic engineering from the University of Padova, Padova, Italy, in 2007 and the Ph.D. degree in neuroscience from the University of Verona, Verona, Italy in 2012.

She was a Postdoctoral Research Fellow at the Department of Neurological and Movement Sciences (2012–2013) and since 2014 at the Department of Computer Science, University of Verona. She is coauthor of 38 international papers and Guest Associate Editor for *Frontiers in Neurology (Epilepsy)*.

Her research interests include methods and models for EEG-fMRI, EEG-TMS, high-density EEG, ECoG data, and brain connectivity analysis for clinical applications.