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Research Paper

Disruptions in brain functional connectivity: The hidden risk for oxygen-intolerant professional divers in simulated deep water☆

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ABSTRACT

In this study, we investigated the effects of oxygen toxicity on brain activity and functional connectivity (FC) in divers using a closed-circuit oxygen breathing apparatus. We acquired and analyzed electroencephalographic (EEG) signals from a group of normal professional divers (PD) and a group that developed oxygen intolerance, i.e., oxygen-intolerant professional divers (OPD), to evaluate the potential risk of a dive and understand the physiological mechanisms involved. The results highlighted a significant difference in the baseline levels of α rhythm between PD and OPD, with PD exhibiting a lower level to counteract the effects of increased O_2 inhalation, while OPD showed a higher level that resulted in a pathological state. Connectivity analysis revealed a strong correlation between cognitive and motor regions, and high levels of α synchronization at rest in OPDs. Our findings suggest that a pathological condition may underlie the higher α levels observed in these individuals when facing the stress of high O_2 inhalation. These findings support the hypothesis that oxygen modulates brain networks, and have important implications for understanding the neural mechanisms involved in oxygen toxicity. The study also provides a unique opportunity to investigate the impact of neurophysiological activity in simulated critical scenarios, and opens up new perspectives in the screening and monitoring of divers.

1. Introduction

Oxygen toxicity is one of the most dangerous factors in underwater operations, representing the harmful consequences of prolonged exposure to elevated levels of oxygen on body tissues. Specifically, exposure to elevated partial pressures of oxygen (PO_2) can have a toxic effect on body tissue, depending on both the magnitude of the partial pressure and duration of exposure. The tolerance range of divers is wide, making it crucial to implement preventive monitoring of brain electrical activity to identify those individuals who are more sensitive to oxygen and monitor them over time [1,2]. Despite the widespread use of oxygen, the effect of O_2 gas modulation on the brain is not well understood, and the relationship between oxygen assumption, hyperbaric condition, and toxicity, as well as its effect on brain electric activity, is still an open problem that requires further research.

Pioneering works on the subject were published in the 1990s [3], but the literature is not very rich, and only more recently additional studies have emerged due to the use of modern and sophisticated technologies [1,4]. In this context, electroencephalography (EEG) has become a suitable tool for studying the temporal dynamics of brain activity thanks to its high temporal resolution (millisecond time scale). The application of EEG spectral analysis is rooted in its association with different brain states reflecting mental states, physiological and cognitive processes. Quantitative EEG in the frequency domain is a well-established approach for identifying rhythms and analyzing cerebral modifications. The recorded EEG encompasses distinct rhythms, namely δ, θ, α, β, and γ, each characterized by a unique frequency range (Hz or cycles/seconds) and amplitude that reflect specific functional states. δ (1–4 Hz) and θ (4–8 Hz) are low-frequency EEG patterns that

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increase during sleep in the normal adult (deep sleep and deep relaxation or light deep, respectively). These patterns play a significant role in neurological disorders, such as those affecting consciousness. The α rhythm (8–12 Hz) is the prominent EEG wave pattern of an awake and relaxed adult, and its amplitudes decrease upon eyes opening and during movement. β rhythm (13–30 Hz) occurs during alertness, attentiveness, and specific mental efforts and, finally γ (30–70 Hz) is correlated with cognitive processes. The power spectral density (PSD) of the EEG signal is a key measure that reflects its frequency content, enhancing our understanding of the brain underlying dynamics. Some studies have been conducted to assess the effects of pure oxygen assumption on EEG brain activity under normobaric conditions, while others have been carry out at specific pressures. In 2007 Seo and colleagues [5] used EEG and the relative spectral analysis to assess the effect of 35% O_2 in normobaric condition on cognitive performance and brain activity. They observed a decrease in β and γ power, an increase in δ power in the left and right hemispheres, and a reduction in α power in the left side of the brain [5]. However, hyperoxia, characterized by an excess of oxygen supplied to the tissues and organs, has also shown contradictory results in the underlying neuronal activity [6,7]. In 2008, Kaskinoro and colleagues conducted a study on the effects of normobaric oxygenation on brain activity and observed an increase in total power in the slow δ and θ frequency bands. However, it should be noted that these alterations were not statistically significant [6]. More recently, in 2017, Sheng and colleagues investigated the influence of normobaric hyperoxia on neural electrophysiological activity in the brain in resting state and during the performance of a visual task. The results suggest that hyperoxia, via inhalation of O_2 or O_2-enriched gas, has a pronounced effect on neural activity. Specifically, neural activity showed a decrease in the α and β band power under hyperoxic conditions. For task-evoked neural activity, hyperoxia resulted in a slower appearance of event-related potential (ERP) signal peaks compared to normoxia [7].

Hyperoxia can occur in either normobaric or hyperbaric conditions. Although neural activity can change in normobaric hyperoxia, as previously described, it is important to say that this alone will not lead to central nervous system (CNS) oxygen toxicity. In contrast, the risk of CNS oxygen toxicity is present in hyperbaric hyperoxia [8]. The impact of pressure on brain rhythms becomes especially notable when simulating high depths, and the severity of CNS toxicity is contingent on both the partial pressure of oxygen (PO_2) and exposure time. In the context of hyperbaric conditions, human tolerance to oxygen in dry dives is higher than in wet dives, and symptoms can range from nausea to convulsions at PO_2 above 1.4 atmosphere absolute pressure (ATA) in an immersed setting. Oxygen-induced convulsions have not been reported in humans below PO_2 levels of 1.3 ATA, but susceptibility to toxicity can vary between individuals [9]. For example, Rostain and colleagues [3] found that during a dive to 450 m seawater with the helium–nitrogen–oxygen gas mixture, there was a decrease in α frequencies around 100 m and an increase in θ frequencies in the frontal area around 200 m. Similarly, pressure can also affect somatosensory evoked potentials. A shorter latency of peaks following initial cortical P1 has been linked to a state of hyperexcitability in the brain [10]. The interaction between hyperoxia and hyperbaric pressure is complex, exerting unique impacts on physiological processes. Visser et al. [11] observed a similar trend to the study by Kaskinoro et al. [6], but in a hyperbaric environment.

They described the effects of hyperbaric hyperoxia (2.8 bar pure oxygen for 30 min) on quantitative EEG observing an increase in total power due to equal increases across all main frequency band spectra. Our previous findings support this, demonstrating that exposure to hyperbaric pressure and hyperoxia, via inhalation of pure oxygen, has a pronounced effect on neural activity in healthy divers. This included an increase in α and β power band and a decrease in δ of spontaneous neural activity [2,4]. Very recently, Vrijdag and colleagues evaluated the effects of hyperbaric oxygen on psychometric performance, EEG, and also task load perception in human participants. They found that while hyperbaric oxygen did not cause changes in EEG global efficiency or psychometric test results, it did cause a reduction in default-mode-network (DMN) complexity (i.e. the measurement of the variability in the repetition of EEG states within the DMN of the brain) and task load perception. The DMN complexity analysis quantifies the complexity of the time-evolution of brain patterns, and a decrease in entropy (complexity) suggests a decrease in the diversity of evolution of brain states, typically a loss of slowly-evolving states. However, the results of the study demonstrate that hyperbaric oxygen does not cause similar narcotic EEG effects to those induced by hyperbaric nitrogen, but produces a disturbance in the time evolution of EEG patterns as evidence of early oxygen-induced cortical hyperexcitability [12].

Despite some contradictory results that may be primary due to different experimental conditions, some indirect confirmations of our previous results [2,4] can be found in studies examining the effects of hypoxia, i.e. low oxygen levels. During hypobaric hypoxia, which simulates high altitude, there is a significant decrease of brain activity in 10–11 Hz range, suggesting suppression of α activity [13,14]. These findings highlight the importance of considering both the duration of hyperoxia exposure and the pressure when investigating the effects of oxygen on neural activity. Moreover, it is crucial to understand the effects of hyperoxia on brain function, even in the absence of clinical symptoms, such as seizures.

The studies introduced so far have shed light on the impact of oxygen on brain function, especially in the context of hyperoxia. Concurrently, high-pressure neurological syndrome (HPNS) poses a significant challenge in deep diving, leading to different neurophysiological changes, including modifications in EEG patterns and evoked potentials. However, it is crucial to clarify that the HPNS is not an oxygen-induced phenomenon; rather, it is mediated by high pressure and helium. HPNS occurs when the pressure exceeds 150 meters in depth and manifests through symptoms such as nausea, ataxia, and tremors when breathing the helium-oxygen (heliox) mixture [15,16]. The EEG changes are typically characterized by a marked reduction of α waves in the posterior region and an increase in the slow waves, such as θ and δ, in the anterior derivatives [17,18]. This is in contrast to the changes caused by oxygen intake, but it is observed only at very high pressures, such as those reached by [18] at a depth of 302 m.

While some research has been conducted on brain rhythm changes in simulated environments [2,12], there is still limited understanding of functional brain connectivity (FC) in extreme conditions. FC refers to the interactions between brain regions and reflects the temporal synchrony of neurophysiological responses within a network, providing insight into cortico-cortical interactions [19]. This approach is a useful tool for understanding brain reorganization during a task or following certain events. On the contrary, by analyzing resting-state EEG data, researchers can gain insight into functional connections between different brain regions and how these connections change over time. An effective approach in this case is the frequency domain analysis [20]. This method typically involves measuring linear dependence between fluctuations in activity recorded from different brain regions, with coherence and phase synchronization commonly used to interpret these relationships as “connectivity” between locations [21]. Compared to other methods, lagged linear connectivity (LLC) is a powerful measure of connectivity that can provide accurate and detailed information on the interdependence of resting state EEG sources [22]. This measure is advantageous over other connectivity metrics because it excludes zero phase contributions, and is robust to the effects of volume conduction.

Only a few studies have investigated FC in healthy subjects in simulated deep see-environments [1,23], and to the best of our knowledge, no studies have provided evidence of FC alterations within and/or between multiple brain networks in oxygen-intolerant subjects.

The study aims to uncover the neural underpinnings of hyperoxia by investigating the differences in EEG oscillatory activity and FC between professional divers (PD) and professional divers with a history

of oxygen toxicity (OPD), providing new insights into the effects of oxygen toxicity on brain function. Moreover, the broader objective of demonstrating the effectiveness of EEG as a method, even in individuals experiencing issues during hyperbaric oxygen breathing, is crucial. This study aspires to contribute to the construction of a database that can offer indications of the diver's state. The selected method, connectivity analysis, provides richer information compared to other analytical approaches. It allows the assessment of how different brain regions are active and connected during various EEG frequency rhythms. This comprehensive evaluation improves our understanding of the complex dynamics within the brain, especially during hyperoxic conditions, and underscores the significance of the chosen methodology in achieving our research objectives.

2. Material and methods

2.1. Participants

Eleven healthy professional divers (PD, age range 38–53 years) [2] and four professional divers with a history of hyperoxic episodes (OPD, age range 26–30 years) were recruited. For the PD exclusion criteria comprised a medical history of respiratory problems, sleep disturbances, smoking, and overweight. All participants were skilled divers with a minimum of 15 years of experience. They had undergone Oxygen Tolerance Tests (OTTs), and none had ever displayed signs of CNS toxicity, whether during an OTT or operational diving involving 100% O_2, nitrox, or other gas mixtures. Four PD subjects were part of the Operational Divers Group, specializing in free diving with air up to 60 msw, nitrox up to 54 msw, heliox up to 150 msw, and diving to 250 msw with a mini-submarine or a special suit. Moderately experienced divers in the group held licenses for diving up to 60 msw.

Regarding the OPD, OPD-1 (Height: 182 cm, Weight: 76 kg) had a history of a hyperoxia crisis, which refers to a hyperoxic seizure characterized by tonic-clonic contractions during hyperbaric oxygen inhalation. In OPD1's clinical history, there has been no occurrence of convulsive episodes resulting from traumatic or febrile events. OPD1 had been breathing oxygen (O_2) for three months, 4 to 5 h per week, at a depth of 3 m (1.3 ATA) for approximately two years. The hyperoxic seizure experience reported in the study was a singular event during hyperbaric oxygen breathing. OPD-2 (Height: 182 cm, Weight: 70 kg) had also breathed O_2 for three months, 4/5 h per week, at a depth of 3 m (1.3 ATA), but this activity was performed sporadically for one year. He had experienced two episodes of headache after breathing O_2 at 3 m depth. The intensity of the headache during the second episode was greater than the first and, in the second episode, had experienced right ear pain with erythematous but intact tympanic membrane. After the second episode, he was hospitalized for two days. Cranial computer tomography was normal, with no signs of intracranial or extracranial hemorrhages. After 11 days from the second episode, he underwent an EEG test while breathing O_2 at 2.8 ATA, which showed abnormal reactivity. During breathing O_2 at 2.8 ATA, a lack of α band activation was observed, with increased θ band activity, particularly on central derivations. The presence of δ activity was observed in the posterior area, and it increased during the first and second minutes of the decompression period, also spreading to the anterior derivations. OPD-3 (Height: 175 cm, Weight: 70 kg) had breathed O_2 for three months, 4/5 h per week, at a depth of 3 m (1.3 ATA) sporadically for four years and experienced a loss of consciousness and inhaled water, presumably related to hyperoxia crisis. Our confidence in attributing OPD-03's blackout to CNS-OT is reasonable, given the antecedent occurrence of tonic-clonic contractions, especially in the upper limbs. OPD-4 (Height: 176 cm, Weight: 80 kg) had also breathed O_2 for three months, 4/5 h per week, at a depth of 3 m (1.3 ATA) sporadically for three years. He had a history of experiencing nausea and dizziness after diving with O_2, and at times, he had more severe vertigo symptoms with occasional muscle twitching. This diver experienced episodes of nausea, dryness of the mouth, and severe vertigo with muscle twitching, especially in the limbs, after hyperbaric oxygen breathing. These episodes, believed to be associated with hyperoxia, were accompanied by tonic-clonic muscle contractions, albeit not of severe intensity, and a slight clouding of consciousness. We are confident that the observed changes in the EEG spectrum are attributed to hyperbaric oxygen breathing, as they occurred during oxygen inhalation and subsided during breathing ambient air.

2.2. EEG data acquisition

A thirty-two channel EEG was recorded using a Bluetooth system (EBNeuro S.P.A., Verona, Italy) [2,4]. The recording lasted 20 min and was conducted in a hyperbaric chamber under three different conditions: (i) baseline at sea level with an open chamber (AIRpre); (ii) simulated depth of 18 msw with 2.8 ATA and oxygen breathing after 2-min of compression stage (O_2); (iii) post-decompression at 1 ATA and breathing air again (AIRpost) (Fig. 1).

The participants lay down on a cot with their eyes closed while their brain activity was recorded, with an expert in neurophysiology responsible for identifying any abnormal event. The research program has been approved by Igesan (Direzione Generale di Sanità Militare) and the Stato Maggiore and their internal ethics-committee (approval Number: cp 1322 (L. 023)). Informed consent was obtained from the subjects, according to the ethical standards of the Declaration of Helsinki [24].

2.3. EEG source imaging

The EEG data were analyzed as reported in [1]. The EEG data were pre-processed using Matlab (MathWorks, Natick, MA), EEGLAB (https://sccn.ucsd.edu/eeglab/index.php) and a custom-made code. Then, a distributed source model, the standardized low-resolution brain electromagnetic tomography (sLORETA; http://www.unizh.ch/keyinst, [25]) method, was used for estimating brain electrical sources.

The EEG recordings were band-pass filtered from 1 to 30 Hz (the optimal Chebyshev finite impulse response filters were designed using Parks–McClellan algorithm, the order was customized to minimize the error in the pass and stop bands), and any visible artifacts were removed using independent component analysis (FastICA algorithm). The data were then re-referenced to a common average reference. The PSD of the scalp EEG signal was computed by Fast Fourier Transform. This allowed for a detailed analysis of the frequency distribution of the EEG signal.

sLORETA [26] was applied to 2-s EEG epochs for each frequency band (δ: 1.5–6 Hz, θ: 6–8 Hz, α: 8–12 Hz, β_1: 12–18 Hz, β_2: 18–21 Hz, β_3: 21–30 Hz) for each subject, under the three conditions. The Montreal Neurological Institute brain template (MNI152) [27] was used as a realistic head model, and the solution space was restricted to the cortical gray matter.

A lagged linear connectivity [28] analysis was used to calculate functional connections between different regions of the brain. The analysis was performed in several frequency ranges, and 42-Brodmann areas (BAs) in each hemisphere were selected as Regions of Interests (ROIs) for the connectivity analysis. ROIs were grouped according to their functional roles: somatosensory, motor, executive, emotional regulation, memory, attention, sound, visual, olfactory, and not well studied [29]. Connectivity contrast maps were created by comparing the connections between the different ROIs using t-statistics, and a significance threshold of $p < 0.05$ with a permutation test of 5,000 permutations. The significant connections were then plotted onto an MRI template.

The interpretation of scalp FC results in EEG studies is often limited by several factors. Firstly, not all electric sources of the brain are radially oriented towards the scalp, which causes issues with localization. Secondly, the choice of EEG reference (average or bipolar) can affect

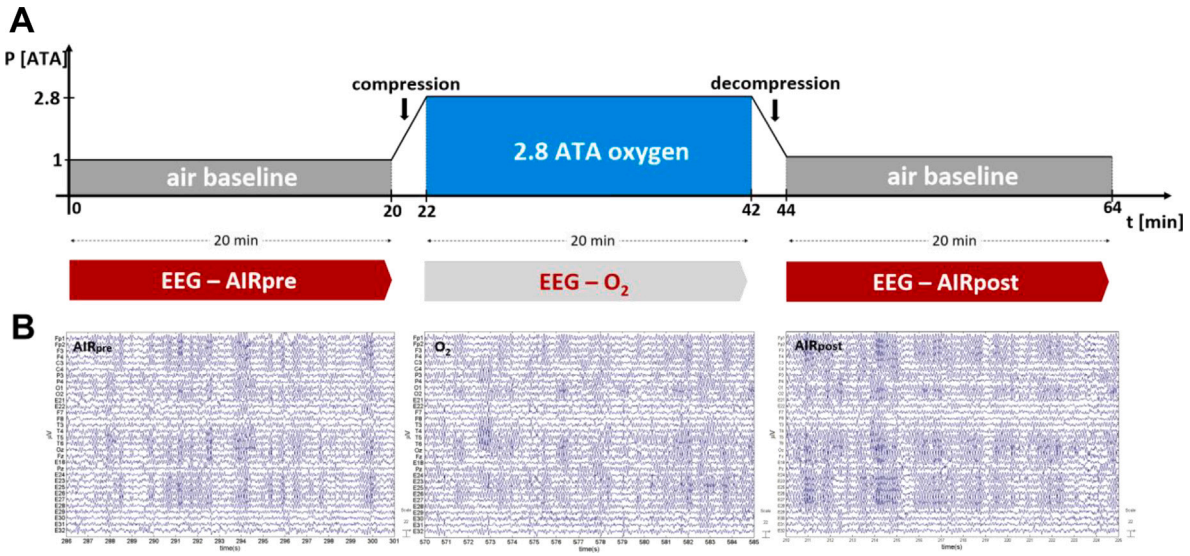

Fig. 1. (A) Dive profile and recording sessions. A 20-min baseline EEG recording was made at 1 ATA breathing air in the open hyperbaric chamber. In the closed chamber, a 2-min compression profile (descent rate 9 m min^{-1}) breathing air was used to reach the next stage at a pressure of 2.8 ATA (compression). At this pressure, the subject breathed pure oxygen via an oronasal mask and a 20-min EEG was acquired. After decompression, back on air breathing, the EEG of each subject was recorded for 20 min, discarding the first 2 min (ascent rate 9 m min^{-1}) (decompression). (B) The bottom row represents the EEG recordings at each exposure in OPD-3: air (AIRpre), oxygen breathing (O_2), and end of air breathing after decompression (AIRpost).

the measured surface potential. Lastly, volume conduction problems can lead to non-physiological FC values due to zero phase lag between data time series. To address these limitations, a source model has been adopted that solves the inverse problem from scalp EEG to cortical source distribution independently of the reference electrode's position. This approach provides a more accurate estimation, enhancing our understanding of the functional connectivity between different brain regions [30,31].

3. Results

3.1. Power spectral density results

The power in the α band remained higher in OPD compared to PD subjects in all three conditions (Fig. 2). The main difference was observed during AIRpre over fronto-central, central-parietal and occipital electrodes. The same, albeit less pronounced, pattern persisted during the O_2 and AIRpost conditions. This is due to the fact that PD showed increased α band power in these two conditions, leading to smaller differences between the two groups.

3.2. Functional connectivity results

We examined the FC between ROIs in PD and OPD under simulated deep-water conditions. In Fig. 3 one can see the mean FC for each condition across participants, with PD group marked by a green box and OPD group marked by a red box. On the other hand, Fig. 4 displays the significant FC connections identified though group comparisons.

The first interesting and unexpected finding is that connectivity analysis reveals a strong difference between PD and OPD, during air breathing in an open hyperbaric chamber (AIRpre). α band activity is significantly higher in oxygen-intolerant subjects compared to healthy professional divers (Fig. 2, Fig. 3), indicating strong alterations that involve the emotional regulation network and the visual, sound, olfactory, somatosensory networks (Fig. 4).

As seen in Fig. 3, α remains altered between the two groups ($p < 0.05$) even during the pure oxygen intake phase minutes 2 and 5, although to a lesser extent. This means that the behavior of α in PD is highly responsive to the uptake of oxygen. Significant differences were also observed in beta 3 (minutes 10–11) and delta (minutes 18–20). Conversely, it is evident that this frequency band in OPD maintains a relatively stable pattern between the baseline, the oxygenation phase, and the baseline after the decompression stage, as demonstrated by the average connectivity maps. Fig. 1 provides an example of EEG recordings from subject OPD-3 for the three conditions, which visually demonstrates the stability of the activity over the time and across conditions. At a glance, it is evident that the α wave, for instance, persists and is broad in all three recordings.

The difference in α band between the two groups becomes less pronounced in the first few minutes of recording (at minutes 2 and 5), although some significant differences remain. As previously demonstrated, the activity of PDs exhibits a strong response in the α frequency band, with a significant increase observed between the AIRpre and oxygen phases.

The connections ($p < 0.05$) that showed a significant difference between the two groups (OPD > PD) include those between the somatosensory and not well-studied networks in the 2nd minute of EEG recording during oxygen inhalation, as well as connections between the memory and executive, and memory and visual areas at the 5th minute (Fig. 4). Even the δ (minutes 18–20) and β_3 (minutes 10–11) bands showed few significant alterations ($p < 0.05$) during the oxygen phase. Mostly, the connections between the motor and executive networks were greater in oxygen-intolerant subjects in the minutes from 10th to 11th during oxygen intake for the β_3 band. In the minutes from 18th to 20th between the executive and not well-studied network, there was an increase in connectivity in the intolerant subjects for the δ band. During the 1 ATA air-breathing phase post-decompression (AIRpost), the differences between the two groups become less pronounced, and no significant difference is observed in the α band. The only significant difference found was observed between minutes 15 and 17 in δ band, where an increase in connectivity between PD and OPD between the visual and executive regions was recorded. This difference was statistically significant according to the permutation test ($p < 0.05$).

4. Discussion

One of the key contributions of this study lies in the exploration of neurophysiological activity in subjects experiencing oxygen toxicity — an area of research that is both rare and underexplored [32].

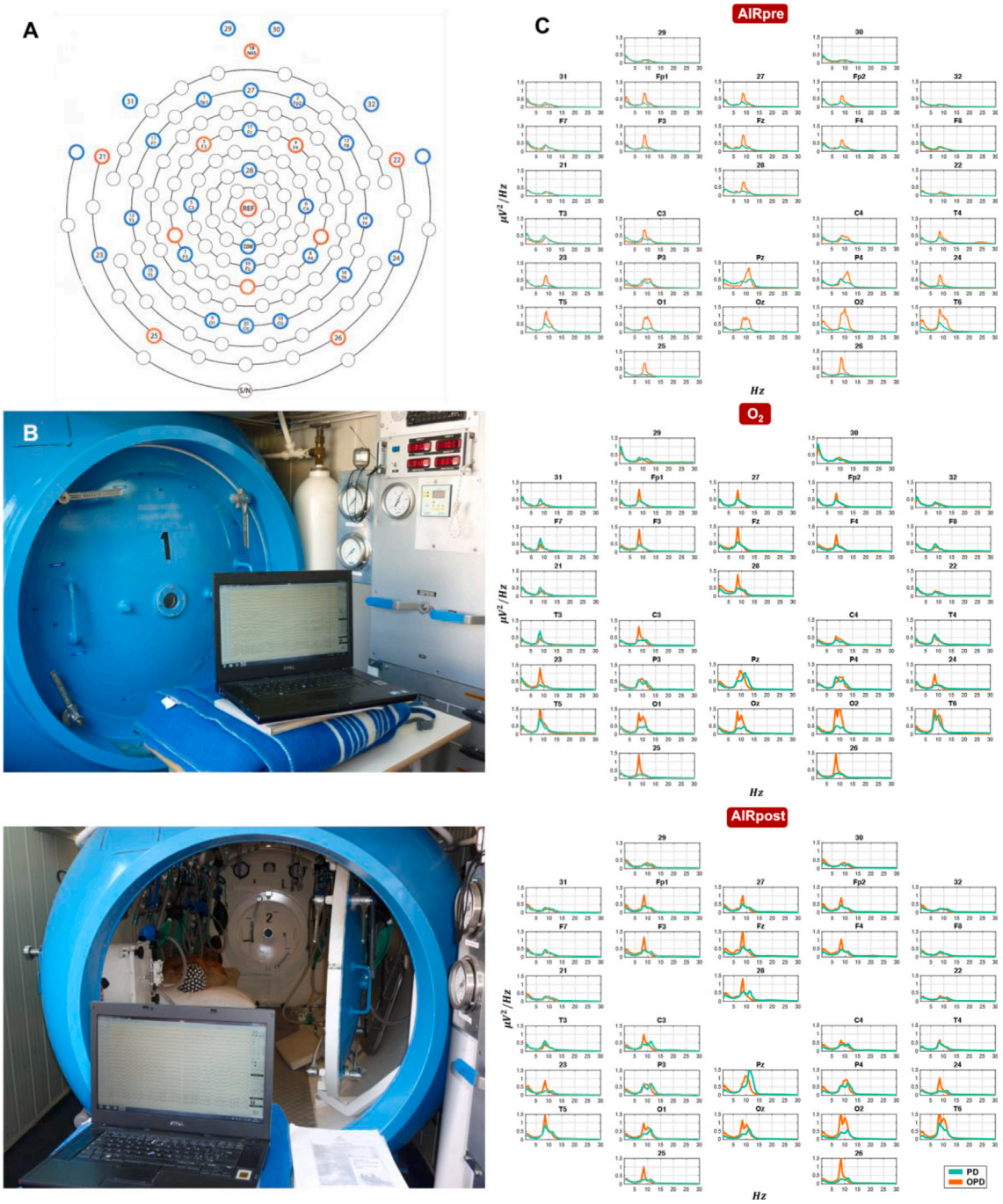

Fig. 2. A. Configuration of the 32-channel HydroCel Geodesic Sensor Net Map. Each blue and red sensor corresponds to an amplifier channel, while the white sensors serve as stabilizer electrodes. B. Experimental Setup. The participant reclines on a cot with closed eyes. The EEG system establishes a wireless connection to a laptop positioned outside the hyperbaric chamber, minimizing signal interference. The EEG recording system is strategically located near the porthole, the sole point enabling signal transmission. C. PSD [μV/Hz]. Average PSD over a 20-min recording period in three conditions: air, oxygen breathing, and air after decompression. Green corresponds to PD, and red to OPD.

Conducting experiments in a hyperbaric chamber allowed us to reveal mechanisms not observable in normal laboratory conditions. This approach provides unique insights and presents significant technical challenges, such as recording inside a Faraday cage and using equipment powered by direct current to avoid sparks. However, we overcome these limitations by using Bluetooth technology, enabling the recording of biological signals inside a hyperbaric chamber [4].

This study reports for the first time EEG brain connectivity results derived from professional divers with a history of oxygen toxicity under simulated deep-sea conditions. Our results revealed significant differences in functional brain networks between PD and OPD. Connectivity analysis showed changes in brain activity during hyperbaric oxygen exposure, suggesting that prolonged oxygen breathing and deep-sea diving may have altered the baseline state and affected their typical response to oxygen intake. Specifically, the results revealed a marked difference in baseline levels of α between PD and OPD, with PD exhibiting a lower level to counteract the effects of increased O_2 inhalation [1]. In comparison, OPD displayed a higher level that resulted in a pathological state. Moreover, connectivity analysis revealed a strong correlation between cognitive and somatosensory regions, along with high levels of α synchronization at rest in OPD. Our findings suggest that a pathological condition may be responsible for the higher levels

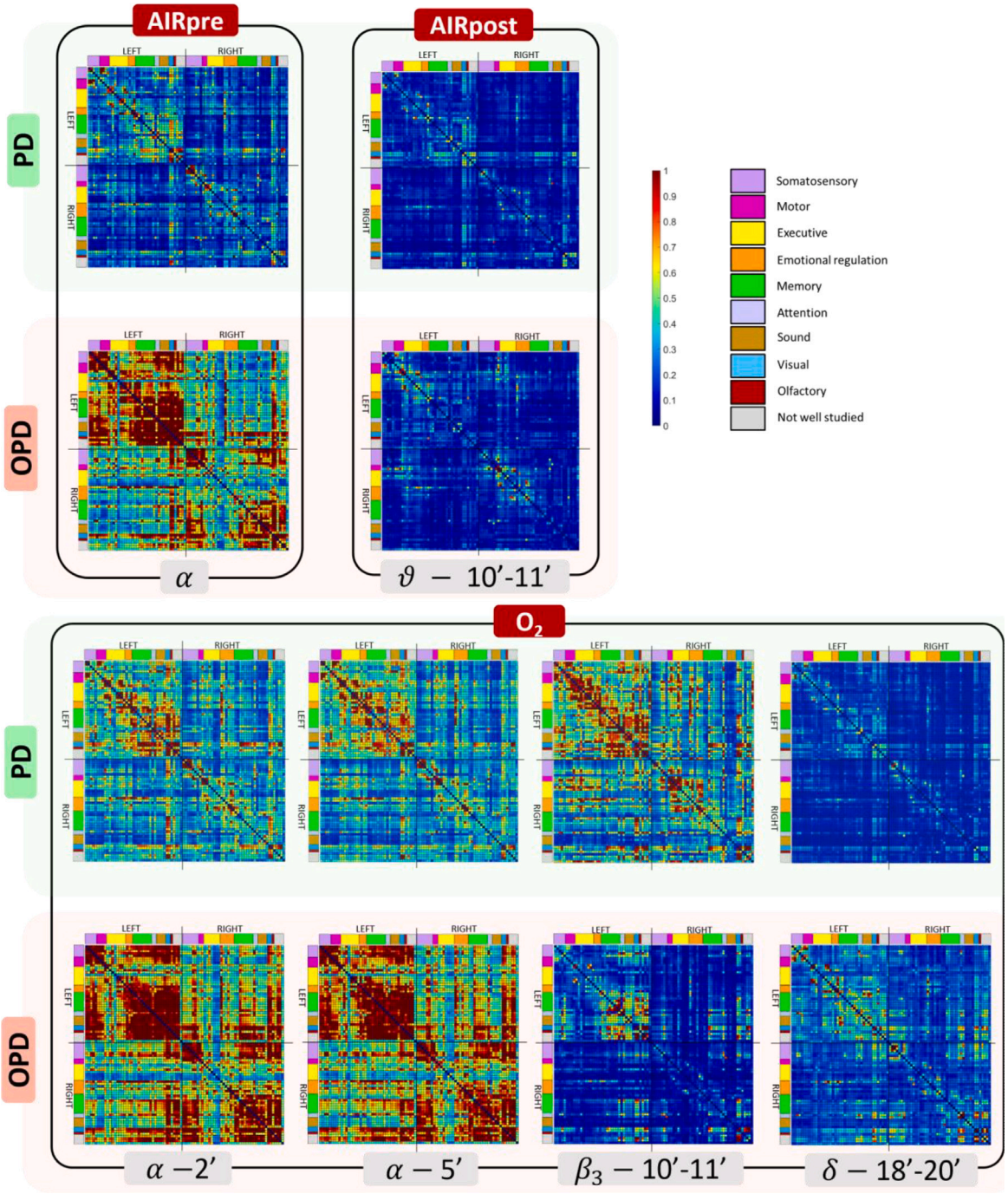

Fig. 3. Functional connectivity in AIRpre (top-left), in O_2 minute 2, minute 5, minutes 10–12, minutes 18–20 (bottom), and in AIRpost (top-right) averaged across subjects: PD (group highlighted by a green box) and OPD (group highlighted by a red box) and shown only for the conditions and minutes identified as significant by the statistical test. Brodmann areas are classified according to their functional role, represented by colors and highlighted in the legend on the side. The colorbar indicated on the side is common to all matrices.

of α observed in these individuals when facing the stress of high O_2 inhalation.

Although the small sample size (four oxygen-intolerant subjects) may limit the statistical power of analysis, it is important to note that obtaining such data is very rare. So far, in our previous studies, we have only been able to examine physiological changes in healthy professional divers. In these works, we observed a marked increase in the amplitude of the α and β sources in parietal and occipital areas, and a decrease in the amplitude of the δ and θ sources in the occipital area during and after oxygen breathing [4]. Furthermore, a disconnection of α and upper β frontal-parietal links has been observed in the early minutes of O_2 breathing [33].

Although some studies have investigated neurophysiological patterns, no data has been gathered yet on individuals with a history of oxygen toxicity. There is a main reason for this lack of research. This kind of screening on military divers is not commonly conducted, and our center in Italy (Comsubin) is one of the few that have undertaken such studies and focused specifically on this issue. These new data collected over years of observation by the medical team of the Italian Navy, although few and rare, could prove to be extremely valuable

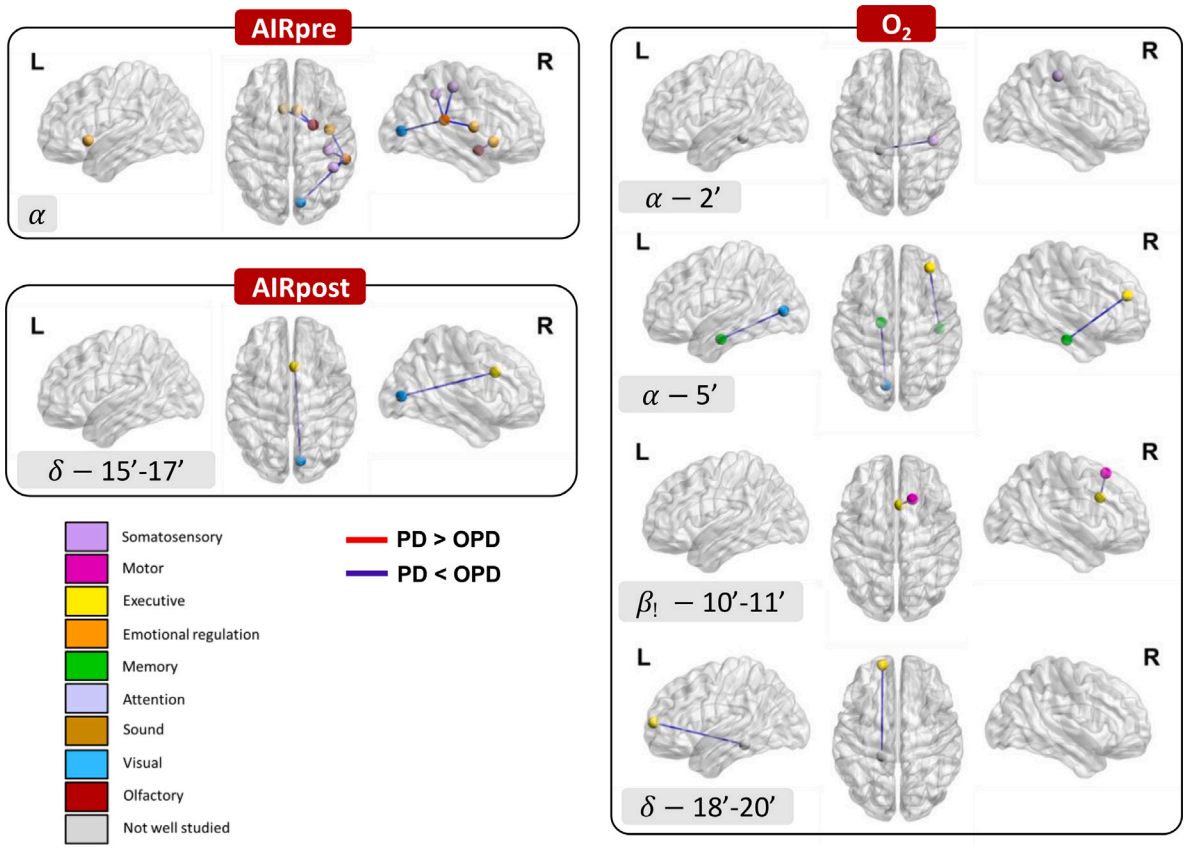

Fig. 4. Significant differences between groups in functional connectivity links (PD > OPD: red color; PD < OPD: blue color). The brain figures were visualized using the BrainNet Viewer (http://nitrc.org/projects/bnv/). The nodes were extracted from the BA template and the ROIs are highlighted in the legend on the side.

because they could serve as a basis for developing a standard, low-cost, and preventive screening.

Previous observations revealed an amplitude increase of α sources in non-expert divers compared to professional divers [34]. We explained this difference by the "neural efficiency" hypothesis [35], which suggests that experts exhibit more efficient cortical function in cognitive and sensorimotor tasks. A previous study has also identified a reduced cortical activity among experts before engaging in tasks that require high precision. This finding has been interpreted as an indicator of selective event- or task-related cortical activation [36]. The behavior we found here, however, presents a contrast between a very low level of α synchronization in professional divers and a high level of α in divers with pathological conditions at baseline.

The α rhythm, dominant during rest periods in human EEG, is typically observed when the eyes are closed, mainly in the posterior area of the scalp. When the eyes are opened, α power suppression occurs, which can be interpreted as a top-down activation process [37]. This basic finding, dating from the earliest work in electroencephalography, demonstrates that α suppression, in terms of desynchronization, may primarily reflect top-down sensory processing. α event-related desynchronization seems to be associated with activated cortical networks related to information processing [38], selective attention [39], and motor preparation [40]. In patients with dementia, the α frequency band spreads to the frontal regions [41], indicating that these areas play an important role in higher cognitive functions, which are impaired in this pathological condition. In healthy individuals, during the resting condition, the α rhythm remains confined to the posterior regions, signaling that the frontal regions are normally active.

Our results show an increase in α power in OPD subjects, already during AIRpre evaluation. This activity is concentrated in central regions associated with emotional regulation and characterized by many functional connections. This pattern suggests that OPD subjects may experience emotional difficulties in achieving cognitive-behavioral goals, as they may be less efficient in specific cortical functions. Moreover, PD subjects may have developed greater adaptation to the new conditions compared to OPD subjects, reflected by α desynchronization, while OPDs have remained in their initial state. Future studies could explore this hypothesis by administering psychological tests to highlight the emotional difficulties affecting cognitive-behavioral goals for these individuals.

Our findings are supported by the work of Grabot and Kayser [42]. Their research shows a connection between spontaneous α brain activity and the level of bias in human choices during a time perception task. This suggests that α power is an index for the cognitive effort required to overcome an individual's idiosyncratic bias.

The modulation of α in OPD differs from other conditions, as it remains high in the first few minutes of hyperbaric O_2 breathing and then decreases in the last few minutes. This highlights how the CNS functions differently depending on initial conditions, as seen in EEG patterns observed in professional divers, non-expert divers, and oxygen-intolerant professional divers when exposed to stimuli such as hyperbaric O_2.

Oxygen toxicity involves the formation of reactive oxygen species (ROS) during oxidative damage to cell membranes. Free radicals, generated during normal metabolism, can cause harm if not neutralized by antioxidants. The severity of damage depends on the balance between ROS formation and elimination by antioxidants. Oxygen toxicity is influenced by both PO_2 and the duration of O_2 exposure, with studies showcasing increased ROS and lipid peroxidation using HBO_2 exposure of pressure $\geq$ 3.0 ATA [43].

The brain, susceptible to lipid peroxidation, is at risk due to its limited natural defense against free radicals and high oxygen consumption–markers like F2-isoprostanes aid in evaluating potential oxidative damage in the brain. Alterations in brain activity, particularly in oxygen-rich environments, may escalate ROS production, leading to lipid peroxidation and oxygen toxicity. Thoughtful oxygen exposure management is essential for risk mitigation and introducing breaks between treatments improves oxygen tolerance and safety.

Despite extensive animal research, understanding oxygen toxicity in human diving remain incomplete. The time of symptom onset depends on both PO_2 and time, with symptoms appearing sooner at higher PO_2 [44]. Prediction models for estimating the risk of oxygen toxicity exist, but their validity in human still needs to be tested. A prediction model for assessing the risk of oxygen toxicity in closed-circuit rebreather dives was first published by Harabin et al. in 1995 [45] and later refined by Arieli et al. in 2002 [44] based on a larger dataset of 2,039 dives, making it the most accurate model to date. The model uses two variables, PO_2 (in kPa) and dive time (in min), to estimate the Z-score of a normal distribution. The equation, which considers PO_2 and dive time as variables, estimates the Z-score of a normal distribution: $Z = \frac{[\ln(t)-9.63+3.38\cdot\ln(PO_2)]}{2.02}$. The recovery time for a diver to neutralize oxygen stress is estimated based on experiments conducted on rats. However, no studies have tested the validity of these models in humans.

Despite the sound methodology of the model, a significant amount of intra- and interpersonal variability in oxygen toxicity persists. The "oxygen tolerance test", which exposed subjects to 100% oxygen at 2.8 ATA for 30 min, was once advocated to identify military divers at risk, but has proven ineffective and been discontinued by many navies [11,46,47]. Similarly, the "Read test" for CO_2 has also been found ineffective [48]. Although diver's ability to detect CO_2 can be trained, it is unknown if this reduces the incidence of oxygen toxicity [49]. To date, no valid test exists to screen for oxygen tolerance [9].

In 2017 Wingelaar et al.highlighted that while the underlying mechanisms and risk factors are not yet fully understood, there is a clear correlation between PO_2 and time [9]. Recommendations for PO_2 limits in sports diving range from 1.4 to 1.6 ATA, while military special operations forces (SOF) diving limits vary according to equipment and acceptable risk. The Arieli model predicts the risk of CNS toxicity with a maximum exposure of 24 min at 2.5 ATA and a 5% risk. However, these high-risk exposures are only taken under specific circumstances and with proper training and equipment. Wingelaar et al. note a lack of incidence reports using the Arieli model, which may be due to the covert nature of SOF diving [9].

Our findings suggest that oxygen toxicity significantly impacts brain FC. The observed neural reorganization during oxygen assumption, detected by EEG, presents new opportunities for screening and monitoring strategies for OPD. Future studies, particularly longitudinal ones, will be crucial in understanding of how brain networks change over time and in identifying the underlying factors of these changes. Additionally, our experiments have provided a valuable opportunity to study the modulation of neurophysiological activity in simulated critical conditions before applying these findings to real-world scenarios. Our research has revealed a potential non-invasive approach for identifying distinct diving patterns and assessing the oxygen-related risk during a dive by identifying specific EEG implications.

5. Strengths and limitations

Our study, like any research pursuit, is not without its constraints. Specifically, the sample size is modest, comprising only four oxygen-intolerant subjects. This could limit the statistical power of the analyses. Nevertheless, it is crucial to note the rarity of obtaining data from individuals with a history of oxygen toxicity, contributing valuable and unique data within the confines of the limited existing research in this field. In addition, CNS-OT is a complex syndrome that appears with many symptoms, and the correlation between EEG rhythms changes and each symptom is difficult to obtain.

In the future, with increased awareness in the scientific community and a more in-depth study of CNS OT cases, it may be possible to identify those cases with unambiguous symptoms (i.e., monosymptomatic). Subsequent analysis of high-density EEG data could then facilitate precise localization of the brain area associated with specific EEG rhythms. It should be noted that none of the participants in our study manifested clinical signs of CNS-OT during or after the exposure. Consequently, our observations were limited to neurophysiological alterations in the EEG. We are also aware that nuanced effects arising from various factors may have influenced the results obtained. Factors that include individual variability, physiological adaptations, and other environmental influences have the potential to provide a nuanced interpretation of our findings. This highlights the need to interpret our findings with caution, mainly when exclusively attributing the observed EEG changes to CNS-OT [4]. Innovative in its methodology, this work uses Bluetooth technology to record biological signals inside a hyperbaric chamber. Although this approach is innovative, it does bring about specific technical constraints that should be recognized.

Furthermore, a significant challenge in this study, as well as in the broader field of diving physiology, is the need of a universally validated test for screening oxygen tolerance. This ongoing challenge underscores the complexity of assessing such physiological parameters and highlights the need for continued exploration in this domain.

6. Conclusion

Our findings indicate that α activity could be a valuable indicator for identifying individuals at risk of oxygen intolerance. To further validate these results and gain a deeper understanding of the effects of hyperbaric oxygen on the brain, it is essential to engage the scientific community in collecting new data using EEG techniques, which are cost-effective and portable. The limited and conflicting data currently available call for further research to develop a predictive model of brain neurophysiology under hyperbaric oxygen conditions. Functional connectivity studies, in particular, could significantly contribute to understanding the complex dynamics of brain activity in response to hyperbaric oxygen.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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