Sensation, Perception, and Cognition in Patients with Completely Locked-In Syndrome (CLIS): First experimental steps towards a psychophysiological description

Dissertation

der Mathematisch-Naturwissenschaftlichen Fakultät

der Eberhard Karls Universität Tübingen

zur Erlangung des Grades eines

Doktors der Naturwissenschaften

(Dr. rer. nat.)

vorgelegt von

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Tübingen

2020

Gedruckt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Eberhard Karls Universität Tübingen.

Tag der mündlichen Qualifikation:	02.03.2021
Stellvertretender Dekan:	Prof. Dr. József Fortágh
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Abbreviations

AEP	Auditory evoked potential
ALS	Amyoterapic lateral Scelorosis
BCI	Brain-Computer Interface
CLIS	Completely Locked-In Syndrome
DoC	Disorders of Consciousness
EEG	Electroencephalography
EOG	Electrooculugraphy
fMRI	Functional Magnetic Resonance Imaging
fNIRS	functional Near-Infrared Spectroscopy
GCS	Glasgow Coma Scale
GUI	Graphical User Interface
GUI	Graphical User Interface
HCI	Human-Computer Interface
LG	Local-Global
LIS	Locked-In Syndrome
MCS	Minimally conscious state
MND	Motor Neuron Disease
OOP	Object-Oriented Programming
SEP	Somatosensory evoked potential
UWS	Unresponsive wakefulness state

Summary (German)

Menschen, welche an einem Komplett-Eingeschlossenem-Syndrom (CLIS, completely locked in syndrome) leiden, das vor allem im Endstadium der Amyotrophen Lateralsklerose (ALS) eintritt, haben keinerlei Möglichkeit einer Kommunikation, da alle motorischen Outputkanäle gelähmt sind, einschleisslich Augenmuskel. Daher ist auch keinerlei subjektive oder verhaltensbezogene Messung von Bewusstseinsvorgängen möglich und es bleibt unklar, ob und auf welchem Niveau Bewusstsein ,Denken und Emotionen existiert. Die einzige Möglichkeit der Kommunikation und damit eines Kontaktes ist über sogenante Brain Computer Interfaces (BCI) möglich, bei denen der /die Patient(in) mit der eigenen Gehirntätigkeit einen Computer ansteuern kann. Da diese Patienten selten untersucht wurden, gibt es fast keinerlei gesicherte Erkenntnisse über ihre verbliebenen kognitiven Fertigkeitrn und Bewusstseinsvorgänge. In der vorliegenden Dissertation werden über einen längeren Zeitraum wiederholt mehrere Patienten in CLIS sowohl mit BCI wie auch mit neurophysiologischen Massen untersucht, um Erkenntnisse über etwaige Bewusstseinvorgänge zu gewinnen. Es handelt sich um eine explorative, Hypothesen generierende wissenschaftliche Arbeit, die eine systematische Erforschung dieses gravierenden klinischen und psychologischen und ethischen Problems anregen soll. Den Patienten wurden einfache und komplex sensorische und kognitive Aufgaben präsentiert und die hirnelektrischen Antworten in Form evozierter Potentiale und Frequenzspektren des EEGs zu mehreren Zeitpunkten über mehr als ein Jahr in der häuslichen Umgebung der CLIS Patienten erhoben. Alle vier Patienten zeigten zwar episodisch eine ja-nein Kommunikation im BCI auf einfache Fragen, konnten aber keine stabile Kommunikation über den Zeitraum entwickeln. Die neurophysiologischen Resultate zeichnen sich durch extreme Heterogenität und Variabilität sowohl innerhalb wie auch zwischen den Patienten aus. Die einfache Regel, dass komplexe kognitive Aufgaben an das Vorhandensein einfacher kognitiver Leistungen gebunden sind gilt ebensowenig wie der Umkehrschluss. Selbst nach völligen Ausbleiben kognitiver Zeichen auf sensorische reize treten komplexe Korrelate auf komplex Aufgaben auf.

Die Arbeit zeigt, dass man zum jetzigen Zeitpunkt grundsätzlich vom Vorhandensein von Bewusstsein bei CLIS Patienten mit ALS ausgehen sollte, aber keine konsistente Antworten auf Fragen nach seiner spezifischen Leistungen und Komplexität erwarten darf und auch die Konstruktion eines BCIs auf die individuellen neurophysiologischen Leistungsprofile auszurichten hat.

Summary (English)

Patients with completely locked-in syndrome (CLIS) suffer from complete paralysis and lack of any communication channel but are assumed to be cognitively intact. The intactness of cognition is a deductive argument mainly based on the observations before transition to the CLIS, but difficult to prove in CLIS. In this dissertation, I describe clinical observations on the capability of the brain in such patients at a sensory, perceptual, and cognitive level, by providing evidence from behavioral and neurophysiological assessments. Behavioral assessments were performed using Brain-Computer Interface (BCI) technology in patients with amyotrophic lateral sclerosis (ALS), before and after the transition to CLIS, using invasive and noninvasive brain imagining techniques. Neurophysiological assessments were based on the observation of the brain reactivity to the presented sensory stimulus. Findings were reported in several scientific papers and revealed that the different levels of cognitive functionalities should be differentiated from the presence or absence of consciousness in unresponsive patients, it is possible that while a neural index within an experimental paradigm indicates presence of complex cognitive processing of a stimulus another measure might deny it in the same patient. Therefore assessments of cognitive function should be fundamentally differentiated from the assumed presence of conscious experiences in unresponsive patients and interpretation of the neurophysiological assessment should be limited to the measures to avoid overinterpretation of the results. On the other hand, findings revealed that although all CLIS patients have the same behavioral symptoms the neurophysiological characteristics of the patients are significantly different between them. Thus, in any attempt to achieve a BCI for communication, which is the only hope for communication in such patients, these neurophysiological differences should be addressed and built into a personal BCI system.

List of Publications

Accepted Publications

I. **Khalili-Ardali, M.**, Rana, A., Pourmohammad, M., Birbaumer, N., & Chaudhary, U. (2019). Semantic and BCI-performance in completely paralyzed patients: Possibility of language attrition in completely locked-in syndrome. *Brain and Language, 194, 93-97*.

II. Martínez-Cerveró, J., **Khalili-Ardali, M.**, Jaramillo-Gonzalez, A., Wu, S., Tonin, A., Birbaumer, N., & Chaudhary, U. (2020). Open Software/Hardware Platform for Human-Computer Interface Based on Electrooculography (EOG) Signal Classification. Sensors, 20(9), 2443.

III. Tonin, A., Jaramillo-Gonzalez, A., Rana, A., **Khalili-Ardali, M.**, Birbaumer, N., & Chaudhary, U. (2020). Auditory Electrooculogram-based Communication System for ALS Patients in Transition from Locked-in to Complete Locked-in State. *Scientific Reports, 10(1), 1-10*.

IV. **Khalili-Ardali, M.**, Wu, S., Tonin, A., Birbaumer, N., Chaudhary, U. (2020). Neurophysiology of the the completely locked-in syndrome in patients with advanced amytrophic lateral sclerosis, *Clinical Neurophysiology* (In Press).

V. Jaramillo-Gonzalez, Tonin, A., A. Rana, A. **Khalili-Ardali**, M., Birbaumer, N., & Chaudhary, U. (2020). A Dataset of an Auditory Electrooculogram-based Communication System for Patients in Locked-In State. *Scientific data*, *8*(1), 1-10.

VI. Secco, A., Tonin, A., Rana, A., Jaramillo-Gonzalez, A., **Khalili-Ardali, M.**, Birbaumer, N.n Chaudhary, U. (2020). EEG Power Spectral Density in Locked-In and Completely Locked-In State Patients: a longitudinal study. *Cognitive Neurodynamics*, 1-8.

Submitted manuscripts

VII. **Khalili-Ardali, M.**, Martínez-Cerveró, J., Tonin, A., Jaramillo-Gonzalez, A., Wu, S., Zanella, G., Corniani, G., Montoya-Soderberg, A., Birbaumer, N., Chaudhary, U. (2020). A General-Purpose Framework for a Hybrid EEG-NIRS-BCI, *SoftwareX (In revision).*

VIII. Chaudhary, U. Vlachos, I. Zimmermann, J. Espinosa, Tonin A., A. Jaramillo-Gonzalez, A. **Khalili-Ardali, M.** Topka, H. Lehmberg, J. Friehs, G. Woodtli, A. Donoghue, J. Birbaumer, N. (2020). Communication using intracortical signals in a completely locked in-patient. *Nature Communication (In revision)*.

Manuscripts ready for submission

IX. **Khalili-Ardali, M.,** Martínez-Cerveró, J., Birbaumer, N., Chaudhary, U. (2020). A dataset of Neurophysiological recordings for ALS patients in CLIS.

X. Wu, S., **Khalili-Ardali, M.**, Jaramillo-Gonzalez, A., Tonin, A., Martínez-Cerveró, J., Rana, A., Birbaumer, N., & Chaudhary, U. (2020). EEG Spectral Entropy and Power during Brain-Computer Interface (BCI) based Communication in Completely Locked-in State (CLIS).

Contribution Declaration

Nr.	Accepted	Position of candidate in list of authors *	Candidate's Contribution**
1	Yes	1st	Conceptualization, analysis, and paper writing.
2	Yes	2nd	Methodology: J.M.-C., **M.K.-A**., A.J.-G., and U.C.; Original draft: J.M.-C., S.W., **M.K.-A.,** and U.C.;
3	Yes	4th	Data analysis.
4	Yes	1st	Design of the study, data acquisition, analysis, and paper writing.
5	Yes	5th	Discussions in Lab.
6	Yes	5th	Data analysis discussion
7	No	1st	Software design, core structure implementation, and paper writing.
8	No	7th	Software development (graphical user interface).
9	No	1th	Experiment design, performing experiments, data collection, manuscript writing.
10	No	2nd	Methodology: S. W., **M. K. -A.**, A. J.-G. Investigation: S. W., **M. K. -A.**, A. J. -G., A. T., A. R., U. C. Software: S. W., **M. K.-A.**, A. J.-G., A. T., J. M.-C., U. C.

*** List of authors is presented in the above-mentioned list.**
**** As specified in the manuscripts.**

Personal Contribution

I hereby declare that I am the sole author of this Ph.D. thesis. I only made use of the cited sources and permitted resources, and marked literal or paraphrased passages as such. I declare that I abided by the guidelines for safeguarding good scientific practice (Richtlinien zur Sicherung guter wissenschaftlicher Praxis) at the University of Tübingen (conclusion of the Academic Senate of 25 May 2000). I hereby make an affirmation in lieu of an oath (Eidesstattliche Versicherung) that all of the above-stated declarations are true and that I did not withhold or conceal anything. I am well aware that false affidavit are punishable by a prison sentence up to three years or by a monetary penalty under German law.

1. Introduction

Is consciousness lost, or simply no longer apparent in an unresponsive human being? The question caused many philosophical arguments for thousands of years, and became a clinical challenge for physicians, since 1950 when artificial breathing was introduced and used to prolong the life of unresponsive patients with severe brain damage. With this intervention, the patients with severe brain damage unable to control vital functions were now able to live with the aim of assistive tools. Almost a decade later, Plum and Posner defined the clinical entity Coma (Plum & Posner, 1972), and soon a clinical assessment was proposed to monitor the progress of comatose patients in intensive care units. This assessment was mainly based on the level of motor and cognitive responses of patients, known as the Glasgow Coma Scale (GCS) (Teasdale & Jennett, 1974). The emergence of neuroimaging techniques in the late 1990s opened up new opportunities to study brain reactivity in unresponsive patients. With the advances in brain studies and the increasing number of surviving patients after severe brain injuries, a new field of study emerged as the study of consciousness in patients who are behaviourally unable to express themselves, referred to as the study of patients with disorders of consciousness (DoC). However, despite the medical advances, the clinical assessment of 'consciousness' remained a difficult and delicate task.

1.1. Disorders of Consciousness

Soon, a wide range of clinical disorders, such as patients recovering from a coma with sustained brain impairments including patients in the minimally conscious state (MCS), and unresponsive wakefulness state (UWS) as well as the patients with locked-in syndrome (LIS) and completely locked-in syndrome (CLIS), grouped into the category of DoC patients. Initially, LIS and CLIS were used to refer to those patients who suffer from partial (LIS) or total (CLIS) motor paralysis with intact cognition. The two main criteria initially proposed for CLIS were: total loss of motor functions, and intactness of cognition (Bauer, Gerstenbrand, & Rumpl, 1979); Neither of them is experimentally measurable in unresponsive patients with reproducible methods in clinical observations thus precludes any clinical diagnosis. It has been reported that 43% of patients who were misdiagnosed as UWS were in fact able to communicate if properly assisted with assistive augmentative communication (AAC) tools (Andrews, Murphy, Munday, & Littlewood, 1996) and thus being in LIS. Distinguishing between C/LIS and other types of DoC needs considerable clinical skills and experiences and is quantitatively and reproducibly impossible at present. With technological advances both in hardware devices and software applications, the limit to communication is pushed further, and therefore a standardized procedure to uncover a remaining communication channel with all available tools is a challenge and a standard procedure is not established yet. On the other hand, by definition, C/LIS are cognitively intact and should not be categorized as DoC patients, but since their behavioral profile is consistent with coma patients, i.e. eyes closed and behaviourally unresponsive to external stimuli, they were initially categorized along with other disorders of consciousness (Schiff & Fins, 2016).

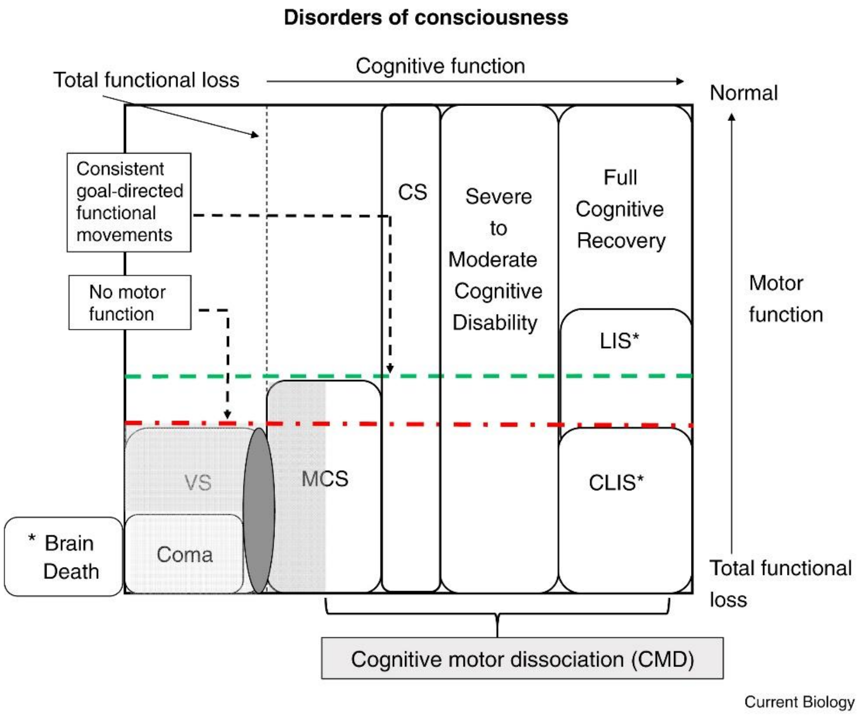

Figure 1. Locked-In syndrome (LIS) and Completely Locked-In Syndrome (CLIS) among disorders of consciousness (DoC): C/LIS are presented with other DoC patients due to the similarity of the behavioral symptoms (i.e. no goal-directed motor function), but usually excluded from DoC patients (astricts) since they are considered to be cognitively intact. Patients in Coma, Vegetative State (VS), and some patients in Minimally Conscious State (MCS) are considered to be suffering from total to severe cognitive functional loss (light gray zone). Though that some MCS patients have some motor functions they do not show consistent goal-directed functional movements (dashed green line). Contrary to C/LIS patients, where recovery is not possible, in other DoC patients transition between states is a possibility, and patients might regain cognitive and motor functions and transit between the states, therefore there are conditions that patients cannot be formally diagnosed and are in Confusional State (CS). Coma and UWS are presented as extremes where there is total cognitive (gray dashed line) and motor (red dashed line) loss, on the other hand, CLIS is considered as total

cognitive functionality with no motor function (red dashed line). Reprinted from "Brain death and disorders of consciousness", by N. D. Schiff 1 and J. J. Fins, 2016, Current Biology, 26, R573.

1.2. Locked-In Syndrome

C/LIS might have various etiologies such as suffering from hypotensive and hypoxic insults (i.e. ischemic), pons related hemorrhages, brain stem contusion due to traumatic injuries, tumors in the ventral pons, central pontine myelinolysis (i.e. metabolic), multiple sclerosis affecting the ventral pons (i.e. demyelination) and even brain stem encephalitis as a result of infectious diseases (Smith & Delargy, 2005). Regardless of the etiology, all of these patients have behaviorally similar symptoms, they are simply unresponsive and assumed to be conscious in most of the cases. These similarities of the symptoms became the main reason why different diseases/disorders such as motor neuron disease (MND) like amyotrophic lateral sclerosis (ALS) and traumatic, infectious, and ischemic patients to be classified with the same terminology of "CLIS". This caused ambiguities in the interpretation of findings in unresponsive patients, particularly generalizing the findings from MND patients in the latest stage of the disease who are behaviorally unresponsive but show significant signs of intact cognition, to traumatic brain-injured patients, or brain infectious patients with a complete lack of motor movements. For instance, in an early review of patients in LIS, among 139 patients, 75% had vascular etiologies, and the rest nonvascular, and only one case of ALS was described (Patterson & Grabois, 1986). In a recent review on the cognitive function in LIS, in which 50% of the patients showed cognitive deficits, there was not even a single case of ALS (Schnakers et al., 2008). In a meta-analysis review of all reported patients in LIS, less than 30% of patients had non-vascular etiologies with an unclear report and distinction between MND-CLIS to other types of CLIS (Laureys et al., 2005). While, analogously to the reports of comatose patients in which the etiology plays a predictive prognostic role (Estraneo & Trojano, 2017), it seems that in the cognitive assessments of C/LIS patients, the etiology should be considered as a critical parameter, particularly once comparing traumatic brain-injured patients to patients with MND, such as ALS. However, the notion that unresponsive patients can retain consciousness without necessarily being able to express it can be found in all DoC patients and in different reports on different diseases/disorders.

1.3. Amyotrophic Lateral Sclerosis

ALS is an MND that is caused due to the degeneration of motor neurons either in neural pathways that carry information from the cerebral cortex and brain stem to the spinal cord (i.e. upper motor neuron, UMN) or in the neurons that carry neural commands from UMN to associated muscles (i.e. lower motor neuron, LMN) (Thorns et al., 2010). In either case, the disease is progressive and eventually affects the entire motor control, and completely paralyzes the patient with respiratory failure as the leading cause of death. Decisions of life-prolongation in CLIS is very difficult and is based on the health care regulatory systems and legal and ethical opinions in different countries and not on scientific grounds (Niels Birbaumer & Zittlau, 2017). In Germany, where all our patients live, before the transition to CLIS, when patients are still able to communicate, patients can decide whether they accept artificial ventilation and want to be kept alive after transitioning to CLIS. Almost, half of the ALS patients can decide decisively on the life-prolonging treatments at a very early time after diagnosis, and the other half of undecided or the ones with negative decisions, show significantly increasing desire to continue living even with the aim of invasive life-prolonging treatments (Lulé et al., 2014). When such patients access a communication channel with AAC tools, their will to live increases significantly (Linse, Aust, Joos, & Hermann, 2018), since the patient attributes him/herself as an active agent in the social environment rather than a passive victim, thus the quality of life increases (Kuzma-Kozakiewicz et al., 2019; Linse et al., 2017). Although the above-mentioned studies are all about patients in LIS, a dramatic change of attitude after the transition to the CLIS is not expected, especially if a channel of communication can be provided after transitioning to the CLIS with BCI systems.

1.4. Need for a BCI

Since in ALS-CLIS patients all motor neurons controlling voluntary movements are lost, the only hope to achieve communication is through invasive or non-invasive BCI (Niels Birbaumer, 2006; Chaudhary, Mrachacz-Kersting, & Birbaumer, 2020). So far, invasive BCIs using intracortical or subdural electrodes (Chaudhary, Birbaumer, & Ramos-Murguialday, 2016; Milekovic et al., 2018) are not tried with patients in CLIS yet, except one from our group (Paper VI), and the few patients using a non-invasive BCI, either NIRS-based (Borgheai et al., 2020; Chaudhary, Xia, Silvoni, Cohen, & Birbaumer, 2017; Fuchino et al., 2008; G. Gallegos-Ayala et al., 2014; Ozawa, Naito, Tanaka, &

Wada, 2020) or EEG-based (Okahara et al., 2018) were able to answer only yes and no questions but never achieved free spelling where patients can freely communicate their desires. These reports rely only on "yes" and "no" answers to simple questions using brain waves and they are still in an experimental phase, not robust enough for everyday use. Whether other modalities or autonomic-vegetative functions, such as heart rate changes, skin conductance, saliva PH, etc., can be used as a voluntary communication channel is a matter of debate (Niels Birbaumer, Ruiz, & Sitaram, 2013; Wilhelm, Jordan, & Birbaumer, 2006). In any case, whether it is invasive or non-invasive, whether it is yes/no questions or free spelling, with such tools and technologies it is possible to behaviourally assess the cognition in these unresponsive patients. However, because of the limited BCI communication performance in CLIS, there is no quantitative and reliable data available on the cognitive processing in ALS patients after transitioning to CLIS.

1.5. Cognition in ALS-CLIS

In ALS patients before transitioning to CLIS, the progress of the disease is not usually correlated with cognitive dysfunction (Schnakers et al., 2008), although the development of frontotemporal dementia is sometimes reported in some cases (Stanton et al., 2007). It has been proposed that sensory perception and processing are preserved and not affected by the disease even after transitioning to CLIS (Kübler & Birbaumer, 2008). These pieces of evidence are the main reasons why it is believed that even in the final stage of the disease when the patient is completely paralyzed and unable to express herself/himself, the patient is still cognitively intact. ALS selectively affects motor neurons thus the sensory processing is expected to be intact even after transitioning to CLIS. Of course, this is only a deductive and speculative argument, and clinical experiments needed to be performed to validate such claims. It has been postulated that one of the reasons for the failures of noninvasive BCI in ALS patients long after transiting to CLIS might be the lack of contingent reinforcement of intentions, which can lead to the 'extinction of goal-directed thinking' (Kübler & Birbaumer, 2008).

The literature on the cognitive assessment of LIS and CLIS patients is controversial, in the sense that by definition the patients are supposed to be cognitively intact (Bauer et al., 1979), but sometimes cognitive deficits are reported in some cases even before the transition to the CLIS (Conson, Pistoia, Sarà, Grossi, & Trojano, 2010; Sacco et al., 2008; Sarà et al., 2018; Schnakers et al., 2008; Zago, Poletti, Morelli, Doretti, &

Silani, 2011). On the other hand, cognition cannot be measured with behavioral means, which is exactly what is missing in CLIS. This controversy partially comes from the fact that the same label (LIS/CLIS) is being used for traumatic and non-traumatic patients. For instance, as previously mentioned, in a report of the cognitive deficit in the LIS patients, not even a single case of ALS patient was among the reported patients (Schnakers et al., 2008). In addition, reports on cognitive deficits such as dementia in the lastest stage of ALS are not exclusively for ALS-LIS patients and overlap with other etiologies. One of the main obstacles for achieving a comprehensive conclusion is inaccessibility to a large number of patients with almost the same etiology and in the same state of the disease in a particular research group. This is mainly because of the small number of surviving patients in CLIS and difficulties in accessing them; they are usually kept at home-care and no official records are available. Hence, case reports are usually provided unsystematically in ALS-C/LIS patients or are usually reported among other patients as a subcategory of patients with DoC. These case reports are valuable but only with accumulation of such case reports a clear picture can be provided.

1.6. From LIS to CLIS

Another challenge in the discussion on the LIS and CLIS patients in general, and ALS patients in particular, is where to draw a line to separate LIS from CLIS. The initially proposed definition by Bauer et al., 1979 is only based on the presence of behavioral responses in patients with the assumption of the intactness of consciousness. While with new findings and advances of the AAC tools, the limits of communication are pushed further and the borders to be in CLIS are enlarged, thus a standard clinical routine to distinguish LIS and CLIS from each other is not defined yet, and the only available criteria is now the possibility to communicate with eyes or another muscle. However, although in ALIS patients, eyes are assumed to be the last muscle that stops working, some evidence indicates that show in some patients other muscles such as the anal sphincter can be voluntarily controlled even once the eyes cannot be moved intentionally(Murguialday et al., 2011). Recently it has been proposed to use the lack of communication with any assistive tools as the main criteria for being in a CLIS (Chaudhary et al., 2020), but still, by this definition, the intactness of the cognition remains undefined; also no clinical procedure for evaluation is proposed.

1.7. Cognitive Assessments in Unresponsive patients

In general, there are three main approaches in the assessment of cognition and/or consciousness in DoC patients. First: the behavioral assessment by overcoming the obstacles to communicate with patients, using BCI, and directly asking questions to patients and evaluate their cognitive state; Second: to evaluate the brain reactivity in the presence of external stimuli and based on the nature of the stimuli and the design of the experiment uncover underlying mechanisms and their correlation with cognitive functionalities at a system level; Third: to analyze the physical structure of a brain in the absence of any external stimuli, i.e. spontaneous brain activity in the resting state, which may give us some insight on the remaining capacity for cognition and/or consciousness (Schnakers & Majerus, 2012).

In this dissertation, I refer to the first approach (behavioral assessment) as active assessment since the patient is actively engaging in performing a task, and the second two approaches (stimulus-dependent and resting) as passive or neurophysiological assessments, since they do not necessarily need the patient to actively engage in a cognitive experimental task.

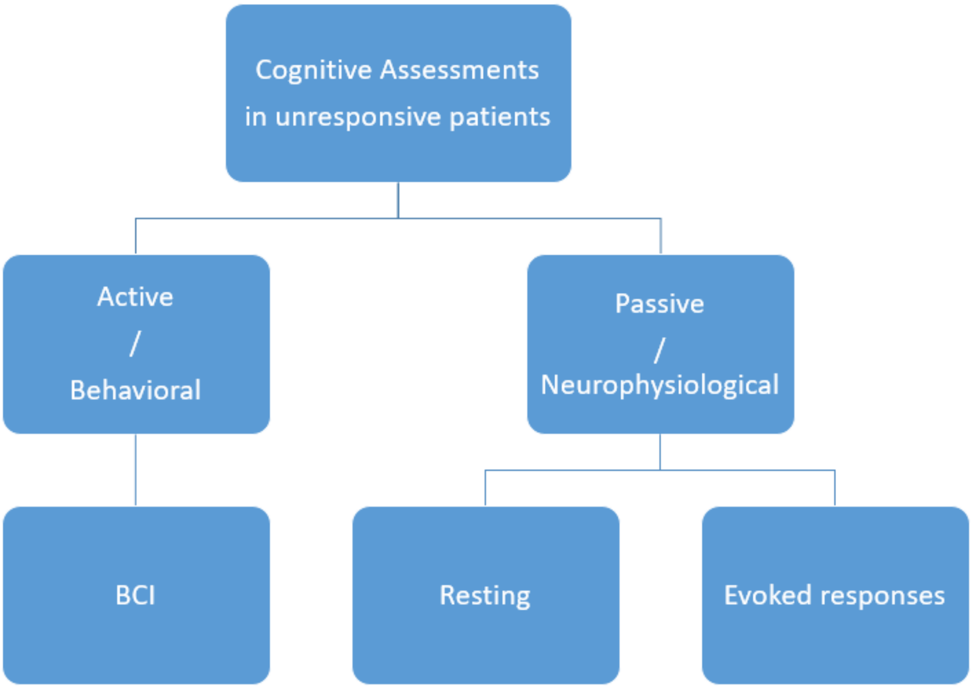

Figure 2 Different approaches to assess cognition in unresponsive patients in this study.

1.8. Overview

In this dissertation, I subcategorized the LIS/CLIS patients into progressive MND patients and traumatic brain-injured patients, and only focused on ALS-C/LIS patients and provided a neurophysiological assessment at a sensory, perceptual, and cognitive level, as well as providing accessibility to the hardly obtained data and analysis toolboxes in such patients to the scientific community. In addition, I provided several first pilot pieces of evidence from a behavioral perspective on how the conscious state of the patient might change over the course of the disease. To some extent, all of the three mentioned approaches were used to assess the sensation, perception, and cognition in C/LIS patients, results are all published and submitted in scientific journals, and in this dissertation, results are being presented and discussed to provide a big picture of the brain capacity in patients with CLIS.

On a behavioral level, in Paper VII, entitled "A General-Purpose Framework for a Hybrid EEG-NIRS-BCI", we proposed a general framework for non-invasive BCI applications using fNIRS and EEG, in non-communicative patients. This framework was evaluated in LIS patients on the verge from LIS to CLIS with no other means of communication and reported in Paper III, entitled "Auditory Electrooculogram-based Communication System for ALS Patients in Transition from Locked-in to Complete Locked-in State". The acquired dataset was reported in Paper V, entitled "A Dataset of an Auditory Electrooculogram-based Communication System for Patients in Locked-In State". A prototype of open-source hardware/software design of a system be used by the LIS patients after losing the ability to control eye-tracking devices was proposed and reported in Paper II, entitled, "Open Software/Hardware Platform for Human-Computer Interface Based on Electrooculography (EOG) Signal Classification". Although using the proposed BCI framework, successful free spelling communication with LIS patients was achieved, CLIS patients were not able to use it for even 'yes' / 'no' communication. To find out whether the failure in CLIS patients comes from the patients or the design of the system, cognitive-related brain responses to the presentation of the questions in the BCI experiment were investigated without trying to distinguishing yes from no answers, and reported in Paper X, entitled "EEG Spectral Entropy and Power during Brain-Computer Interface (BCI) based Communication in Completely Locked-in State (CLIS)". In addition, to investigate the possibility of the language attrition due to the total loss of motor assemblies in CLIS, the correlation between the semantic content of the sentence presented to CLIS patient and the performance of the BCI were analyzed and reported in Paper I, entitled “Semantic and BCI-performance in completely paralyzed patients: Possibility of language attrition in completely locked-in syndrome”. To overcome the problems of noninvasive BCIs and investigate the possibility of free spelling communication in ALS patients after transitioning to CLIS, one of the patients who was using the BCI before transitioning to the CLIS and had learned the experimental paradigm was implanted with microelectrode arrays and using intracortical recordings a BCI application was developed and reported in the Paper VII, entitled “Communication using intracortical signals in a completely locked in-patient”. On the neurophysiological level, the progressive evolution of neurophysiological changes in neuroimaging recordings of patients from LIS to CLIS was analyzed and reported in Paper VI, entitled “EEG Power Spectral Density in Locked-In and Completely Locked-In State Patients: a longitudinal study”. In a systematic review, several neurophysiological aspects of patients in CLIS were investigated and reported in Paper IV, entitled “Neurophysiology of patients with Completely locked-in syndrome”, and the dataset was released in Paper IX, entitled “A dataset of Neurophysiological recordings for ALS patients in CLIS”.

2. Objectives

The main objective of this thesis is to assess sensation, perception, and cognition in patients in CLIS. In this regard, several experimental paradigms in an active and passive condition have been employed. By active, I refer to the experiment in which the patient actively participates in experimental tasks, and by passive I refer to experimental paradigms in which the patient does not actively perform any particular cognitive task. In both cases, a set of toolboxes and datasets which are not easily accessible for other researches are published to be used by the scientific and clinical community. Since the only hope for ALS patients to communicate is through a BCI and it is dependent on the patient's physiological condition, it is expected that the results can help to achieve a functioning BCI to be used by non-communicative patients at the latest stage of the ALS disease. Particularly, it is expected that the behavioral assessment and neurophysiological measurement of ALS-CLIS patients and longitudinal investigation of ALS-LIS patients on the verge from LIS to the CLIS, can bring us a perspective for defining consciousness and the possibility to measure cognitive deficit at the latest stage of the disease.

3. Results and Discussion

Findings obtained from several experiments are summarized into two sections, First: Active Brain Assessments In CLIS where results obtained from the behavioural experimental paradigms, in which patients actively engaged in performing a cognitive task such as BCI, are presented; And second: Passive Brain Assessments in CLIS where the neurophysiological findings of passive experimental paradigms, in which patients did not need to perform any cognitive task, are presented. In the end, in the Summary of Findings section, all findings are converged to bring a perspective on sensation, perception, and cognition in ALS-CLIS patients.

3.1. Active Brain Assessments In CLIS

At the behavioral level, the only possible solution to directly assess cognitive functions in patients in CLIS is through a BCI, which allows communication (Niels Birbaumer, 2006). Since visual perception eventually will be lost in ALS-CLIS patients, an auditory version of a BCI is required both in CLIS and in LIS. Previous findings have proposed that using EEG (N. Birbaumer et al., 1999) and NIRS (Guillermo Gallegos-Ayala et al., 2014) it is possible to perform BCI communication with patients in C/LIS, for answering yes-no questions with brain responses. It was speculated that the combination of EEG and NIRS might be beneficial towards achieving improved BCI performance (Pfurtscheller, 2010). A challenge for a BCI in CLIS is the huge inter-individual difference among patients, which precludes a unique analysis pipeline for all patients. Another challenge is the few numbers of trials in the BCI experiment in CLIS. The reason for this is that auditory based BCIs take significantly more time in comparison to visual based BCIs, and therefore even with extended long-time recordings, the number of experimental trials is too small in comparison to the number of features extracted from the brain signals. This leads to a problem in the classification algorithms, known as overfitting of a classifier, and although there are mathematical algorithms to avoid it, the complex setup for simultaneous recording of the EEG and NIRS as well as human and technical errors while performing the experiments entails visual inspection and supervision of the analysis pipeline for each patient individually at the patient bedside. To minimize the human error in this procedure as well as allowing enough flexibility to the BCI system, a need for a hybrid BCI to perform systematic analysis in CLIS patients was raised. To address this issue, in the first step, I formalized and developed a structured framework of auditory BCI that can be used in visually impaired patients, including LIS and CLIS patients called HybridBCI, which reported in the Paper V. The conceptualized framework was then used in a non-invasive and invasive BCI to communicate with LIS and CLIS patients, each of which are explained below.

3.1.1. The HybridBCI

The HybridBCI framework is designed based on the object-oriented programming (OOP) principles which allow the necessary flexibility and extendability of the system (Lee, Fazli, Kim, & Lee, 2016) for further developments. This is crucial since new methodologies need structural software design concepts. Visits to the CLIS patients usually take place within a week, and each visit consists of several days of recordings, and based on the condition of the patient there is usually a need for the last minutes' changes at the bedside to adapt it to patients' condition, thus, applications that need to be compiled after each minor modification, such as BCI2000 (Schalk, McFarland, Hinterberger, Birbaumer, & Wolpaw, 2004), are not suitable options for this application. To avoid this issue, Matlab (The MathWorks Inc, 2019) was chosen as the programming language of the HybridBCI and a file-based structure was used to implement it. Using the provided graphical user interface (GUI) most of the modification needed to adapt the system for patient's problems and the recordings situation can be performed without the need to change the core structure of the code; Also, further development can be implemented by following the standards of the software design.

With the code of HybridBCI, a standard experimental paradigm is conceptualized that can be used with or without the code for other purposes, such as neurofeedback based training paradigms. This experimental design is based on presenting questions with known answers to the patient and then by extracting features from obtained brain signals, using machine learning algorithms to classify trials with yes answer from no answer. This process includes three sequential phases, and each of them can be one to several experimental blocks. In the initial phase, the training phase, HybridBCI learns the pattern of brain signals for yes and no responses for each subject. This happens through presenting several trials (i.e. questions with known answers to the experimenters) and acquiring brain signal and then analyzing it all the way from pre-processing to classification. Once a proper model that can classify yes trials from no trials more than the chance level is achieved, it goes to the second phase, the feedback phase, in which the patient learns how the system works. This step is basically the same as the previous one, only that the HybridBCI gives feedback to the patient (closed to loop) on which answers (yes or no) have been detected by the system. This auditory feedback serves as a reward and reinforces the thinking strategy. In this stage, the previously built model is also validated, and if the feedback accuracy more than chance level is achieved the system goes to the third phase, the application phase. In this step, the BCI can be used to freely communicate with patients and asking questions whose answers are not priory known for experimenters. The model achieved from the first two phases (training and feedback blocks) now can be used for a variety of applications. A typical application is asking open questions, in which the answers are not known by the experimenters and families (e.g. Are you in pain?). The HybridBCI is designed in such a way that the same model can be used for spelling, in which an optimum auditory based speller, presents the alphabet letter by letter to patients for spelling and communication.

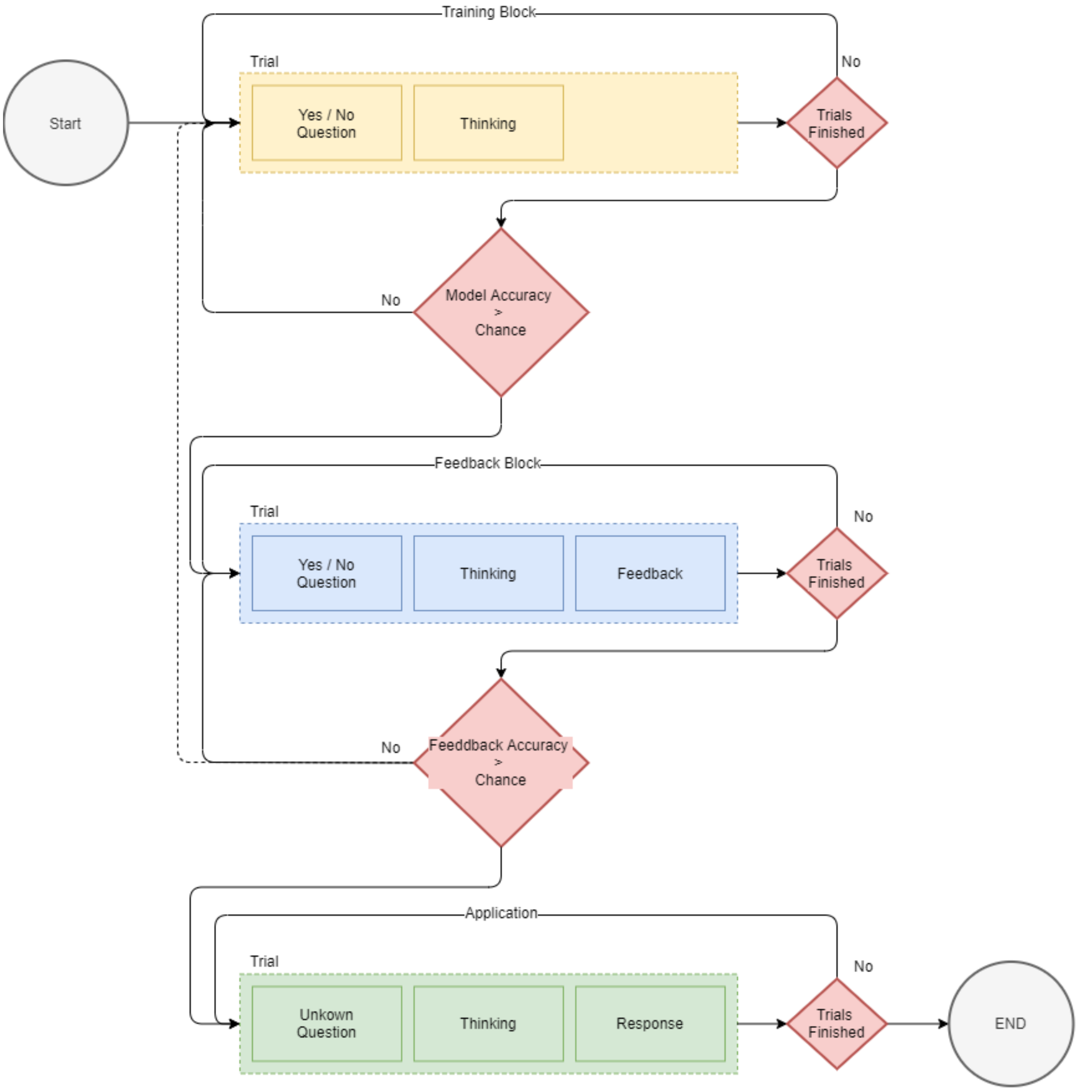

Figure 3. HybridBCI experimental paradigm: HybridBCI works in three phases, in each step if the classification accuracy is more than chance level the system transits to the next step. In training blocks, HybridBCI learns the brain signal to classify yes and no questions. Then in feedback blocks, auditory feedback reinforces proper thinking strategies in subjects. Finally, a model is achieved that can be used for free spelling communication with patients.

3.1.2. Non-Invasive BCI in C/LIS

HybridBCI was used in ALS patients on the verge from LIS to CLIS and revealed that there is a time span during the progress of the disease in which the patient loses his/her communication ability with commercial eye-tracking devices, but still able to use ocular muscles to execute commands, and therefore prolong the duration of the communication. As reported in Paper III, in this timespan when a patient is considered to be non-communicative (i.e. CLIS), it is still possible to use electrooculogram (EOG) to run BCI systems to freely communicate and spell (back to LIS!). In this study, four patients on the verge from LIS to CLIS underwent several experimental sessions performing BCI, using HybridBCI illustrated in Figure 3. All four patients achieved free spelling communication, although in one patient it gradually decayed and was not possible to communicate anymore after a certain time. Contrary to what might be perceived as an observer, patients showed satisfaction and high quality of life once they achieved free spelling communication. HybridBCI was used in these patients only after losing their only communication channel (i.e. eye trackers) and in the first attempts of free spelling, patients 13 and 15 spelled “Danke" to thank experimenters for their efforts. Patient 16 started expressing his feelings by saying "I am happy" and it was similar for patient 11, who was later implanted with microelectrode arrays for communication.

3.1.3. Open-source Hardware/Software for communication

The machinery used in this experiment is expensive, while the technology required for this application was less. Although the insurance pays for the BCI communication devices in the C/LIS patients in Germany, the expensive setup used in the reported experiments prevents other patients from using such vital technologies, particularly because they need technical assistance. The term vital was used not to refer to the possibility of prolonging breathing, rather to express the necessity of communication and increase the quality of life. Therefore, as a proof of concept, in Paper II, " Open Software/Hardware Platform for Human-Computer Interface Based on Electrooculography (EOG) Signal Classification", we proposed an open-source Hardware/Software with less than 1000 € cost that can be used with the same experimental paradigm as HybridBCI and can bring communication for patients with no other means of communication. The proposed platform is based on an open-source platform and consisted of a Raspberry Pi (a commercial single board computer) as the main processing unit for performing the experiment and analyzing; OpenBCI (an open-source EEG device) was used for signal acquisition, and a set of open-source Python libraries were used to implement the software. The system provided a cheap and compact device with a mean of 90% classification accuracy in 7 out of 10 healthy subjects for online classification in a four conditioned experimental paradigm.

3.1.4. Brain responses in BCI experiments in CLIS

Although the HybridBCI could have been used in LIS and healthy subjects, we were never able to successfully use it in patients over extended periods with CLIS, however, some of the patients were previously able to achieve some level of communication with other versions of a BCI (Guillermo Gallegos-Ayala et al., 2014). As it is also described in Passive Brain Assessments in CLIS, the fact that neurophysiological characteristics of patients change over time and dramatically changes after transitioning to CLIS, this along with the failure of BCI communication, raises the question of whether the cognitive functions, such as attention, comprehension, command following, and goal-directed thinking, as well as the learning of new paradigms, cease to function after the transition to the CLIS, or it is just that the BCI is not sensitive enough to capture related brain signals and to distinguish yes from no brain patterns? To answer this question, we investigated two aspects of the acquired signal from the BCI experiments in CLIS patients: first the correlation between the semantic content (motor vs non-motor, etc.) of the presented questions and the performance of the BCI. Second the changes in the brain pattern in response to a cognitive stimulus (i.e. questions), regardless of the performance of BCI in distinguishing the difference between questions with yes and no answers.

3.1.5. Semantic comprehension long after transitioning to CLIS

In Paper I, "Semantic and BCI-performance in completely paralyzed patients: Possibility of language attrition in completely locked-in syndrome", on a meta-analysis of a single case subject in the BCI experiment, we reported that the performance of the fNIRS based BCI is correlated with particular semantic contents of questions presented in the experiment. In particular, we found that motion-related content can influence the BCI performance and argued that it can be due to the degradation or non-use of motor-related brain assemblies and altered processing of such contents. It has been shown that the motor-related brain regions are not only involved in the acquisition of new concepts/words but also they are actively engaged while retrieving those concepts/words (Pulvermüller, 1999; Pulvermüller, Lutzenberger, & Preissl, 1999; Pulvermüller et al., 1994). Hence it is possible that in ALS patients long after transitioning to CLIS motor neurons processing of such words/concepts are affected and new reorganizational processes are required in order to properly comprehend and respond to them, and this won't happen unless there is a training phase with positive feedbacks to reinforce retrieval networks. The correlation between the content of the sentence during a BCI experiment and the performance of the BCI, particularly in the concepts related to the physical condition of the patient, might indicate some language attrition after total paralysis. Although the linguistic differences did not change the state of BCI performance in general, a significant difference was observed in the processing of such concepts. These results might indicate that some semantic contents presented during the BCI can be used in the future as a feature in the BCI in completely paralyzed patients.

3.1.6. Sign of semantic processing in BCI in CLIS

In Paper X, "EEG Spectral Entropy and Power during Brain-Computer Interface (BCI) based Communication in Completely Locked-in State", we focused on the patients in CLIS who could not use the HybridBCI for communication and analyzed the signal acquired during the BCI experiments to investigate if there remains brain reactivity to the presentation of the question to patients even if no communication with BCI is possible. We calculated spectral entropy of the EEG during baseline, presentation of questions, and thinking period. Spectral entropy of spontaneous EEG has been previously found as an indicator of cognitive and neurophysiological complexity and thus could serve as an indicator of brain status in CLIS (Elbert et al., 1994). Interestingly, in some patients who did not have any significant BCI performance, we found a variation of the EEG spectral entropy in response to the presentation of a question, in specific frequency bands. In fact, it was discovered that in some patients, although the HybridBCI could not discriminate between yes and no questions, there was a significant difference between the baseline and thinking periods in the EEG spectral entropy. This might indicate that features extracted from the brain signals in the HybridBCI are not receptive enough in patients in CLIS. It also emphasizes the fact that the findings of healthy and even LIS patients before the transition to the CLIS cannot be generalized to CLIS.

3.1.5. Invasive BCI in CLIS

Since the noninvasive recording did not lead to free spelling communication in any of our patients in CLIS in this study, one patient was selected to be implanted with microelectrode arrays in order to assess whether free spelling after transitioning to CLIS is possible at all. The patient was using a specific version of the HybridBCI for communication and had already learned the spelling paradigm, which was also later used after implantation. He was one of the patients reported in Paper II, who used the HybridBCI for communication after the loss of the eye-tracking communication channel.

As reported in Paper VIII, "Communication using intracortical signals in a completely locked in-patient", two 64 channel microelectrode arrays ("Utha array") were implemented for intracortical recording in supplementary motor and primary motor hand area. One day after the implantation, attempts were initiated to establish communication by performing motor imagery tasks like movements of the eyes, hand, tongue, and foot movement. No significant difference could have been found between different imagery conditions. After 86 days of unsuccessful attempts, the communication strategy was changed to a neurofeedback paradigm, in which a pure tone was presented to the patient and he was trained to increase or decrease the frequency of the auditory stimulus by controlling the spike rates of the recording channels. Once the desired spike rate above a certain threshold was achieved, the patient was rewarded with auditory feedback to reinforce the strategy of controlling spike rates. Already on the first day of neurofeedback, the patient was able to volitionally control his spike rate on a particular recording channel and thereby selectively switch between two different brain states (spike rate below and above the threshold). This binary switch was then used in the previously learned experimental paradigm to answer yes/no questions. And afterward was used for free spelling as before the transition to CLIS (Paper III). The patient achieved free spelling communication 106 days after the implantation and spelled his very first words letter by letter, with a speed rate of almost a character per minute.

These results supported intact consciousness and cognition in ALS patients after total loss of motor control and indicated that these patients can comprehend their surroundings and are able to perform cognitive tasks after total loss of motor neurons/control at the latest stage of the disease (i.e. CLIS). We don't know yet what will happen after several years in this state and have to wait how long he can retain this communication channel and to see if the degenerative progress of the ALS disease affects it.

Although these behavioral results of the BCI experiments demonstrate intact consciousness and preserved cognitive functionalities in a single-case in CLIS, it doesn’t tell much about those patients who failed the non-invasive BCI experiments. A failure in the noninvasive BCI experiments does not necessarily mean a lack of consciousness. It could be that the system is not well adjusted for these patients, or that because of the neuropsychological condition of patients, they may not actively engage in the experiment for a long period of time, which is required for non-invasive BCI experiments. It might even be that after transitioning to the CLIS, patients are incapable of learning new paradigms due to the extinction of goal-directed thinking (Kübler & Birbaumer, 2008). Which one of them or the combination of them is the main reason for the failure in the noninvasive BCI cannot be answered only from the BCI experiments. Hence we performed several passive experiments in which the patients do not need to actively perform any cognitive task, and by analyzing the fundamental brain structures we tried to address these questions. These findings along with the behavioral information can bring a better understanding of the brains of patients in CLIS.

3.2. Passive Brain Assessments in CLIS

The global neuroelectric cortical activity of the brain measured with the EEG in the absence of task performance or sensory stimulation can be used as an approximation to indicate the level of tonic arousal in patients with disorders of consciousness (DoC) or under general anesthesia (Colombo et al., 2019; Kreuzer, Kochs, Schneider, & Jordan, 2014; Zhang, Roy, & Jensen, 2001). The EEG pattern of spontaneous brain activity reflects the global intactness of anatomical brain structures (Biswal, Zerrin Yetkin, Haughton, & Hyde, 1995; Custo et al., 2017). It has been shown that EEG in CLIS significantly deviates from healthy and slow frequencies appears after the transition to the CLIS (Hohmann et al., 2018; Maruyama et al., 2020), but due to the need for medical care and attached life-saving devices MRI recordings are difficult or impossible and thus the source for this slow dominant brain activity during waking hours remains unclear. However, it is possible to investigate this source of activity from EEG recordings and solving the inverse problem. But the accuracy of neuroelectric source localization methods in the EEG depends on the distribution of EEG electrodes over the scalp, and without a proper setup using many electrodes and a valid analysis pipeline, results are prone to error and misinterpretation. Especially that patients in CLIS are most of the time in the supine position and it is a challenge to record posterior brain regions.

3.2.1 Evolution of EEG in C/LIS

In Paper VI, “EEG Power Spectral Density in Locked-In and Completely Locked-In State Patients: a longitudinal study”, we investigated the evolution of the EEG pattern during rest over time and confirmed what has been reported previously in the literature that in almost all patients in CLIS the EEG power spectrum gradually shifts towards the lower bands. Also, as reported in Paper IV as well, we observed that in some patients, once the alpha band power disappears and the power of the delta band increases, a new peak in the power spectrum emerges, with increased power at around 4 Hz. In these patients, EEG is highly synchronized among all recording channels, with episodic burst-like signals, very similar to a triphasic wave in vegetative state patients and anoxic coma. In fact, in terms of EEG traces, two categories of patients were observed. First, patients with slowed EEG and attenuated alpha peak at around 8 Hz. Second, patients with delta dominant EEG, in which a new peak with increased power at around 4Hz emerges that dominates the whole spectrum. In the second group, any activity above this dominant frequency is superimposed and cannot be visually inspected in the EEG traces. This raises questions about the underlying mechanisms and their correlation with the conscious state of the patient.

3.2.2 Tracing slow dominant frequency during the Night

To investigate the underlying mechanism for this abnormal EEG pattern, the EEG was traced during the night and in sleep. Results are reported in Paper IV, “Neurophysiology of patients with Completely locked-in syndrome”, and it shows that the power of 4Hz frequency is correlated with the arousal state of the patient, and its’ power is lost in the “deep” sleep (stage 3) during night time recordings. Some of these patients have been recently reported to retain circadian rhythms and have intact sleep cycles (Malekshahi et al., 2019). We came to the conclusion that this dominant slow frequency at 4Hz cannot be interpreted as pathological noise, however, the underlying mechanism and the reason why only some patients show such a pattern remains unclear. Since no structural brain imaging data, such as MRI, is available from patients in CLIS, the source of this slow activation and its corresponding brain regions is unknown yet.

3.2.3 Source of slow dominant frequency in CLIS

We used source localization techniques to find the anatomical source of this slow dominant frequency and found different brain regions in different patients, however, in all patients the source of slow activity was located in deeper structures. This was not surprising, since the time-domain EEG was highly synchronized with no phase lag in all recording channels. It has been reported that the functional connectivity of the sensorimotor cortex (SMC) to the cingulate cortex is increased in ALS patients prior to the transition to CLIS (Agosta et al., 2011), at the same time the default mode network connectivity is reported to be increased (Chenji et al., 2016). This might be due to the loss of inhibitory neurons in the cortico-striato-pallido-thalamo-cortical network (Lloyd, Richardson, Brooks, Al-Chalabi, & Leigh, 2000) which significantly decreases the overall brain activity and might be the reason behind the observed synchronization pattern in the EEG of patients in CLIS. This is in line with different observations reporting atrophy and shape changes of the cerebral and corticospinal tract in ALS patients before transitioning to the CLIS (Kumar, Aga, Gupta, & Kohli, 2016; Mioshi et al., 2013; Rajagopalan et al., 2013). These observations support the findings of Paper IV, that the slow dominant frequencies in ALS patients after transitioning to CLIS originates in deep structures of the brain, however, EEG source localization cannot solely be used to prove this hypothesis.

3.2.4 Stimulus processing in CLIS

Common biomarkers in EEG of healthy subjects are not valid for ALS-CLIS patients with altered EEG. However, after revealing the correlation of the EEG power at very slow frequencies in delta range with the arousal state of the patients in sleep, and showing that deep sleep has a much slower frequency than the slow waking EEG, the question remained whether these abnormal “waking” EEG is compatible with conscious processing in the presence of a stimulus? Considering that patients did not show any positive classification results in the BCI experiments, the reactivity of the brain in the presence of the external stimuli was investigated in ALS patients in CLIS and reported in Paper IV, “Neurophysiology of patients with completely locked-in syndrome”. In this paper, we showed that in all patients there is a clear brain response to opening the eyes as compared to eyes closed. This might indicate some level of visual processing in patients or can only be due to a basic attentional mechanism which is represented in the alpha suppression mechanisms in healthy subjects (Niels Birbaumer & Schmidt, 2014). It has been reported that even in total darkness with no visual input, eyes condition (open or closed) can be distinguished from EEG pattern (Boytsova & Danko, 2010) showing that input of light alone cannot solely explain the phenomenon. This finding is crucial since it indicates that even in patients with no positive BCI results, and abnormal EEG, some level of stimulus processing is present, which could be correlated with conscious processing as well.

In addition, we investigated the brain response to stimulation of the somatosensory neural pathways. Only some patients showed detectable cortical evoked responses in response to the electrical stimulation of the median nerve on their wrist. In some patients, the stimulation did not even travel through the peripheral nerves and did not reach the brain, thus no cortical evoked responses were recorded. Lack of peripheral neural responses in some patients might be due to experimental failures, or a strong increase in the sensory threshold due to deformation of the skin as a consequence of years of immobility. Without repetition of the experiments with the same patients, with higher intensities, no further speculation can be given. However, we abstain from higher intensities because of patients' inability to report pain. In some patients, where the stimulation reached the brain, the very early cortical evoked responses of healthy subjects were absent. The earliest significant response to the electrical stimulation in these patients was a relatively large peak at around 50ms after the stimulation, which was bilaterally distributed on both hemispheres over the centroparietal fissure. Eventual disorders of consciousness may be worse in these patients with missing early cortical responses since the bilateral absence of N20 in somatosensory evoked potentials (SEPs) is not usually a good prognosis in anoxic coma patients (Cruccu et al., 2008). The patients missing the early cortical responses had also a very slow dominant frequency at 4 Hz with a highly synchronized redundant EEG. Previous findings in ALS patients before transitioning to CLIS have also shown a pathological slowing of SEP responses (Hamada et al., 2007) and which in addition to the possibility of the atrophy of the brain might be enough reasons to alter the recorded SEPs. Thus, any diagnosis solely based on the SEP was avoided.

3.2.5 Experiments with a cognitive paradigm

In a more complex experimental paradigm (in terms of cognitive functions involved), a modified version of the local-global (LG) experimental paradigm proposed by Bekinschtein et. al. was used to assess cognitive processing in ALS-CLIS patients (Bekinschtein et al., 2009). The LG paradigm is an auditory evoked potential (AEP) based paradigm to assess consciousness in unresponsive patients. The main idea behind this paradigm is that, in a set of consecutive sensory stimuli with Local (order of consecutive stimuli within a trial) and Global (order of trials within an experimental block) pattern changes, two different types of ERP can be detected. Based on previous neurophysiological findings of event-related potentials (Squires, Squires, & Hillyard, 1975; Sutton, Braren, Zubin, & John, 1965; Ulanovsky, Las, & Nelken, 2003), it has been argued that detection of any violation in the order of stimuli within a trial (i.e. local pattern) only requires pre-attentive mechanisms and elicits the early evoked response (before 200ms), called local effect (LE), while detecting a violation in the order of trials within an experimental block (i.e. global pattern) requires attention and memory updating and is reflected in the later evoked potentials components (after 200ms), called global effect (GE).

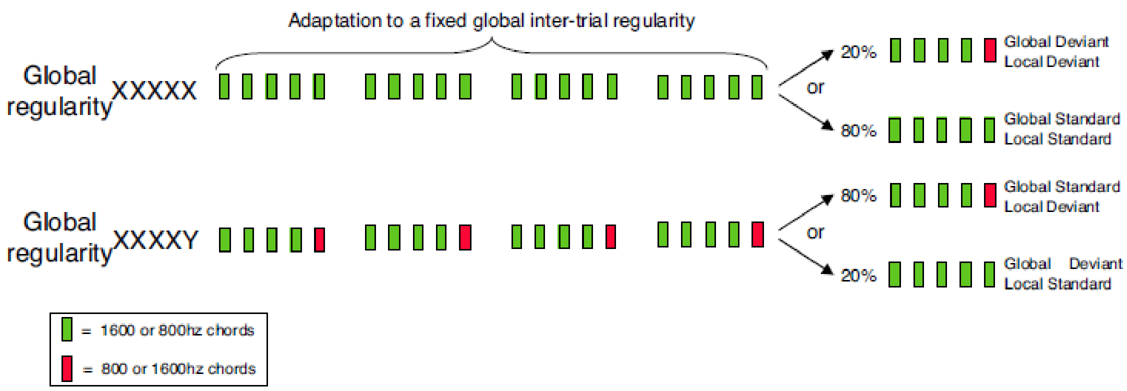

Figure 4 Block Design of Local-Global experiment design. Two patterns for global regularities and two patterns of local regularities creates four experimental conditions: Global Deviant Local Deviant (GDLD), Global Standard Local Standard (GSLS), Global Deviant Local Deviant (GSLD), and Global Deviant Local Standard (GDLS). Detection of local deviations only involves a pre-attentive mechanism, thus produce early evoked responses (before 200 ms), while detection of the violation in global patterns requires conscious processing and produces later evoked responses (after 200ms). Reprinted from "Neuralsignature of conscious processing of auditory regularities", by Beckinschtein et. al., PNAS February 3, 2009, 106 (5) 1672-1677.

We used a modified version of the LG experimental paradigm to assess cognitive processing in patients in CLIS. In all patients, the brain responses to the auditory stimuli were detected, however, the local and global effects were significantly deviant from the healthy population. In particular, the early evoked responses of the local effect and the later evoked potentials of the global effect were either missing or delayed in time. In some patients, the LE was completely missing while GE was delayed in time. This phenomenon is similar to DoC patients in which the P300 is shifted later in time up to 600ms, while no early evoked potentials can be detected and the mismatch negativity is missing (Kotchoubey, Lang, Winter, & Birbaumer, 2003); Interestingly, in patients with autism spectrum disorders, early components can be easily detected and are increased while later conscious components are missing (Niels Birbaumer, 1999).

These results are important from two aspects, first, they show that in CLIS some fundamental changes in the timing of the processing of external stimuli exists but that they still show processing of auditory material at some level, which is crucial for performing in an auditory based BCI. Second, it showed that the altered neural signature of cognitive functions (i.e. later evoked responses) are similarly affected as in patients with DoC.

4. Summary of Findings

The heterogeneity of the results and inconsistency of the neural signatures in the same patients at the same time and between patients asks for different and speculative explanations. Since these studies are the first in the literature that are being performed at the same time on the same patient and despite the small sample only very tentative hypotheses can be formulated. Patients in CLIS suffering from ALS are not always accessible at the same time and their medical conditions are constantly changing, and it is very rare that standardized experiments can be performed as in a psychophysiological laboratory working with healthy persons or in a clinic with a stable experimental room and stable environment. This condition prevents large dataset acquisitions in a short period of time, and several generations of studies are required to organize a proper sized dataset. In addition, in the unresponsive patient, the arousal state of the patient cannot easily be evaluated, therefore the behavioral assessments are vulnerable to inconsistency among different experimental sessions. The patient may be in a sleeplike state in one session and awake in another, the two different states cannot be inferred from any behavioral change such as eye closing or different repiratory pattern, or any type of movements. The progressive nature of the disease causes constant changes in the neurophysiological condition of the patients, which is also reflected in the EEG (Paper IV and Paper VIII), therefore findings of different sessions at different times cannot be grouped together. Another issue is that patients are usually in home-care and performing a systematic assessment in a single experimental visit is usually too much workload for the family, patients and experimenters. Therefore, properly adjusted experimental paradigms adapted to the conditions of the patients for neural and behavioral assessments are required. Overall, our results strongly indicate the possibility to retain cognition and consciousness after total loss of motor control, while it is also possible to lose even the most basic sensory and perceptual processing, at the latest stage of the disease.

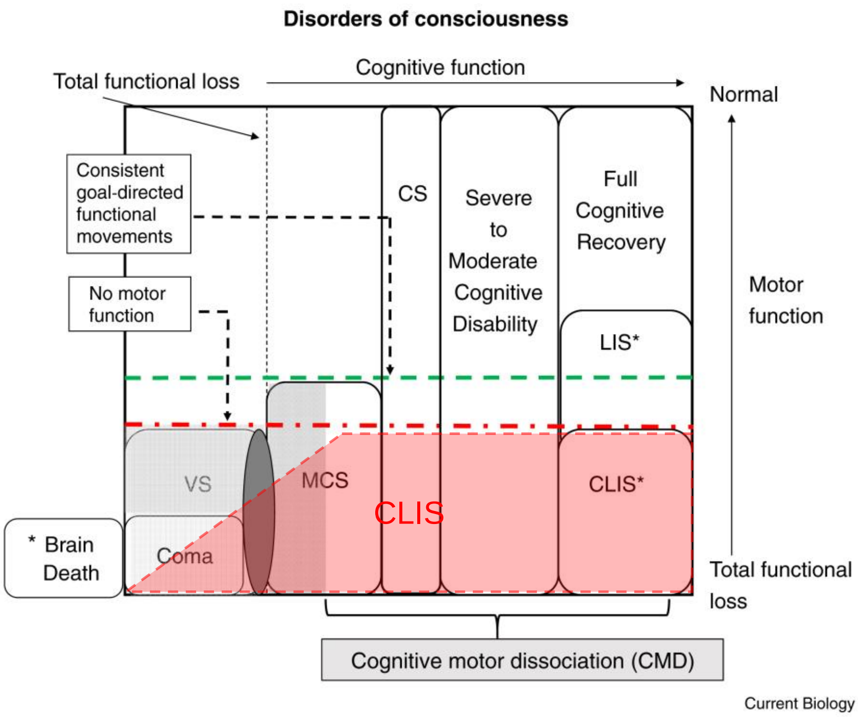

Figure 5 Disorders of Consciousness - CLIS might be represented as a heterogeneous spectrum from intact cognition to severe cognitive disorders: Locked-In Syndrome (LIS) and Completely Locked-In Syndrome (CLIS) are usually illustrated with patients with Disorders of Consciousness (DoC) including unresponsive wakefulness (UWS), Coma, and Confusional State (CS), because of the similarity of symptoms (i.e. being unresponsive), but excluded from them, due to the assumption of intact cognition (LIS and CLIS in black with astricts). However, we provided evidence that patients in CLIS due to ALS show gradient heterogonous results from lack of basic sensory response to complex brain responses. Thus when all different perceptual and cognitive functionalities are mapped into a one-dimensional scalar of cognition, a patient in CLIS might be anywhere in the spectrum from severe loss of cognitive function to intact cognition (highlighted in red). Modified from "Brain death and disorders of consciousness", by N. D. Schiff 1 and J. J. Fins, 2016, *Current Biology*, 26, R573.

These findings raise serious and critical issues and demonstrate that a single or one-dimensional index of consciousness and awareness is scientifically and clinically impossible and can lead to misdiagnosis. Different experimental paradigms are measuring different brain mechanisms and therefore heterogeneity of the results might represent different sensory, perceptual, or cognitive processing, which are often correlated in healthy human beings but might be dissociated in brain-injured, DoC, and the completely paralyzed patients. No single metrics of consciousness exists and if one claims such an index in unresponsive patients including CLIS needs additional neuronal and behavioral metrics. The extreme heterogeneity of the results of different brain measures in CLIS reflecting different neural and therefore different cognitive processes remind us of a similar dilemma in the diagnosis of conscious processes in the severe brain-damaged (Laureys & Boly, 2007; Majerus, Gill-Thwaites, Andrews, &

Laureys, 2005; Real et al., 2016); while one measure (i.e. SEP) denies any conscious or cognitive process the other index points to the existence of highly complex semantic processing and reasoning in the same patient at the same time or at different times. This warns us not only of seemingly consistent theoretical explanations and theories in CLIS and DoC but also of any clinical diagnostic statements, even brain death; we just don’t know what’s possible, yet. With findings on the patients in the present report, who were never investigated with such measures before, we are forced to conclude that we have some neural indices indicating intact or only deviant cognitive processing while others suggest severe disorders and/or absence of perception and reasoning. The ethical and therapeutic consequences of such results are clear: never give up hope, there is not a single patient reported in the literature with CLIS or MCS who would not show "islands" of cognitive processing, but nobody can conclude much about the capacities and nature of these "islands".

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Appendix

Appendix A

Paper I: Semantic and BCI-performance in completely paralyzed patients: Possibility of language attrition in completely locked-in syndrome.

Brain and Language 194 (2019) 93–97

Contents lists available at ScienceDirect

Brain and Language

journal homepage: www.elsevier.com/locate/b&l

Semantic and BCI-performance in completely paralyzed patients: Possibility of language attrition in completely locked in syndrome

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ARTICLE INFO

Keywords:
Language comprehension
Completely locked-in syndrome
Brain computer interface
Embodied cognition

ABSTRACT

Patients with completely locked-in syndrome (CLIS) are incapable of any voluntary muscle movement and do not have any means of communication. Recently functional near infrared spectroscopy (fNIRS) based brain computer interface (BCI) has been successfully used to enable communication with these patients. The developed fNIRS-BCI system relies on the intactness of language comprehension in these patients in all dimensions of language. Interwoven language and motor cortex in brain, and lack of muscular activity in long run, can cause language attrition due to complete immobility in CLIS patients. In this study we have investigated effects of semantic content of sentences presented to a CLIS patient on the performance of the BCI system during a YES/NO paradigm. Comparison of communication success rate in BCI classification between different semantic categories indicate that semantic content of sentences presented to a CLIS patient can affect the BCI performance. Affected concepts are mostly associated with executive words. These findings can be beneficial towards development of more reliable communication device for patients in CLIS. In addition, these results may assist in elucidating the cognitive changes in completely paralyzed patients with the passage of time since the onset of total immovability.

1. Introduction

"*Sight and hearing were the only senses remaining, and they, like two solitary sparks, remained to animate the miserable body which seemed fit for nothing but the grave; it was only, however, by means of one of these senses that he could reveal the thoughts and feelings that still occupied his mind, and the look by which he gave expression to his inner life was like the distant gleam of a candle which a traveler sees by night across some desert place, and knows that a living being dwells beyond the silence and obscurity.*"

This poetic definition of a locked-in patient was given by Alexander Dumas in his book *The Count of Monte Cristo,* long before the Locked-In Syndrome (LIS) was introduced in the scientific literature (Dumas & Sante, 2004). Later, Bauer et al defined LIS in which patient is completely immobile except for voluntarily eye movement or blinking (Bauer, Gerstenbrand, & Rumpl, 1979). Patients with neurodegenerative disease like motor neuron disease (MND), lose the voluntarily control of their muscles and transits to LIS as time passes. When the patient loses the control of their eye movement, which is usually the last active muscle (Murguialday et al., 2011), the patient is in the completely locked-in state (CLIS) (Smith & Delargy, 2005). The patients in CLIS are then left without any means of communication. Ned Block, philosopher of mind, has argued that despite the lack of capabilities to self-report in patients in CLIS there is no magical reason to believe that the conscious state has disappeared (Block, 2007), but these arguments have always been challenged due to the unclear definition of the consciousness. "Consciousness" is an ill-defined umbrella-term and can cause ambiguity while shifting from one paradigm (e.g. philosophy of mind) to another (e.g. clinical psychology). To be precise, here we only used the term consciousness in order to refer to the subjective experience of self and the ability to voluntarily respond to environmental stimuli, which can be gained by processing external stimuli using sensory information and by modulating the brain response.

Empirical evidence has demonstrated that verbal comprehension in these patients are still present and patient can intentionally modulate thinking process in experimental tasks (Chaudhary, Xia, Silvoni, Cohen, & Birbaumer, 2017; Sellers, Ryan, & Hauser, 2014; Wilhelm, Jordan, &

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https://doi.org/10.1016/j.bandl.2019.05.004
Received 14 August 2018; Received in revised form 14 January 2019; Accepted 23 May 2019
Available online 28 May 2019

Birbaumer, 2006). In an interesting experiment, a CLIS patient was asked YES/NO questions and instructed to think of lemon or milk corresponding to YES and NO, respectively. Significant difference in level of salivary pH between this two conditions was reported (Wilhelm et al., 2006), which can be interpreted as proper comprehension of the conditioning task by patient. These findings led to an extensive research toward development of a BCI system to achieve a reliable communication with these patients. Different brain imaging techniques such as electroencephalography (EEG), functional near infrared spectroscopy (fNIRS) and functional magnetic resonance imaging (fMRI) and also the combination were used to achieve this goal (Chaudhary, Birbaumer, & Curado, 2015; Chaudhary, Birbaumer, & Ramos-Murguialday, 2016; Gibson et al., 2014; Hinterberger, Kübler, Kaiser, Neumann, & Birbaumer, 2003; Sellers et al., 2014). Brain implants using microelectrodes were particularly successful in achieving BCI commination in paralyzed patients (Hochberg et al., 2006; Milekovic et al., 2018). Brain imaging is sometimes accompanied with other experimental features like electrical stimulation (De Massari et al., 2013) or classical conditioning (Ruf et al., 2013). The ideal system would be the one that can discriminate between two different mental states, so that it can be used as binary switch for a speller system. Even though different computational methods and psychological methodologies have been applied and considering impressive improvement of imaging techniques during recent years, a reliable voluntarily communication has not been established yet, and the best BCI communication performance reported so far is 70% in binary YES/NO questions paradigm (Chaudhary et al., 2017) and though that is more than chance level, it is not good enough to achieve reliable online communication. Still, BCI provides the only approach to end this silence and enable communication in patients in CLIS.

To achieve BCI based communication, experimenters usually present a sentence with known Yes or No answer (e.g. "Your name is Majid") and instruct the patient to respond by performing particular thinking activity. This mental activities could be imagining of playing tennis and imagining to move around the room (Gibson et al., 2014) or simply thinking yes or no (Chaudhary et al., 2016, 2017; Gallegos-Ayala et al., 2014) or doing a mental calculation and resting. Different features are extracted from the source signal, and using machine learning algorithms a classifier is trained to classify two conditions and then the same model is being used to predict the outcome of open question answered, where the answer is not priori known for experimenter (e.g. Do you have pain in your back?).

In all the experimental paradigm mentioned above, intactness of linguistics abilities in all dimensions of the language is assumed while evidences indicate that neural networks involved in linguistic tasks, overlap with neural networks for movement and motor actions (Hadar, Wenkert-Olenik, Krauss, & Soroker, 1998; Iverson & Goldin-Meadow, 1998; Rizzolatti & Rozzi, 2016). 'Action perception theory' purports that during linguistic tasks, sensorimotor circuits become active (Pulvermüller, Moseley, Egorova, Shebani, & Boulenger, 2014; Rizzolatti & Rozzi, 2016) and lowers the threshold for post activations. For instance, when the healthy participants are reading aloud the functional links between hand motor area of the language dominant hemisphere and language processing areas in brain, lowers excitation threshold in transcranial magnetic stimulations (TMS) (Meister et al., 2003). Based on motor theory of speech, motor activation plays a critical role in speech perception (Liberman & Mattingly, 1985) and there is ample evidence to state that, language does not only require the activity of motor system but is deeply embedded in motor organization (Rizzolatti & Rozzi, 2016). Overlapping motor cortical area and language functioning networks (Binder et al., 1997; Hadar et al., 1998; Hickok & Small, n.d.; Huth, De Heer, Griffiths, Theunissen, & Gallant, 2016) are not only effective in the process of language production but also in the process of perceiving any speech signal (Pulvermüller, 1999). During perception of the semantic content of a word, cortical motor area is activated even if the participant is not actively attending to the language input. Importantly, this brain activation is not post comprehension thought process but is involved in the semantic comprehension process (Moseley & Pulvermüller, 2018).

Neural substrates for thought, language and movement are intrinsically interwoven and functionally interdependent (Moseley & Pulvermüller, 2018). Cognition is the product of reactivation of the neural basis acquired during initial learning process in sensorimotor network (Barsalou, 2008; Gallese & Lakoff, 2005; Moseley & Pulvermüller, 2018; Thompson, 2014). During acquisition of new words, depending on the semantic content of that word, a particular combination of neural basis are activated in the sensorimotor network, and while retrieving that word, most of that network is activated (Pulvermüller, 1999) and as a result during the perception of different semantic content, activation in different neural pathways are required (Binder, Westbury, McKiernan, Possing, & Medler, 2005). In the most rigorous perspective, sensorimotor approach implies that LIS patient's perception should be severely deficient since they are unable to move and proprioceptive afferents are absent (Kyselo & Di Paolo, 2015) but empirical finding demonstrates absence of extensive cognitive deficits in these patients (Schnakers et al., 2008). However, as different concepts are acquired by different mechanisms, it is very unlikely that perception and ability to comprehend various concepts are uniformly preserved after the onset of total immobility and changes in neural activation thresholds. In this study we have investigated effects of linguistic features on the success rate in a BCI system during communication with CLIS patients. In accordance with a Hebbian associative theory of language formalized by Pulvermueller et al. (1994)) following a model articulated first by Braitenberg (1996), we hypothesize that, "non-use" of motor semantic networks in complete paralysis and lack of appropriate proprioceptive input from relevant muscle groups, the excitation threshold for semantic nodes for action words and action phrases is increased in CLIS, hence performance of the BCI to questions containing action words in comparison to object words is decreased.

2. Methods

The data for this report was acquired by Chaudhary et al., 2017, during the BCI study with four different patients in CLIS. During each session, patients were presented 10 questions with "Yes" answer and 10 questions with "No" answer, randomly. Meanwhile, BCI system using fNIRS signal and support vector machine (SVM) classifier detected Yes or No answers and gave feedback to patient. For more details on the BCI study see Chaudhary et al. (2017). Here we analyzed results from 11 feedback sessions performed by Patient B (61 Old, male) reported in the same publication. For the other 3 patients reported in Chaudhary et al. (2017) the analysis reported in this paper could not be carried out because of the continuous update of the sentence database.

For patient B, in total, 174 different sentences were presented for 218 times. Each sentence was presented to the patient at least once. The sentences were constructed by the experimenter based on the information obtained from patient's family and were recorded by a family member of the patient. The sentences were typically about the patient himself (e.g. Do you have brown hair?), the biography of the patient (e.g. You have two daughters, or You have been to China.) or general knowledge information (e.g. Berlin is the capital of Germany).

Sentences presented were categorized based on their linguistic features. Six different categories were applied and each category consisted of two opposite attributes. Each sentence comprised of attribute in each category. For instance, in the first category, two attributes are "Question" and "Statement" and each sentence belongs only to one of them. Finally, performance of BCI was defined as the ratio of correct answer detected by the BCI system for each attribute to total questions presented to the patient in the same category.

2.1. Linguistics features

Category 1: Statement versus Questions - Based on the syntactical structure of each sentence, sentences were categorized in statements and questions. For instance, “Your name is Majid” categorized as statement and “Is your name Majid?” categorized as question.

Category 2: Frequent versus Occasional - Word frequency is an indicator of how frequent a particular word is used in general discourses in a particular language. “German dictionary of word frequencies, Leipzig Corpora Collection” (Goldhahn, Eckart, & Quasthoff, 2012) was used to calculate frequency of the words. For each sentence, the frequency index was defined as the summation of word frequencies in that sentence divided by the total number of words in the sentence (Eq. (1)). Sentences with frequency index higher than the mean of frequency indexes were ranked as Frequent and the rest as Occasional.

$$FrequncyIndex = \frac{\sum_{word}^{sentence} frequncy \ \ of \ \ word}{Number \ \ of \ \ words \ \ in \ \ sentence} \tag{1}$$

Category 3: Emotional versus Non-Emotional - A sentence can carry positive, negative or neutral emotional content, which can regulate neural pathway of perceiving and analyzing that sentence in brain (Vuilleumier, 2005). In this study, dictionary of sentiment, Germanlex which comes from PolArt (Klenner, Fahrni, & Petrakis, 2009) and was used at Polcla[1] project (Wiegand, Wolf, & Ruppenhofer, 2017) was used. It contains information about the sentiment that each word expresses, the polarity of that sentiment (positive, negative or neutral) as well as intensity of sentiment which is a value between -1 and 1 (Klenner et al., 2009). We defined emotional index of a sentence as sum of positive and negative values minus neutral value of all the words in that sentence (Eq. (2)). Sentences with emotional index more than 0.5 were ranked as emotional and the rest as non-emotional sentences.

$$EmotionalIndex = \sum_{word}^{sentence} |NEG| + |POS| - |NEU| \tag{2}$$

Category 4: Abstract versus Concrete - It has been demonstrated that there are distinct brain systems for processing abstract and concrete words and there are disparate neural networks for processing them (Binder et al., 2005; Pulvermüller, 2013; Scorolli et al., 2011; Wiemer-Hastings, Krug, Xu, & Com, 2001). In this study, we borrowed the definition by Scorolli et al and ranked the nouns and verbs. Nouns that refer to graspable objects were ranked as concrete and nouns that do not refer to any manipulable objects were ranked as abstract words. Verbs that refer to hand actions were ranked as concrete and verbs that do not refer to any motor actions were ranked as abstract (Scorolli et al., 2011). Then sentences containing abstract noun and abstract verbs were ranked as abstract and sentences with concrete verbs and concrete nouns ranked as concrete. Sentences with the combination of concrete and abstract verb and nouns were excluded from the analysis in this category. For instance, “Did you break the vase?” was ranked as concrete and “Sky is blue” was ranked as abstract.

Category 5: Motion versus Motionless - Using the concept of action, words which only refer to movements of the one’s body (Pulvermüller, 1999), motion words are defined as words with executive affordance representing any kind of motion or movements, sentences including these words were ranked as motion otherwise as motionless. For instance, “Airplanes move faster than trains” was ranked as Motion and “Do you wear glasses?” was ranked as Motionless.

Category 6: Self-Related versus Not Self-Related - If a sentence represents any type of information about the patient identity it was ranked as self-related otherwise as not self-related, for instance “You have brown hair” was ranked as Self-Related and “Birds can fly” was ranked as Not Self-Related.

[1] A Polarity Classifier Incorporating Polarity Shifting for German.

2.2. Analysis

Each sentence presented to the patient was labeled and categorized based on the linguistic features defined above and BCI performance was calculated. The BCI performance was defined as the percentage of correctly classified answer of the presented sentence in a category. For each category, performance of the BCI was compared between two attributes in the same category. e.g. in the first category, performance of BCI system was compared between questions and statements and so on for different categories as defined above. For comparison chi-square statistics was used. Chi-square was calculated using online free statistical calculator for comparison of proportion, provided by MEDCALC (“MedCalc Software bvba, Ostend, Belgium;”, 2016). In addition to the comparison of single features, sentences with two different linguistic features were compared. e.g., among the sentence with frequent words, performance of the BCI was compared between emotional and non-emotional sentences. Categories with less than 10 sentences were excluded from analysis.

3. Results

For each complimentary pair of features Table 1 shows the total number of sentences in each group, BCI performance for each feature, chi-square and level of significance for difference between two categories. Results indicate that there is no significant difference in the BCI performance between two attributes in one category, although in category 1 there is a near significant ($p = 0.06$) difference in the performance of the BCI with better performance in statements in compare to questions.

Imbalanced distribution of linguistics features among the sentences makes it impossible to compare all possible combination for paired attributes and categories with less than 10 sentences were eliminated from analysis. Table 2 shows the categories for which the BCI performance was significantly ($p < 0.05$) different between two attributes. There was significant difference ($p = 0.001$) in BCI performance in sentences with abstract content versus concrete ones, when the sentence represented to the patient has motion contents. The second group of features for which the BCI performance was significantly different ($p = 0.02$), was when the sentence presented was about the patient himself (i.e. self-related). Among all the self-related sentences, there was clear difference in the BCI performance in motion sentences versus motionless ones. For other combination of linguistics attributes there was no significant results.

4. Discussion

The difference between BCI performance in two groups of sentences with different linguistic features suggest that semantic information of sentences presented to a CLIS patients plays an important role in control of BCI. It is evident that neural process of motor related networks plays a significant role in the processing of sentences by the CLIS patient, because only with inclusion of motor related words significant differences emerged. The “preference” of motionless self-related sentences could reflect the transfer of motor paralysis state of the patient’s body into the cognitive imagery domain. Associative connections between motionless contents related to one’s own body are strengthened in CLIS because associative pairings with body-motions extinguishes. We predicted this results (Birbaumer, 2006; Kübler & Birbaumer, 2008) and speculated (Chaudhary et al., submitted) that sufficient quality of life rating in chronic paralysis could be a consequence of extinction of voluntary, goal directed thinking due to the absence of contingent “fulfilment” of expectancies and consequences in a motionless, non-communicative state such as CLIS. Frustrative non-reward, one of the most frequent causes of human stress becomes highly unlikely with the lack of contingent expectancies probably contributing to the sufficient quality of life in LIS (Linse et al., 2017).

Table 1
Comparison of BCI performance in different linguistic categories with two attributes for each category. Chi-square and p-Value indicates difference of BCI performance between two attributes. Performance are presented in percentage.

Feature	Number of Questions	Performance	Chi-Square	p-Value
Abstract/Concrete	142/49	47.2/55.1	0.905	0.34
Frequent/Occasional	82/136	51.2/46.3	0.490	0.48
Question/Statement	99/119	41.4/53.8	3.313	0.06
Motion/Motionless	73/145	43.8/50.3	0.818	0.36
Emotional/Non-Emotional	28/190	50/47.9	0.043	0.83
Self-Related/Not Self-Related	92 / 126	42.4/52.4	2.12	0.14

Table 2
Comparison of BCI performance in different linguistic categories with two attributes for each sentence. Chi-square and p-Value indicates difference of BCI performance between two attributes. Performance are presented in percentage. Results with p-Value > 0.05 are not presented.

Feature	Number of Questions	Performance	Chi-Square	p-Value
Motion – Abstract/Motion - Concrete	28/39	25/56.9	6.646	0.001
Self-Related – Motion/Self-Related - Motionless	48/44	31.3/54.5	5.004	0.02

The Hebbian “rules” of learning state that coactivation of neurons determines association of cells and leads to the development of functional units called cell assemblies (Hebb, 1967). Electrophysiological studies demonstrate that frequent activation of cell assemblies, strengthens the synaptic connection between these two cells. More importantly, activation of presynaptic neuron without the activation of post synaptic neuron leads to the weakening of their synaptic connection (Artola, Bröcher, & Singer, 1990). For action words (words that refer to body movements), perisylvian assembly representing the word form would become linked to neurons in motor, premotor and prefrontal cortices related to motor programs (Hauk, Johnsrude, & Pulvermüller, 2004; Pulvermüller, 1999). Hence, extensive reduction in activation of motor cortex neurons after the onset of CLIS in patient’s brain can increase the activation threshold in perisylvian assembly, as a results while recalling concepts that requires activation of this network more input trigger is need to activate the conceptual motor related nodes, otherwise it can reduce the “ability” of perceiving and comprehending executive words. Thereby sentences with action words presented to a CLIS patient might appear ambiguous to them. It could be one of the reasons why no reliable communication with these patients have been achieved yet (Kübler & Birbaumer, 2008).

Neural basis for comprehension and production of words in different semantic domains are structurally different and different pattern of neural activity are generated during the processing of words in different semantic domains. For instance, concrete words are expected to be stored in the brain contralateral while abstract words are more ipsilateral, therefore process of words in these two categories require different neural pathways (Binder et al., 2005; Pulvermüller, 1999; Scorolli et al., 2011). Considering these linguistics fractures while training a classifier might help to discriminate different pattern of signal and increase classification accuracies.

5. Conclusion

Investigation on the effects of semantic content of sentence presented to a CLIS patients shows clear discrimination in BCI performance among different semantic domains. This could suggest changes in language comprehension and production in CLIS after the onset of total immobility and difficulties in perception and/or production of words in executive concepts highly related to the patient’s body. Our findings also suggest that the semantic aspects of sentences used in such tasks must be considered in future BCI communication experiments. In the present study, imbalance of syntactical features distribution among all the sentences, made it impossible to analyses effects of syntactic aspects of a sentence presented to the patient on BCI performance, but in our future research we intend to include syntactic analysis too. On the basis of the promising findings presented in this paper, work on the remaining issues is continuing and will be presented in future papers.

Statement of significance

In the current manuscript we investigated the reorganizational changes in language comprehension by a patient with complete paralysis. We elucidate the importance of intactness of motor system for processing the semantic meaning of language; where in a patient in CLIS process self-related and motion contents, better than self-related and motionless contents.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Acknowledgements

Supported by Deutsche Forschungsgemeinschaft (DFG, BI 195/77-1), BMBF (German Ministry of Education and Research) 16SV7701 CoMiCon, Deutsche Forschungsgemeinschaft (DFG, Bi195, Kosellek), LUMINOUS-H2020- FETOPEN-2014- 2015-RIA (686764), and Wyss Center for Bio and Neuroengineering, Geneva.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bandl.2019.05.004.

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Appendix B

Paper II: Open Software/Hardware Platform for Human-Computer Interface Based on Electrooculography (EOG) Signal Classification.

sensors

MDPI

Article

Open Software/Hardware Platform for Human-Computer Interface Based on Electrooculography (EOG) Signal Classification

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Received: 21 February 2020; Accepted: 23 April 2020; Published: 25 April 2020

Abstract: Electrooculography (EOG) signals have been widely used in Human-Computer Interfaces (HCI). The HCI systems proposed in the literature make use of self-designed or closed environments, which restrict the number of potential users and applications. Here, we present a system for classifying four directions of eye movements employing EOG signals. The system is based on open source ecosystems, the Raspberry Pi single-board computer, the OpenBCI biosignal acquisition device, and an open-source python library. The designed system provides a cheap, compact, and easy to carry system that can be replicated or modified. We used Maximum, Minimum, and Median trial values as features to create a Support Vector Machine (SVM) classifier. A mean of 90% accuracy was obtained from 7 out of 10 subjects for online classification of Up, Down, Left, and Right movements. This classification system can be used as an input for an HCI, i.e., for assisted communication in paralyzed people.

Keywords: electrooculography (EOG); Human-Computer Interface (HCI); Support Vector Machine (SVM)

1. Introduction

In the past few years, we have seen an exponential growth in the development of Human-Computer Interface (HCI) systems. These systems have been applied for a wide range of purposes like controlling a computer cursor [1], a virtual keyboard [2], a prosthesis [3], or a wheelchair [4–7]. They could also be used for patient rehabilitation and communication [8–11]. HCI systems can make use of different input signals such as voice [7], electromyography (EMG) [12], electroencephalography (EEG) [13], near-infrared spectroscopy (NIRS) [14–16] or electrooculography (EOG) [5].

In this paper, we describe an EOG classification system capable of accurately and consistently classifying Up, Down, Left, and Right eye movements. The system is small, easy to carry, with considerable autonomy, and economical. It was developed using open hardware and software, not only because of economic reasons, but also to ensure that the system could reach as many people as possible and could be improved and adapted in the future by anyone with the required skills.

The end goal of this work is to build a system that could be easily connected to a communication or movement assistance device like a wheelchair, any kind of speller application, or merely a computer mouse and a virtual keyboard.

To achieve these objectives, we have developed and integrated the code needed for:

- Acquiring the Electrooculography (EOG) signals.

- Processing these signals.
- Extracting the signal features.
- Classifying the features previously extracted.

EOG measures the dipole direction changes of the eyeball, with the positive pole in the front [17]. The technique of recording these potentials was introduced for diagnostic purposes in the 1930s by R. Jung [18]. The presence of electrically active nerves in the posterior part of the eyeball, where the retina is placed, and the front part, mainly the cornea, creates the difference in potential on which EOG is based [19]. This creates an electrical dipole between the cornea and the retina, and its movements generates the potential differences that we can record in an EOG.

There are several EOG HCI solutions present in the literature. One of the issues with current HCI systems is their size and lack of autonomy, the use of proprietary software, or being based on self-designed acquisition and processing devices. Regarding the acquisition system, the most common approach is to use a self-designed acquisition device [1,4,20–22]. In our view, this solution dramatically restricts the number of users who can adopt this system. Other proposed systems make use of commercial amplifiers [23,24], which in turn make use of proprietary software and require robust processing systems, mainly laptops. This also reduces the number of potential users of the system and its applications since it increases the cost of the system and reduces its flexibility, portability, and autonomy. As far as signal processing is concerned, most systems choose to use a laptop to carry out these calculations [1,20–22,24,25], but we can also find the use of self-designed boards [6,26]. Table 1 shows the characteristics of some solutions present in literature as a representation of the current state of the art. The goal of our work is to achieve results equivalent to the present state of the art using an open paradigm, demonstrating that it is possible to arrive at a solution using cheaper components that could be modified to build a tailored solution. As far as we know, this is the first time that an open system is presented in this scope.

Table 1. Comparison of results between different studies.

Study	Movements	Acquisition	Processing	Method	Accuracy
Qi et al. [27]	Up, Down, Left, Right	Commercial	-	Offline	70%
Guo et al. [28]	Up, Down, Blink	Commercial	Laptop	Online	84%
Kherlopian et al. [24]	Left, Right, Center	Commercial	Laptop	Online	80%
Wu et al. [20]	Up, Down, Left, Right, Up-Right, Up-Left, Down-Right, Down-Left	Self-designed	Laptop	Online	88.59%
Heo et al. [26]	Up, Down, Left, Right, Blink	Self-designed	Self-designed + Laptop	Online	91.25%
Heo et al. [26]	Double Blink	Self-designed	Self-designed + Laptop	Online	95.12%
Erkaymaz et al. [29]	Up, Down, Left, Right, Blink, Tic	Commercial	Laptop	Offline	93.82%
Merino et al. [27]	Up, Down, Left, Right	Commercial	Laptop	Online	94.11%
Huang et al. [21]	Blink	Self-designed	Laptop	Online	96.7%
Lv et al. [19]	Up, Down, Left, Right	Commercial	Laptop	Offline	99%
Yathunanthan et al. [6]	Up, Down, Left, Right	Self-designed	Self-designed	Online	99%

In our system, the signal is acquired using the OpenBCI Cyton Board (Raspberry Pi 3B+ official website), a low-cost open software/hardware biosensing device, resulting in an open hardware/software-based system that is portable, with considerable autonomy and flexibility.

Once we have the EOG signal, this is processed using a Raspberry Pi (OpenBCI Cyton official website), a single board computer that allows installing a Linux-based distribution, which is small, cheap, and gives us the option to use non-proprietary software.

Features are then extracted from the acquired signal and classified employing a machine learning algorithm. The feature extraction process aims to reduce the dimensionality of the input data without losing relevant information for classification [28] and maximizing the separation between elements of different classes by minimizing it between elements of the same class [27]. To achieve this, several models have been proposed on EOG feature extraction [29–32]. We employed Support Vector Machine (SVM) to classify the data [33,34], which creates a boundary to split the given data points into two different groups.

The result of this process, in the context of signal (EOG) mentioned in this article, is the classification of the subject's eye movement to be used as input commands for further systems. This process and the tools used for it are explained in detail in Section 2. The Section 3 shows the performance achieved by the system. Finally, in the Section 4, we discuss the designed system and compare our system with existing related work along with the limitations of our system and future work.

2. Materials and Methods

2.1. Hardware-Software Integration

In the present study, OpenBCI Cyton board was used for the signal acquisition. This board contains a PIC32MX250F128B microcontroller, a Texas Instruments ADS1299 analog/digital converter, a signal amplifier and an eight-channel neural interface. This device is distributed by OpenBCI (USA). Figure 1 depicts the layout of the system.

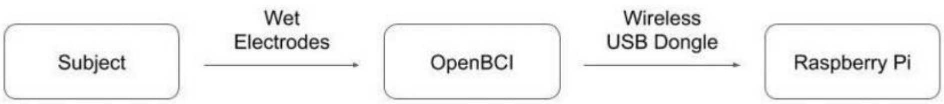

Figure 1. Block diagram with system connection.

This device gives us enough precision and sampling rate (250 Hz) for our needs, it has an open-source environment (including a Python library to work with the boards (OpenBCI Python repository)), it has an active and large community' and it can be powered with a power bank, which is a light and mobile solution. Attached to the board, we have 4 wet electrodes connected to two channels on the board in a differential mode. Differential mode computes the voltage difference between the two electrodes connected to the channel and doesn't need a reference electrode. The two channels correspond to the horizontal and vertical components of the signal.

The acquisition board is connected to a Raspberry Pi, a single-board computer developed by the Raspberry Pi company based in the United Kingdom. Although its firmware is not open source, it allows installing a Linux-based distribution keeping the open paradigm in our system. In this case, we chose to install Raspbian, a Debian-based distribution. The hardware connection between the OpenBCI board and the Raspberry Pi is made using a wireless RFDuino USB dongle. On the software side, we used an open Python library released by OpenBCI. To run this library over the Raspberry Pi, the source code of the mentioned library has been partially modified. It has also been necessary to recompile some third-party libraries so that they could run on the Raspberry Pi. We decided to power both the OpenBCI board and the Raspberry Pi via a USB connection to a power bank (20,000 mAh) to maximize the system autonomy and mobility.

This hardware configuration offers us all the characteristics that we were looking for: it has enough computational power to carry our calculations, it's small and light, it allows us to use free and open-source software, and is economical. It should be noted that although we have used the OpenBCI board as acquisition system there are some other solutions that fit our needs like the BITalino biosignal acquisition board. This board offers an EOG acquisition module and an open environment which includes a Python-based API for connection and signal acquisition over Raspberry Pi.

It should be mentioned that the data presented in this article have been processed using a conventional laptop instead of the Raspberry Pi, just for the convenience of the experimenters. During the development of the research, several tests were carried out that did not show any difference in the data or the results depending on the platform used.

We decided to use EOG over other eye movement detection techniques like Infrared Reflection Oculography (IROG) [35] or video-based systems [25], as the EOG technique does not require the placement of any device that could obstruct the subjects' visual field. Four electrodes were placed in contact with the skin close to the eyes to record both the horizontal and the vertical components of the eye movements [36,37].

2.2. Experimental Paradigm

Ten healthy subjects between 24 and 35 years old participated in the study and gave their informed consent for inclusion. The signal acquisition was performed in two stages: training and online prediction. For both stages, we asked the subjects to perform four different movements: Up, Down, Left, and Right. Each movement should start with the subject looking forward and then look at one of these four points already mentioned and look again at the center. For the training stage, we acquired two blocks of 20 trials, 5 trials per movement. In these blocks, five "beep" tones were presented to the subject at the beginning of each block in 3 s intervals to indicate the subject the interval that they had to perform the requested action. After these initial tones, the desired action was presented via audio, and a "beep" tone was presented as a cue to perform the action. The system recorded during the 3 s after this tone was presented, and the system presented again another action to be performed. For some of the subjects, these two training blocks were appended in a single data file. The schematic of the training paradigm (offline acquisition) is shown in Figure 2a.

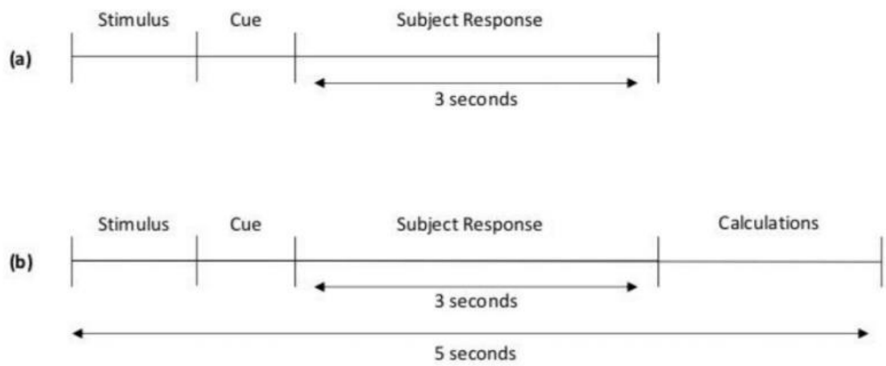

Figure 2. Acquisition paradigm. (**a**) Offline acquisition. (**b**) Online acquisition.

The online classification was performed with a block of 40 trials, 10 per movement, on Subject 1. After this experiment, we decided to reduce the number of trials per block to 20, 5 per movement, for the convenience of the subject. This online block had the same characteristics as the classification blocks except that the five initial tones were not presented, and the actions to be performed were separated by 5 s interval to have enough time for the prediction tasks. Furthermore, in these blocks, the system recorded only during the 3 s after the cue tone was presented. During this stage, we generate two auxiliary files: one with the acquired data and the other containing the action that the user should perform and the action predicted. We only considered predicted actions with a prediction probability higher than a certain threshold. For the first subject, we set this threshold as 0.7, but after that experiment, we changed the threshold to 0.5. In this case, the auxiliary file corresponding to subject 1 contains the predictions made using 0.7 as a prediction probability threshold. Figure 2b depicts the schematic of the online prediction paradigm.

2.3. Signal Processing

A second-order 20 Hz lowpass Butterworth filer [37] was used to remove the artifacts arising from electrodes or head movements and illumination changes [19,27,38]. A 20 Hz lowpass filter was used because the artifacts, as mentioned earlier, appear in the high frequencies [17], and the EOG signal information is contained mainly in low frequencies [30]. The irregularities in the signal after the lowpass filter were removed using a smoothing filter [30]. For applying these filters, we used the SciPy library. This library is commonly used and has a big community supporting it.

The last step in pre-processing was to standardize the data. This is done to remove the baseline of EOG signals [27]. The standardization was done using the following formula:

$$X_t = \frac{x_t - \mu_i}{\sigma_i}, \tag{1}$$

where i is the sample that we are processing, t corresponds to a single datapoint inside a sample, X_t is the resulting datapoint, x_t is the data point value before standardization, μ_i is the mean value of the whole sample and σ_i is the standard deviation of the whole sample. An example of the processed signal can be seen in Figure 3, which shows a single Down trial extracted from a classification block of Subject 5.

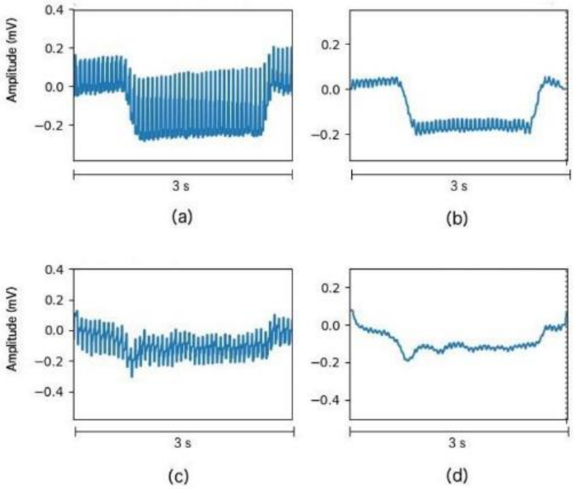

Figure 3. Down movement example taken from Subject 5. The x-axis depicts time (in seconds), and Y-axis represents the signal amplitude (in millivolts). (**a**) Unfiltered vertical component. (**b**) Filtered vertical component. (**c**) Unfiltered horizontal component. (**d**) Filtered horizontal component.

Figure 4 depicts the vertical and horizontal component for four different eye movement tasks performed by subject 5.

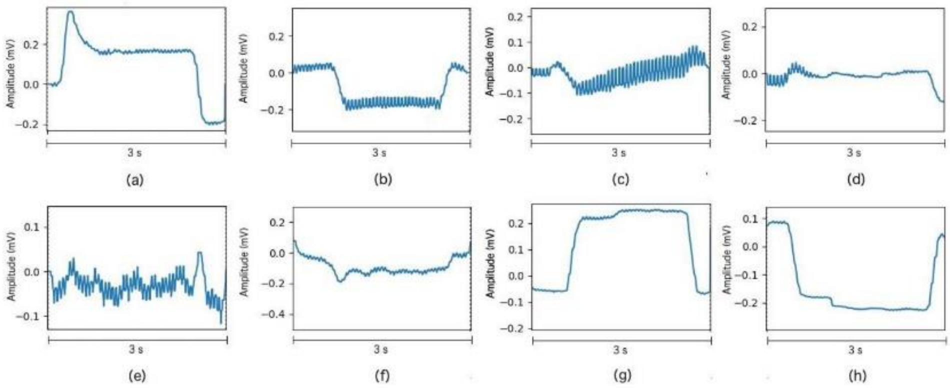

Figure 4. Processed signals examples taken from Subject 5. The x-axis depicts time (in seconds), and Y-axis represents the signal amplitude (in millivolts). (**a**) Vertical component for Up movement. (**b**) Vertical component for Down Movement. (**c**) Vertical component for Left movement. (**d**) Vertical component for the Right movement. (**e**) Horizontal component for Up movement. (**f**) Horizontal component for Down movement. (**g**) Horizontal component for Left movement. (**h**) Horizontal component for Right movement.

2.4. Feature Extraction

An essential step in our system's signal processing pipeline is feature extraction, which for each sample, calculates specific characteristics that will allow us to maximize the distance between elements in different classes and the similarity between those that belong to the same category. We use a model based on the calculation of 3 features for the horizontal and vertical components of our signal, i.e., 6 total features per sample. The features are the following:

- Min: The minimum amplitude value during the eye movement.
- Max: The maximum amplitude value during the eye movement.
- Median: The amplitude value during the eye movement that has 50% values above as below.

2.5. Classification

Once we have calculated the features of each sample, we create a model using that feature values and its class labels. Even though some biosignal-based HCI use other machine learning techniques, such as artificial neural networks [29,36] or other statistical techniques [19], most of the HCI present in the literature use the machine learning technique called Support Vector Machine. We have decided to use SVM because of its simplicity over other techniques, which results in a lower computational cost and excellent performance.

In this study, we have used the implementation of the SVM of Scikit-Learn, a free and open-source Machine Learning Python library. This library has a high reputation in Machine Learning, and it has been widely used. The selected parameters for creating the model are a Radial Basis Function (RBF) as kernel [39], which allows us to create a model using data points that are not linearly separable [40], and a One vs. One strategy [41], i.e., creating a classifier for each pair of movement classes. Finally, we have performed 5-fold cross-validation [42], splitting the training dataset into 5 mutually exclusive subsets and also creating 5 models, each one using one of these subsets to test the model and the other four to create it. Our model accuracy is calculated as the mean of these 5 models.

3. Results

The acquired signal is processed to remove those signal components that contain no information, resulting in a clearer signal. The data were acquired from 10 healthy subjects between 24 and 35 years old. The result of signal processing can be seen in Figures 3 and 4, which shows the single trials of a training block performed by Subject 5. As Figures 3 and 4 show, the result of this step is the one expected. For Subject 8, we found flat or poor-quality signals in the vertical and horizontal component, so we decided to stop the acquisition and discard these data. Some trials extracted from this discarded block can be seen in Figure 5 which shows no clear steps or any other patterns for the four movements. This situation is probably due to an electrode movement, detachment, or misplacement that could not be solved during the experiment.

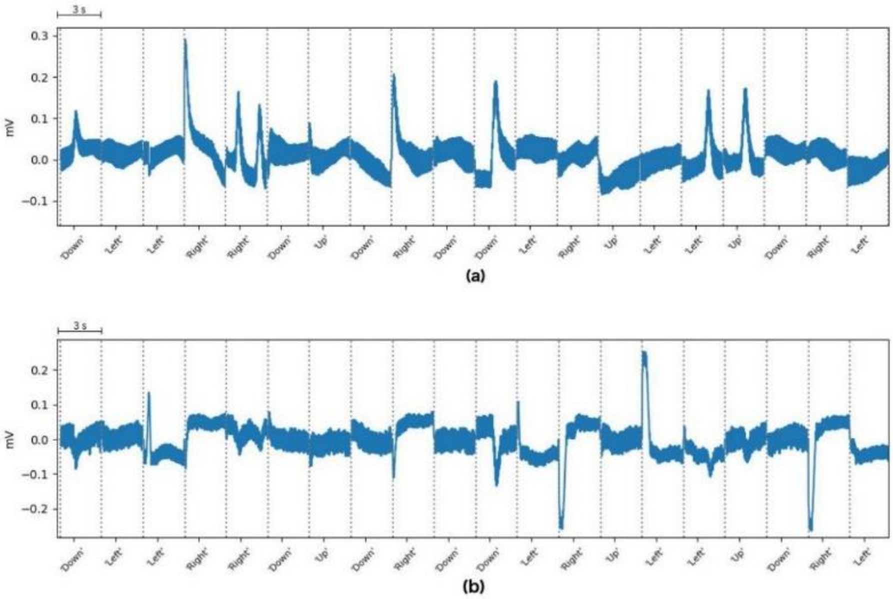

Figure 5. Example trials taken from Subject 8. The x-axis depicts time (each trial is 3 s), and Y-axis represents the signal amplitude (in millivolts). (**a**) Vertical component. (**b**) Horizontal component.

After artifact removal, feature extraction is performed to reduce the dimensionality in input, leading to characteristics that define the signal without information loss. As mentioned above, the features used were Maximum, Minimum, and Median. It should be noticed that Up and Down movements have relevant information only for the vertical channel of our signal as well as Left and Right movements have this relevant information in the horizontal component. Figures 6 and 7 present an example of this feature extraction process over two blocks of 20 trials, each corresponding to the training data of Subject 5, who ended up with 100% accuracy. Figures 8 and 9 present an example of the same feature extraction process over two blocks of 20 trials performed by Subject 6, who ended up with 78.7% accuracy. In these figures, we can appreciate that Subject 5, with 100% accuracy, shows a more evident difference in the data values than Subject 6, with 78.7% accuracy. Figures 8 and 9 show some overlapping in the data values, which explains the lower classification accuracy achieved.

The last step in our pipeline is to build a model and perform an online classification of the subject's eye movements. As we mentioned before, we build our model using 5-fold cross-validation. Table 2 shows the model accuracy, the accuracy-related on how good the model has been classifying the training data, as the mean of these five models for each subject. For the prediction accuracy—the accuracy related to the prediction of unseen data—we have asked the subject to perform 20 movements per block (five of each movement), as is explained in Section 2.2. We predicted those movements using the pre-built model and, finally, validated how accurate that prediction was.

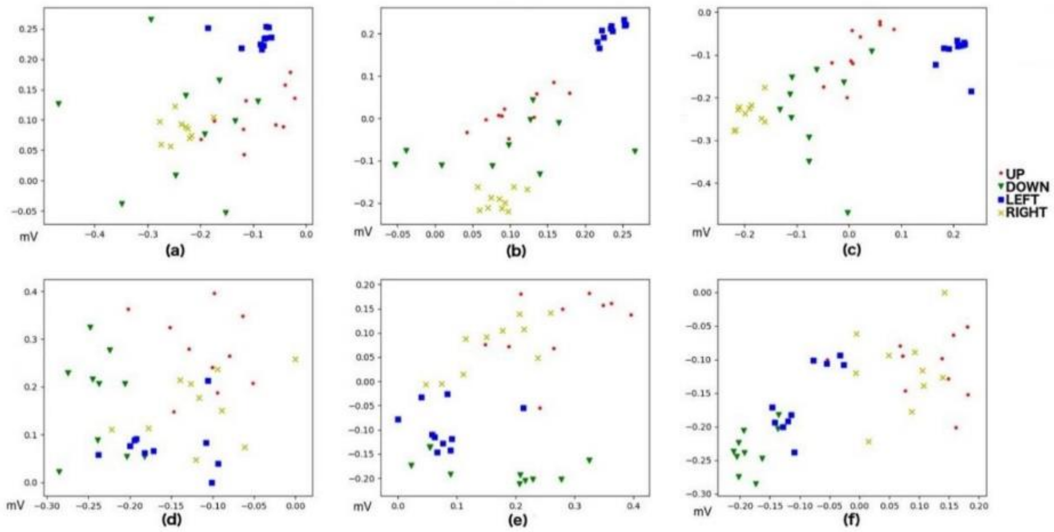

Figure 6. Values after Feature Extraction for Up, Down, Left, and Right movements performed by Subject 5 (100% model accuracy). Both X-axis and Y-axis depict signal values (in millivolts). (**a**) Horizontal Min vs. Max. (**b**) Horizontal Max vs. Median. (**c**) Horizontal Median vs. Min. (**d**) Vertical Min vs. Max. (**e**) Vertical Max vs. Median. (**f**) Median vs. Min.

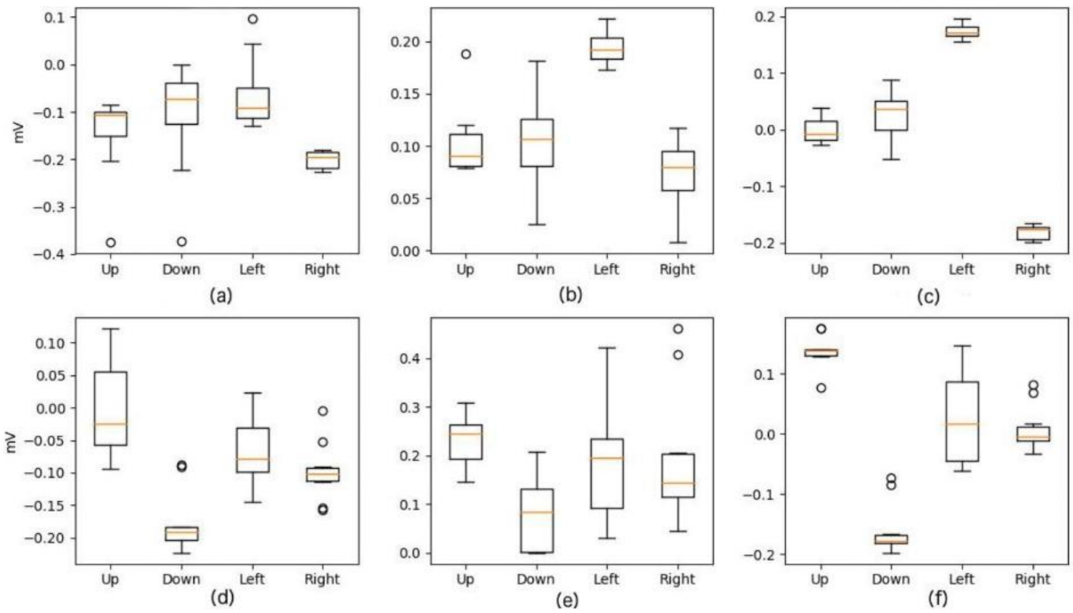

Figure 7. Values after Feature Extraction for Up, Down, Left, and Right Movements performed by Subject 5 (100% model accuracy). The x-axis depicts movement class, and Y-axis depicts signal amplitude (in millivolts). (**a**) Horizontal Min. (**b**) Horizontal Max. (**c**) Horizontal Median. (**d**) Vertical Min. (**e**) Vertical Max. (**f**) Vertical Median.

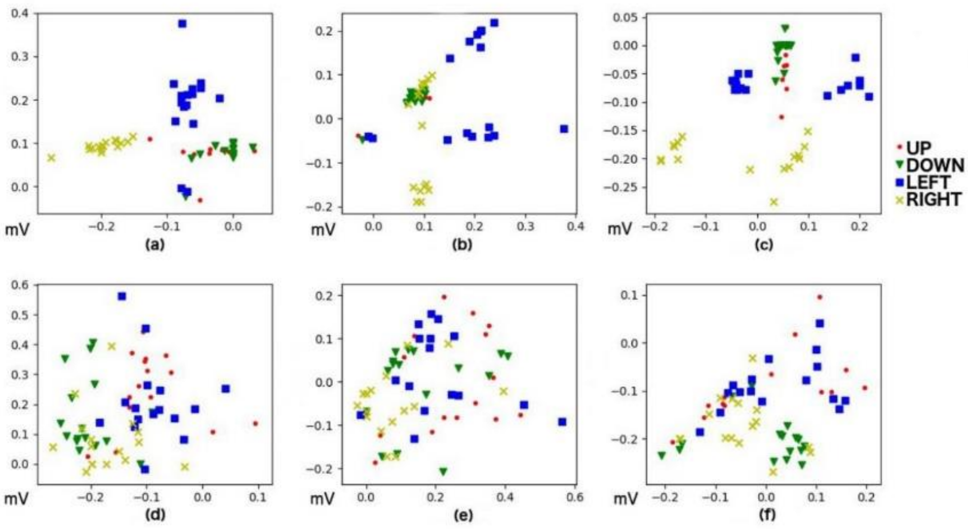

Figure 8. Values after Feature Extraction for Up, Down, Left, and Right movements performed by Subject 6 (78.7% model accuracy). Both *X*-axis and *Y*-axis depict signal values (in millivolts). (**a**) Horizontal Min vs. Max. (**b**) Horizontal Max vs. Median. (**c**) Horizontal Median vs. Min. (**d**) Vertical Min vs. Max. (**e**) Vertical Max vs. Median. (**f**) Median vs. Min.

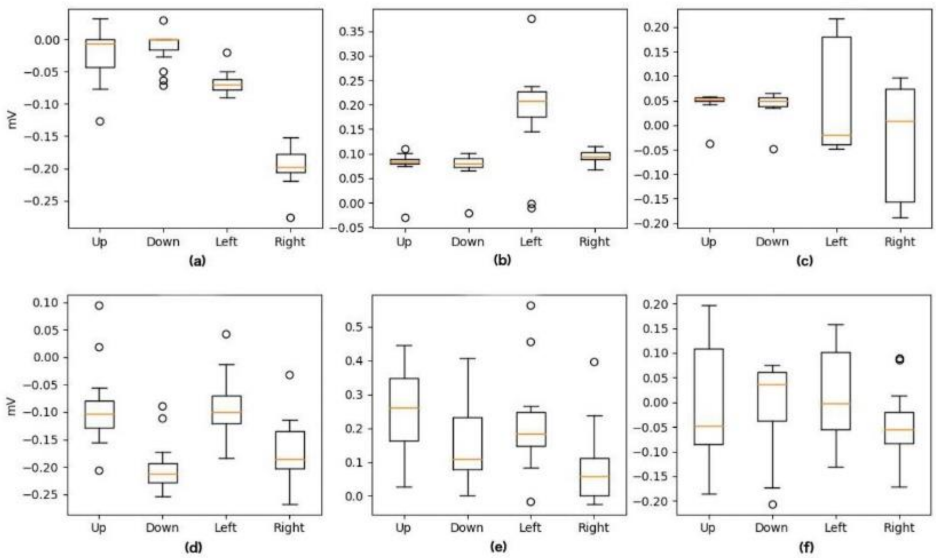

Figure 9. Values after Feature Extraction for Up, Down, Left, and Right Movements performed by Subject 6 (78.7% model accuracy). The *x*-axis depicts movement class, and *Y*-axis depicts signal amplitude (in millivolts). (**a**) Horizontal Min. (**b**) Horizontal Max. (**c**) Horizontal Median. (**d**) Vertical Min. (**e**) Vertical Max. (**f**) Vertical Median.

Table 2. Model and Prediction Accuracies.

Subject	Model Mean Accuracy	Online Accuracy
Subject 1	100%	90%
Subject 2	100%	95%
Subject 3	92.5%	85%
Subject 5	100%	100%
Subject 6	78.7%	85%
Subject 7	97.5%	95%
Subject 10	90.8%	80%
MEAN	94.21%	90%

As mentioned in Section 2.2., we only consider those predicted actions with a prediction probability higher than 0.5. For subject 1, the prediction probability threshold was set to 0.7 during the online acquisition, so the auxiliary file with the predictions corresponds to this threshold, and after experimenting, we re-analyzed the online data using a 0.5 threshold.

We acquired one single online block for subjects 1, 2, 5 and 7. For subject 3, we acquired three online blocks with 50%, 80%, and 85% accuracy. For subject 6, we acquired two online blocks with 80% and 85% accuracy. For subject 10, we acquired three online blocks with 55%, 70%, and 80% accuracy. It can be seen that for all subjects, the online accuracy increases with each block acquisition. The accuracy shown in Table 1 corresponds to those online blocks with the highest accuracy for each subject. For subject 4, the training and online data have poor quality (66.7% accuracy for the model and 20% accuracy for the online prediction). Subject 9 had a good model accuracy (95%) but poor-quality signals during online acquisition (50% and 20% accuracy). Post-experimental analysis of the data revealed noisy and flat signals, showing no clear pattern in the signal acquired from subjects 4 and 9, similar to the signal acquired from Subject 8 (Please see Figure 5 for the signal from patient 8). These distortions may have arisen due to the probable electrode movement, detachment, or misplacement. Thus, we decided to discard the data from Subjects 4, 8 and 9.

4. Discussion

It must be clear that in order to make a completely fair comparison between our system and the state-of-the-art systems, some extensive testing would be required. These tests should process the data acquired in this study with other processing pipelines, run our pipeline over the data acquired in other studies, and adapt our acquisition and processing modules to be connected to further systems found in the literature. The results obtained after this process would give us a full picture of the differences between our system and those already in place. Unfortunately, due to lack of time and materials, these tests could not be carried out.

Concentration loss and tiredness are two of the biggest challenges when it comes to EOG-based HCI. As reported in Barea et al. [43], the number of failures using this kind of system increases over time after a specific period of use. This has been seen during the development of this study, where long periods of system use have led to the appearance of irritation and watery eyes. This could be a problem for subjects who use the system for a long time. In the paper above mentioned [43], the researchers deal with this problem by retraining the system.

Another challenge related to our system is the presence of unintentional eye blinks. Eye blinks create artifacts in the EOG signal and, also, during the eye blinks, there is a slight eye movement [37]. The trials containing eye blinks can lead to a reduced model accuracy if it occurs in the training stage or to a trial misclassification if it is in the online acquisition stage. Pander et al. [44], and Merino et al. [30] have proposed methods to detect spontaneous blinks so these trials can be rejected. Yathunanthan et al. [6] proposed a system where eye blinks are automatically discarded.

Our system, like most of the available systems in the literature [19–21,29,30,38,43], uses a discrete approach, i.e., the user is not free to perform an action when desired, but the action must be performed at a specific time. This affects the agility of the system by increasing the time needed to perform an action. Barea et al. [38,43] and Arai et al. [25] have proposed systems with a continuous approach where the subject has no time restrictions to perform an action.

There are different ways to improve our system in future work. First, we could put in place a mechanism to detect and remove unintentional blinks. This would prevent us discarding training blocks, or could improve the training accuracy in the cases in which these unintentional blinks occur. In some cases, a continuous online classification means a considerable advantage. Therefore, it would be interesting to add the necessary strategies to perform this type of classification. Finally, by combining our system with further communication or movement assistance systems, we could check its performance in a complete HCI loop.

5. Conclusions

We have presented an EOG signal classification system that can achieve a 90% mean accuracy in online classifications. These results are equivalent to other state-of-the-art systems. Our system is built using only open components, showing that it is possible to avoid the usage of expensive and proprietary tools in this scope. As intended, the system is small, easy to carry, and has complete autonomy. This is achieved using OpenBCI and Raspberry Pi as hardware, connected to a power bank as a power source.

Because of the use of open hardware and software technologies, the system is also open, easy to replicate, and can be improved or modified by someone with the required skills to build a tailored solution. The use of open technologies also helps us to obtain a cheap platform.

Finally, the resulting system is easy to connect to subsequent communication or movement assistance systems.

Author Contributions: Conceptualization, J.M.-C., N.B., and U.C.; methodology, J.M.-C., M.K.A., A.J.-G. and U.C.; software, J.M.-C.; validation, A.J.-G.; formal analysis, J.M-C.; data curation, J.M.-C. and S.W.; writing—Original draft preparation, J.M.-C., S.W., M.K.A. and U.C.; writing—Review and editing, J.M.-C., A.T., N.B., and U.C.; supervision—U.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Deutsche Forschungsgemeinschaft (DFG) DFG BI 195/77-1, BMBF (German Ministry of Education and Research) 16SV7701 CoMiCon, LUMINOUS-H2020-FETOPEN-2014-2015-RIA (686764), and Wyss Center for Bio and Neuroengineering, Geneva.

Conflicts of Interest: The authors declare no conflict of interest.

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Sample Availability: Corresponding code and data are available at https://github.com/JayroMartinez/EOG-Classification.

Appendix C

Paper III: Auditory Electrooculogram-based Communication System for ALS Patients in Transition from Locked-in to Complete Locked-in State.

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Auditory Electrooculogram-based Communication System for ALS Patients in Transition from Locked-in to Complete Locked-in State

Alessandro Tonin[1,3], Andres Jaramillo-Gonzalez[1,3], Aygul Rana[1], Majid Khalili-Ardali[1], Niels Birbaumer[1] & Ujwal Chaudhary[1,2] ✉

Patients in the transition from locked-in (i.e., a state of almost complete paralysis with voluntary eye movement control, eye blinks or twitches of face muscles, and preserved consciousness) to complete locked-in state (i.e., total paralysis including paralysis of eye-muscles and loss of gaze-fixation, combined with preserved consciousness) are left without any means of communication. An auditory communication system based on electrooculogram (EOG) was developed to enable such patients to communicate. Four amyotrophic lateral sclerosis patients in transition from locked-in state to completely locked-in state, with ALSFRS-R score of 0, unable to use eye trackers for communication, learned to use an auditory EOG-based communication system. The patients, with eye-movement amplitude between the range of $\pm 200\mu$V and $\pm 40\mu$V, were able to form complete sentences and communicate independently and freely, selecting letters from an auditory speller system. A follow-up of one year with one patient shows the feasibility of the proposed system in long-term use and the correlation between speller performance and eye-movement decay. The results of the auditory speller system have the potential to provide a means of communication to patient populations without gaze fixation ability and with low eye-movement amplitude range.

Swiss philosopher Ludwig Hohl stated that "The Human being lives according to its capacity to communicate, losing communication means losing life"[1]. Our ability to communicate ideas, thoughts, desires, and emotions shapes and ensures our existence in a social environment. There are several neuronal disorders, such as amyotrophic lateral sclerosis (ALS), or brain stem stroke, among others, which paralyzes the affected individuals severely impairing their communication capacity[2–5]. The affected paralyzed individuals with intact consciousness, voluntary eye movement control, eye blinks, or twitches of other muscles are said to be in locked-in state (LIS)[6–11].

Early and modern descriptions of ALS disease emphasize that oculomotor functions are either spared or resistant to the progression of the disease[9], and consequently, eye-tracking devices can be used to enable patients in the advanced state of ALS to communicate[12,13]. Besides, longitudinal studies evaluating eye-tracking as a tool for cognitive assessment report that the progression of the disease does not affect eye-tracking performance[9]. Nevertheless, a subset of the literature reports a wide range of oculomotor dysfunctions in these patients[14–17] that might prevent the use of eye-tracking devices[18]. The most used metric to evaluate the patient's degree of functional impairment is the revised ALS functional rating scale (ALSFRS-R)[19], which is not a precise measure of the ability to communicate. A patient with an ALSFRS-R score of zero can still have eye-movement capability or control over some other muscles of the body, which can be used for communication[8].

CLIS is an extreme type of LIS, which leads to complete body paralysis, including paralysis of eye-muscles combined with preserved consciousness[6,20]; therefore, even if the individuals are incapable of voluntary control of any muscular channels of the body, they might remain cognitively intact[9]. Several systemic or traumatic

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neurological diseases may result in a LIS with the potential to progress towards CLIS, such as ALS, Guillain-Barré, pontine stroke, end-stage Parkinson disease, multiple sclerosis, traumatic brain injury and others with different etiological and neuropathological features[4,7,10]. In the case of ALS patients in LIS who survive longer attached to life-support systems, the disease progression might ultimately destroy the oculomotor control in many patients, leading to the loss of gaze-fixation[17]. Thus, patients become unable to use eye-tracker-based communication technologies and are therefore left without any means of communication. This raises the question, what happens with those ALS patients in transition from LIS to CLIS with highly compromised oculomotor skills unable to retain gaze-fixation, and therefore unable to use eye-tracking systems to communicate?

There is a considerable amount of research related to patients in the early stages of ALS who can successfully achieve communication by using gaze-fixation-based assistive and augmentative communication (AAC) technologies or brain-computer interfaces (BCIs). These patients have intact cognitive skills, residual voluntary movements, intact or partial vision with complete gaze-fixation capabilities. Several examples of communication technologies for ALS patients in LIS can be found in the literature. Concerning BCI-based communication, different types of systems have been developed to provide a means of communication to LIS patients[4,10,11], among the most recognized are the ones based in features of the EEG, as the slow cortical potential[21], or evoked potentials, mainly the P300[22–26] or SSVEP[27]; or the BCIs based in metabolic features, as NIRS[28–31]. Concerning the use of eye-tracking systems, as long as the patients have intact vision and control gaze fixation, commercial systems are an accessible and reliable option to allow them limited communication[32]. Other types of eye-tracking technologies as the scleral search soils, infrared reflection oculography, or video-oculography (or video-based eye-tracking)[33], have not yet been tested on LIS patients to our knowledge. Except for two studies[28,29], all the developed BCIs for ALS patients describe patients with remnant muscular activity, remnant eye movement control, or even without assisted ventilation, and in general with ALSFSR-R score above 15.

The progress of ALS often, if not always, diminishes the general capabilities of the patients making BCI-based communication impossible[7,24]. On the other hand, even though eye movement might be the last remnant voluntary movement before CLIS[34], during this transitional state from LIS to CLIS, patients become unable to maintain gaze-fixation and, unable to use eye-tracking AAC technologies.

Some electrooculogram (EOG) -based systems have been presented to overcome the limitations of other AAC technologies. However, most of the studies were performed on healthy participants or tested in LIS patients in the early stages of ALS, reporting results of single sessions or sessions performed closely in time, allowing the patients in advanced LIS to reply yes/no questions, but without the feasibility of freely communicate spelling sentences[35,36]. To our knowledge, no studies report on the long-term use of EOG or eye-tracking for patients on the transition from LIS to CLIS, and how this progression affects communication capabilities using these AAC technologies. Either in clinical descriptions or technical applications, very little is known about how this LIS to CLIS progression affects the oculomotor capabilities precluding the patient's communication.

Considering ALS patients in an advanced state, a first meta-analysis has shown that there is a correlation between the progression of physical impairment and BCI performance[7], and a recent one has suggested that the performance of CLIS patients using BCI cannot be differentiated from chance[37]. The only available long-term studies are either single cases for patients able to perform with a P300-based BCI[38,39] or a thoroughly home-based BCI longitudinal study[40] that shows favorable results. However, these studies do not provide details on how the progression affected the performance, particularly for the patients with the lowest ALSFSR-R score.

It has been shown in a single case report[34], that during the transition from LIS to CLIS, despite compromised vision due to the dryness and necrosis of the cornea and inability to fixate, some remaining controllable muscles of the eyes continue to function. Hence, there is an opportunity to develop a technology to provide a means of communication in this critical transition. Such a technology would extend these patients' communication capacities until the point the disease progression destroys any volitional motor control. Pursuing that goal, an EOG-based auditory communication system was developed, which enabled patients to communicate independent of their gaze fixation ability and independent of intact vision. This study was performed with four ALS patients in transition from the locked-in state to the completely locked-in state, with ALSFRS-R score of 0, and unable to use eye-trackers effectively for communication, i.e., without any other means of communication. The patients, with eye-movement amplitude between the range of $\pm 200\,\mu V$ and $\pm 40\,\mu V$, were able to form complete sentences and communicate independently and freely, selecting letters from an auditory speller system. Moreover, the study shows the possibility of using the proposed system for a long-term period, and, for one patient, it shows the decay in the oculomotor control, as reflected in EOG signals, until the complete loss of eye control. Such a communication device will have a significant positive effect on the quality of life of completely paralyzed patients and improve mandatory 24-hours-care.

Results

Four advanced ALS patients (P11, P13, P15, and P16) in the transition from LIS to CLIS, all native German speakers (Table 1), used the developed auditory communication system to select letters to form words and hence sentences. All the patients attended to four different types of auditory sessions: training, feedback, copy spelling, and free spelling session. Each training and feedback sessions consisted of 20 questions with known answers (10 questions with "yes" answer and 10 questions with "no" answer, presented in random order), for example, "Berlin is the capital of Germany" vs. "Paris is the capital of Germany". All the questions were presented auditorily. While in the copy and free spelling sessions, the patients were presented the group of characters and each character auditorily (see "Methods" section for the details). Patients were instructed to move the eyes ("eye-movement") to say "yes" and not to move the eyes ("no eye-movement") to say "no". Features of the EOG signal corresponding to "eye-movement" and "no eye-movement" or "yes" and "no" were extracted to train a binary support vector machine (SVM) to identify "yes" and "no" response. This "yes" and "no" response was then used by the patient to auditorily select letters to form words and hence sentences during the feedback and spelling sessions. Due

Patient	Gender/ Age	ALS type	Medical history	Visits
P11	M/33	Non-bulbar	Aug 2015: Diagnosis	10 visits over 13 months from March 2018 onwards
			Aug 2017: Last use of AAC	
P13	M/58	Bulbar	Jan 2011: Diagnosis	4 visits over 12 months from Jun 2018 onwards
			Jan 2018: Last use of AAC	
P15	F/63	Lower motor neuron predominant (ICD-10: G12.2)	Feb 2017: Diagnosis	2 visits over 5 months from Feb 2019 onwards
			Nov 2018: Last use of AAC	
P16	M/56	Lower motor neuron	Dec 2012: Diagnosis	2 visits over 3 months from March 2019 onwards
			Jun 2018: Last use of AAC	

Table 1. List of participants. The table lists the patient's number, the gender and the age at the time of the first visit, the type of diagnosed ALS, year of diagnosis, and the last use of assistive and augmentative communication (AAC) technologies, and the number of performed visits and their time range.

to the degradation of vision in ALS patients[14,16,17], the system was designed to work only in the auditory mode without any video support. We frequently traveled to the patient's home to perform the communication sessions. Each visit (V) lasted for a few days (D), during which the patient performed different session (S) as listed in Supplementary Tables S1–S4.

Eye movement. According to the literature, in healthy subjects, the amplitude of the EOG signal varies from 50 to 3500 µV, and its behavior is practically linear for gaze angles of ± 30 degrees and changes approximately 20 µV for each degree of eye movement[41,42]. Nevertheless, like any other biopotential, EOG is rarely deterministic; its behavior might vary due to physiological and instrumental factors[32]. For LIS patients in the transition to CLIS, the range and angle of movement are affected by the progress of the ALS disease, affecting the range of voltage amplitude as well. Figure 1 depicts the horizontal eye movement of P11, P13, P15, and P16 during one of the feedback sessions of their first visit (V01). In each plot, for a particular feedback session, all the questions' responses classified as "yes" or "no" by the SVM models were grouped and averaged. Figure 1 elucidates the differences in the dynamics of the signals corresponding to the "yes" and "no" responses, and it can be observed that each patient used different dynamics to control the auditory communication system.

Figure 2 depicts a decrease in horizontal eye-movement amplitude of P11 over 13 months. During the 12 months period, from March 2018 (V01) to February 2019 (V09), P11 performed feedback sessions with a prediction accuracy above chance level, in which small eye-movements recorded with EOG allow classification of "yes" and "no" signals. Employing the same eye-movement dynamics with an approximate amplitude range smaller than ± 40 µV over 4 months, from V06 (November 2018) to V09 (February 2019), P11 was able to select letters, words and form sentences using the speller. The eye-movement amplitude range decreased to ± 30 µV during V10, i.e., 12 months after the first BCI sessions, because of the progressive paralysis typical of ALS. During V10, model-building for prediction during feedback and spelling sessions was unsuccessful. Thus, V10 was the last visit for a communication attempt by P11 using this paradigm. During this visit, even if this training session allowed to build a model of 80% of cross-validation accuracy (Supplementary Table S1), it proved unsuccessful for predicting any classes from the data (50% accuracy).

In the case of P13, the progression of the disorder has been slower, which can be ascertained by the relatively high and constant amplitude of EOG, in an approximate range of ± 300 µV, but still, he was unable to communicate with the commercial eye-tracker technology. Employing the eye-movement strategy, as shown in Fig. 1B, P13 was able to maintain a constant dynamic to control the auditory communication system for feedback and spelling sessions (see Supplementary Table S2). Similar observations can be drawn for P15 and P16 EOG plots in Fig. 1C,D. During two visits each, they achieved successful performance for feedback and spelling sessions (see Supplementary Tables S3 and S4), with stable eye-movement dynamics.

Speller results. The performance of the SVM during all the feedback sessions by each patient is reported in Fig. 3 as a Receiver-Operating Characteristic (ROC) space. The ROC space of P11, who was followed for one year from March 2018 to March 2019, shows a trend in the performance of the feedback sessions. As shown in Fig. 3A, during the initial visits P11 exhibited a successful feedback performance (markers located in the upper-left corner in the ROC space), while during the later visits, particularly V08 and V09, P11 exhibited a decrease in feedback performance and ultimately by V10, it was impossible to perform a successful feedback session. This negative trend is due to the progressive neurodegeneration associated with ALS[8], which leads to the complete paralysis of all muscles, including eyes muscles. For each of the three patients P13, P15, and P16, the feedback sessions' performances are located mostly in the upper-left region of the ROC space, which means successful feedback performance. Nevertheless, for each of these three patients, a few feedback sessions also fall in the lower-right region of the ROC space. This might be due to a learning process of the patients in which they improved or adjusted their eye-movement strategy or due to the suboptimal performance of the SVM classifier during the first few feedback sessions.

The patients were asked to attempt a spelling session when a model was validated with a successful feedback session, i.e., results above random[43]. After the feedback session, patients performed two different types of spelling sessions: copy spelling and free spelling, i.e., sessions in which the patient was asked to spell a predetermined phrase, and sessions in which the patient spelled the sentence she/he desired.

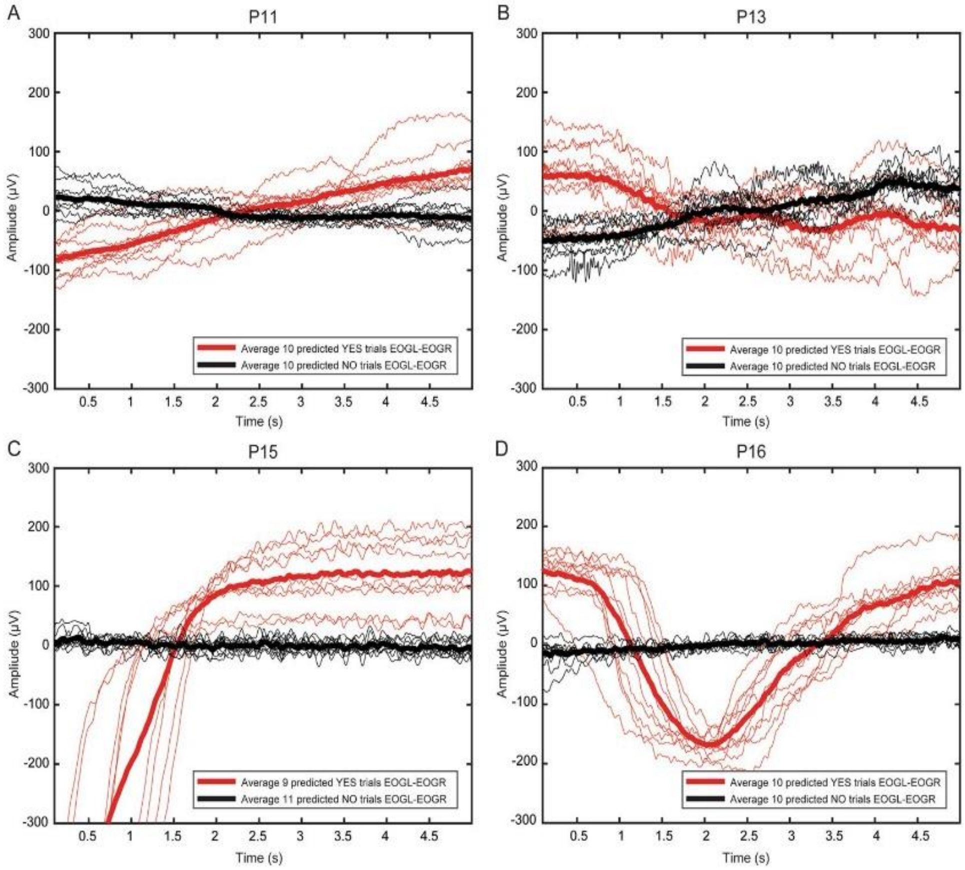

Figure 1. Horizontal eye movement during feedback sessions for all patients. Differential channel EOGL-EOGR for a particular feedback session performed by (**A**) P11, (**B**) P13, (**C**) P15, and (**D**) P16 during the first visit. In each subfigure, the x-axis is the response time in seconds, and the y-axis is the amplitude of the eye-movement in microvolts (μV). The thin and thick red trace corresponds to a single "yes" response and average of all the "yes" responses, respectively. The black thin and thick trace corresponds to a single "no" response and average of all the "no" response. The box at the bottom right of each subfigure lists the number of trials classified as "yes" and "no" by the SVM classifier for that particular session.

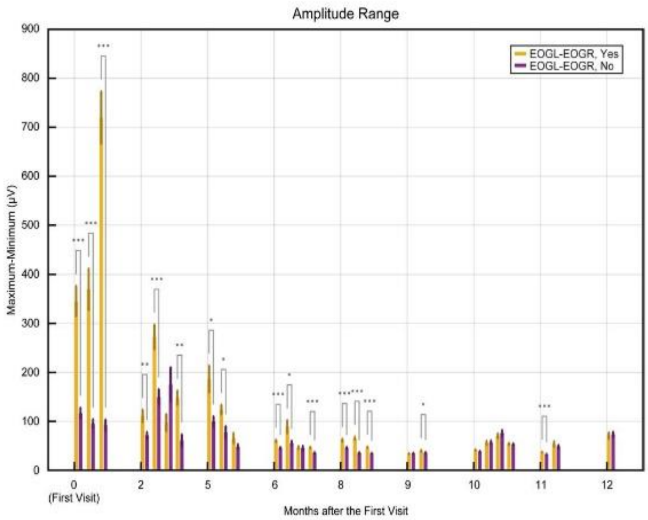

Figure 2. Progressive decline of the eye-movement amplitude along the visits for P11. Depicts the trend of decline in the range of the amplitude of the EOG signal for yes/no questions answered by the patient during the period March 2018 to March 2019. The figure shows the mean and the standard error of the mean of the extracted range of the amplitude of the horizontal EOG signal across each day for yes and no trials. The x-axis represents the month of the sessions, and the y-axis represents the amplitude in microvolts. The asterisk (* - p-value less than 0.05; ** - p-value less than 0.01; *** - p-value less than 0.001) in the figure represents the results of the significance test performed between yes and no for horizontal EOG employing the Mann-Whitney U-test.

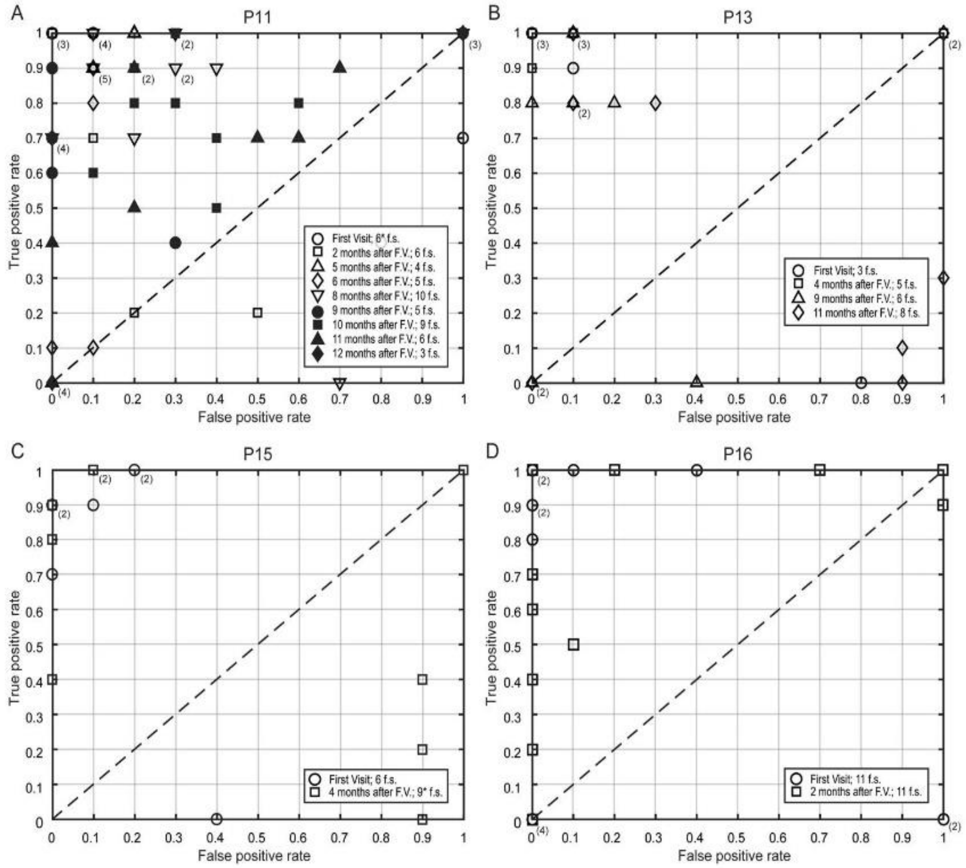

Figure 3. ROC space of feedback sessions for the four patients. Receiver operating characteristic (ROC) space for the performance of the binary support vector machine (SVM) classifier during the total number of feedback sessions performed by (A) P11, (B) P13, (C) P15, and (D) P16. In the figures, the x-axis is the false positive rate (FPR), and the y-axis is the true positive rate (TPR). The diagonal line dividing the ROC space represents a 50% level. Points above the diagonal represent good classification results (accuracy better than 50%), points below the line represent poor classification results (accuracy worse than 50%). In each subfigure, FPR vs. TPR for the feedback sessions are indicated by different arbitrary symbols according to the visit (V) they belong and the date, as defined in the legend at the bottom right side of each subfigure. The rectangular box at the bottom right of each subfigure lists the visit's month and the number of feedback sessions performed during each visit. Some feedback sessions have the same coordinate values in the ROC space, and their symbols overlapped; in these cases, the number of overlapped symbols is specified in parenthesis close to the symbols.

In the developed auditory communication system, the letters have been grouped in different sectors in a layout that was personalized for each patient to match the paper-based layout developed independently by each family (Supplementary Fig. S1). To select one letter, every sector was sequentially presented to the patient and the patient auditorily selected or skipped a sector, once a sector was selected the letters inside the sector were presented auditorily. This select/skip paradigm (i.e., yes/no answer to auditory stimuli) allows the system to work using just a binary yes/no response. The patient could form words by selecting every single letter, but the speed of the system was improved by a word predictor, which, based on the previous selections, suggested the completion of a word whenever it was probable. The speller algorithm is described in detail in the section Speller algorithm.

The results of the copy spelling sessions performed by all the patients are reported in Supplementary Table S5. As shown in Table 2, P11 performed 14 copy-spelling sessions out of which 7 times he correctly copy-spelled the target phrase. Moreover, in one of the other cases, he just miss-selected one letter, and in another one, he selected only one of the two requested words. P13 over 8 sessions copy spelled correctly the target word 6 times. P15 selected correctly the target phrase 3 times out of 5 sessions. Finally, P16 was able to correctly copy spell a target phrase 3 out of 5 sessions. The typing speed achieved by each patient is shown in Table 2.

The system can present one question every 9 seconds, which implies an information transfer rate of 6.7 bits/min. The optimal speed of the speller, along with the user accuracy, depends on the two factors mentioned: first, the speller design for the letter selection (Supplementary Fig. S1); second, the collection of stored sentences (i.e., corpus) needed for the word prediction. In order to describe and evaluate the performance, the sentence "Ich bin" (German for "I am") followed by the name of the patient was considered as a standard example for P11 and P13, while the name of the spouse was considered for P15 and P16. These standard example sentences are composed of 13, 11, 5, and 6 characters for P11, P13, P15, and P16, respectively. Therefore, considering no errors in the answers' classification, the average typing speed for the sentence mentioned above is 1.14 char/min for P11, 1.19 char/min for P13, 1.08 char/min for P15, and 0.87 char/min for P16. These theoretical results show that, due

Type	Patient	Number of sessions	Characters selected	Speed (char/min)
Copy	P11	7/14	5,28 ± 3,59	0,54 ± 0,30
	P13	6/8	4,00 ± 1,67	0,50 ± 0,35
	P15	3/5	5,00 ± 0,00	0,49 ± 0,30
	P16	3/5	5,00 ± 1,73	0,69 ± 0,14
Free	P11	5/9	11,60 ± 8,79	0,57 ± 0,29
	P13	10/11	13,00 ± 10,34	0,48 ± 0,24
	P15	4/5	26,67 ± 19,14	0,68 ± 0,13
	P16	3/3	14,00 ± 4,36	0,64 ± 0,13

Table 2. Results of the spelling sessions performed by the four patients. The columns indicate the type of sessions, the patient, the number of considered sessions over the total number of sessions, the number of characters selected (mean ± standard deviation), and the typing speed in characters per minute (mean ± standard deviation). For the copy and free spelling sessions, only the sessions in which the target was spelled correctly, and the spelled sentence was meaningful, respectively, have been considered. Sessions have been excluded a priori if an error in the code occurred, if the signal was noisy, if they terminated before 15 trials, if the patient did not select any letter, or if all the answers have been classified only as "yes" or only as "no": in total 6 sessions from P11, 2 sessions from P13, 10 sessions from P15, and 3 sessions from P16 were excluded.

to the word prediction, the performance of the speller improves when the patient auditorily spells a complete sentence rather than a single word. The difference between the theoretical and the real typing speed is due to the nature of the speller that requires many inputs to correct a mistake, e.g., if a sector is wrongly skipped, to select that sector again the patient must first skip all the other sectors.

After successful copy spelling sessions, the patients were free to form words and sentences of their choice. The results of these free spelling sessions performed by all the patients are reported in Supplementary Table S6. The typing speed in these sessions is similar to the speed achieved by each patient during the copy spelling; one exception is P13 who due to the low number of errors and to better words' prediction reached the speed of 1.02 char/min during one of the sessions shown in Supplementary Table S6. In most of the sessions, the patients were able to form complete sentences communicating their feelings and their needs. Nonetheless, some of the performed sessions were not successful. Videos of selected spelling sessions are available in Supplementary Videos S1–S3.

Discussion

The auditory communication system enabled four ALS patients, with ALSFRS-R score of 0, on the verge to CLIS to select letters and words to form sentences. Three out of the four patients (P13, P15, and P16) showed, during all the sessions, a preserved eye movement. One patient (P11), followed over one year from March 2018 to March 2019, demonstrated an effective eye-movement control until the penultimate visit (V09 in February 2019), despite August 2017 being the last successful communication with a commercial AAC device. However, the progression of the disease varies from patient to patient.

Nonetheless, P11's successful results of V09, even if not perfect, are very encouraging since they show the possibility of communicating even with an eye-movement amplitude range of $\pm 30\ \mu V$. Even if the developed auditory communication system was used only from V06, the evolution of eye movements of P11 (Fig. 2) indicates that the eye signal was clear enough to be used for communication purposes since the first visit in March 2018. The results of the feedback sessions confirm this during the initial five visits (Fig. 3A). Speed is the main limitation of the developed system since the spelling of one single word could take up to 10 minutes. In the literature, other spelling systems have been successfully tested with ALS patients, and they achieved an information transfer rate of 16.2 bits/min[44] and 19.95 bits/min[45]. However, since all of them are based on visual paradigms, except a single study where the patients communicated just "yes" or "no" using an auditory system[46], comparison with the here proposed system is difficult. The slow speed of our system is an intrinsic characteristic of its auditory nature, even though the spelling time can be reduced by optimizing the speller schema and improving the word prediction with the creation of a corpus of words personalized for each patient. Even though the user experience was not assessed with a questionnaire, it is vital to notice that the patients showed no frustration for this slow speed, which they indicated by moving their eyes when questioned, "Would you like to continue?". The patient formed sentences like, "I am Happy", "I am happy to see my grandchildren growing up", and "I look forward to a vacation" indicating their willingness to communicate. From these, we infer speculatively that the slow speed did not frustrate the patients, probably because even this slow communication is preferred and valued in comparison to the isolation experienced without a functioning eye-tracker. It is essential to employ such a paradigm and follow these patients regularly to elucidate their eye-movement dynamics further and provide them a means of communication.

In conclusion, the long-term viability of an EOG based auditory speller system in ALS patients on the verge of CLIS (with ALSFSR-R score of 0) unable to use eye-tracking based AAC technologies were explored. For one of the patients, it was possible to perform a long-term recording, capturing the changes in the EOG signal, evidencing a correlation between speller performance and progressive degeneration of the oculomotor control. After a follow-up of one year, the patient was unable to take advantage of the spelling system proposed because of the complete loss of oculomotor control. Although the reported system cannot be considered as the ultimate communication solution for these patients according to the best of the authors' knowledge, this is the only system that, during the period of transition from LIS to CLIS, might offer a means of communication that otherwise is not possible. Nevertheless, whether this can be generalized to other patient populations or not is an empirical question.

Methods

The Internal Review Board of the Medical Faculty of the University of Tubingen approved the experiment reported in this study. The study was performed per the guideline established by the Medical Faculty of the University of Tubingen. The patient or the patients' legal representative gave informed consent with permission to publish the results and to publish videos and pictures of patients. The clinical trial registration number is ClinicalTrials.gov Identifier: NCT02980380.

Instrumentation. During all the sessions, EOG channels were recorded with a 16 channel EEG amplifier (V-Amp DC, Brain Products, Germany) with Ag/AgCl active electrodes. A total of four EOG electrodes were recorded (positions SO1 and IO1 for vertical eye movement, and LO1 and LO2 for horizontal eye movement). During some sessions, a minimum of seven EEG channels were recorded for analysis, not directly related to the purpose of this paper. All the channels were referenced to an electrode on the right mastoid and grounded to the electrode placed at the FPz location on the scalp. For the montage, electrode impedances were kept below 10 kΩ. The sampling frequency was 500 Hz.

Patients. Four ALS patients with ALSFRS-R score of 0 participated in this study. Table 1 summarizes the clinical history of each patient and lists the number of visits (V). After the last successful use of AAC, all the four patients were still communicating with the relatives saying "yes" and "no" by moving and not moving the eyes. Using this technique, patients P11, P13, and P15 were forming words by selecting letters from a paper-based layout (Supplementary Fig. S1A–C) developed, independently, by each family. These same layouts were integrated into our developed system to provide each patient with a personalized schema for selecting letters. For patient P16, based on the feedback and suggestions of the family members, we proposed and tested the spelling schema shown in Supplementary Fig. S1D.

Paradigm. The developed paradigm is based on a binary system, in which a patient is asked to reply to an auditorily presented question by moving the eyes to say "yes" and by not moving the eyes to say "no". The paradigm includes four different types of sessions: training, feedback, copy spelling, and free spelling session. Each training and feedback session consists of 10 questions with a "yes" answer and 10 questions with a "no" answer well known by the patient. Each question represents a trial. Copy and free spelling sessions consist of yes/no questions (i.e., trial) in which a patient is asked whether he wants to select a particular letter or group of letters (see the below paragraph Speller algorithm). Each of these trials consists of the baseline (i.e., no sound presented), the stimulus (i.e., auditory presentation of the question and the speller options), the response time (i.e., time for the patient to move or not move the eyes), and feedback (i.e., auditory feedback to the patient to indicate the end of the response time). The training sessions differ from the feedback sessions in terms of the feedback that is provided to the patient. During the training sessions, the feedback is a neutral stimulus ("Danke" – "Thank you" in English) to indicate the end of the response time, while during the feedback sessions, the feedback is the answer that the program classifies (see Online analysis for details). Copy and free spelling sessions differ in terms of the instruction given to the patient. During the copy spelling sessions, the patient was asked to spell a specific sentence, while during the free spelling sessions, the patient was asked to spell whatever he desired.

The length of the response time-window was determined according to the progress and performance of the patient as described in the Supplementary Tables S1–S4 and varies between 3 and 10 seconds. The duration of each trial varies accordingly between 9 and 20 seconds. Therefore, each training and feedback session lasted for 3–7 minutes. The spelling sessions were usually longer (up to 57 minutes), but no fixed time can be indicated since the number of trials is different from session to session; on average, the copy and free spelling sessions lasted respectively for 10 and 27 minutes.

Speller algorithm. After the patients were unable to communicate with the commercial eye-tracker based AACs devices, the primary caretakers developed a speller design/layout. The auditory speller layouts used here were developed by optimizing and automatizing the schematics already used by the primary caretakers. The spellers used by the patients are shown in Supplementary Fig. S1. The spellers consist of letters grouped in different sectors, plus one sector with some special characters ("space", "backspace" for P11 and P15, and for P13 and P16 along with these the additional option, "delete the word"). Despite the different layouts, the same algorithm, as described below, drives all the spellers. The spellers enable the patients to auditorily select letters and compose words. To increase the speed of the sentence completion, the speller predicts and proposes words based on the letters previously chosen. The auditory speller developed to enable patients without any means of communication to spell letters, words freely, and form sentences auditorily have two main components called "Letter selection" and "Word prediction", which are described below.

Letter selection. The patient, in order to select a letter, first must select the corresponding sector, and only once he is inside the sector, he can select the letter. The selection is made, answering "yes" or "no" to the auditory presentation of a sector or a letter. As schematized in the diagram in Supplementary Fig. S2, to avoid false positives, the speller uses a single-no/double-yes strategy. If the recognized answer is "no" the sector is not selected, and the following sector will be asked, if the answer is "yes" the same sector is asked a second time as a confirmation: the sector is selected if the patient replies "yes" also the second time. If the last sector is not selected, the program asks the patient whether he wants to quit the program. If he replies "yes", and confirms the answer, the program is quit.

Otherwise, the algorithm restarts from the first sector. Once a sector is selected, the paradigm for selecting a letter (or a special character) uses the same single-no/double-yes strategy as described above. If none of the letters in a sector is selected, the patient is asked to exit the sector. If he replies a confirmed "yes" the speller goes back asking the sectors starting from the one after the current, otherwise, if he replies "no", the algorithm asks the letters of the current sector again starting from the first one. Whenever a letter or a special character is selected, the speller updates the current string and gives auditory feedback reading the words already completed (i.e., followed by a space) and spelling the last one if it is not complete. After every selected letter, the speller searches for probable words based on the current string (the details are explained in the paragraph below). If a word is probable, the program presents that word auditorily. Otherwise, it starts the letter selection algorithm from the first sector again.

Word prediction. To speed up the formulation of sentences, the speller is provided with a word predictor that compares the current string with a language corpus to find if there is any word that has a high probability of being the desired one. To have a complete and reliable vocabulary, the German general corpus of 10000 sentences compiled by the Leipzig University[47] was used. Since the developed speller contains only English letters, firstly the corpus is normalized converting the special German graphemes (ä, ö, ü, ß) with their usual substitutions (ae, oe, ue, ss). Thus, a conditional frequency distribution (CFD) is created based on the n-gram analysis of the normalized corpus; for word prediction, we considered the frequencies of the single words (i.e., unigrams), of two consecutive words (i.e., bigrams), and three consecutive words (i.e., trigrams). Whenever a letter is added to the current string, the program returns the CFD of all the words starting with the current non-completed word (if the last word is complete, it considers all the possible words). For these words first, the frequency value is considered concerning the two previous words, i.e., trigram frequency. Then, if for the current string, there is any stored trigram in the corpus, the program considers only the last complete word and checks the bigram frequency. Finally, if it is not possible to find any bigram, it considers the overall frequency value of the single word, i.e., unigram frequency. Once all the frequency values of the words are stored, considered as trigrams, bigrams, or unigrams, the program establishes if any of these words are highly probable comparing their values to a predefined threshold. To predict words, we considered a word as probable if its frequency value is more significant than half of the sum of all the frequency values of the possible words. If a word is detected as probable, the speller, after a letter is selected, instead of restarting the algorithm from the first sector, proposes that word to the patient, and if a confirmed "yes" is answered it adds the word followed by a space to the current string.

Online analysis. The EOG data were acquired online in real-time throughout all the sessions. During all the trials belonging to a session (except for the training sessions), the signal of the response time was processed in real-time to extract features to be fed to a classification algorithm for classifying the "yes" and "no" answers. Features computed from the trials of the training sessions were used to train an SVM classifier that was validated through 5-fold cross-validation. The obtained SVM classifier was used to classify feedback and speller sessions only if its accuracy was higher than the upper threshold of chance-level[43].

To extract the features from the signal during the response time, the time-series were first preprocessed with a digital finite impulse response (FIR) filter in the passband of 0.1 to 35 Hz and with a notch filter at 50 Hz. The first 50 data points were removed to eliminate filtering-related transitory border effects at the beginning of the signal. Then all the channels were standardized to have a mean of zero and a standard deviation of one. Subsequently, features were extracted from all the data series from the "yes" and "no" answer for all the channels.

Different features were extracted for the different patients: for P11 and P13 the maximum and minimum amplitude and their respective value of time occurrence feature were used; while for P15 and P16 the range of the amplitude (i.e., the difference between the values of maximum and minimum amplitude) feature was used.

The code was developed and run in Matlab_R2017a. For the SVM classification, the library LibSVM[48] was used. The detailed list of sessions used for building the model and, therefore, perform feedback and spelling sessions are described in the Supplementary Tables S1–S4.

Receiver-operating characteristic space. For binary classifiers in which the result is only positive or negative, there are four possible outcomes. When the outcome of the prediction of the answer is yes (positive), and the actual value is positive, it is called True Positive (TP); however, if the actual answer to a positive question response is negative, then it is a False Negative (FN). Complementarily, when the predicted answer is negative, and the actual answer is also negative, this is a True Negative (TN), and if the prediction outcome is negative and the actual answer is positive, it is a False Negative (FN). With these values, it is possible to formulate a confusion or contingency matrix, which is useful to describe the performance of the classifier employing its tradeoffs between sensitivity and specificity. The contingency matrix can be used to derive several evaluation metrics, but it is particularly useful for describing and visualizing the performance of classifiers via the Receiver-Operating Characteristic (ROC) space[49]. A ROC space depicts the relationship between the True Positive Rate (TPR) and the False Positive Rate (FPR). TPR and FPR were calculated for each feedback session, and they were then used to draw ROC space, as shown in Fig. 3.

Data availability

The data and the scripts are available without any restrictions. The correspondence between sessions and the corresponding raw files are listed in Supplementary Table S7. Data link: https://doi.org/10.5281/zenodo.3605395.

Received: 2 October 2019; Accepted: 30 April 2020;
Published online: 21 May 2020

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Acknowledgements
Deutsche Forschungsgemeinschaft (DFG) DFG BI 195/77-1, BMBF (German Ministry of Education and Research) 16SV7701 CoMiCon, LUMINOUS-H2020-FETOPEN-2014-2015-RIA (686764), and Wyss Center for Bio and Neuroengineering, Geneva.

Author contributions
Alessandro Tonin – Performed 35% of the BCI sessions and data collection; Data analysis; Manuscript writing. Andres Jaramillo-Gonzalez – Performed 35% of the BCI sessions and data collection; Data analysis; Manuscript writing. Aygul Rana – Performed 35% of the BCI sessions and data collection. Majid Khalili Ardali – Data analysis. Niels Birbaumer – Study design and conceptualization; Manuscript correction. Ujwal Chaudhary – Study design and conceptualization; Performed 65% of the BCI sessions and data collection; Data analysis supervision; Manuscript writing.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-65333-1.

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Appendix D

Paper IV: Neurophysiology of patients with Completely locked-in syndrome.

Clinical Neurophysiology

Neurophysiological aspects of the completely locked-in syndrome

--Manuscript Draft--

Manuscript Number:	CLINPH-D-20-13603
Article Type:	Full Length Article
Section/Category:	Sleep and Disorders of Consciousness
Keywords:	Amyotrophic lateral sclerosis; Completely locked-in syndrome; Resting state, Somatosensory evoked potential; auditory evoked potential
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	Ujwal Chaudhary

Abstract:

Objective

Amyotrophic lateral sclerosis (ALS) patients in completely locked-in syndrome (CLIS) are incapable of expressing themselves, and their state of consciousness and awareness is difficult to evaluate. Due to the complete paralysis, any assessment on the perceptual and cognitive level can only be implemented in passive experimental paradigms with neurophysiological recordings.

Methods

Four patients in CLIS were investigated in several experiments including: resting state, visual stimulus (eyes open vs eyes closed), auditory (modified local-global paradigm), somatosensory (electrical stimulation of the median nerve), and during the sleep.

Results

All patients showed altered neurophysiology, but a unique pattern could not be found among patients. However, slowing of the EEG and attenuation or diminish of the alpha waves was common in all patients. In some patient, a slow dominant frequency emerged at 4 Hz which synchronized EEG at all channels. EEG of eyes open and closed were significantly different in all patients. The dominant frequency during the day lost its power during the night and sleep. SEPs were lost or significantly altered in compare to healthy subjects, similarly for AEPs.

Conclusions

The heterogeneity of the results underscores the fact that no single criteria or measure is available to assess cognitive changes at the neurophysiological level. Behavioral assessment of cognition with the aim of a brain-computer interface based communication might solve this problem.

Significance

Most of the studies on the neurophysiology of ALS patients is on the early stage of the disease and there are very few studies on the latest stage when patients are completely paralyzed with no means of communication (i.e., CLIS). This study provides different neurophysiological aspects of these patients at the sensory and cognitive level at the same time in the same patient.

Neurophysiological aspects of the completely locked-in syndrome

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Keywords: Amyotrophic lateral sclerosis; Completely locked-in syndrome; Resting state, Somatosensory evoked potential; Auditory evoked potential.

Highlights

- EEG significantly changes after complete paralysis in the latest stage of the ALS disease.
- Neurophysiological indexes of patients in with completely locked in syndrome (CLIS) is significantly altered with no unique pattern among patients.
- Heterogeneity of the results in the same patient at the same time precludes use of a single criteria to assess cognition in CLIS.

Abstract

Objective

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Most of the studies on the neurophysiology of ALS patients is on the early stage of the disease and there are very few studies on the latest stage when patients are completely paralyzed with no means of communication (i.e., CLIS). This study provides different neurophysiological aspects of these patients at the sensory and cognitive level at the same time in the same patient.

1. Introduction

Completely locked-in syndrome (CLIS) was initially defined as total immobility with intact cognitive processing (Bauer, Gerstenbrand, & Rumpl, 1979) in which the patient is fully conscious but unable to express herself/himself (Hayashi & Kato, 1989; Smith & Delargy, 2005). By this definition, any disease such as quadriplegia and anarthria or neurodegenerative motor neuron disease (MND) like amyotrophic lateral sclerosis (ALS) that is accompanied by total immobility is categorized as CLIS as long as conscious awareness is assumed to be intact (Patterson & Grabois, 1986). It has recently been proposed to only use a lack of communication as the main criteria for CLIS (Chaudhary, Mrachacz- Kersting, & Birbaumer, 2020), which we also use in this manuscript. However, with this definition, the patients' conscious and cognitive state remains undefined, and differentiation to other non-responsive states such as unresponsive wakefulness state (UWS) is not possible. Existence of different approaches in the assessment of consciousness precludes a clinical definition of CLIS in unresponsive patients, hence a clinical assessment of CLIS is not established yet and attempts to differentiate unresponsive disorders of consciousness from CLIS with neurophysiological measures and neuroimaging were mostly unsuccessful (Kotchoubey, Lang, Winter, & Birbaumer, 2003; Kübler, Kotchoubey, Kaiser, Birbaumer, & Wolpaw, 2001).

ALS is a progressive MND that causes loss of motor neurons and eventually completely paralyzes the patient and leads to CLIS (Thorns et al., 2010). In ALS patients, like all the patients reported here, the progression of the disease is not necessarily correlated with a cognitive deficit (Schnakers et al., 2008), although cognitive dysfunction is reported in some cases (Huynh et al., 2020; Stanton et al., 2007). It has been proposed that somatosensory and auditory perception, as well as cognitive processing, are preserved and not affected even after the transition to CLIS (Kübler & Birbaumer, 2008). These shreds of evidence are the main reasons why it is hypothesized that in the final stage of the disease, when the patient is completely paralyzed and unable to express herself/himself, s/he is still cognitively intact with preserved consciousness. Of course, this is only a deductive argument, and valid and reliable experimental clinical observations are required to validate this claim. However, experimental findings have sometimes challenged the idea of intact sensory processing of patients in CLIS. A case study on a patient in CLIS with intracranial recordings reported selective somatosensory dysfunction in joint-mechanoreceptor pathways and raised doubts about the intactness of proprioception in CLIS (Murguialday et al., 2011). Also, vision is said to be impaired or absent in most patients in LIS and CLIS with ALS due to drying and necrosis of the cornea (Tonin et al., 2020). A theoretical analysis of cognition in CLIS based on a motor theory of thinking (Ferster & Skinner, 2005; Washburn, 1916) has speculated "extinction of goal-directed thinking" due to the lack of contingent reinforcement after the transition to CLIS (Kübler & Birbaumer, 2008). The only way to behaviourally assess the perception and cognition in CLIS is through a brain-computer interface (BCI). So far, no case of non-invasive BCI was reported with the ability of free spelling communication in CLIS (De Massari et al., 2013). An invasive approach, resulted in free spelling communication with a patient in CLIS and demonstrated the preservation of cognitive functionality several months after transitioning to CLIS (Chaudhary, Vlachos, et al., 2020). Since the only way for patients in CLIS to communicate is thorough BCI (Birbaumer, 2006; Chaudhary, Birbaumer, & Curado, 2015; Chaudhary, Birbaumer, & Ramos-Murguialday, 2016) and it relies on preserved auditory perception and cognitive functions, we performed a systematic neurophysiological assessment at a perceptual and a cognitive level in patients in CLIS. Several experimental paradigms were used to investigate sensory processing on a neurophysiological level in four ALS patients in CLIS.

We investigated the resting-state electroencephalogram (EEG) of four patients in CLIS and identified the sources of the dominant slow oscillations previously reported in the literature using source localization techniques. To validate the accuracy of the used technique, we compared it with the anatomical source of EEG activity in healthy participants. Altered resting EEG in CLIS with a slow dominant frequency, which synchronizes all recording channels, evokes the question of whether this slow oscillation is constant pathological noise or varies in different arousal states. Therefore, we monitored the variation of the dominant slow EEG frequency during the night. A recent study reported intact sleep cycles in all patients in CLIS, including 3 patients re-recorded for this study, despite pathological slowing during daytime (Malekshahi et al., 2019). In this report, we focused on the variation of the dominant slow frequency during the night without defining the sleep stages. Altered EEG in CLIS makes it excessively difficult to classify different sleep stages, and modified criteria are proposed for classifying sleep stages (For a detailed sleep analysis, see Malekshahi et al., 2019). Along with resting-state EEG, brain reactivity in response to an external stimulus was used to asses brain reactivity to external stimuli. EEG signal was recorded during eyes open (EO) condition as compared to eyes closed (EC) to investigate the alpha wave suppression mechanism as an indicator of basic attentional mechanism in the brain after total immobility since it is known as a neural signature of an attentional arousal mechanism (Danko, 2006; Toscani, Marzi, Righi, Viggiano, & Baldassi, 2010) and can even be detected in complete darkness with no visual input (Boytsova & Danko, 2010).

In addition, evoked related potentials (ERPs) can give us some insight into brain functions at the perceptual level and higher-level cognitive processing that involves selective attention, memory updating, semantic comprehension, and other cognitive functionalities (Duncan et al., 2009). Early ERPs (until 200ms latency) are correlated with automatic, unconscious processing of the stimuli in the brain, while later components are usually a sign of conscious processing of an event. The early and late components of ERPs are not mutually exclusive events; rather they follow each other in a proper experimental paradigm, but there are occasions when they vary independently, particularly in neuropathological cases (Kotchoubey et al., 2005). Bekinschtein et al. proposed an experimental paradigm known as Local-Global (LG) processing, in which the distinction between the unconscious and conscious processing in ERP events is easier to distinguish (Bekinschtein et al., 2009) and several versions of it have been modified and validated on healthy and DoC patients (Rohaut & Naccache, 2017). The main idea behind this paradigm is that, in a set of consecutive sensory stimuli with Local and Global pattern changes, two different types of ERP can be detected. The Local pattern refers to the order of consecutive stimuli within a trial, and the Global pattern refers to the order of trials within an experimental block. Detecting any violation in the order of stimuli within a trial (i.e., local pattern) only requires pre-attentive mechanism and elicits the early evoked response (before 200ms), called local effect (LE), while detecting a violation in the order of presented trials within an experimental block (i.e., global pattern) requires attention and memory updating and is reflected in the later evoked potentials components (after 200ms), called global effect (GE). Based on this principle, a modified version of LG was used to assess auditory perception and cognitive capacities of patients in CLIS and compared to healthy subjects. Also, somatosensory evoked potentials (SEPs) were investigated to assess sensory processing in CLIS. SEPs are used to assess the functional status of the somatosensory pathways and to identify the sensory portion of the sensorimotor cortex (Toleikis, 2005) and are expected to remain intact in CLIS due to ALS. However, studies on ALS patients before the transition to CLIS suggested a pathological slowing of the conduction along central sensory pathways (Constantinovici, 1993; Cosi, Poloni, Mazzini, & Callicco, 1984; Murguialday et al., 2011). Particularly, abnormal somatosensory evoked potentials (SEPs) are reported in LIS and patients in CLIS with no specific pattern of SEP abnormality (Bassetti, Mathis, & Hess, 1994; Gütling, Isenmann, & Wichmann, 1996). Here we report SEPs after transitioning to CLIS.

2. Materials and Methods

A four days visit occurred with all four patients in CLIS. On the first day, a semantic comprehension experiment was performed using jokes as auditory cognitive stimulation followed by somatosensory evoked potentials (SEPs) stimulation. On the second day, auditory evoked potentials (AEPs) with a modified local-global paradigm (Bekinschtein, Davis, Rodd, & Owen, 2011) was performed. On both days, resting-state EEG was recorded while eyes were closed (EC) and open (EO). On the remaining two days, simultaneous EEG and fNIRS recordings were performed in a BCI experiment. Two nights of sleep recording were performed dependent upon the clinical status of the patient and her/his family estimates of the patient's status. In this article, we are reporting the resting state analysis, the EEG changes during sleep, and the brain response to SEPs and AEPs, and each of them is described in detail below. No reliable BCI communication was achieved with none of the patients, except for P1 who previously showed was able to respond to yes-no questions in an fNIRS based BCI (Gallegos-Ayala et al., 2014).

All the EEG was recordings reported here were performed using BrainAmp device (Brain Products Inc, Gmbh, Munich, Germany). The recording sites were based on the 10-10 international systems for electrode placement, using actiCAP from the same company, and by manually adding some extra electrodes, including Fp1, Fp2, F7, F3, Fz, F4, F8, FC5, FC3, FC1, FC2, FC4, FC6, T7, C3, Cz, C4, T8, CP5, CP1, CPz, CP2, CP6 channels. Also, mastoids were recorded to be later used as a reference programmatically. Bilateral Erb's points 2-3 cm above the clavicle were recorded for the SEP analysis. Recordings were performed with a 500 Hz sampling rate referenced to FCz and grounded to Fpz. In addition, two electrodes were used above and below the same eye to record eye movements. Ten healthy subjects underwent the same protocol, with a 3000Hz sampling rate and programmatically resampled to the same frequency as the patient group. For the sleep recordings, eight passive electrodes were placed over Cz, C3, C4, Fz, F3, F4, AF3, and AF4, referenced to the forehead and grounded to a mastoid. Also, four electrodes were used to monitor EOG activity. Two electrodes were placed on either side of the eyes close to lateral canthus, and two electrodes were placed above and below one of the eyes, depending on the physical accessibility.

All the analyses reported in this paper are performed using Matlab (Mathworks, 2018). The data was analyzed using Fieldtrip toolbox (Oostenveld, Fries, Maris, & Schoffelen, 2011), and EEGLab (Delorme & Makeig, 2004) for some plots.

2.1. Ethical Approval

The Internal Review Board of the Medical Faculty of the University of Tubingen approved the experiment reported in this study. The study was performed per the guideline established by the Medical Faculty of the University of Tubingen. The patient or the patients' legal representative gave informed consent with permission to publish the data. The clinical trial registration number is: ClinicalTrials.gov - Identifier: NCT02980380.

2.2. Participants

Four patients reported here are also being reported in other experimental contexts by the same authors, so to keep consistency, the indexes of patients' numbers are not necessarily sequential. Reports on the same patients are also reported in (Malekshahi et al., 2019; Maruyama et al., 2020). In addition, 10 healthy subjects (20% female) with mean age of 28.8 years old and standard deviation of 4.5 underwent the same producer as patients for comparison.

Patient 1 (P1), female, 75 years old, and CLIS, was diagnosed with sporadic bulbar ALS in May 2007, was diagnosed as locked-in in 2009, and as completely locked-in May 2010, based on the diagnosis of experienced neurologists. She has been artificially ventilated since September 2007, fed through a percutaneous endoscopic gastrostomy tube since October 2007, and was in homecare. No communication with eye movements, other muscles, or assistive communication devices was possible. She passed away in 2019.

Patient 4 (P4), female, 29 years old, and CLIS, was diagnosed with juvenile ALS in December 2012. She was completely paralyzed within half a year after diagnosis and has been artificially ventilated since March 2013, fed through a percutaneous endoscopic gastrostomy tube since April 2013, and is in homecare. She was able to communicate with the eye-tracking device from early 2013 to August 2014 and was unable to use the eye-tracking device after the loss of eye control in August 2014. After August 2014, family members were able to communicate with her by training her to move her eyes to the right to answer "yes" and to the left to answer "no" questions until December 2014. In January 2015, eye control was completely lost, and she tried to answer "yes" by twitching the right corner of her mouth, and that too varied considerably, and parents lost reliable communication contact.

Patient 9 (P9), male, 25 years old, and CLIS, was diagnosed with juvenile ALS with FUS mutation heterozygote on Exon 14: c.1504delG, gene mutation diagnosed in 2013. He has been artificially ventilated since August 2014 and is in homecare. He started communication using Tobii eye-tracking device (Tobii Dynavox, Dnderyd, Sweden) in January 2015. He was able to communicate until December 2015, after which the family members attempted to communicate by training him to move his facial muscles to answer "yes" but the response was unreliable. No communication with eye movements, other muscles, or assistive communication devices was possible since 2016.

Patient 17 (P17), male, 63 years old, and CLIS, was diagnosed with ALS of the second motor neuron in spring 2009. He was not able to breathe after one year, is artificially ventilated, and fed through a percutaneous endoscopic gastrostomy tube since spring 2010. He was able to communicate with the Tobii eye tracking device (Tobii Dynavox, Dnderyd, Sweden) until 2014. After that the family was trying to communicate based on his eye-movements. Family members were able to communicate with him by training him to move his eyes to the right to answer "yes" and to the left to answer "no" to the questions. For the last 2-3 years, it is almost not possible to recognize any voluntary response. He had constant involuntary eye movements even when eyes were closed. Movements were similar to horizontal optokinetic nystagmus with a large range of motion. He passed away in 2019.

2.3. Resting-State EEG

The data acquired from each patient was filtered using a notch filter at 50 Hz, and bandpass filtered between 0.5 Hz to 45 Hz. Noisy channels were interpolated, and the signal was re-referenced to both earlobes. ICA components were extracted from EEG, and noise components were removed manually by visual inspection. This signal is referred to as "cleaned data" in this text. For each patient, the second 64 seconds of cleaned data in EC condition are plotted, and abnormalities of the time domain data are highlighted. Independent component analysis (ICA) was used to remove artifacts and plotted around the EEG trace to illustrate the spatial distribution of the source of the EEG activity. The power spectral density (PSD) of four representative EEG channels are plotted to compare eyes open and close.

2.4. Source Localization

For healthy subjects, the individual alpha peak frequency of EC resting, and for patients, the dominant slow frequency of EC was selected as the frequencies of the interest (FOI). Dynamic Imaging of Coherent Sources (DICS), as an optimal Beamforming technique for solving the inverse problem (Fuchs, 2007; Gross et al., 2001; Jonmohamadi et al., 2014), was used to localize the FOI for each subject. Partial Canonical Coherence (PCC) which essentially implements DICS and provides more flexibility in data handling (REF; FieldTrip, 2011) was used to calculate neural activity index using the frequency transformation of the signal. For the forward model, the standard head volume conduction model proposed by Oostenveld et al. was used (Oostenveld, Stegeman, Praamstra, & Van Oosterom, 2003).

2.5. Eyes Open vs. Eyes Closed

The EEG of patients in CLIS is significantly different from healthy, and the alpha power in the range defined for healthy subjects (8 to 13Hz) tends to be zero in both EO and EC conditions. Besides, in the recordings of patients in this study, the occipital region was not covered. To overcome these issues, in addition to PSD comparison, another metric based on the comparison of the variability of the signals in the two conditions, using second-order plots (SOP) of time-domain EEG data was used, which is proposed to be more subtle even for healthy subjects, due to its' sensitivity to the EEG changes without the need to specify a particular frequency range for comparison (Thuraisingham, Tran, Boord, & Craig, 2007).

All subjects were recorded 3 minutes in EO, followed by 3 minutes EC. In patients for EO recordings, the eyelids were taped open, and using eye drops, the corneas were kept wet, and for EC condition, the eyelids were closed and covered with dark covers. In some recording of patients, the time of EO was reduced by caretakers and varied between 2 to 3 minutes.

2.5.1. PSD Comparison

The power spectrums were calculated on the cleaned EEG for both conditions using a Welch's overlapping window of 5 seconds and 30% overlap, using multitaper frequency transformation and the boxcar taper. For each single frequency bin, a student t-test was performed to compare EC and EO with 0.01 significant level, corrected for multiple comparisons using the Bonferroni method. Each channel is marked as different if it contained at least 10 consecutive frequency bins (equivalent to 2 Hz) with a significant difference between the two conditions below 25 Hz.

2.5.2. Second-order plots

For a cleaned EEG of each condition, the time domain EEG is shifted one data point and subtracted from the original signal, and denoted as X. Similarly, the signal is shifted two data points and is subtracted from X and denoted as Y. Plotting X over Y represents the variability of the original signal in three consecutive data points (embedding dimension equals to three). Measure for central tendency was defined as the minimum radius for a circle that holds at least 90% of all the data points in it and denoted as r. Finally, r is calculated for every time window in the cleaned data, epoched to five seconds with a 30% overlap. The variability of the r in EO and EC is compared using student t-test with 0.01 significant level, corrected for multiple comparisons, for details, see (Thuraisingham et al., 2007).

2.6. Sleep

Patients in CLIS need constant medical care also during the night, and patients are repositioned by caretakers during the night to avoid decubitus. Thus displacement or detachment of recoding electrodes are very common in sleep recordings. Therefore, two nights of sleep are recorded, and by visual inspection of the raw data, one of the recordings with fewer artifacts, preferably the second night, is analyzed and reported here. Selected recordings are then separated between EEG and EOG signals for visualization. EEG signal is filtered between 0.5 Hz to 30 Hz and the EOG signal is filtered between 5 Hz to 75 Hz. Noisy channels in EEG were interpolated. Noisy channels in EOG data were rejected. The filtered data is segmented to 30 seconds data with no overlap, and power spectral density is computed. For each frequency in the 30-second window, the median of absolute power in all channels is calculated then normalized and plotted over the night.

2.7. Somatosensory evoked potentials

Monophasic electrical square pulse with 200 µs pulse width at the rate of ~3 Hz was applied with the intensity of stimulation range proposed by the American Clinical Neurophysiology Society (ACNS) at 30 mA (American Clinical Neurophysiology Society, 2009). For P4, P9, and P17 for whom caretakers believed communication with the patient seemed possible from time to time by movements of a facial muscle or eyes at a very slow rate, the stimulation intensity was determined with caretakers but still in range proposed by ACNS. However, no reliable communication was ever achieved with these patients. The stimulation was performed on two blocks with 250 repetitions. Between the stimulation of the median nerve, the tibial nerve of the contralateral site was stimulated with the same protocol. Since the recording of spinal cord evoked potentials (SCEPs) was not possible, the tibial stimulation is not reported here. For the stimulation, bar electrodes with 8 mm diameter and 3 cm distance by Technomed (Technomed, The Netherlands) were placed over the wrists, cathode proximal, and connected to a D188 digital switch connected to DS7A constant current Stimulator (Digitimer Ltd., Welwyn Garden City, UK). Ten healthy subjects underwent the same procedure for comparison.

Data were resampled to 3000Hz and the electrical noise of the stimulation was removed by replacing the data points between the beginning of stimulation (zero lag) to 7 ms after the stimulation by a flat line (Waterstraat, Fedele, Burghoff, Scheer, & Curio, 2015) and then filtered between 3 to 1000 Hz. Epochs from -15ms to +150ms were extracted and averaged over all 500 trails. Representative channel was manually selected from ERP images among the CP5/6, CP3/2, C1/2, or C3/C4 channels and referenced to F1/F3. For plotting N9, Erb's point ipsilateral to the stimulation was referenced to the contralateral Erb's point. Topoplots for the P50 is plotted as the most significant lateralized response to the stimulation.

2.8. Auditory evoked potentials

Auditory stimuli consisted of two pure tones with 500Hz and 1000Hz frequency with 50ms duration, 5ms rise up, and 5ms rise down. Five sets of auditory stimuli were presented with 100ms inter stimulus interval (ISI) and referred to as a stimulation epoch in this text. A trial was defined as a repetition of 5 epochs with 650ms ISI (Fig. 7). Brain response to the changes in the order of auditory stimuli within an epoch was defined as a local effect (LE) and the brain response to the change in the order of trials within a block was defined as a global effect (GE). As depicted in Fig. 7, two different global patterns in two experimental blocks were used. Each block consisted of 120 trials with the first 20 as training trials for the participant to learn the rule of global regularity within that block. In each block, the order of presenting trials was randomized with an 80% probability of the trials that were not violating the global pattern (i.e., globally similar) and 20% for the ones that were violating the global regularity (i.e., globally deviant). The difference between the two blocks was in the pattern of stimuli in one epoch (i.e., local pattern). In the first experimental block, in the first four epochs, all the auditory stimuli are the same (i.e., locally similar), while in the second experimental block, the last auditory stimulus is different from the first four (i.e., locally deviant). In summary, as depicted in Fig. 7, four different conditions resulted in two blocks, Globally Similar Locally Similar (GSLS), Globally Similar Locally Deviant (GSLD), Globally Deviant Locally Similar (GDLS), and Globally Deviant Locally Deviant (GDLD). GSLS and GDLD are used as a control condition for GDLS and GSLD, respectively. For details, see (Bekinschtein et al., 2009).

The significant difference between the first four epochs in the first and second block, which is only due to the violation of local pattern, was calculated as LE. The significant difference between the two conditions in the second block and their control condition in the first block, which had the same local pattern while their global pattern was changing, was calculated as the GE. The p-Value obtained from the t-test comparison for LE and GE is scaled in 10 gradual levels and plotted for comparison, in which 0 indicates no significant difference and 9 indicates a significant difference at 0.01 level corrected for multiple comparisons. For healthy subjects' plot, LE and GE are averaged across all subjects.

For the ERPs, the signal was resampled to 500Hz and filtered between 1Hz to 20Hz, noisy channels were interpolated, trials averaged across conditions, and the baseline was removed. ICA ran on epoched trials and noise components were removed, by visual inspection, outliers were rejected, using the FieldTrip visual inspection toolbox.

3. Results

A figure with the same structure is provided that summarizes all the experimental findings for each patient. Fig.1 for P1, Fig.2 for P4, Fig.3 for P9, and Fig.4 for P17. Descriptive and analytic findings of the most important findings for each experimental paradigm in each patient are reported separately below.

3.1. Resting-State

All patients had an EEG amplitude range of ± 50 μV. Alpha peak was completely missing in P1 and P9, but a very small peak at around 8 Hz can be detected in P4 and P17. In P4 and P9 a slow oscillation at around 4 Hz with a huge power is emerged and can be detected both in time domain and frequency domain plots (Fig.2a&b and Fig.3a&b). Although the patient does not show any visible and detectable eye movements for communication purposes with caretakers, there exists eye activity in the list of ICA components of all patients, except for P9, speculating the presence of attenuated remaining eye activity after the patient is considered to be CLIS. PSD pattern did not significantly change between eyes open and eyes closed conditions in none of the patients (Fig.1-4b), which is described in detail in the ' Altered resting EEG in CLIS with a slow dominant frequency, which synchronizes all recording channels, evokes the question of whether this slow oscillation is constant pathological noise or varies in different arousal states. Therefore, we monitored the variation of the dominant slow EEG frequency during the night. A recent study reported intact sleep cycles in all patients in CLIS, including 3 patients re-recorded for this study, despite pathological slowing during daytime (Malekshahi et al., 2019). In this report, we focused on the variation of the dominant slow frequency during the night without defining the sleep stages. Altered EEG in CLIS makes it excessively difficult to classify different sleep stages, and modified criteria are proposed for classifying sleep stages (For a detailed sleep analysis, see Malekshahi et al., 2019). ' section.

In P4 and P9 abnormal EEG patterns can be detected that synchronizes EEG in all channels at the same time and lasts for almost two seconds (Fig. 2-A). This pattern repeats periodically every five to eight seconds and has a similar morphology to the burst suppression and triphasic waves that can be found in coma patients and anesthetic subjects (Emilia Cosenza Andraus, Fantezia Andraus, & Vieira Alves-Leon, 2011; Niedermeyer, 2009). This synchronization signal at ~4 Hz dominates the background activity at all channels at the same time with progressive increase and decrease of the amplitude (Fig. 2,3a&b) and activities above 4Hz that are superimposed with the dominant slow frequency are not appearing in EEG plots. Thus the EEG of these two patients shows more unpredictability than the EEG of P1 and P17.

3.2. Source Localization

As demonstrated in Fig. 5-A, in every healthy subject, the source of alpha activity is reconstructed over the posterior cortices, even though there was no electrode covering the occipital region. This result validates the mathematically hired method as a proper tool for localizing the source of a particular frequency of interest in EEG activity with the recording montage used in this study. Source localization results for patients (Fig. 6) demonstrate that in all patients, deeper structures of the brain are the origin of the slow dominant frequency. However, the exact location varied among patients, and no common pattern could be found among patients.

3.3. Sleep

For P1 due to the family's request, only one night was recorded, which contained a continuous recording noise that could not be removed. In the other three patients, the dominant slow frequency in the EEG loses power periodically during the episodes at night concurrent with an increase of the power in the EOG channels. It should be noted that no voluntary EOG activity can be visually or instrumentally detected for communication during the day. In P4, who got benzodiazepines (Lorazepam, 0.5 mg) at the time of the experiment, these episodes repeated 3 times during the night with the duration of almost an hour (Fig.2f) while in P9 these episodes had a lower duration of approximately 15 minutes but got more frequent (Fig.3f). In P9, EOG data was not recorded and its' correlation to EEG cannot be reported. In P17 attenuated alpha activity at 8 Hz vanishes during episodes during the night at the same time with losing the power of dominant slow frequency during the day at 2Hz and EEG gets as slow as 1 Hz; During these episodes at night, the EOG activity significantly increases, which indicates a similar pattern with other patients but with a different dominant frequency.

3.4. Eyes Open vs. Eyes Closed

Using bootstrapping, 95% confidence interval for the number of channels that showed a significant difference between EO and EC, in the healthy subjects, was calculated between 20.4% to 45.6% using PSD comparison, and between 39.6% and 66.4% using SOP method (Fig. 5-c). Although the alpha peak was present in the PSD in one healthy subject, no significant difference was detected in the PSD comparison method in none of the channels, while using the SOP method significant difference between the two conditions could be detected in some channels. This subject had no history of visual, neurological, or psychiatric disorders and was excluded from the PSD method's bootstrapping iterations. None of the patients showed any EEG changes while opening the eyes using the PSD comparison method, while the SOP method captured significant differences at least in four channels in all patients (Fig. 1:4b&d).

3.5. Somatosensory evoked potentials

In all healthy subjects, the N9 component on Erb's indicated intact peripheral somatosensory pathway. The early and lateralized component before 50ms were detected in all subjects. Although the SEP patterns were different among subjects and inter-individual variability was high. Nevertheless, the presence of the non-cephalic N9, and cortical P50, and the propagation of the stimulation response in the brain was found in all healthy subjects.

In P1 and P17, the N9 is missing on the Erb's point (Fig 1,4c). This might indicate the absence of the sensory neural pathways or a huge increase in the sensory threshold for stimulation intensity. However, the possibility of experimental failure cannot be excluded. Repetition of the experiment with higher stimulation intensity can rule out the speculation of the increase in threshold or experimental failure hypothesizes. Unfortunately, both patients passed away and it was not possible to replicate the experiment. In P4 and P9 that intactness of afferent neurons could be detected with N9 response on Erb's points, the N20 was missing bilaterally and P50 was the most significant earliest brain response (Figure 2-3c), which propagated over the sensory cortex bilaterally with a uniform spatial distribution.

3.6. Auditory evoked potentials

As presented in Fig. 5-b results from healthy subjects indicate that LE can be detected in earlier latencies (before 150 ms) in comparison to GE, which appears at latencies after almost 200ms. However, the inter-individual variability was high due to the small number of subjects in the healthy group.

In P1, no LE was detected, while very late GE appeared after 500ms in only some frontal channels. A closer look at the ERP pattern in Fig. 1-d indicates that in the deviant condition in block 1 (i.e., GDLD) a clear P3 peak can be found. However, this peak is not statistically significantly different from the baseline due to the large variance of the baseline activity. In P9, also no LE could be detected but there seems to be a strong GE. A closer look at the ERP raw signal in Fig 3-d clarifies that the highly synchronous sinusoidal background EEG is the main reason behind this statistical difference, while no physiologically relevant response can be detected, which means that a phase shift in the raw EEG signal is causing a significant difference between any two conditions independently from experimental conditions. In P17, both LE and GE are present, however, the responses are delayed to 500ms after the stimulation onset. For P4, the experiment was terminated due to technical issues no report is available.

4. Discussion

Results from each experiment are discussed individually below, and a summary discussion of all the findings is provided at the end.

4.1. Resting-State EEG

Given that ALS patients before the transition to CLIS show close to normal EEG (Hohmann et al., 2018), the large difference found here in each patient after the transition to CLIS is considerable. This is not the first time that the slowing of EEG activity is reported in patients in CLIS (Hohmann et al., 2018; Malekshahi et al., 2019; Maruyama et al., 2020). Although EEG changes seem to be different from patient to patient, the slowing of the signal is common in all of them. The slowing of the EEG and lack of alpha peak is not limited to patients in CLIS and is found also in aging (Scally, Burke, Bunce, & Delvenne, 2018), Alzheimer (Cantero et al., 2009), attention-deficit /hyperactivity disorders (Lansbergen, Arns, van Dongen-Boomsma

Martine, Spronk, & Buitelaar, 2011). However, a large increase in the power in a particular frequency band below 5Hz and synchronization of the signal in all channels is unique in patients in CLIS and to some extent similar to coma patients (Hofmeijer, Tjepkema-Cloostermans, & van Putten, 2014; Niedermeyer, 2009), particularly for P4 and P9. Due to the neurodegenerative nature of ALS, loss of motor neurons in the central nervous system, loss of mass and volume, and atrophy of the brain are frequently reported (Kassubek et al., 2005; Mezzapesa et al., n.d.; Mioshi et al., 2013a), which alone can effectively change the EEG pattern in the latest stage of the disease in CLIS. Unfortunately, due to these patient's physical condition, there is no structural MRI available of a CLIS to validate this speculation. Overall, it seems that there are two distinct EEG patterns in CLIS, in the first group EEG characteristics are attenuation or loss of power in the alpha band, decrease in the EEG amplitude, more complex and not predictable EEG, and weak alpha-like activity at around 8 Hz, such as P1 and P17. The second group is the diminishing of alpha waves and emergence of a very slow and high power at around 4 Hz, which dominates the whole spectrum and is phase synchronized in all the channels, such as P4 and P9. Of course, a small sample of four patients is not enough to generalize to all patients in CLIS but most of the patients reported by authors can be classified in one of the two mentioned categories (Hohmann et al., 2018; Malekshahi et al., 2019; Maruyama et al., 2020; Ramos-Murguialday et al., 2011, 2013).

4.2. Source Localization

Different criticism could be raised against source localization techniques, especially when the source of the activity is located in the deeper structure of the brain, and when only a few electrodes are used. The algorithm is forced to locate the source of activity in such a way that the input data can be reproduced, and the easiest way (in terms of computational costs) would be to assume the source of the common activity in the middle of the head model. However, anatomically is also more plausible to find the source of common slow activity that synchronizes all EEG recording channels at the same time with no phase lag in a deeper structure of the brain with symmetric cortical accessibility. Particularly in P4 and P9 that all EEG channels are episodically synchronized in one particular low frequency without any phase shift between all channels, it is highly probable that the source of activity is subcortical, with all other probability, to be thalamic. In ALS patients, after total loss of upper or lower motor neurons, brain networks with presynaptic and postsynaptic connections to the motor assemblies in the diencephalon such as the subthalamic network that together with striatum are controlling skeletal muscle movements might also be disrupted. This is in line with different observations reporting atrophy and shape changes of the cerebral and corticospinal tract in ALS patients before transitioning to the CLIS (Kumar, Aga, Gupta, & Kohli, 2016; Mioshi et al., 2013b; Rajagopalan et al., 2013). It has been reported that the functional connectivity of the sensorimotor cortex (SMC) to the cingulate cortex is increased in ALS patients prior to the transition to CLIS (Agosta et al., 2011). Additionally, default mode network connectivity is reported to be increased in ALS patients before the transition to CLIS (Chenji et al., 2016). Loss of inhibitory motor neurons in ALS patients (Lloyd, Richardson, Brooks, Al-Chalabi, & Leigh, 2000) is proposed as the main reason for the increase in brain connectivity and baseline activity (Chenji et al., 2016; Douaud, Filippini, Knight, Talbot, & Turner, 2011) and are expected to be even stronger after the transition to CLIS and might be the reason behind the emergence of high power and slow activity in EEG of patients in CLIS.

4.3. Sleep

Authors have recently reported that patients in CLIS have similar to normal sleep behavior and circadian rhythms, including 3 patients reported here (Malekshahi et al., 2019). Continuous several days recording of a single patient in CLIS have shown that the sleep cycles are distributed during the day and are not only limited to the night (Ramos-Murguialday et al., 2013). In this report, we investigated the fluctuation of the dominant slow frequency in the EEG data during the nighttime recordings and demonstrated that in all patients the dominant frequency of the day is slower at night, down to 1 Hz in some patients. We also demonstrated cyclic changes in the EEG band power during the night that might be correlated with different slow-wave sleep stages. However, due to patient's significant changes in the EEG pattern, the classification of different sleep stages with criteria from healthy subjects is not possible in any of the patients. Overall, in all patients, the power of slow dominant frequency in the EEG during the day is lost in those cyclic episodes of ultraslow waves during the night. This may suggest that the slow dominant background EEG that is common in all patients in CLIS should not necessarily be interpreted pathological and the ultraslow activity during the night might indicate the typical slow-wave sleep episodes (Sleep stage 3). For the sleep analysis see Malekshahi et. al., 2019.

4.4. Eyes Open vs. Eyes Closed

The increase of the confidence interval to detect EO vs. EC in healthy subjects while using the SOP method in comparison to the PSD comparison method supports the idea that SOP is more sensitive than PSD comparison in detecting brain reactivity while opening the eyes. Besides, lost or attenuated alpha peak in the patients and failure of the PSD comparison method to detect the EO condition indicates that the methodologies used healthy subjects' analysis might be insufficient in patients with significantly altered EEG. Although for P4 and P9, the number of channels that showed significant differences using the SOP method, are smaller than in healthy subjects, the fact that there is some difference at all might indicate some level of visual processing in patients. Also, in these two patients only in one of the two days recordings, brain reactivity to the opening of the eyes could have been detected. With non-responsive patients like CLIS, it is a challenge to detect arousal changes during an experiment; here, we assume that the difference between the two days is due to the level of arousal in the recording of these days. Studies have shown that episodes of sleep during the daytime are a common behavior in patients in CLIS and it is a possibility that the patient is not attentively aroused during an experiment.

4.5. Somatosensory evoked potentials

Diagnosis is not the primary function of SEPs, yet, loss of all cortical and subcortical SEP components is usually associated with brain death in anoxic coma (Cruccu et al., 2008). On the other hand, ALS patients before the transition to CLIS show altered SEPs probably because of the compensatory activity of the sensory-motor cortex (Hamada et al., 2007). Considering that the pathology of sensory pathways in ALS patients is not well known yet, any diagnosis based only on SEPs in ALS-CLIS patients should be avoided.

Absence of the N9 response on the Erb's point in P1 and P17, which only requires proper functioning of the peripheral somatosensory neural pathway, may represent the dysfunction of sensory pathways in the latest stage of the disease. Although, experimental failure cannot be ruled out and no further hypotheses can be given before repetition in the same subjects and others. In P4 and P9, with the presence of N9 and bilaterally absence of cortical responses before 50 ms, the patients' condition might be more critical, since the absence of bilateral N20 in median nerve stimulation of anoxic coma has always been associated with severe brain damage (Cruccu et al., 2008). But, as mentioned before, due to the alteration of the SEPs in ALS before the transition to CLIS, any diagnosis solely based on the SEPs should be avoided.

4.6. Auditory evoked potentials

Patients in CLIS, with altered background EEG, did not show similar ERP response to auditory stimuli compared to healthy people and each patient had a unique brain response. Although, lack of LE and delayed GE was common in all patients, which is in line with previous findings reporting delayed auditory ERP response in CLIS (Kotchoubey et al., 2003). Even with insufficient attentional early analysis of the stimulus characteristics like modality and intensity, a cognitive differentiation of differential characteristics (global or local without the presence of both) is possible late in the processing stream. Similar paradoxical findings were reported by Kotchoubey et al. in severe brain-damaged patients, where a semantic mismatch potential (N400) was present late in the processing stream (at around 600ms) while all early components such as the mismatch negativity after 150 to 200ms were completely absent (Kotchoubey et al., 2005). Whether this indicates conscious processing in the absence or only rudimentary early automatic differentiation of stimulus characteristics is more a basic theoretical question than an empirical question, but such a separation seems possible. A similar paradox was observed in autistic savant patients, where early (unconscious) processing ERP components are excessively large while late (conscious) components are completely absent (Birbaumer, 1999).

5. Conclusion

Resting-state EEG of four patients in CLIS was analyzed in EC and EO condition. In all patients, the EEG was significantly slower than in healthy subjects. Although slowing of the EEG was common in all patients, two different patterns of slow EEG were found. Two of the patients had a slow EEG activity, with slow alpha-like activity around 8 Hz with attenuated power. The other two had as slow EEG, with a higher amplitude, highly synchronized in all recording channels, and with a huge peak at around 4 Hz. The slow and high power 4 Hz signal cannot be compared with sleep slow-wave activity in these patients because the sleep slow-wave episodes were slower around 1,5 Hz. Reduction of power in the dominant frequency was usually correlated with an increase in EOG activity, although for communication with the patient in BCI experiments, no voluntary EOG activity could be detected in any of the patients. The source of the dominant slow activity in the resting state recordings during the day might be localized in subcortical regions and thalamic. All patients showed a small but detectable significant difference in the resting state EEG while opening the eyes. The two patients with slow and high amplitude EEG with high synchronization in all the recording channels were bilaterally missing the N20 in the electrical stimulation of the median nerves (SEPs) and the first peak was found at 50ms, and the SEPs were significantly different from the healthy. In the other two, no response could be detected, and electrical stimulation did not reach to the Erb's point, consequently, did not cause any cortical evoked response, reasons remained unclear. In the two patients with dominant slow frequency at 4 Hz, no AEPs could be found. While in the other two patients, pre-attentive evoked responses were absent, and attentive late evoked responses were retained but delayed to 500ms. We proposed a pathology in the early automatic perceptual sensory systems with an intact but delayed higher cognitive processing system analogously to findings in autistic spectrum disorder (Birbaumer, 1999). " Extinction of goal-directed thinking" (Kübler & Birbaumer, 2008) and lack of contingent reinforcement and rewards in the absence of any type of communication from a behavioral point of view, might reduce cognitive capability after longer periods in CLIS. Due to the small number of patients in this report, results are vulnerable to bias and need to be validated with more patients. It is important to follow patients longitudinally before the transition to CLIS until months after that, to elucidate the relation between the progress of the disease and changes in brain responses. It is unclear yet if these changes are gradual or occur in a rapid stepwise fashion.

The extreme heterogeneity of the results of different brain measures in CLIS reflecting different neural and therefore different cognitive processes remind us of a similar dilemma in the diagnosis of conscious processes in the severe brain-damaged patients (Laureys & Boly, 2007; Majerus, Gill-Thwaites, Andrews, & Laureys, 2005; Real et al., 2016); while one measure (i.e. SEP) denies any conscious or cognitive process the other measurement points to the existence of highly complex semantic thinking and reasoning in the same patient at the same time. This warns us not only of seemingly consistent theoretical explanations and theories in CLIS and disorders of consciousness (DoC) but also of any clinical diagnostic statements and assurances; we just do not know what is possible yet. From this work on patients in CLIS who were never investigated with such measurements before we are forced to conclude that we have some neural indices indicating intact or only deviant cognitive processing while others suggest severe disorders of perception and reasoning. The ethical and therapeutic consequences of such results are clear: never give up hope, there is not a single patient reported in the literature with CLIS or minimally conscious state (MCS) who would not show "islands" of cognitive processing, but nobody can say much about the capacities and nature of these "islands".

Acknowledgments

Supported by Deutsche Forschungsgemeinschaft (DFG, BI 195/77-1), BMBF (German Ministry of Education and Research) 16SV7701 CoMiCon, Deutsche Forschungsgemeinschaft (DFG, Bi195, Kosellek), LUMINOUS-H2020- FETOPEN-2014- 2015-RIA (686764), and Wyss Center for Bio and Neuroengineering, Geneva.

Competing interests

None of the authors have potential conflicts of interest to be disclosed.

Authors Contribution

MKH designed the study, acquired the data, performed the analysis and wrote the first draft of the paper. SW verified the analysis and interpretation of the result and acquired the data. AT helped in the implementation of the experimental paradigms. NB conceptualized the worked, designed the experiments and substantially revised the paper. UC accumulated the funding ins and revised the paper.

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Figures

Figure 1: Neurophysiological map of P1 a) 60 seconds of resting state EEG while EC with the amplitude range of ± 31 μV; C7&C9 ICA components might indicate remaining eye activity b) PSD of four representative central and parietal EEG channels in EC (red) vs. EO (blue) c) Significant differences between EO vs. EC using PSD comparison (top) and SOP (down). d) SEPs for the stimulation of the right and left median nerves; topo plots of most significant evoked response at 50 (P50); N9 responses recorded from Erb's points; cortical SEPs of right (top) and left (bottom) median nerves. e) The significance level of local (Top-Left) and global (Top-Right) effects in AEPs of local-global paradigm and their corresponding ERPs in four conditions: GSLS(black), GDLD(red), GSLD(green), and GDLS(blue).

Figure 2: Neurophysiological map of P4 a) 60 seconds of resting state EEG while EC with the amplitude range of ± 36 μV; C7 ICA component might indicate remaining eye activity b) PSD of four representative central and parietal EEG channels in EC (red) vs. EO (blue) c) Significant differences between EO vs. EC using PSD comparison (top) and SOP (down). d) SEPs for the stimulation of the right and left median nerves; topo plots of most significant evoked response at 50 (P50); N9 responses recorded from Erb's points; cortical SEPs of right (top) and left (bottom) median nerves. f) Periodic decrease in the power dominant frequency in the sleep EEG (top) and increase of EOG activity (bottom) during a night. Vertical lines represent environmental noises such as truing lights on or off, medication, or auditory noises.

Figure 3: Neurophysiological map of P9 a) 60 seconds of resting state EEG while EC with the amplitude range of ± 30 μV; b) PSD of four representative central and parietal EEG channels in EC (red) vs EO (blue) c) Significant differences between EO vs EC using PSD comparison (top) and SOP (down). d) SEPs for the stimulation of the right and left median nerves; topo plots of most significant evoked response at 50 (P50); N9 responses recorded from Erb's points; cortical SEPs of right (top) and left (bottom) median nerves. e) The significance level of local (Top-Left) and global (Top-Right) effects in AEPs of local-global paradigm and their corresponding ERPs in four conditions: GSLS(black), GDLD(red), GSLD(green), and GDLS(blue). f) Periodic decrease in the of power dominant frequency in the sleep EEG during a night. Vertical lines represent environmental noises such as truing lights on or off, medication, or auditory noises.

Figure 4: Neurophysiological map of P17 a) 60 seconds of resting state EEG while EC with the amplitude range of ± 45 μV; C2, C4, & C17 ICA components might indicate remaining eye activity b) PSD of four representative central and parietal EEG channels in EC (red) vs. EO (blue) c) Significant differences between EO vs. EC using PSD comparison (top) and SOP (down). d) SEPs for the stimulation of the right and left median nerves; topo plots of most significant evoked response at 50 (P50); N9 responses recorded from Erb's points; cortical SEPs of right (top) and left (bottom) median nerves. e) The significance level of local (Top-Left) and global (Top-Right) effects in AEPs of local-global paradigm and their corresponding ERPs in four conditions: GSLS(black), GDLD(red), GSLD(green), and GDLS(blue). f) Periodic decrease in the power dominant frequency in the sleep EEG (top) and increase of EOG activity (bottom) during a night. Vertical lines represent environmental noises such as truing lights on or off, medication, or auditory noises.

Figure 5: Resting and Auditory evoked response in Healthy controls. A) source of individual alpha peak frequency sum of all subjects. B) The local (Top-Left) and global (Top-Right) effect in auditory evoked potentials due to the auditory stimulation averaged of all subjects. C) The number of healthy subjects

showing a significant difference between eyes open and eyes closed conditions, using two different metrics: PSD comparison (left) and second-order plots (right).

Figure 6: Source of slow activity in patients.

Figure 7: Design of experiment for modified local-global paradigm used for auditory stimulation in patients and healthy.

Figure 1

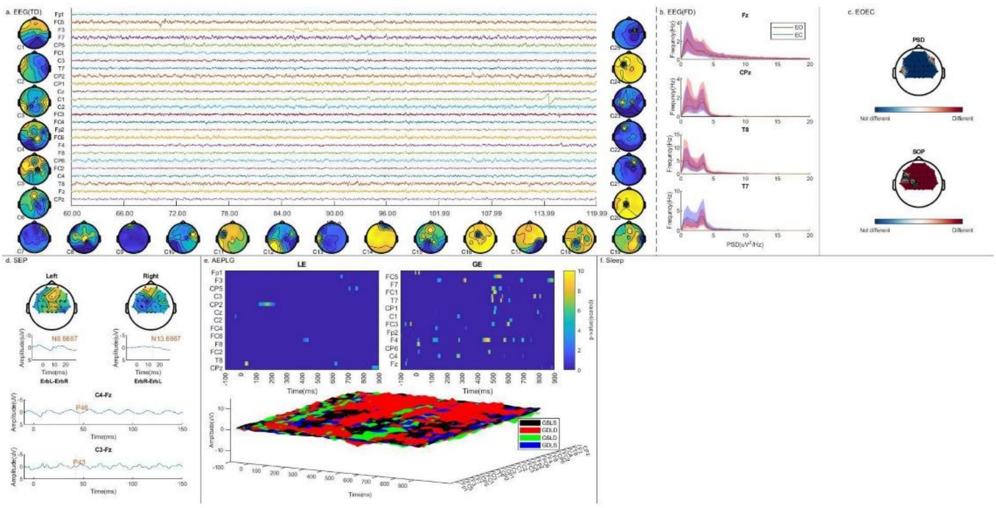
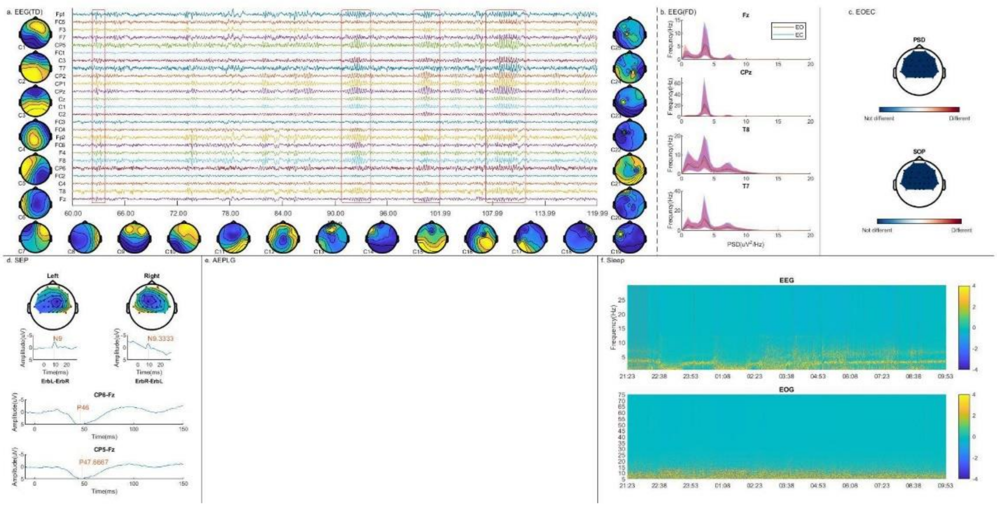

a. EEG(TD)
b. EEG(FD)
c. EOEC
PSD
SOP
Not different
Different
d. SEP
Left
Right
e. AEPLG
LE
GE
Time(ms)
f. Sleep
C4-Fz
C3-Fz

Figure 2


a. EEG(TD)
b. EEG(FD)
c. EOEC
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Different
d. SEP
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e. AEPLG
f. Sleep
EEG
EOG

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Figure 3

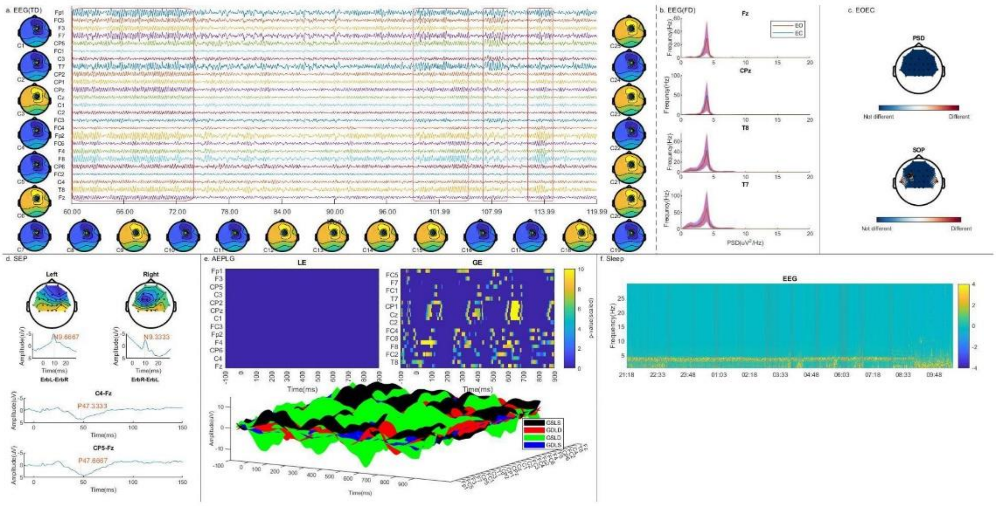

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Figure 4

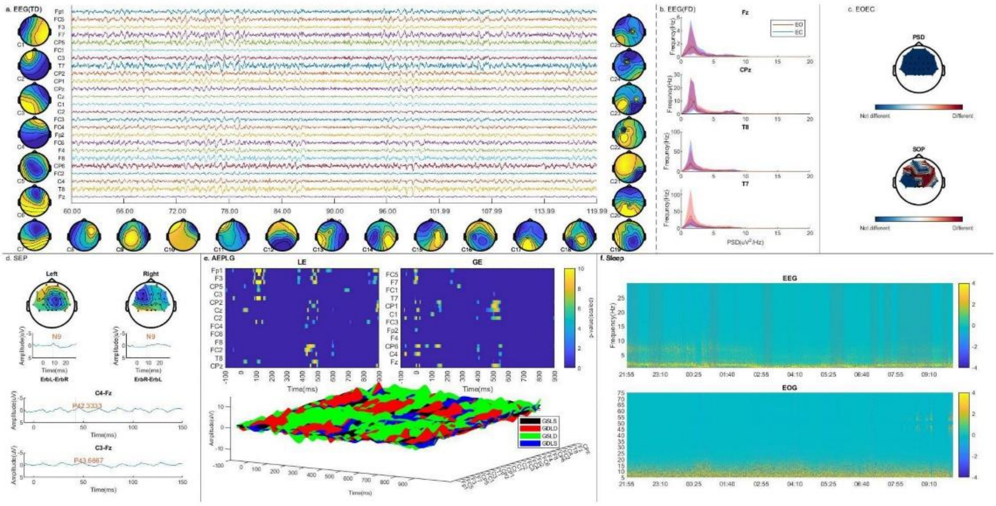

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Figure 5

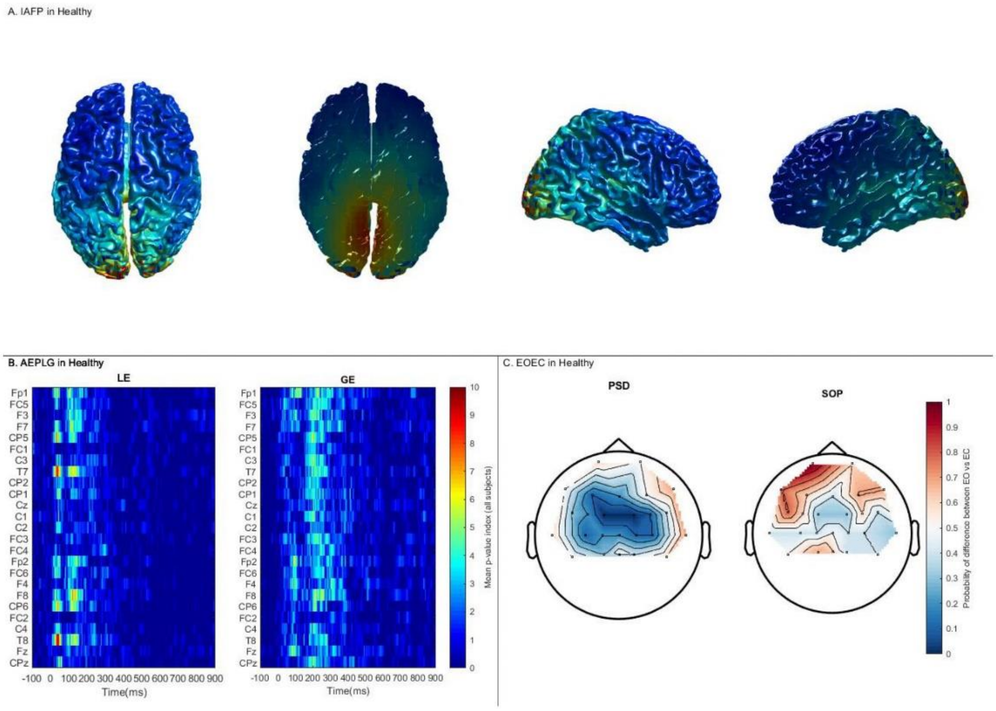

A. IAFP in Healthy
B. AEPLG in Healthy
LE
GE
Fp1
FC5
F3
F7
CP5
FC1
C3
T7
CP2
CP1
Cz
C1
C2
FC3
FC4
Fp2
FC6
F4
F8
CP6
FC2
C4
T8
Fz
CPz
-100 0 100 200 300 400 500 600 700 800 900
Time(ms)
Mean p-value index (all subjects)
C. EOEC in Healthy
PSD
SOP
Probability of difference between EO vs EC

Figure 6

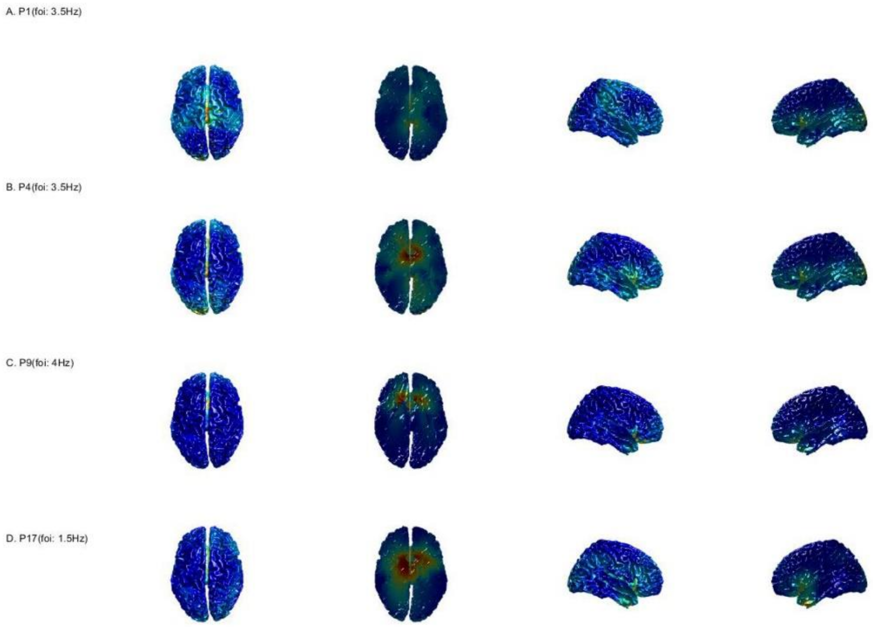
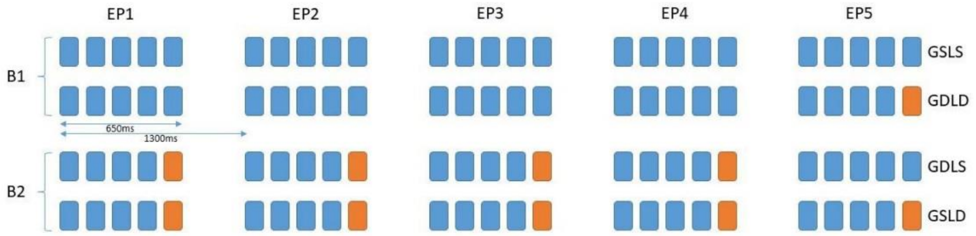


Figure 7



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Appendix E

Paper V: A Dataset of an Auditory Electrooculogram-based Communication System for Patients in Locked-In State.

SCIENTIFIC DATA

Check for updates

OPEN

DATA DESCRIPTOR

A dataset of EEG and EOG from an auditory EOG-based communication system for patients in locked-in state

Andres Jaramillo-Gonzalez[1], Shizhe Wu[1], Alessandro Tonin[2], Aygul Rana[1], Majid Khalili Ardali[1], Niels Birbaumer[1,3] & Ujwal Chaudhary[1,2]

The dataset presented here contains recordings of electroencephalogram (EEG) and electrooculogram (EOG) from four advanced locked-in state (LIS) patients suffering from ALS (amyotrophic lateral sclerosis). These patients could no longer use commercial eye-trackers, but they could still move their eyes and used the remnant oculomotor activity to select letters to form words and sentences using a novel auditory communication system. Data were recorded from four patients during a variable range of visits (from 2 to 10), each visit comprised of 3.22 ± 1.21 days and consisted of 5.57 ± 2.61 sessions recorded per day. The patients performed a succession of different sessions, namely, Training, Feedback, Copy spelling, and Free spelling. The dataset provides an insight into the progression of ALS and presents a valuable opportunity to design and improve assistive and alternative communication technologies and brain-computer interfaces. It might also help redefine the course of progression in ALS, thereby improving clinical judgement and treatment.

Background & Summary

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that, in its final stages, paralyzes affected individuals impairing their ability to communicate[1–4]. Those patients with intact consciousness, voluntary eye movement control, who can blink their eyes or twitch their muscles are said to be in a locked-in state (LIS)[5,6]. Patients in LIS rely on eye-tracking based assistive and augmentative communication (AAC) technologies to communicate[7,8]. In the case of patients who survive attached to life-support systems, the progression of the disease ultimately destroys oculomotor control, leading to the loss of gaze-fixation and impeding the use of eye-tracking based communication technologies[9–11]. Nevertheless, even in the late stages of this condition, some remaining controllable muscles of the eyes continue to function for an unspecified length of time, which can be used to provide a means of communication to these patients[11,12].

An auditory electrooculogram (EOG) based communication system[12] was developed to provide a means of communication to ALS patients without gaze-fixation and who were unable to use the commercial AAC eye-tracking devices, but who had remnant oculomotor control to form words, phrases, and sentences using the system described in Tonin & Jaramillo-Gonzalez *et al.*[12]. Four ALS patients with progressively decreasing EOG signal amplitude in the range of $\pm 200\,\mu V$ to $\pm 40\,\mu V$ were able to select letters to construct words to form sentences and hence communicate freely using an auditory speller system. The auditory speller system is based on a binary system in which a patient is asked to respond to auditory questions by moving the eyes to say "yes" and not moving the eyes to say "no". The system must use the auditory modality because, in these patients, vision is often impaired due to drying and necrosis of the cornea and the partly or fully paralyzed eye-muscles. The study design and paradigm are described in detail in the Methods section.

This data descriptor outlines the EEG and EOG recordings from four different patients recorded during their use of the auditory communication system, having first trained progressively, and then ultimately controlling the

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system to communicate. Electromyography (EMG) recordings are available for some sessions, according to the clinical conditions.

There have been other studies with similar goals, but only one has an available online dataset[13], with different features. To our knowledge, in the available open-access specialized repositories[14–16], there are no datasets with similar properties to the one described here. It must be emphasized that the data described here are both the EOG and EEG signals recorded with a dedicated set of electrodes for each type of signal simultaneously. These EEG and EOG data are recorded from patients with ALS in the most advanced stage, whose disease progression is not well defined and is, to a certain extent, unknown. The data highlight a phase in ALS where communication becomes difficult and gradually impossible with existing commercial AAC. It includes recordings of over the course of a year during which one of the users became unable to use the system because of disease progression. As a consequence, we believe that the study of this dataset might help towards improving the clinical definition of ALS in its very advanced state, the testing of hypotheses on the brain's electrophysiological changes during this progression and evaluating the impact of advanced ALS on the cognitive state of the patients. Nevertheless, even though the data is quite specific, further investigation of the data can support novel clinical and therapeutic practices. It could help develop augmentative and alternative assistive communication technologies and brain-computer interfaces that can be generalized to other types of disorders and patients with pervasive communication deficits and motor impairments due to CNS damage, such as stroke or high spinal cord injury. Lastly, although the system can be considered successful in enabling communication, other analytical methods can still improve the system's speed and efficiency, for example, offline testing of other feature extraction methods or testing and comparing the performance with different machine-learning methods to classify the patients' response.

Methods

The Internal Review Board of the Medical Faculty of the University of Tubingen approved the experiment reported in this study. The study was performed according to guidelines established by the Medical Faculty of the University of Tubingen. The patient, or the patient's legal representative, gave informed consent with permission to publish the data. The clinical trial registration number is: ClinicalTrials.gov - Identifier: NCT02980380. The methods described here are complementary to an in-depth description of the results derived from this dataset that have been presented in related work[12].

Participating patients. Four ALS patients with amyotrophic lateral sclerosis with a functional rating scale revised (ALSFRS-R)[17] score of 0 in the locked-in state (LIS) were visited on subsequent months starting from Feb 2018 to May 2019. Team members travelled to the patient's home to perform the communication sessions, depending on the health status and convenience of the patient. The medical history of patients is described in our related work[12]. Every visit (V), lasted for a few days (D), during which the patient performed different session (S), as detailed in the Online-only Tables 1–4, with the precise dates of all the visits and details of the sessions.

Auditory communication system. *Prerequisites for performing the study.* In agreement with the patients' caretakers and considering the patients' health and wellness and optimization of resources, it was established that the visits should be performed every two months approximately, with each visit no longer than four days. However, on some occasions the condition of the patients led to shorter visits, from three days to a single day. (see Online-only Tables 1–4). For each visit, guided by the same criteria of health and wellness of the patient, two team members transported all equipment and set up all systems in the patient's home or accommodation.

Before the beginning of the study, at least 100 questions with known "yes" or "no" answers were formulated and recorded by a family member or caretaker in their own voice, in close proximity to the patient. Each question with a "yes" answer is paired with a similar question with "no" answer (e.g., "Paris is the capital of France" and "Berlin is the capital of France"). Each question is saved as an audio file with an explicit identifier, a question with a "yes" answer is saved with a 001_NUMBER identifier, and a question with "no" answer is saved with a 002_NUMBER identifier. The value of the label NUMBER is the same for a semantically paired sentence. The same procedure was repeated with biographical-related questions with at least 100 for every patient. Sentences are then stored on a laptop and accessed and played by the communication system during the sessions.

Study and paradigm. The study consisted of patients performing four different types of sessions, namely, Training, Feedback, Copy speller, and Free speller session, to train and enable the patient to employ an oculomotor strategy to control the spelling system successfully. During the visit, the patient performed different sessions, as depicted in Fig. 1. The patients developed a strategy to respond during successive trials to an auditory question (the questions previously recorded) by moving the eyes to say "yes" and by not moving the eyes to say "no". To control the activities during the trials, specific paradigms were designed for the different sessions, as depicted in Fig. 2.

The different sessions performed by the patients are described below.

a. Training session
The study on a single day always started with Training sessions during which the patients were instructed to listen to a sequence of 20 personal questions consisting of 10 sentences with a "yes" answer and 10 with "no" answer, presented in pseudo-random order. After the system presents an auditory question, patients are asked to move their eyes to respond "yes" and not to move the eyes to respond "no" during a response time window. The duration of the response segment depended on the patient's performance, i.e., if the patient could move his/her eye with ease, the duration was kept shorter and vice versa. Therefore, this window has a range from 3 to 10 seconds. For each Training session, the set of triggers indicating the sequence of events were recorded on the raw file, using the labels shown in Fig. 2a. Alongside, the system creates a questions sequence text file (Block_X_senlist.txt) that includes the list of identifiers of the presented audio/

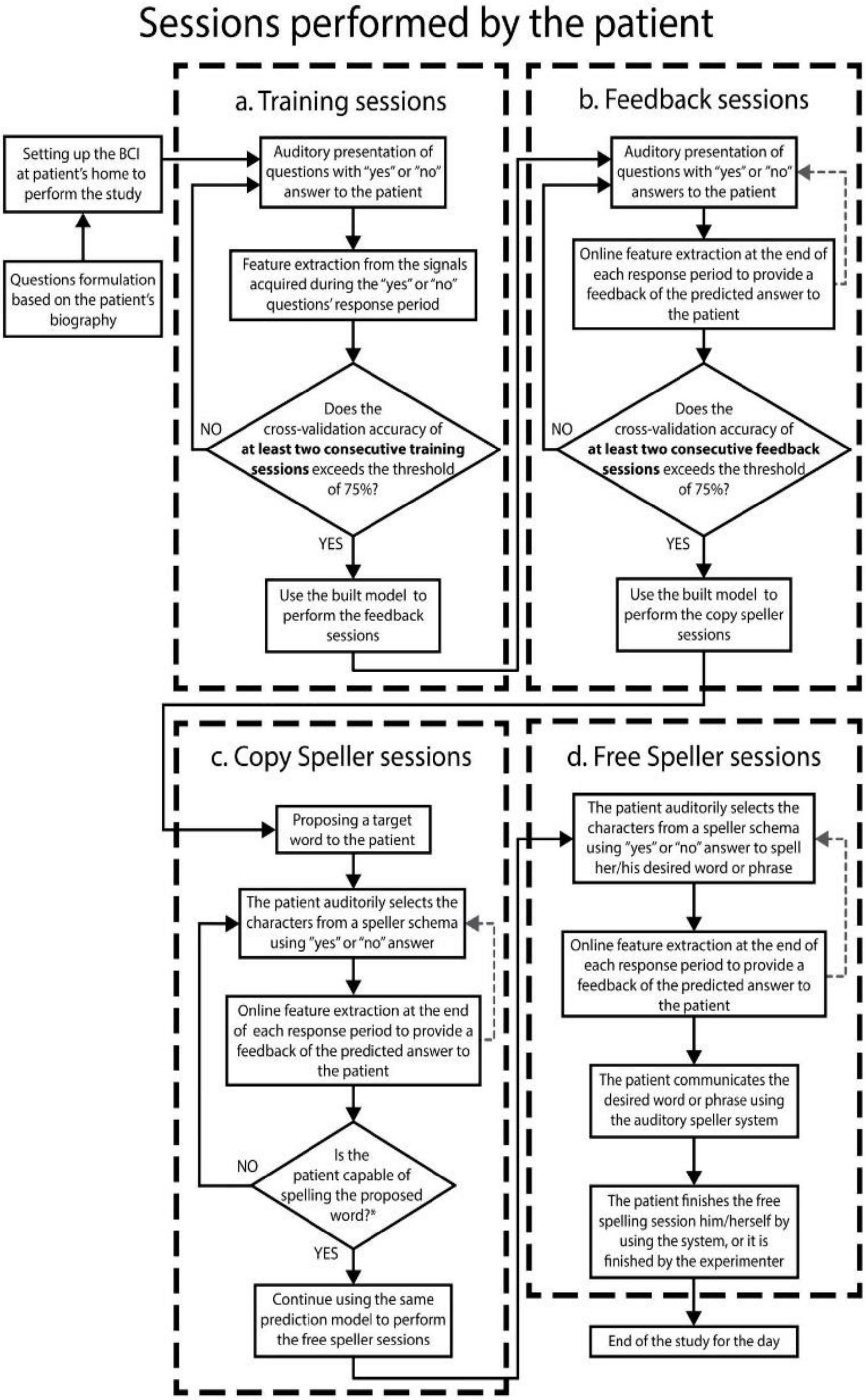

Fig. 1 The procedure performed during a single day. The figure depicts the sequence of the types of sessions performed by patients and the criteria to progress from one type of session to the next. The patients first performed the Training sessions during which the patient learned to move his/her eyes to generate the signal to control the auditory communication system. At the end of the Training session, a classification model was built, and when the accuracy of the built model was greater than 75% the patients performed the feedback session. During the feedback sessions the patients were provided the feedback of their response, i.e., whether their answer was classified as "yes" or "no". When the feedback accuracy exceeded 75% the patients first performed a copy speller session and then a free speller during which they could spell whatever they desired.

question files during the session (e.g., 001_13012a.wav), including also the label of the corresponding type of answer ("0" for sentences with "no" as an answer, and "1" for sentences with "yes" as an answer). The. txt lists are included inside the raw data folder structure, as described in the section Data Records. After at least two consecutive Training sessions with a classification accuracy result greater than 75%, the patient progresses to the Feedback sessions (see Fig. 1).

b. Feedback session

As in the Training session, the patients were presented with a sequence of a familiar question, but, at the end of the response segment, they were provided with auditory feedback as to whether their answer was

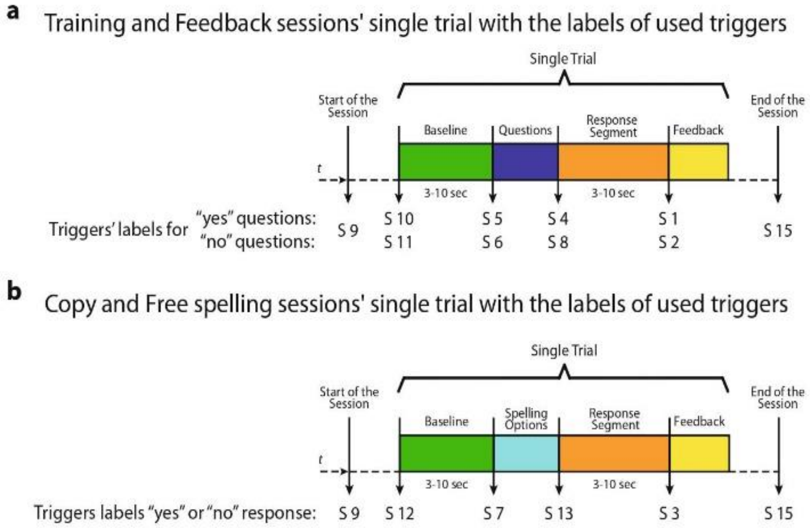

Fig. 2 Different types of trials in the study. (**a**) Paradigm describing the sequence of events and sequence of the triggers' labels used in a single trial for the Training and Feedback sessions. In these types of sessions, 20 questions with "yes" and "no" answers, known by the patient, are presented in a pseudo-random order. (**b**) Paradigm describing the sequence of events and sequence of the triggers' labels used during a single trial for the Copy and Free spelling sessions. In these sessions, instead of questions, the patient is presented with options that allow him/her to navigate through his/her predetermined spelling scheme (e.g., sectors, letters). For both spelling sessions, the limit in the number of trials depends only on the patient's attempts to spell the given target (i.e., Copy speller sessions) or her/his desired sentence (i.e., Open speller sessions). For any type of session recorded, the recording's start and end are indicated by an "S 9" and an "S 15" trigger.

recognized as "yes" or "no" by the system. For each Feedback session, the triggers indicating the events' sequence were recorded on the raw file, using the labels shown in Fig. 2a. The system creates a sentence list (Block_X_senlist.txt) in the same way it was created for the Training sessions. In the case of Feedback sessions, in addition to the sequence of the questions text file, the system creates a result file (Date_result_f1_X.txt) listing the predicted results, i.e., "1" if the answer was recognized as "no", "0" if the answer was recognized as "yes", and "2" if the answer was unable to be classified by the system. The system gives the patient auditory feedback with the sentence: "Your answer was classified as yes/no". Both.txt lists are also included inside the raw data folder structure, as described in the section Data Records. After at least two consecutive Feedback sessions with a classification accuracy result greater than 75%, the patient progresses to the Copy spelling sessions (see Fig. 1).
The sequence of events and triggers (with their labels) for a single trial of the Training and Feedback sessions is depicted in Fig. 2a. Each of these trials consists of the segment of baseline (i.e., no sound presented), stimulus, during which the question is presented auditorily to the patients, followed by the segment of response time, in which the patient moves or does not move the eye according to his/her answer, and lastly the segment of feedback. For a Training session trail, the feedback is "thank you" to mark the end of the response while for a Feedback session trail, the feedback is "yes" or "no" depending on the answer classified by the system.

c. Copy spelling session
During the Copy spelling sessions, the patients were asked to spell a specific word described in our previous work[12]. For each Copy spelling session, the set of triggers indicating the sequence of events was recorded on the raw file, as shown in Fig. 2b. For the Spelling sessions, there are no questions sequence text files, but there are results files (Date_result_f1_X.txt) with the label of the predicted answer, listing the predicted results as "1" if the answer was recognized as "no", "0" if the answer was recognized as "yes", and "2" if the system was unable to classify the answer. The.txt lists are also included in the raw data folder structure described in the section Data Records.
d. Free spelling session
After completing the Copy spelling session, the patients were asked to spell whatever he/she desired. For each Free spelling session, the set of triggers indicating the sequence of events were recorded on the raw file, as shown in Fig. 2b. As in the Copy spelling case, each Free spelling session created a result file (Date_result_f1_X.txt) using the same label code. The.txt lists are also included in the raw data folder structure described in the section Data Records.

The trials for the Copy and Free spelling sessions do not consist of the pre-recorded personal questions, but instead, of "yes"/"no" questions asking the patient whether to select or not, a particular letter, group of letters, or command, from his/her particular speller scheme[12]. Copy and Free spelling sessions differ in terms of the instruction given to the patient. During the Copy spelling sessions, the patient was asked to spell a specific word, while during the Free spelling sessions, the patient was asked to spell whatever he/she desired. Consequently, instead

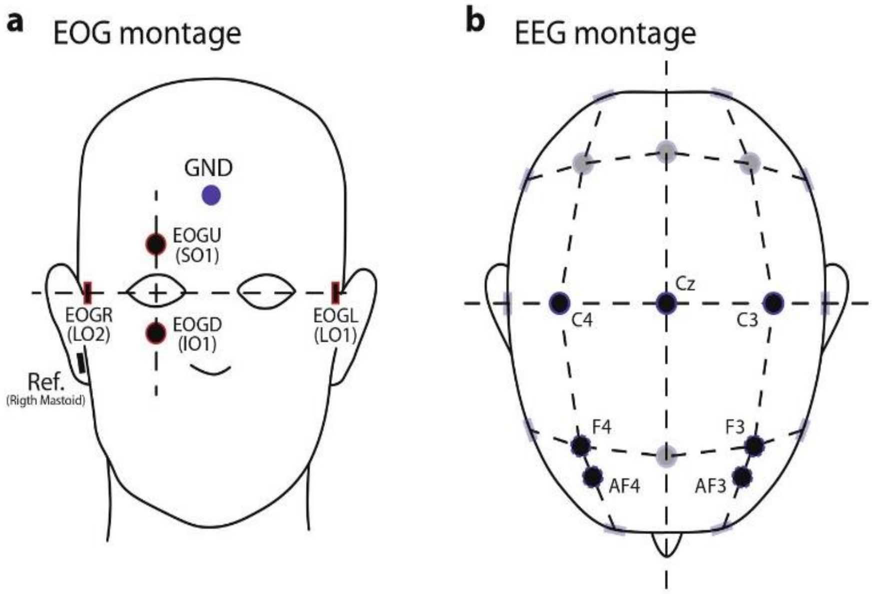

Fig. 3 EOG and EEG setup. (**a**) Montage for the minimum number of EOG channels for each recorded session, using the locations LO1 (left cantus) and LO2 (right cantus) for horizontal eye movement, and SO1 (above superior orbit) and IO1 (below inferior orbit) for vertical eye movement. We used the labels EOGL, EOGR, EOGU, and EOGD, respectively, for the *online* study. (**b**) Montage for the minimum number of EEG electrodes for each recorded session, emphasizing the central motor (C4, Cz, C3) and prefrontal areas. In this latter case, the location of used electrodes might vary between F3 and F4, or Af4 and Af3. Nevertheless, the total number of electrodes might vary between days of the visits due to the patient's wellness conditions. The exact number of electrodes and labels used can be verified in the Online-only Tables S1–S4.

of being a fixed number, the number of trials in these sessions depends on the number of attempts performed by the patient to spell the given target (for the Copy speller sessions) or his/her desired sentence (for the Free speller sessions).

The sequence of events and triggers (with their labels) for a single trial for the Copy and Free spelling sessions is depicted in Fig. 2b. The trials consist of the segment of baseline (i.e., no sound presented), stimulus, where instead of questions the patient is presented with auditory options that allow him/her to navigate through his/her predetermined spelling scheme[12] (e.g., sectors, characters, letters), followed by the response time segment in which the patient move or not move the eye according to his/her answer. Lastly, the feedback segment, during which depending on the answer classified by the system, "yes" or "no" auditory feedback is given to the patient.

Regardless of the session type, the recording time's start and end are labeled by "S 9" and "S 15" triggers. During each trial, the sequence of events is presented to the patient and simultaneously, in a synchronized manner, a system of digital triggers is created by a Matlab script interacting with the V-Amp amplifier, to indicate the onset of each event in the time series. Both Fig. 2a,b show the sequence of triggers (their labels) as used in each trial. Information on the onset and labels of each event is also provided (see section Data Records).

We have to add that during the setting up of the system or the sessions' execution, patients' care and wellness were a high priority; therefore, under any request or signal of unease, sessions or even the day's study were stopped.

System for data acquisition. The communication system is composed of the different elements described below.

- Laptop: The present setup uses a laptop with 8 GB RAM, Windows 7 operating system, and 3.3 GHz processor.
- EEG amplifier and recorder: For each session, EEG and EOG channels were recorded according to the 10-20 EEG electrode positioning system, with a 16 channel EEG amplifier (V-Amp DC, Brain Products, Germany) with Ag/AgCl active electrodes.
- EOG channels: at least four electrodes were recorded (positions SO1 and IO1 for vertical eye movement, and LO1 and LO2 for horizontal eye movement).
- EEG channels: at least seven channels located in central and prefrontal areas were recorded (exact locations per day in the Online-only Tables 1–4).
- EMG channels: on a limited number of sessions electrodes located on the chin of the patient or any other face muscle with assumed remaining function.

All the channels were referenced to an electrode on the right mastoid and grounded to electrode FPz on the forehead. For the montage, electrode impedances were kept below 10 kΩ. The sampling frequency was 500 Hz. The standard montage for the minimum number of available EOG and EEG electrodes is specified in Fig. 3. The precise number and location of electrodes available for each session are detailed in the Online-only Tables 1–4, including recording EMG electrodes.

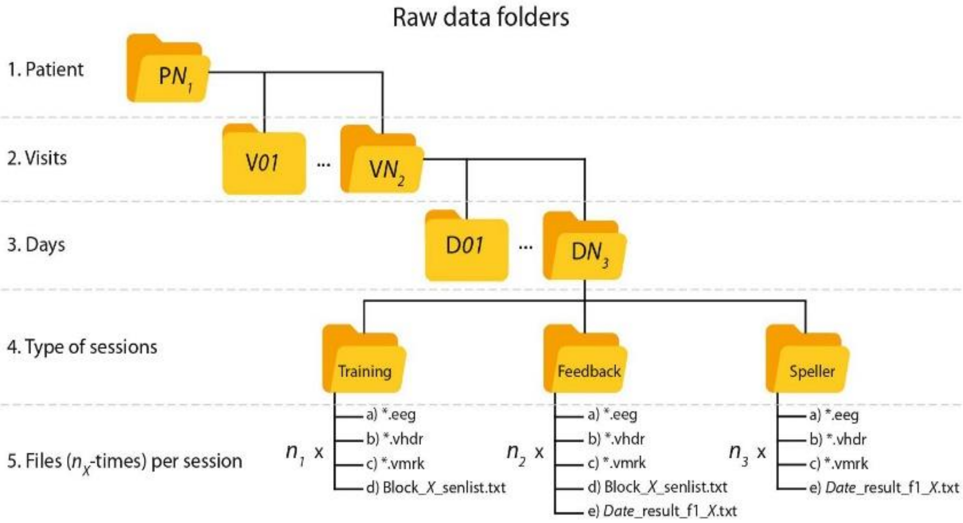

Fig. 4 Raw data folder structure. Structure of nested folders containing the raw recordings of the study. According to the patient identifier, the upper level is the folder, which can be $PN_1 = 11,13,15$ or 16. In the next level, VN_2 indicates the total number of visits available for that patient, and inside it, D$N3$ indicates the number of days that the visit lasted. Each day's folder stores subfolders for the Training, Feedback, and spelling (that stores recordings from both the Copy and Free speller sessions). Each of these folders contains a set of files that are the outcome of a recorded session (detailed in the section Data Records), times the number that particular type of session (i.e., n_1, n_2, and n_3) was respectively performed during the day.

- Serial cable: This cable is used to connect the Laptop and the EEG amplifier to send the triggers with the custom Matlab code to mark the EEG-EOG recording with the different segments' starting point.
- Loudspeakers: Loudspeakers connected to the laptop performs the function of delivering the audio stimuli to patients during the Training/Feedback/Copy spelling/Free spelling sessions, as described below.

Data Records

Raw data folders. The data stream was recorded directly from the EEG amplifier and stored with the proprietary BrainVision Recorder format[18,19] during the sessions. According to the dongle key available during the visit, the data were stored in two possible formats, necessary to access and use BrainVision Recorder, 42% of data were recorded in *.ahdr and the rest 58% in *.vhdr format. For consistency here, we present the data in *.vdhr after converting the other 42% of *.ahdr format data also to *.vhdr format. Thus, as an output of this recording scheme, three output files per recording had the same name but different extension:

a. Header file (*.vhdr), containing recording parameters and further meta-information, as the scaling factor necessary to convert the recorded raw amplitude to milivolts.
b. Marker file (*.vmrk) describes the events and their onset during the data recording, in this case, the sequence of triggers.
c. Raw EEG data file (*.eeg) is a binary file containing the EEG and EOG data and additional recorded signals.

Nevertheless, to assist with handling the unmodified raw data, we have used the BrainVision Analyzer[20] software to export all the recordings to the more accessible.vhdr format, but without altering anyhow the content of the data itself.

For storing the raw data, a database was created using a nested structure of five levels (see Fig. 4), from the top:

1. Patient folder, where PN_1 can be either P11, P13, P15, or P16.
2. Visits folder, where VN_2 indicates the total number of visits available for each particular patient.
3. Day folder, where DN_3 indicates the number of days that the particular visit lasted.
4. Type of sessions, where data has been separated according to the type of sessions. Training, Feedback, and Spelling sessions (consisting of both Copy and Free spelling sessions).

At the 5th level, according to the type of session, there might be up to five types of files stored, times the number of that particular session recorded on the day, i.e., n_1, n_2, and n_3 (see Fig. 4). Namely, the hosted files can be:

(a) *.vhdr, the exported version of the *.ahdr file.
(b) *.vmrk, the exported version of the *. amrk file.
(c) *.eeg, that is a binary file with the recorded data.
(d) Block sentence list (Block_X_senlist.txt), where X is the counter of the number of ongoing sessions. This type of *.txt file was only created for Training and Feedback sessions.
(e) Result list (*Date*_result_f1_*X*.txt), with the *Date* in which the recording was made, and X is the counter of the ongoing sessions. This type of *.txt file was only created for Feedback and both Speller sessions.

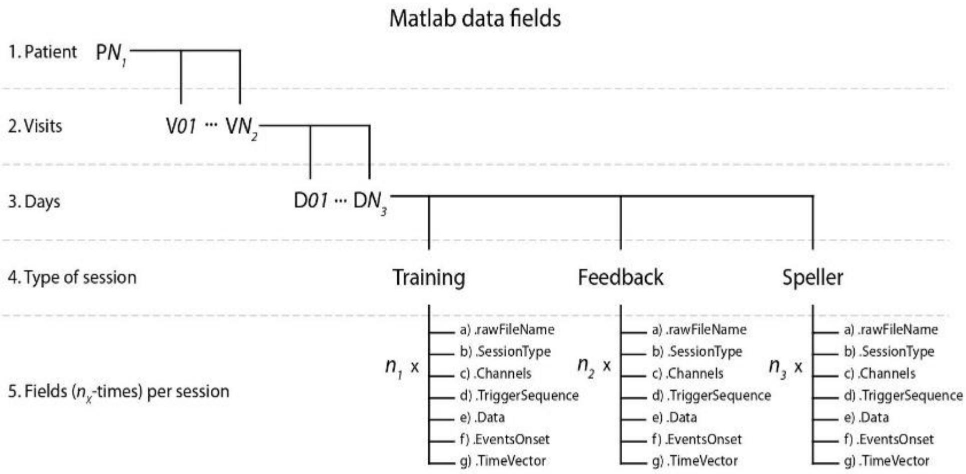

Fig. 5 Matlab data fields structure. Nested structure elements containing the values and features of recordings from the study. According to the patient identifier, the upper level is the main structure, which can be $PN_1 = 11,13,15$ or 16. In the next level, VN_2 indicates the total number of visits available for that patient, and inside it, D*N3* indicates the number of days that the visit lasted. Inside each day, there are structures for the Training and Feedback sessions and the spelling sessions (containing recordings from both the Copy and Free speller sessions). Each of these contains a set of structures that result from exporting the *.vhdr raw files for each recorded session, times the number of that particular session type (i.e., n_1, n_2, and n_3) was performed during the day. Read the Data Records section for details on the data exporting.

Matlab data fields. Additionally, another format has been chosen to present and share the data obtained from exporting the original raw files (details in the section Usage Notes). In this rectangular form, a Matlab variable is stored (*.mat), corresponding to the patient's name, i.e., P11. In the variable, nested structures were created using a somehow similar architecture for the raw files, as detailed in Fig. 5. The levels, from upper to lower, are:

1. Patient structure, where PN_1 can be either P11, P13, P15, or P16.
2. Visits structure, where VN_2 indicates the total number of visits available for each particular patient.
3. Day structure, where DN_3 indicates the number of days that the particular visit lasted.
4. Type of sessions structure, where data has been separated according to the type of sessions. Training, Feedback, and Spelling sessions (containing both copy and free spelling sessions).

At the 5th level, according to the type of session, there are seven fields stored, times the number of that particular session recorded on the day, i.e., n_1, n_2, and n_3 (see Fig. 6). The hosted fields are:

(a) .rawFileName: character type variable with the name of the original raw file that was exported
(b) .SessionType: character type variable with the label of the type of session that the data belongs to
(c) .Channels: cell array with *1xK* dimensions, with *K* being the total number of EEG, EOG and EMG channels recorded, where each cell element is the label of a channel.
(d) .TriggerSequence: cell array with *1xM* dimensions, including the *Mth* events of all the trials recorded and the session as a sequence of triggers, with the labels indicated in Fig. 2., e.g., S 9, S 10, S 5, S 4, S 1, S 11, …, S 15
(e) .Data: a *KxR* dimensional matrix of numerical values, being *K* the number of channels recorded, and *R* the number of data points in the time domain of the recording, each element being the amplitude values of the recording. It is highly relevant to consider that the default amplitude of the recording needs to be multiplied for a scaling factor of 0.0488281 (± 410 mV range in 24 bits) to convert to μV[21]. The scaling factor can be verified inside every *.vhdr file produced for every recording
(f) .EventsOnset; a *1xR* dimensional vector of numerical values, being *R* the number of data points in the time domain of the recording, and to each time point we have assigned the numerical value of the trigger labels (see Fig. 2) occurring at that time point, e.g., 9, 10, 5, 4, 1, 11, …, 15, and a value of zero otherwise. This vector aims to help quickly locate each event's onset and nature in the time domain
(g) .TimeVector: a *1xR* dimensional vector of numerical values, being *R* the number of data points in the time domain of the recording, where an element of *R* indicates the time value in seconds of the recording.

As an example of the previous variable description, Fig. 6 illustrates the data structure using P11's data from visit V01 and day D03.

All the datasets described in this section can be freely downloaded from the open access repository[22].

Technical Validation

The raw data referred to in this descriptor was recorded using a Brain Products V-Amp amplifier, without any type of hardware or software filter besides the physical instrumental restriction of the amplifier (wideband filter in the range of 0 Hz (DC) – 320 Hz or 4 kHz for the high-speed mode)[21].

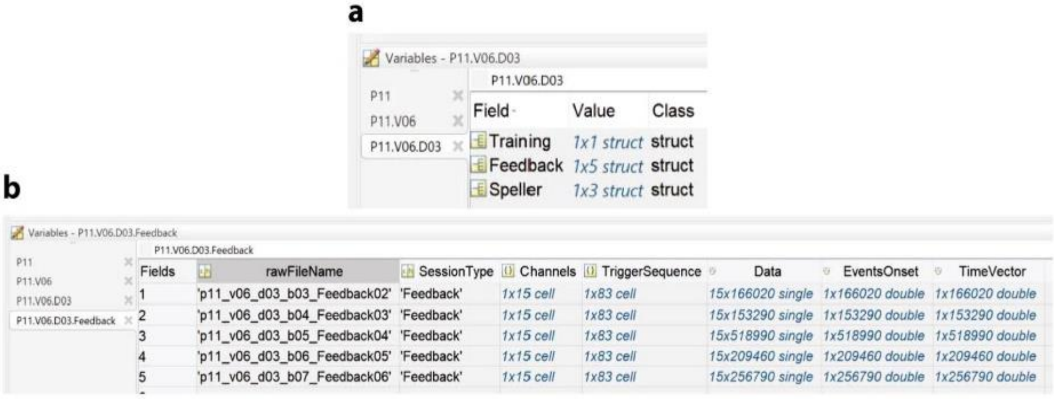

Fig. 6 Example of a Matlab structure of the data using P11's data. The figure illustrates the data structure using P11's data from visit V06 and day D03. (**a**) Indicates the selection of patient variables and the data fields corresponding to a particular visit and day and inside it, the type and number of sessions performed on the given day. (**b**) Depicts the presence of different fields upon selecting a session type, in this case, the number of Feedback sessions performed by P11, upon selection of Field named Feedback, and their different elements, as shown in the figure. Read the Data Records section for the detailed description.

The raw data recorded with BrainVision Recorder software (v2.1.0) in *.ahdr, *.amrk, *.eeg formats were exported using BrainVision Analyzer software[20] (v2.2.0) to obtain the formats[19] *.vhdr, *.vmrk and *.eeg.

The data given as a Matlab format variable (*.mat) has been exported from the raw files taking advantage of the EEGLAB[23] toolbox (https://sccn.ucsd.edu/eeglab/index.php, v2019.0) and the "bva-io" plugin (https://sccn.ucsd.edu/eeglab/plugin_uploader/plugin_list_all.php, v1.5.13), to save in the described variable structure desired features of the original raw file, as detailed in the Data Records section. No special parameter was used for exporting these data, and therefore we consider both raw and exported to the same values. Nevertheless, the amplitude of the recorded data (either.eeg or exported files using the "bva-io" plugin) is defined by the ADC bit resolution of the device, that is a $\pm$410 mV range in 24 bits, and therefore, the amplitude value needs to be multiplied by the scaling factor of 0.0488281[21] to be converted to µV (microvolts) units. The resolution of each recording can be found per channel inside each *.vhdr given file.

The Matlab script used to export the raw files to Matlab variables (see Code Availability section) includes a deactivated code line that can be used to convert to µV the amplitude.

EOG electrodes were located and placed according to the standard 10-20 system with EEG neoprene caps (Neuroelectrics, Barcelona, Spain), inserted in the cap using plastic holders. Once the whole set of electrodes was in place, they were filled with SuperVisc electrolyte gel (Easycap, Germany, GmbH). Impedance was measured on the whole set using an ImpBox (Brain Products, Germany, GmbH), to achieve a target impedance of 10 KΩ. Researchers in charge of the study ensured that the recorded activity had the proper impedance and a clean signal for all the channels. Recordings are not affected by muscular or blinking artifacts, besides eye movements related to the patients' intentions.

Usage Notes

Performance of the communication system. The communication system can present a question every nine seconds with an information transfer rate of 6.7 bits/min. The system's optimal speed can be improved depending on the speller scheme's design for each individual patient and the corpus of sentences stored for word prediction. Descriptive statistics on each patient's performance can be found in the related publication[12].

A minimal criterion for communicating using the system is the presence of eye-movements recordable with sate of the art EOG recording devices in the microvolt range. For one of the patients, the progression of the disease over the course of a year eventually prevented him from controlling his oculomotor activity. He was however capable of producing undifferentiated EOG activity with low amplitude in the range of $\pm$30 µV which reached that minimal criterion. The other patients never arrived at such a total loss of control when the data described here was recorded. Therefore, the duration of the transition period to CLIS, and whether voluntary communication with non-invasive physiological recording technologies, as described here, will be possible in CLIS, is still a matter of future research.

Date and time of the recordings. The original timestamp of the beginning of a recorded session can be found both inside the *.vhdr and the *.vmrk files, as the occurrence of the first marker in the recording. It can also be found as the timestamp of the sentence lists (Block_*X*_senlist.txt), or indicating the end of a session in the results text files (*Date*_result_f1_*X*.txt).

Name of the raw files. During the study, files recorded with BrainVision Recorder software (v2.1.0) (i.e., *.vhdr, *.vmrk and *.eeg) were labeled by the experimenters, and therefore, human error or discrepancies might have been committed during the labeling process. To clarify any possible confusion, the Online-only Tables 1–4 include a set of columns that show the correspondence between the name of the raw file, the session's sequence, and any *.txt files attached to it.

Patient	Visits	Days	Sessions		
			Training	Feedback	Speller
P11	9	27	68 (including 2 lost files)	56	26
P13	4	14	28	22	21
P15	2	7	7	16 (including 1 lost files)	18
P16	2	9	27	22	8

Table 1. The number of sessions in the dataset. Detail of the number of visits and total days of the study, and the total number of different types of sessions recorded for each patient. Indicated in parenthesis are the numbers of lost recordings. Copy speller and free speller sessions are considered in the same column. A more detailed description of the days, dates, and sessions can be verified in the Online-only Tables 1–4.

The text lists (Block_*X*_senlist.txt and *Date*_result_f1_*X*.txt) were created automatically by a Matlab script running during each session.

From the given recordings, either raw or Matlab fields, it can be noticed from the labels that some files or sessions are lacking. This is because during the visits, sessions belonging to another paradigm for a different and unrelated study were also recorded, and they have been deliberately removed from the actual data descriptor to focus on the auditory communication recordings. Removed files are indicated in the Online-only Tables 1–4. Additionally, a number of files were lost or corrupted; the precise number and sessions are indicated in Table 1 and Online-only Tables 1–4.

EEG locations and inconsistency. Working with patients who have critical health conditions means being completely dependent on their current (minute by minute) state. These limitations were considered in the design of the study. The number of EEG electrodes was limited by restrictions of accessibility of some scalp regions. Since the patients lie on their backs most of the time, it is impossible to access occipital areas.

Nevertheless, the most relevant restriction is the time constraint, that is, to place a minimal number of EEG electrodes in appropriate locations, in the minimum possible time, so to maximize the time available to work with the patient before tiredness or another need (for example, sucking of saliva) prevents them from participating in the study. Consequently, the montages of electrodes might be affected by inconsistency in EEG electrode locations, even for the same patient, and different visits, since it is always dependent on changing circumstances of health and time.

Therefore, the criterion we follow aims to reach with the minimal number of electrodes the greatest coverage of the prefrontal and mesial surfaces of the brain (besides the EOG electrodes), under the assumption that the cognitive activity implicated in the processing of these questions might elicit changes in the electrical activity of the aforementioned cortical regions.

Regardless of that, we managed to keep a constant number of seven EEG electrodes and four EOG electrodes for most of the patients, for most of the visits, as can be verified in the Online-only Tables 1–4.

Audio files. The audio files (recorded questions) used in this research contains personal information of the patients and their relatives and consequently, to make these audio files fully open and public will compromise their identities. These data[24] have been uploaded with restricted access, therefore any researcher or laboratory interested in accessing the data to perform the analysis will have to sign an identity protection agreement document provided as a "Data Use Agreement" Supplementary material with this manuscript.

Code availability

The given Matlab data variables were obtained by exporting the raw files (i.e., *.vhdr, *.vmrk, and *.eeg) using the EEGLAB[23] toolbox (v2019.1.0) and exporting the data using the "bva-io" plugin (v1.5.13). We wrote a short Matlab script (ExportingCode_vhdr2mat.m) to export and save the desired features of the recordings, as thoroughly detailed in the section Data Records. The code is included in the same repository as the rest of the data, and it is accompanied by a brief document (ExportingCode_vhdr2mat.docx) explaining details of the code.

Received: 24 April 2020; Accepted: 30 November 2020;
Published online: 11 January 2021

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Acknowledgements

Deutsche Forschungsgemeinschaft (DFG) DFG BI 195/77-1, BMBF (German Ministry of Education and Research) 16SV7701 CoMiCon, and LUMINOUS-H2020-FETOPEN-2014-2015-RIA (686764).

Author contributions

Andres Jaramillo-Gonzalez – Performed 35% of the Auditory communication system (ACS) sessions and data collection; Data curation; Manuscript writing. Shihze Wu – Data curation and validation. Alessandro Tonin – Performed 35% of the ACS sessions and data collection. Aygul Rana – Performed 35% of the ACS sessions and data collection. Majid Khalili Ardali – Discussion in Laboratory. Niels Birbaumer – Study design and conceptualization; Manuscript correction. Ujwal Chaudhary – Study design and conceptualization; Performed 65% of the ACS sessions and data collection; Supervision; Manuscript writing.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41597-020-00789-4.

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Appendix F

Paper VI: EEG Power Spectral Density in Locked-In and Completely Locked-In State Patients: a longitudinal study.

Cognitive Neurodynamics
https://doi.org/10.1007/s11571-020-09639-w

RESEARCH ARTICLE

EEG power spectral density in locked-in and completely locked-in state patients: a longitudinal study

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Received: 1 March 2020 / Revised: 14 August 2020 / Accepted: 30 September 2020

Abstract

Persons with their eye closed and without any means of communication is said to be in a completely locked-in state (CLIS) while when they could still open their eyes actively or passively and have some means of communication are said to be in locked-in state (LIS). Two patients in CLIS without any means of communication, and one patient in the transition from LIS to CLIS with means of communication, who have Amyotrophic Lateral Sclerosis were followed at a regular interval for more than 1 year. During each visit, resting-state EEG was recorded before the brain–computer interface (BCI) based communication sessions. The resting-state EEG of the patients was analyzed to elucidate the evolution of their EEG spectrum over time with the disease's progression to provide future BCI-research with the relevant information to classify changes in EEG evolution. Comparison of power spectral density (PSD) of these patients revealed a significant difference in the PSD's of patients in CLIS without any means of communication and the patient in the transition from LIS to CLIS with means of communication. The EEG of patients without any means of communication is devoid of alpha, beta, and higher frequencies than the patient in transition who still had means of communication. The results show that the change in the EEG frequency spectrum may serve as an indicator of the communication ability of such patients.

Keywords Resting-state electroencephalogram (EEG) · Completely locked-in state (CLIS) · LIS (locked-in state) · Power spectrum density (PSD) · Alpha frequency

Introduction

The cardinal feature of a patient in a locked-in state (LIS) is paralysis of most of the voluntary motor function of the body except the oculomotor function with preserved consciousness (Bauer et al. 1979; Chaudhary et al. 2020a). Because of the preserved oculomotor function and consciousness (Schnakers et al. 2008), patients in LIS have several means of communication (Birbaumer et al. 1999; Wolpaw and McFarland 2004; Kübler et al. 2005; Sellers et al. 2010; Lesenfants et al. 2014; Wolpaw et al. 2018; Tonin et al. 2020). A patient can be in LIS because of the severe brain injury or pontine stroke (Sacco et al. 2008; Sarà et al. 2018; Pistoia et al. 2010; Conson et al. 2010), or progressive neurodegenerative motor neuron disorders (Birbaumer 2006; Birbaumer et al. 2012; Chaudhary et al. 2015, 2016a, b). Amyotrophic lateral sclerosis (ALS) is a severe of all progressive neurodegenerative disorder leading to complete paralysis with symptoms involving both upper and lower motor neurons (Rowland and Shneider 2001). Like any other LIS patient, an ALS patient in LIS are paralyzed with preserved voluntary eye movement control, eye blinks or twitching of other muscles, and intact consciousness. The LIS is not a final state for a patient who has ALS. As the disorder progresses, ALS leads to a state of complete paralysis, including eye movements, transferring patients to the completely locked-in state (CLIS)

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11571-020-09639-w) contains supplementary material, which is available to authorized users.

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Published online: 23 October 2020

(Bauer et al. 1979; Chaudhary et al. 2020a). The transition from LIS to CLIS is usually a gradual process that is patient specific. During this transition phase from LIS to CLIS, the patient starts losing their eye movement control and ultimately losing the ability to open their eyes is lost. ianllt, the patients in CLIS have their eyes closed all the time, even in the CLIS, patients are assumed to preserve their cognitive functions (Kübler and Birbaumer 2008).

Many studies have compared electrophysiological signatures from ALS patients and controls (Jayaram et al. 2015; Nasseroleslami et al. 2019; Dukic et al. 2019; Maruyama et al. 2020), reporting features distinguishing the two groups. The most reliable evidence found is a decrease in alpha relative power, with a shift of the peak in the alpha frequency band (generally present in healthy patients' EEG power spectrum) to lower frequencies (Mai et al. 1998; Hohmann et al. 2018). Several other studies with a different patient population such as depression (Goshvarpour and Goshvarpour 2019), Alzheimer's disease (Nobukawa et al. 2019), stress (Subhani et al. 2018), autism (Gabard-Durnam et al. 2019), epilepsy (Myers and Kozma 2018) and Parkinson's disease (Yi et al. 2017) have shown a difference in EEG spectral power, fractal change, power correlation and complexity of resting-state EEG as compared with the healthy participants (Buiza et al. 2018). However, how these features and biomarkers evolve during the ALS progression, reaching a state where they separate patients in different stages of the disease, is still unclear.

This study aims to perform a longitudinal analysis of EEG frequency in three ALS patients, analyzing how the power spectral densities of EEG resting-state recordings evolve in each patient. Two out of three patients considered here are in CLIS (P6 and P9), while the third patient was first in the transition from LIS to CLIS (P11) and, ultimately, in CLIS. The decrease in relative alpha band power is registered in LIS and CLIS patients with respect to controls (Babiloni et al. 2010) (Maruyama et al. 2020), but a direct comparison between these states is still missing. Investigating whether these conditions differ from the electrophysiological point of view can help understand the effects of the transition and possibly monitor the patients for BCI use. In addition, an earlier report on several CLIS patients (Maruyama et al. 2020) needs replication, finding a reduction of higher frequencies in CLIS in a one-session protocol. Whether such a change in spontaneous EEG frequency spectrums indicates functional changes in the central nervous system is now a question of further investigations.

Materials and methods

The Internal Review Board of the Medical Faculty of the University of Tubingen approved the experiment reported in this study. The study was performed per the guideline established by the Medical Faculty of the University of Tubingen and Helsinki declaration. The patient or the patients' legal representative gave informed consent. The clinical trial registration number is ClinicalTrials.gov Identifier: NCT02980380.

Patients

The patients chosen for this study were selected from the available database if the EEG resting-state recordings were in a sufficient number for a longitudinal comparison and covering a time range of at least 1 year. Table 1 lists the most relevant clinical information for each patient and the dates of the acquired EEG recordings.

EEG data acquisition

EEG resting-state recordings were acquired during visits to the patients for BCI experiments before the experimental sessions started. From now on, "visits" refers to a period of several subsequent days in which acquisitions were performed. Usually, a single visit lasted for 4 to 5 days, and two subsequent visits were at least 30 days apart from each other.

During the resting state recordings, patients were lying in their beds, being instructed to relax. EEG electrodes were attached according to the 10-5 system, with reference and ground channels placed respectively to their right mastoid and the forehead. EEG signals were recorded using a V-Amp amplifier and active electrodes (Brain Products, Germany). The numbers and positions of electrodes were different between patients and visits due to clinical and experimental needs, as outlined in Supplementary Table 1.

EEG preprocessing

EEG data were processed using Matlab R2018_b (The MathWorks, Inc., Natick, Massachusetts, U.S.A.) and EEGLAB 14.1.1 (Delorme and Makeig 2004). First, a windowed band-pass filter at 0.5 to 45 Hz was applied to the raw EEG data, followed by down-sampling to 128 Hz. Data were then cleaned from the ocular signal by removing the artifacts using the AAR plug-in (Gómez-Herrero et al. 2006) of EEGLAB. The AAR toolbox process EEG data by first decomposing the time series into spatial components using a Blind Source Separation (BSS) algorithm, then identifying the artifactual components and finally

Table 1 List of patients—the table lists for each patient the respective ID, the age and gender, the ALS type diagnosed, a short report of the progression of the disease, and the month and year of visits

Patient ID	Birthday/sex	ALS type	Medical history	Resting state data acquisition date
P6	40/M	Bulbar	2009: Diagnosis	May 2017
			Sept 2010: Percutaneous feeding and artificial ventilation	September 2017
			Dec 2010: Lost speech and walk	April 2018
			2012: Transition to CLIS	May 2018
				January 2019
P9	24/M	Juvenile	2013: Diagnosis	June 2017
			Aug 2014: Percutaneous feeding and artificial ventilation	November 2017
			2016: Transition to CLIS	March 2018
				June 2018
P11	35/M	Non-bulbar	Aug 2015: Diagnosis	May 2018
			Dec 2015: Lost of speech and walk	August 2018
			Jul 2016: Percutaneous feeding and artificial ventilation	September 2018
			March 2019: Transition to CLIS	November 2018
				December 2018
				January 2019
				February 2019
				March 2019
				August 2019
				September 2019

reconstructing the signals using the non-artifactual components. For this study, the decomposition in independent components was obtained through second-order blind identification (SOBI) algorithm (Belouchrani et al. 1997), and artifactual components were automatically identified based on the value of the fractal dimension of the waveform (Gómez-Herrero et al. 2006). In particular, each EEG recording (comprehensive of all the channels acquired) was processed on sliding windows of 180 s, with an overlap period equal to 60 s, and the components with smaller fractal dimensions were selected as artifactual as they correspond to the ones with less low-frequency components. After ocular artifacts rejection was applied singularly to each EEG resting-state record on the complete set of channels, the Cz channel was selected for further analysis.

PSD was obtained through Welch's overlapped segments averaging estimator, using windows of 5 s length with an overlap of 2 s on a segment of 180 s extracted from the middle of each recording (samples were taken equally before and after the central sample of the complete EEG recording). Then, each PSD was normalized by its median to reduce the effect of different offsets in the recordings. The representative resting-state PSD of each visit was obtained averaging Cz's PSDs from recordings belonging to the same visit.

The relative band-power was then computed from each PSD (for each visit-wise PSD of each patient) to compare relative power values in the three patients quantitatively. The frequency range was divided into delta (0–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), low beta (12–20 Hz), high beta (20–30 Hz), and gamma (30–45 Hz) bands (Fig. 1).

Results

Statistical tests were applied using Matlab 2018b. Pearson's linear correlation coefficient was computed on subsequent values of relative band-power, obtained for each patient's set of visits, to investigate the correlation with the corresponding timeline. Then, the Mann–Whitney U test was applied to test the power difference between the three patients for each frequency band at the Cz sensor, considering for each of them the whole set of PSDs. The obtained p values were corrected through the False Discovery Rate (FDR) using the Benjamini–Hochberg method (Benjamini and Hochberg 1995) to compensate for the multiple comparisons of 6 frequency bands. The results are reported through the visualization of the PSD profile's evolution within the period of observation for each patient separately. The evolution of PSD of patients 6, 9, and 11 are shown in Figs. 2, 3, and 4, respectively. The results on the variance within visits relative band power and power

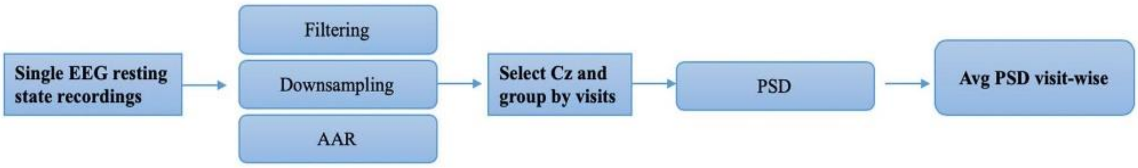


Fig. 1 Schematic workflow showing EEG's processing steps

Fig. 2 EEG power spectral density evolution in Patient 6. The PSDs corresponding to different visits is shown in different colors, as explained in the box in the top right corner of the figure. The x-axis represents the frequency in Hz. The y-axis represents the normalized amplitude of the power spectral densities on a logarithmic scale. In dashed lines are shown the frequency bands of interest. The frequency range analyzed is divided in the canonical frequency bands, represented in dashed lines in the figures: delta (1 to 4 Hz), theta (4 to 8 Hz), alpha (8 to 12 Hz), beta (12 to 30 Hz) and gamma (30 to 45 Hz)

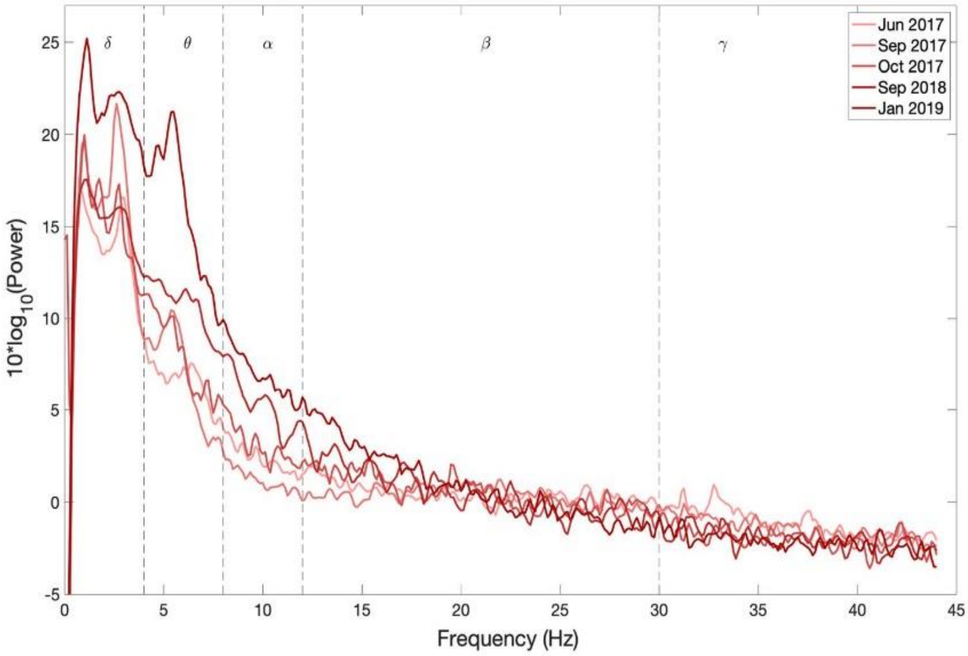
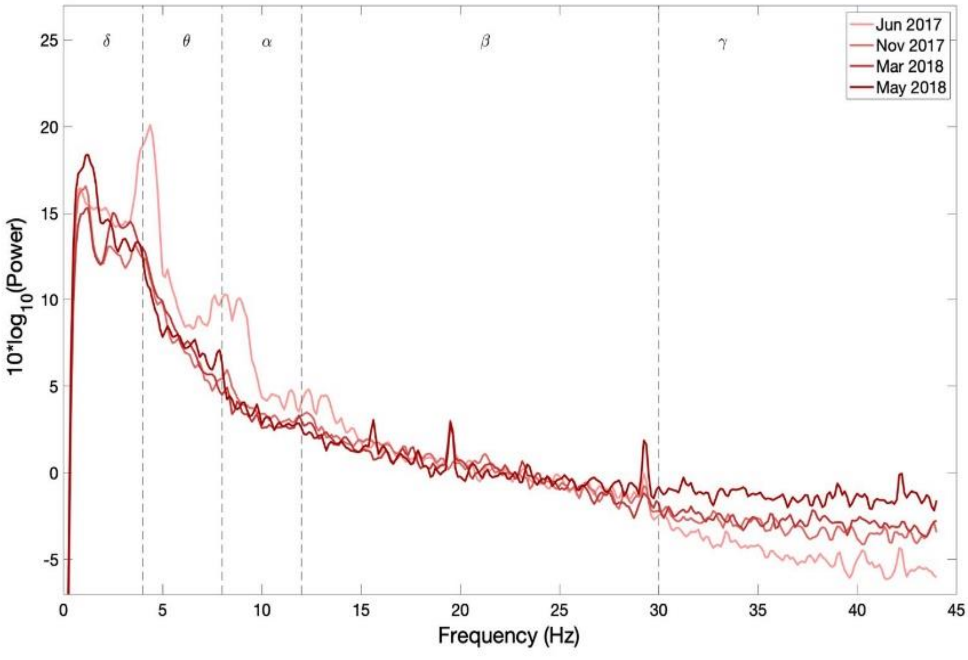


Fig. 3 EEG power spectral density evolution in Patient 9. The details of the figure are the same as explained in the legend of Fig. 2



spectral density of each patient is shown in Supplementary Text 1, where we show that the variance within a visit to be insignificant.

It can be observed from Figs. 2 and 3 that the frequency content of patients 6 and 9, who are in CLIS, are shifted towards delta and theta frequency bands. During the observation period reported in this paper, no general evolution of trends could be seen in patients 6 and 9. While Patient 11 has activity in the alpha band, present in all the recordings within the observation period, as shown in

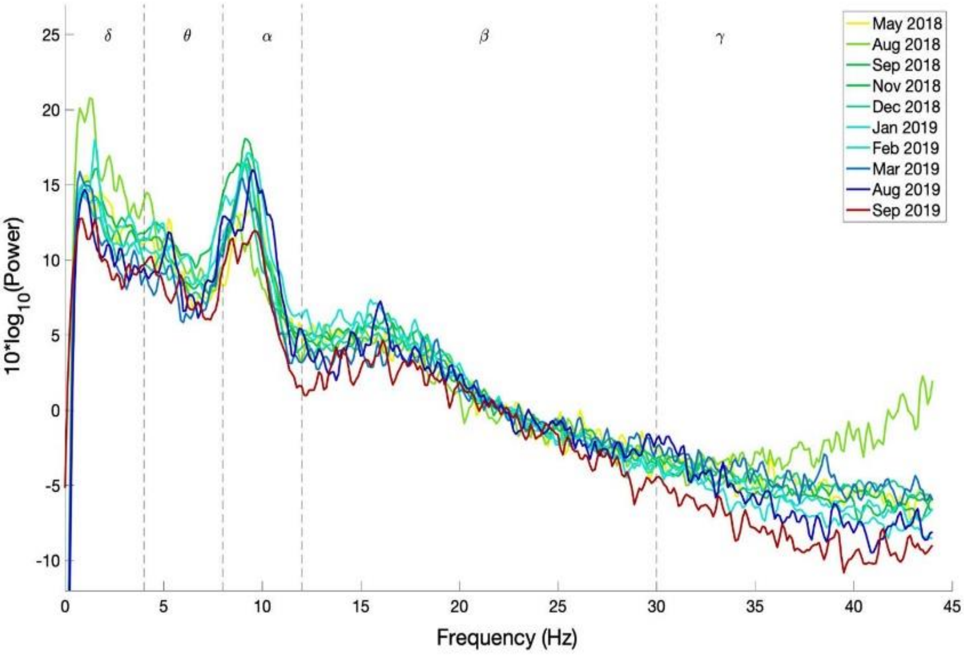


Fig. 4 EEG power spectral density evolution in Patient 11. The details of the figure are the same as explained in the legend of Fig. 2

Fig. 4. Nevertheless, a decrease in the power of the EEG signal as the patient transitioned from LIS to CLIS and, ultimately, in CLIS could be observed. The frequency content of patients' 6 and 9 EEG is very different from the EEG of patient 11. This aspect is more evident in Fig. 5, where the average of the PSDs related to all the visits grouped for patients is presented. These results were confirmed by the results of the Mann–Whitney *U* test shown in Fig. 6, which revealed the significant difference in the relative band power between Patient 11 and the two CLIS patients (Patients 6 and 9) at delta, alpha, and low-beta frequency bands. On the other hand, no significant difference was found over the values of relative power between patients 6 and 9.

Discussion and conclusion

A longitudinal resting-state analysis of patients in LIS and CLIS reveals a trend on the variation of EEG relative band power within the observation period. Patient 6, who is in CLIS since 2012 and was recorded for the first time in May 2017, shows a stable EEG frequency spectrum with dominant frequency in the delta and theta band. Patient 9, who is in CLIS since 2017 and was recorded for the first in June

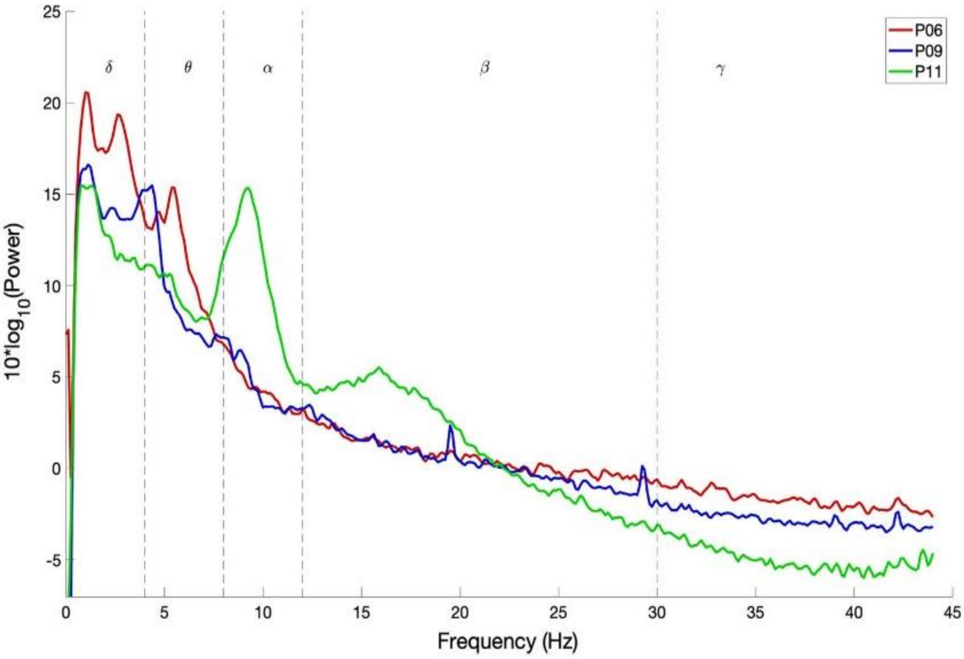


Fig. 5 Comparison of average EEG power spectral densities in Patients 6, 9, and 11. The red, blue, and green traces correspond to the average PSDs at electrode Cz for patients 6, 9, and 11, respectively. The x-axis represents the frequency in Hz. The y-axis represents the normalized amplitude of the power spectral densities in the logarithmic scale. In dashed lines are shown the frequency bands of interest as described in the legend of Fig. 2

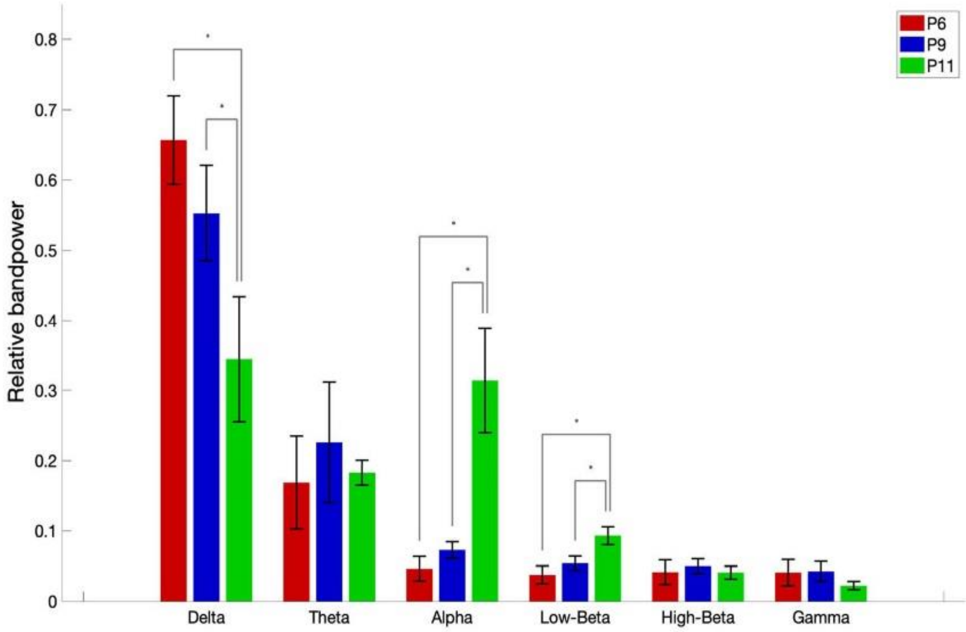


Fig. 6 Relative band power at electrode Cz. Error bars represent standard deviations. The figure depicts the significant power differences between patients 6, 9, and 11 in the two-tailed Wilcoxon rank-sum test with False Discovery Rate correction are marked: *$p < 0.05$. The x-axis represents the different frequency bands in Hz, and the y-axis represents the relative band power

2017 also shows a trend similar to patient 6. When we started recording Patient 11 in May 2018, the patient had control over his eye-movements but was unable to communicate with the eye-tracker based communication system because of his inability to fixate his gaze. During every visit to each patient, brain–computer interface (BCI)-based communication was attempted after resting-state recording. With patients 6 and 9, functional near-infrared spectroscopy (fNIRS) based communication was attempted, except for the visit 1 of patient 6, we were not able to establish a reliable means of communication using fNIRS based BCI communication system (Chaudhary et al. 2017) with these two patients. The fNIRS based BCI communication system was employed for patient 6 and 9 because it was demonstrated earlier that EEG-based BCI system had failed so far to provide a means of communication to the patients in CLIS (Kübler and Birbaumer 2008) except for a short one-session period report (Okahara et al. 2018) while fNIRS based BCI communication system showed some promise (Gallegos-Ayala et al. 2014). Since the patient 11 still had eye-movement an electrooculogram (EOG) based BCI communication was developed and implemented to provide a means of communication to the patient. The EOG-based communication by patient 11 is described in Tonin et al. (2020). As described in Tonin et al. (2020), patient 11 was able to employ his eye movement ability to communicate his thoughts and desires until February 2019, albeit with increasing difficulties due to the progressive paralysis of his eye muscles associated with the progression of the amyotrophic lateral sclerosis. From February 2019, the patient 11 could not employ his eye-movement to drive the EOG-based communication system (please refer to (Tonin et al. 2020) for further details). Patient 11 could not communicate reliably with his eyes from March 2019 onwards. He was implanted with microelectrodes in the motor region to provide him a means of communication (Please refer to Chaudhary et al. 2020b for details). The patient although in CLIS was able to form phrases and sentences to express his desires and wishes (Chaudhary et al. 2020b). His EEG spectrum remained constant throughout the observation period reported in this paper.

Patients 6 and 9, although of different ages and being in CLIS for different time periods, have the same EEG spectrum, which is significantly different from patient 11, who was first in LIS, then in the transition from LIS to CLIS and ultimately in CLIS during the period of observation reported in this paper. The main difference between patients 6 and 9 and patient 11 is that since we started following patients 6 and 9, they never had any means of communication. While we were able to provide a means of communication to patient 11 despite his degrading oculomotor function. It can be stated that from the patients reported in this longitudinal analysis, patients without any means of communication have different EEG spectrums than a patient who, despite being in CLIS, has a means of communication. It can also be hypothesized that if a patient has a means of communication despite being in CLIS the general shift in EEG spectrum to the lower bands might not occur, but to generalize these results to other patients in LIS and CLIS with and without means of communication, there is a need to perform such a longitudinal study on the large patient population. Also, a contrary causality is possible: with loss of normal EEG power spectrum and the underlying neurological functionality a loss of communication may be the consequence.

These results are partly supporting an earlier report from our lab of a remarkable reduction of higher frequencies in CLIS (Maruyama et al. 2020), all without any means of communication. It can also be hypothesized that the reason for the failure to establish communication with patients already in CLIS might be due to general shift of their EEG spectrum to the lower bands and absence of alpha and higher frequency bands since all the current EEG based BCI communication systems rely on the alpha and higher frequency bands (Jayaram et al. 2015; Lazarou et al. 2018). Nevertheless, it can also be argued that lack of alpha, in general, might also indicate reduced cognitive processing or compromised vigilance state of the patient (Klimesch 1999). However, in a recent study reported by Khalili-Ardali et al. (2019), patient in CLIS was shown to have the ability to process sentences with motor semantic content and self-related content better than control sentences indicating comprehension and some level of cognitive processing in CLIS in ALS patients. It can also be argued that the patients might be asleep during the period of data acquisition, but recently we showed in a larger sample of patients in CLIS (Malekshahi et al. 2019) that despite a general decrease in their EEG spectrum, patients in CLIS still have an intact sleep and wake cycle.

Thus, there is a need to perform long-term longitudinal studies with patients in LIS, the transition to LIS, and CLIS and parallel cognitive evaluation with BCI assistance to elucidate the evolution in their EEG signature, which afterward may then be used in the development of more efficient non-invasive BCI-systems.

Acknowledgements Deutsche Forschungsgemeinschaft (DFG) DFG BI 195/77-1, BMBF (German Ministry of Education and Research) 16SV7701 CoMiCon, and. LUMINOUS-H2020-FETOPEN-2014-2015-RIA (686764).

Author's contribution AS: data analysis; manuscript writing. AT: performed 40% of data collection; data analysis discussion. AR: performed 30% of the data collection. AJ-G: data analysis discussion. MK-A: data analysis discussion. NB: study design and conceptualization; manuscript correction. UC: study design and conceptualization; performed 70% of the data collection; data analysis supervision; manuscript writing.

Funding Open Access funding enabled and organized by Projekt DEAL.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Appendix G

Paper VII: A General-Purpose Framework for a Hybrid EEG-NIRS-BCI.

A General-Purpose Framework for a Hybrid EEG-NIRS-BCI

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Current code version

Nr	Code metadata description	*Please fill in this column*
C1	Current Code version	V 1.5.5
C2	Permanent link to code / repository used of this code version	https://github.com/majidkhalili/HybridBCI
C3	Legal Code License	MIT license (MIT)
C4	Code Versioning system used	Git on Github.
C5	Software Code Language used	Matlab
C6	Compilation requirements, Operating environments & dependencies	Matlab R2018a, Psychtoolbox, DSP System Toolbox (Only for EEG), TextAnalytics Toolbox (Only for speller)
C7	If available Link to developer documentation / manual	https://github.com/majidkhalili/HybridBCI
C8	Support email for questions	Majidkhalili89@aim.com

Abstract

Brain-computer interfaces (BCI), use brain signals to generate a control signal to control external devices to assist paralyzed people in movement and communication. Electroencephalography (EEG) and functional near-infrared spectroscopy (fNIRS) are the two most widely non-invasive brain recording techniques to develop BCIs. In this article, we are describing a software tool called "HybridBCI with an open-source framework for NIRS and EEG for a Hybrid BCI" application. This HybridBCI has been successfully used to enable brain- communication in patients without any means of voluntary communication. The results from these patients have been recently reported. This software tool is Matlab based, using object-oriented programming principles modular, and can be used by experimenters with different platforms and hardware to perform their BCI experiments and integrate their custom modules according to their needs.

Keywords

Brain computer interface (BCI), EEG, fNIRS, HybridBCI, Locked-in syndrome (LIS), Completely locked-in syndrome (CLIS)

Introduction

Several research labs have developed versions of BCIs to enable communication with the ALS-patient population, and other patients with heterogeneous results[1–6]. Recently Tonin et al. reported the successful use of an eye-movement based BCI for communication with four patients with amyotrophic lateral sclerosis (ALS) in the transition from LIS to CLIS [7] and the data set was published by Jaramilo-Gonzalez et. al. [8]. These patients had residual miniature eye activity, which they could not use to communicate using augmentative communication devices. Exploiting their residual eye-movements, these patients spelled freely and communicated again expressing their desires. The study was performed using a software tool designed by the authors for the BCI application called HybridBCI. HybridBCI is the result of extensive development of BCI application for communication purposes in LIS and CLIS patients using electroencephalography (EEG), and functional near-infrared spectroscopy (fNIRS) signals [7–9]. With this report, we are providing the source code for with HybridBCI, which can be used for HCI application in patients including but not limited to LIS and CLIS.

HybridBCI is not just a programming toolbox but also consists of an experimental paradigm allowing the user to implement any type of BCI using EEG, EOG, EMG, or fNIRS. HybridBCI benefits from a modular pattern; therefore, if the user wants to implement a particular algorithm, s/he can add only that particular segment without the need to know how the whole system works. HybridBCI is implemented in Matlab [10] widely used by neuroscientists and due to the interpreting nature of the Matlab, once a new functionality is added, there is no need to recompile the code. Besides, in clinical applications, data organization, and experiment logging are very crucial and HybridBCI manages the file organization and properly logs recording sessions.

With this paper, we propose an experimental paradigm with an open-source framework of a BCI with a clear and simple pipeline from data acquisition to signal analysis and classification with a separate implementation of each part. New features at any step of the pipeline can be added to the software by placing newly implemented .m files in the correct folders.

Paradigm

In the HybridBCI, regardless of the cognitive task used (e.g. mental calculation, the imagination of hand/foot movement, covert thinking, etc.), a list of questions/sentences with answers known by the experimenters is presented auditorily to the BCI user, and the user is asked to perform two different tasks i.e. responding mentally " yes" or" no" (i.e., true/false or 1/0). The auditory channel is used because many severely ill chronic patients suffer from impaired vision. HybridBCI uses Psychotolbox [11] in presenting auditory stimuli to minimize the software delay. Questions are repeated to the user with an equal but random distribution of questions with Yes and No answer (i.e.Your name is Majid. Your name is Ujwal) in an experimental block, and blocks are repeated to acquire enough data for classification. The initial experimental blocks are called training blocks since the user is familiarizing with the paradigm, and the data is collected to train a classifier. Once enough trials are acquired, a classifier is trained to classify yes and no answers. If the classification accuracy reaches above chance level, a feedback block is performed in which the user receives feedback of what has been classified, which serves also as a reward. If the feedback accuracy in a feedback block is also higher than chance, the model can be used for other applications with unknown answers. The chance can be calculated as proposed by Müller-Putz et al.[12] based on the number of trials in each block. The proposed block diagram paradigm is depicted in Figure 1. The number of blocks is dependent on the selected task, the number of trials in each block, and the users' condition. The rule of thumb would be to have twenty trials per block in four training blocks and one feedback block, which approximately takes one hour.

Implementation

HybridBCI is implemented in Matlab based on object-oriented programming (OOP) principles and new features can be added by placing the user implemented .m file in the correct path, to guarantee the scalability [5] of the system. As depicted in Figure 2, the core structure of HybridBCI is based on the two main modules named "HybridBCI.mlapp" and "ModelBuilder.mlapp" which do not need to be modified by users and are implemented using the Matlab App Designer[13].

1) Hybrid BCI

This module runs and controls the sequence of running the experiment, controls data acquisition functions, handles the triggering, runs "ModelBuilder" and log the experimental report. HybridBCI has three tabs, 'Configuration', 'Experiments', and 'Applications', which are used in an experiment, respectively. In the first tab, the brain signal measuring techniques and their corresponding recording handlers are selected, and the timing of the experiment is set (Figure 3A). These pieces of information, along with other experimental information such as the name of experimenters, data and time of the experiment, list of auditory stimuli, etc. are saved for each experimental block in a single .mat file.

a. Trigger

The functioning of the HybridBCI system relies on a precise subdivision of the raw data in several events. The correct timing is delivered by HybridBCI module to the acquisition devices through a set of symbols, named triggers. The proper sequence of triggering values for blocks and trials is presented in Table . Any triggering device is an instance of a class derived from 'Device.m' and needs to implement its' abstract functions. With this code, implementation of hardware triggering over the LPT port as well as the software triggering through a TCP/IP protocol for Brain Products GmbH (Germany) for EEG recording and file-based triggering for NIRx Medical Technologies (USA) for NIRS recording are implemented. HybridBCI is designed not to have any cumulative error between blocks and trials.

b. Data acquisition

HybridBCI can run and handles two recording devices simultaneously as needed for NIRS and EEG data acquisition, and it sends triggers to both of them at the same time. For each recording device, a new instance of Matlab is loaded by clicking on the corresponding 'RUN' button in the Experiment tab of the HybridBCI module (Figure 3B). New recording devices can be added by placing their .m file in the "..\lib\DataAcquision\EEG|NIRSDevices\". With this code, also the implementation of data acquisition and/or triggering for BrainVision, StarStim, and NIRx are also implemented and can be provided upon a request.

c. File Management

For each day of recording, a new folder is created in '..\Subjects\XX\' and for each block, a configuration file is saved in it, containing parameters of the performed experiment. Subjects' audio files are stored with .wav extension in '..\Subjects\XX\Audios\Questions\', which is very helpful in experiments with (C)LIS patients, in which audios needed to be recorded by the individuals' family members. Audio question files with yes answers are labeled as '001_FileName.wav' while questions with no answer are labeled as '002_FileName.wav'. Name of the files are optional but are recommend to be named with proper identifiers since they will be stored in the configuration file of each block. A sample of 20 audio files with yes and no answers are provided with this code.

d. Applications

Once a proper classification accuracy above the chance level is achieved, the same model can be used to run different applications that the intention of the user is not priory known for the experimenters. HybridBCI can automatically run and control the state transitions for any application that derives from 'Paradigm.m'. 'Paradigm.m' is a class with abstract methods that are needed for their functionality in HybridBCI. Once a new paradigm class is defined, from the 'Application' tab in the 'HybridBCI' module (Figure 3C). With this code, two main examples of such applications are provided including OpenQuestion in which the user can answer to the questions that the answers are unknown for the experimenters and Speller in which the user can freely spell what he has in mind [7].

2) Model Builder

"ModelBuilder" handles the analysis processing pipeline for NIRS and EEG in six steps (Figure 4) and stores it in a .mat file in the 'Models' folder for each subject to be used for giving feedback, running applications, or to perform offline analysis. Each analysis step has a dedicated tab in the GUI, and are described below.

a. Data Selection

This tab enables users to select data and reject noisy channels before performing any analysis which may arise due to displaced EEG electrodes or noisy NIRS channels or other recording issues (Figure 5A).

b. EEG Preprocessing

EEG analysis pipeline is composed of, Filtering, Band selection, and Inter Preprocessing (Figure 5) to clean the recorded signals from various artifacts [14,15] and improve the signal-to-noise ratio, using filtering [16] and conditioning of the signal by applying several operations to favor further Feature Extraction and Classification [17]. There is no standard sequence for the steps in the pre-processing of EEG [18] and each pre-processing sequence is tailored depending on the nature of the performed operations, on the type of features needed, and the physiological phenomena that are being investigated [18]. The same consideration can be made for the particular pre-processing for EEG recordings for BCIs; and several different EEG analysis pipelines are proposed [14,19–22]. In HybridBCI we use the pipeline that has previously been used in our lab [23], and we stick to the recommendation of performing first the filtering, since it can induce some nonlinearities in the signal, and subsequently any other linear operations without particular emphasis in the order [18]. Thus, the Pre-processing pipeline of this software is determined by the next steps and can be configured in the EEG Preprocessing tab of the ModelBuilder (Figure 5B).

I. Filtering

Previous to the filtering, two sets of options are provided for the user: First, *Source Selection*, in which the user is able to select the types of signals to be considered in the analysis, either EEG, EOG, or EMG channels. Second, *Bands Selections*, in which a group of traditional ranges of oscillations commonly used in EEG analysis [24], Wideband (0.5-30 Hz), Delta (1-4 Hz), Theta (4-7 Hz), Alpha (7-13 Hz) and Beta (13-30Hz), are offered to the user, with the possibility of using the default given ranges, or manually introducing modifications to each range. Then we apply a second-order Infinite Impulse Response (IIR) notch filter at 50 Hz and after that, we included two options for filtering: Finite impulse response (FIR) filter and Butterworth IIR filter design. Both filters are applied in such a way that the phase of the signal is not affected by [25]. We propose these two types of filters to allow the user to consider each according to her/his needs and their particular benefits or drawbacks applied to EEG signals [26].

II. Processing Functions

With this code, is provided three of the most common amplitude correction methods. First, *Detrending*, for rescaling all the data to have a mean of zero, by subtracting the mean of each analyzed segment to itself; In this case, avoiding dividing by the standard deviation to preserve the original amplitudes [27]. Second, *Baseline Correction* which is an operation that assumes the effects of any physiological response recorded post-stimulus in the EEG can be highlighted if compared with a pre-stimulus [28]. It must be considered that the definition of a baseline period varies depending on the physiological and analytical nature of the study [29]. Third, *Common Average Reference (CAR),* which is a technique that allows, if all the channels were recorded respective to the same physical reference, to approach the recordings to an "inactive reference", for details see [30]. From the list of all available functions, the user has the liberty of choosing the order in which they will be applied. User implemented EEG processing functions can be added to the pipeline by placing the .m file in in '..\lib\EEG\Preprocessing\.

c. EEG Feature Extraction

With this code, an initial set of features used in the analysis of EEG in the field of BCI in the time and frequency domain are included [31] including a series of range features for EEG time-series (Figure 5C). Details on the implemented EEG features can be found on supplementary data. User implemented EEG feature can be added to the pipeline by placing the .m file in '..\lib\EEG\Features\'.

d. NIRS Preprocessing

HybridBCI develops the pipeline for the NIRS pre-processing in two steps: first wavelength conversion, second filtering. In the first step, a default basic function is developed to convert the wavelength signal to hemodynamic concentration using the Modified Beer-Lambert Law (MBLL) [32], while in the second group the systemic components can be filtered using a bandpass filter function [33] and the signal can be normalized performing a baseline correction.

The NIRS signal is acquired as a pair of wavelengths belonging to the near-infrared spectral range between 650 nm to 950 nm [34]. To get a physiological signal, the most commonly used technique is the MBLL [32] to convert the wavelength to optical density and the hemodynamic concentrations: oxyhemoglobin (HbO), deoxyhemoglobin (HbR) and total hemoglobin concentration (HbT). In the MBLL two terms of the equation are the molar extinction coefficient and the differential path length factor that accounts for the real distance the light travels due to the scattering [32]; these two terms depend on many factors (e.g. age and gender of the subject), and their values can be found on the literature [33,35]. The observed hemodynamic signal is the result of the sum of neuronal and systemic components, thus to analyze functional changes, many techniques have been developed to separate the different components and to remove external noise (see [36] for an extensive review). With this code, an implementation to convert wavelength data to hemodynamic response is provided, as well as proper filters designed for filtering hemodynamic responses(Figure 5B). User implemented functionalities can be added to the processing pipeline by placing the .m file in '..\Lib\NIRS\PreProcessing\'.

e. NIRS features

Once the HbO, HbR, and HbT are pre-processed, it is possible to extract features to describe the signal synthetically using only relevant characteristics of the data. The developed functions extract the features for each of the three hemodynamic signals working on a single channel level, i.e., without averaging or grouping different channels or different signal types. With this code, preliminary features mostly used for NIRS signal, are provided and listed in supplementary data. Any new feature can be added to the pipeline by placing related m file in '..\Lib\NIRS\Features\'.

f. Dimensionality

Once the pre-processing and feature extraction steps are concluded, the size of the dataset in the feature space increases significantly. At this point, we incorporated two steps to reduce the dataset size keeping only those features that have a greater influence on the final result (Figure 5D).

I. Features consistency

This step is used to test the homogeneity of the features' distribution across the different trials that will be used to train the classifier, and results in a training sample with equal or fewer features than initially. This process keeps only those features that are consistent over time and discards the rest, it also reduces the calculations in future stages. For details see [37,38].

II. Dimension reduction

This step is used to reduce the dimensionality of the training data by selecting an ordered set of the most relevant features for classification [39–42]. Given a training sample with *m* features and a percentage *K* specified by the user, a variable selection method is implemented, resulting in a training sample with a number *k* percent of features. The implemented function is a variation on the popular mRMR method, originally created by *Ding and Peng* [39] and *Peng et al.* [40] but using another association measure instead of "mutual information", as proposed by *Berrendero et al.* [41]. Their approach, which appears to work better on small samples [41] and is thus relevant for BCI applications, consists of using squared "distance correlation", which measures dependency between variables and was introduced by *Székely et al.* [42].

g. Classification

HybridBCI can use any classifier that is derived from the abstract class 'Classifier.m' in '..\Lib\Classifiers\'. Classes inheriting from this class need to implement two abstract functions including training the model with given features as input, and predicting a single trial using the same model. With this code, two well-known classification algorithms are provided: Support Vector Machines (SVM's) and k-Nearest Neighbours (k-NN) [43,44] (Figure 5E). For a review of classification methods for EEG based BCI, see [45] and for hybrid EEG and NIRS see [46].

h. Validation

One of the known issues in the field of machine learning is the problem of overfitting the data, and it refers to the problem that due to the incorrect tuning of the classifier, the model overfits the features space. Therefore, it is necessary to check the performance of the classifier on the data other than the data that has been used for training (simulating the online feedback in BCI experiment). For this reason, the Validation tab in the 'ModelBuilder' is provided to check the performance of each model on other datasets (Figure 5F).

Discussion

During several years of research and development in the field of BCI for communication in DoC patients, several versions of this software have been used to perform studies. This version of HybridBCI is the outcome of this process in our lab. The latest version was successfully used to enable communication with patients with ALS patients when no other communication channels were left for patients [7]. The code is provided in the Matlab which most of the scientists in the field of cognitive science are already familiar with and is easy to develop. HybridBCI has an OOP software design, and due to the interpreting nature of the Matlab programming language, as opposed to compiling based programming languages, new features and functionalities can be added to the system by placing new .m files in the proper path without the need to know the whole system or recompiling the project.

Acknowledgments

Deutsche Forschungsgemeinschaft (DFG), Kosellek and DFG BI 195/77-1, BMBF (German Ministry of Education and Research) 16SV7701 CoMiCon, LUMINOUS-H2020-FETOPEN-2014-2015-RIA (686764), and Wyss Center for Bio and Neuroengineering, Geneva.

Author contributions

MKH designed the software, implemented the core structure, and wrote the first draft of the paper. JMC improved the core structure and combined different software modules. AT implemented NIRS pipeline, presenting paradigm and classification methods, implemented speller, and wrote the NIRS section. AJG implemented the EEG pipeline and wrote the EEG section. SW improved EEG pipeline and test and debug the system. GZ test and reported the triggering, and improved

NIRS pipeline. GC implemented range features. AMS implemented and reported feature consistency and dimension reduction. NB designed the paradigm and provided resources. UC initiated the HybridBCI software, implemented initial paradigms and signal processing pipeline, and supervised.

Competing interests

The author(s) declare no competing interests.

Code availability

Source code and sample data are available at https://github.com/majidkhalili/HybridBCI.

Ethical Approval

The Internal Review Board of the Medical Faculty of the University of Tubingen approved the experiment reported in this study. The study was performed per the guideline established by the Medical Faculty of the University of Tubingen. The patient or the patients' legal representative gave informed consent with permission to publish the data. The clinical trial registration number is: ClinicalTrials.gov - Identifier: NCT02980380.

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List of Tables

Table 1: The table represents the 15 different triggers used by the HybridBCI for managing the different events. These values are sent to the acquisition systems during the experiment and used to keep track of the timing of the different events.

Table1

Event		Trigger values		
		Yes Question	No Question	Open Question
Block Start	9			
		10	11	12
Question		5	6	7
Thinking		4	8	13
Feedback		1	2	3
Block End	15			

List of Figures

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Figure 1

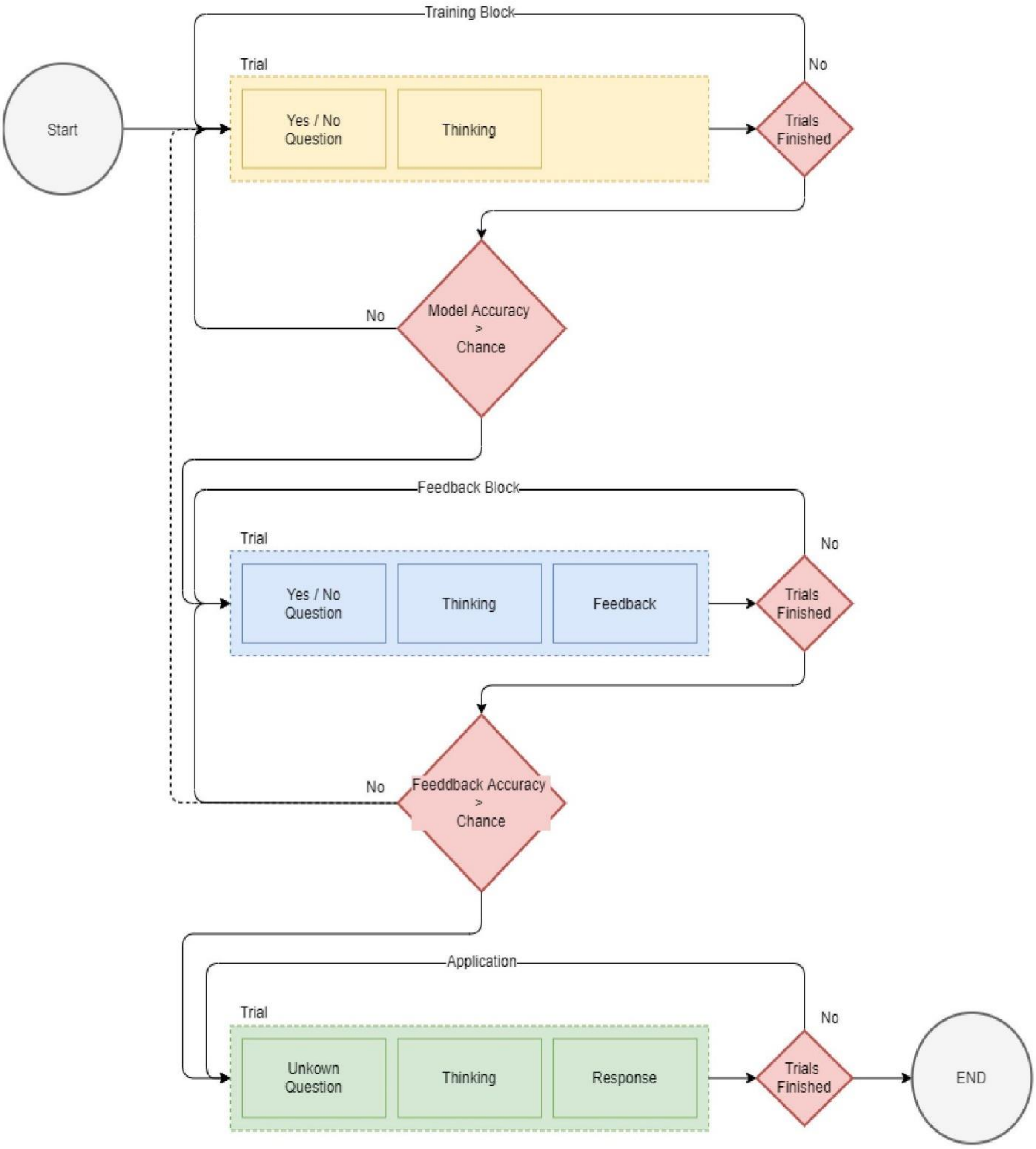
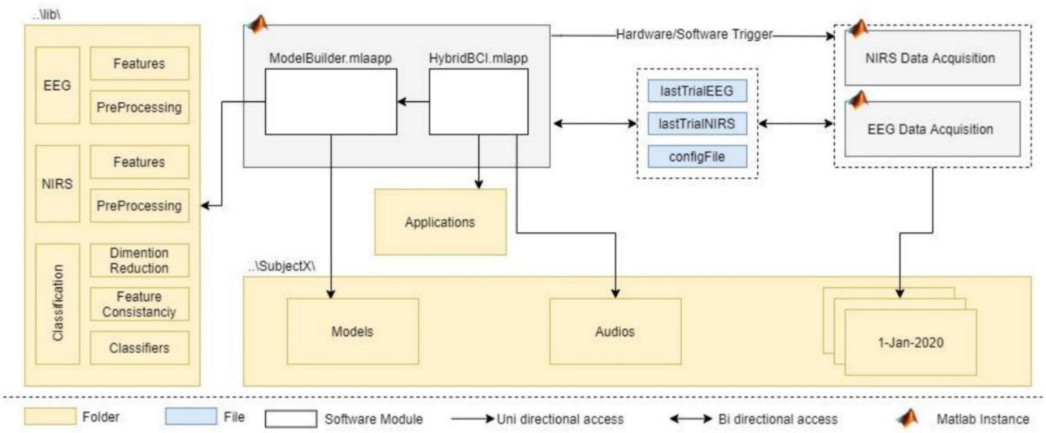

Start
Training Block
Trial
Yes / No Question
Thinking
No
Trials Finished
No
Model Accuracy > Chance
Feedback Block
Trial
Yes / No Question
Thinking
Feedback
No
Trials Finished
No
Feeddback Accuracy > Chance
Application
Trial
Unkown Question
Thinking
Response
No
Trials Finished
END

Figure 2


..\lib\
EEG
Features
PreProcessing
NIRS
Features
PreProcessing
Classification
Dimention Reduction
Feature Consistanciy
Classifiers
ModelBuilder.mlaapp
HybridBCI.mlapp
Hardware/Software Trigger
lastTrialEEG
lastTrialNIRS
configFile
NIRS Data Acquisition
EEG Data Acquisition
Applications
..\SubjectX\
Models
Audios
1-Jan-2020
Folder
File
Software Module
Uni directional access
Bi directional access
Matlab Instance

19

Figure 3

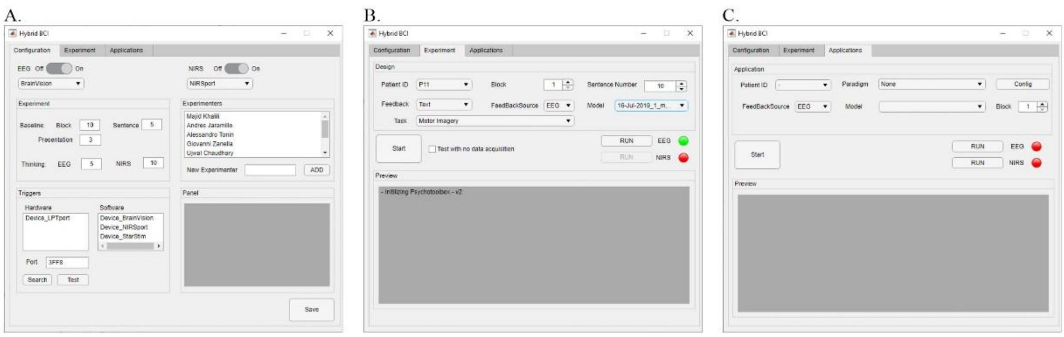


Figure 4

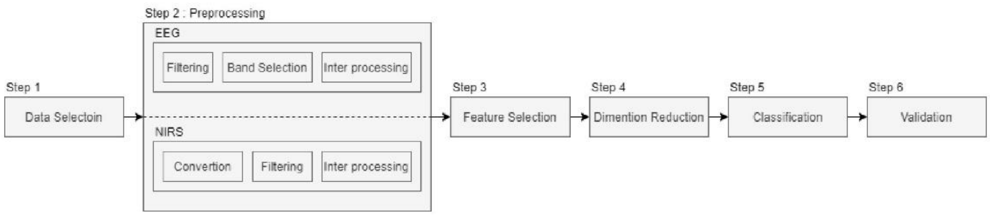
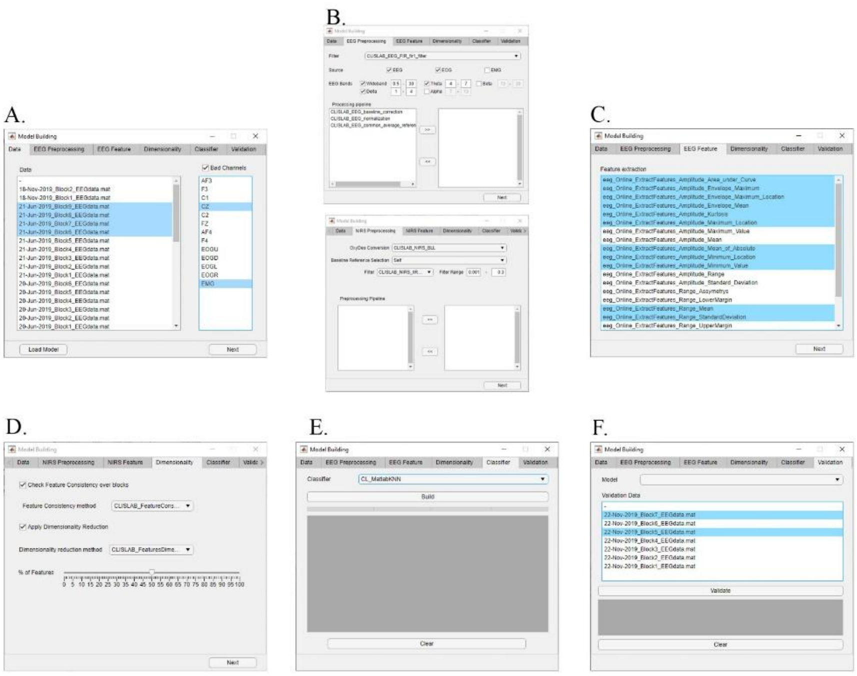

Step 2 : Preprocessing
EEG
Filtering
Band Selection
Inter processing
Step 1
Data Selectoin
NIRS
Convertion
Filtering
Inter processing
Step 3
Feature Selection
Step 4
Dimention Reduction
Step 5
Classification
Step 6
Validation

Figure 5



Appendix H

Paper VIII: Communication using intracortical signals in a completely locked in-patient.

Verbal Communication using Intracortical Signals in a Completely Locked In-Patient

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Abstract

Patients with amyotrophic lateral sclerosis (ALS) can lose all muscle-based routes of communication as motor neuron degeneration progresses, and ultimately, they may be left without any means of communication. While others have evaluated communication in people with remaining muscle control, to the best of our knowledge, it is not known whether neural-based communication remains possible in a completely locked-in state (CLIS). With consent and approval of the participant, family, and legal authority for this study, two 64 microelectrode arrays were implanted in the supplementary and primary motor cortex of a CLIS patient with ALS. The patient modulated neural firing rates based on auditory feedback, and he used this strategy to select letters one at a time to form words and phrases to communicate his needs and experiences. This case study provides novel evidence that brain-based volitional communication is possible even in a completely locked-in state.

Keywords: Amyotrophic lateral sclerosis; Completely locked-in state; Intracortical; Microelectrodes; Communication; Motor cortex.

Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder that leads to the progressive loss of voluntary muscular function of the body[1]. As the disorder typically progresses, the affected individual loses the ability to breathe due to diaphragm paralysis. Upon accepting artificial ventilation and with oro-facial muscle paralysis, the individual in most cases can no longer speak and becomes dependent on assistive and augmentative communication (AAC) devices[2,3], and may progress into the locked-in state (LIS) with intact eye-movement or gaze control[4,5]. Several invasive[6-10] and non-invasive[11-16] brain-computer interfaces (BCIs) have provided communication to individuals in LIS[17-20] using control of remaining eye-movement or (facial) muscles or neural signals. Once the affected individual loses this control to communicate reliably, no existing assistive technology has provided voluntary communication in this completely locked-in state (CLIS)[17-20]. Non-invasive[11-16] and invasive[6-10] BCIs developed for communication have demonstrated successful cursor control and sentence formation by individuals up to the stage of LIS. However, none of these studies has demonstrated communication at the level of voluntary sentence formation in CLIS individuals without stable and reliable eye-movement/muscle control, leaving the possibility open that once all movement - and hence all possibility for communication - is lost, neural mechanisms to produce communication will concurrently fail.

Here, we established that an individual was in the CLIS state and demonstrated that sentence-level communication is possible without relying upon vision. This individual lacked reliable voluntary eye-movement and, consequently, was unable to use an eye-tracker for communication. He also showed increasingly unstable and ultimately failing performance with a non-invasive eye-movement-based communication system. To restore communication in CLIS, this participant was implanted with intracortical microelectrode arrays in two motor cortex areas. The patient then employed an auditory-guided neurofeedback-based strategy to modulate neural firing rates to select letters and to form words and sentences using custom software. This individual, who before implantation was unable to express his needs and wishes through non-invasive methods, including eye-tracking, visible categorization of eye-movements, or an eye movement-based BCI-system, started using the intracortical BCI system for voluntary verbal communication three months after implantation.

Materials and Methods

The medical procedure was approved by the Bundesinstitut für Arzneimittel und Medizinprodukte ("BfArM", The German Federal Institute for Drugs and Medical Devices).

The study was declared as a Single Case Study and has received a special authorization ("Sonderzulassung") by BfArM, according to §11 of the German Medical Device Law ("Medizin-Produkte-Gesetz") on December 20, 2018, with Case Nr. 5640-S-036/18. The Ethical Committee of the Medical Faculty of the Technische Universität München Rechts der Isar provided support to the study on 19 Jan 2019, along with the explicit permission to publish on 17 February 2020. Before the patient transitioned into CLIS, he gave informed consent to the surgical procedure using his eye movements for confirmation. The patient was visited at home by authors HT and JL, and thorough discussions were held with the legally responsible family members in order to establish convincing evidence of the patient's informed consent and firm wish to undergo the procedure. The legally responsible family members then provided written permission to the implantation and the use of photographs, videos, and portions of his protected health information to be published for scientific and educational purposes. In addition, a family judge at patient's home county court gave the permission to proceed with the implantation after reviewing the documented consent and a visit to the patient.

Patient

The patient in mid 30's was diagnosed with progressive muscle atrophy, a clinical variant of non-bulbar ALS, selectively affecting spinal motor neurons approximately 3.5 years before the implantation. He lost verbal communication and the capability to walk 3 years before the implantattion. He has been fed through a percutaneous endoscopic gastrostomy tube and artificially ventilated 2.5 years before the implantation and is in home care. He started using the MyTobii eye-tracking-based assistive and augmentative communication (AAC) device 2.5 years before the implantation. Aprroximately 2 years before the implantation, he could not use the eye-tracker for communication because of his inability to fixate his gaze. Subsequently, the family developed their own paper-based spelling system to communicate with the patient by observing the individual's eye movements. According to their scheme, any visible eye movement was identified as a "yes" response, lack of eye movement as "no". The patient anticipated complete loss of eye control and asked for a new communication system, which motivated the family to contact authors NB and UC for alternative approaches. Initial assessment sessions were performed one year before the implantationin. During this interval, the detection of eye movements by relatives became increasingly difficult, and errors made communication attempts impossible up to the point when communication attempts were abandoned. The patient and family were informed that a BCI-system based on electrooculogram

(EOG) and/or electroencephalogram (EEG) might allow "yes" - "no" communication for a limited period.

The patient began to use the non-invasive eye movement-based BCI-system described in Tonin et al.[21]. The Patient was instructed to move the eyes ("eye-movement") to say "yes" and not to move the eyes ("no eye-movement") to say "no". Features of the EOG signal corresponding to "eye-movement" and "no eye-movement" or "yes" and "no" were extracted to train a binary support vector machine (SVM) to identify "yes" and "no" response. This "yes" and "no" response was then used by the patient to auditorily select letters to form words and hence sentences. The patient and family were also informed that non-invasive BCI-systems might stop functioning satisfactorily, and in particular, selection of letters might not be possible if he became completely locked-in (where no eye movements could be recorded reliably). In that case, implantation of an intracortical BCI-system using neural spike-based recordings might allow for voluntary communication. As the patient's ability to communicate via non-invasive BCI systems deteriorated, in mid of 2018, preparations for the implantation of an intracortical BCI system were initiated. To this end, HT and JL and GF were approached in order to prepare the surgical procedure and ensure clinical care in a hospital close to the patient's home. The patient was able to use the non-invasive BCI system employing eye-movement to select letters, words, and sentences until beginning of 2019, as described in Tonin et al. [21]. By the time of implantation, the EOG/EEG based BCI system failed, as signals could not be used reliably for any form of communication in this investigational setting. The EOG/EEG recordings and their analysis are described in Supplementary text 1 and Supplementary Figure S2. Additionally, the patient reported low visual acuity caused by the drying of the cornea.

Surgical Procedure

A head MRI scan was performed to aid surgical planning for electrode array placement. The MRI scan did not reveal any significant structural abnormalities, in particular no brain atrophy or signs of neural degeneration. A neuronavigation system (Brainlab, Munich, Germany) was used to plan and perform the surgery. In the first quarter of 2019, two microelectrode arrays (8×8 electrodes each, 1.5mm length, 0.4mm electrode pitch; Blackrock Microsystems LLC) were implanted in the dominant left motor cortex under general anesthesia. After a left central and precentral trephination, the implantation sites were identified by neuronavigation and anatomical landmarks of the brain surface. A pneumatic inserter was used (Hochberg et al. 2006) [22] to insert the electrode arrays through the arachnoid mater, where there were no major blood vessels. The pedestal connected to the microelectrode arrays connected via a bundle of fine wires (Blackrock Microsystems LLC), was attached to the calvaria using bone screws and was exited through the skin. The first array was inserted into the hand area region of the primary motor cortex[23], and the second array was placed 2cm anteromedially from the first array into the region of the supplementary motor area (SMA) as anatomically identified. No implant-related medical adverse events were observed. After three days of post-operative recovery, the patient was discharged to his home.

Neural Signal Processing

A digitizing headstage and a Neural Signal Processor (CerePlex E and NSP, Blackrock Microsystems LLC) were used to record and process neural signals. Raw signals sampled at 30kS/s per channel were bandpass filtered with a window of 250-7500 Hz. Single and multi-unit action potentials were extracted from each channel by identifying threshold crossings (4.5 times root-mean-square of each channel's values). Depending on the activity and noise level, thresholds were manually adjusted for those channels used in the BCI sessions after visual inspection of the data to exclude noise but capture all of the visible spikes above the threshold. Neural data were further processed on a separate computer using a modified version of the CereLink library (provided by Blackrock Microsystems LLC) and additional custom software. For communication, we used spike rates from one or more channels. A spike rate metric (SRM) was calculated for each channel by counting threshold crossings in 50 ms bins. The SRM was calculated as the mean of these bins over the past one second.

Neurofeedback communication

The patient was provided auditory feedback of neural activity levels by mapping the SRM for one or more channels to the frequency of an auditory feedback tone, as shown in Figure 1. Single channel spike rates were normalized according to the spike rate distribution of each channel. Selected channels' normalized SRMs were then summed and linearly mapped to the range of 120-480 Hz, determining the frequency of the feedback tone produced by an audio speaker. Feedback tones were updated every 250ms. The firing rate r_i of each selected channel was constrained to the range $[a_i, b_i]$, normalized to the interval $[0,1]$, and optionally inverted, and the resulting rates were averaged:

$$r(t) = \frac{1}{n}\sum_{i}^{n} \frac{1-c_i}{2} + c_i \frac{\max(\min(r_i(t), b_i), a_i)}{b_i - a_i}$$

where $r(t)$ is the overall normalized firing rate, and the c_i are 1 or -1. The normalized rate was then linearly mapped to a frequency between 120 and 480 Hz for auditory feedback. Feedback tones were pure sine waves lasting 250 ms each. Initially, channels were selected randomly for feedback. Then the parameters a_i, b_i, c_i as well as the channels used for control were chosen and iteratively optimized each day in the neurofeedback training paradigms.

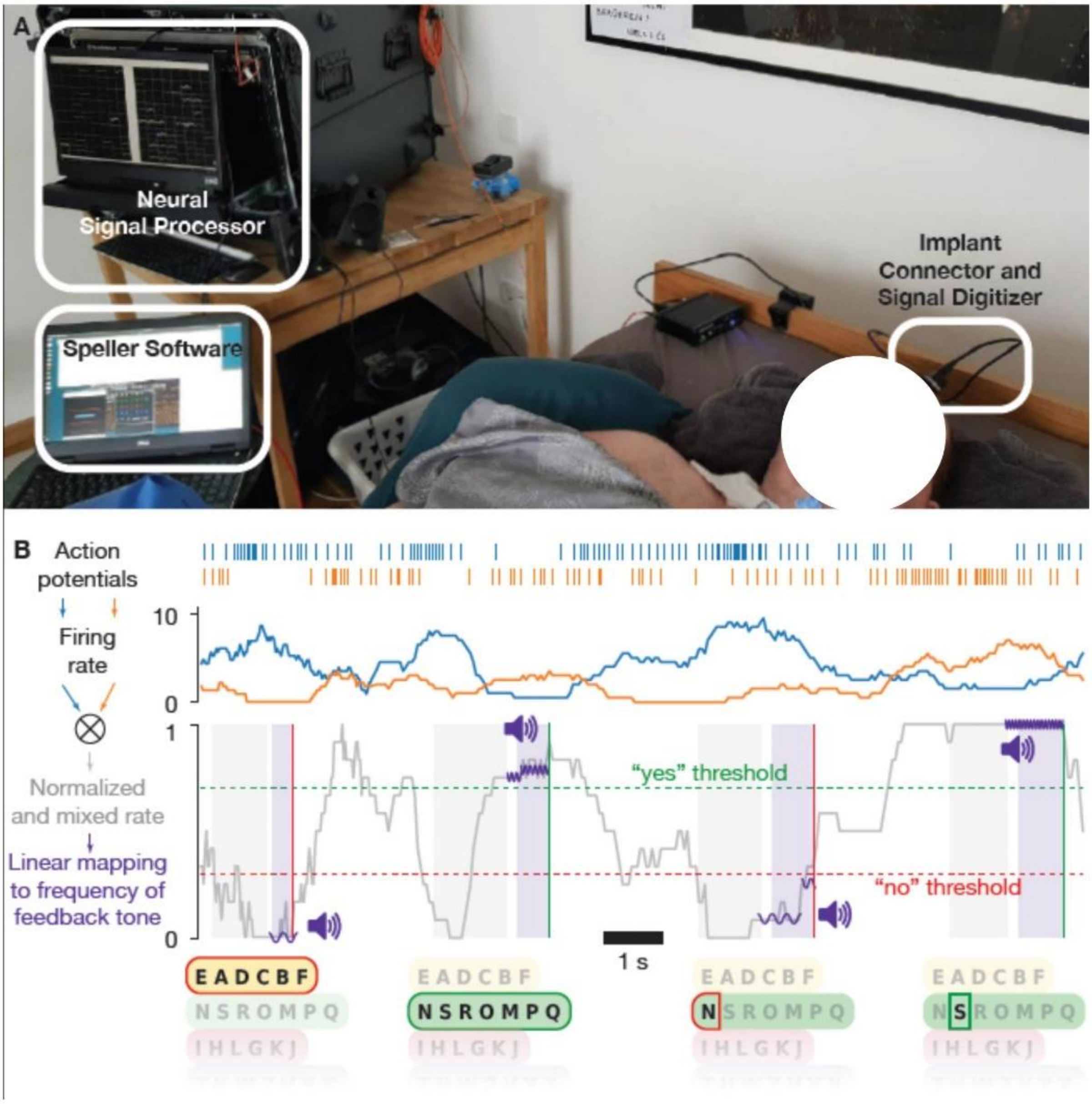

Figure 1: Intracortical auditory speller-based communication – *A) Setup at the patient's home. Signals were recorded from microelectrode arrays implanted in the motor cortex and processed with custom BCI software. B) Schematic representation of auditory neurofeedback and speller. Action potentials were detected and used to estimate neural firing rates. One or several channels were selected, their firing rates normalized and mixed (see Online Methods). Options such as letter groups and letters were presented by a synthesized voice, followed by a response period during which the patient was asked to modulate the normalized and mixed firing rate up for a positive response and down for a negative response. The normalized rate was linearly mapped to the frequency of short tones that were played during the response period, to give feedback to the patient. The patient had to hold the firing rate above (below) a certain threshold for typically 500ms to evoke a "Yes" ("No") response. Control over the neural firing rates was trained in neurofeedback blocks, in which the patient was instructed to match the frequency of target tones.*

The first paradigm ("feedback without reward") provided successive target tones at 120 or 480 Hz, and the patient was asked to match the frequency of the feedback to the target (typically 20 pseudorandom trials per block). In the "feedback with reward" paradigm, was essentially the same, however, upon reaching and holding (during a configurable number of interactions, each interaction lasting 50ms) the feedback tone within a predefined range around the target frequency, an additional reward sound was delivered for 250ms indicating successful performance to the patient. Holding the feedback tone at the high (low) end of the range for 250ms was then interpreted by the patient upon instruction as a Yes (No) response. The "feedback with reward" paradigm served to train and validate the responses.

We also validated the Yes/No responses in a question paradigm, in which the answers were assumed to be known to the patient. Furthermore, we used an 'exploration' paradigm to test if the patient's attempted or imagined movements could lead to modulation of firing rates.

Finally, in an auditory speller paradigm, the patient could select letters and words using the previously trained Yes/No approach. The auditory speller paradigm is depicted in Figure 1C. The speller system described here avoids long adaptation and learning phases because it is identical to the one used previously when he was still in control of eye movements.

The speller's output was rated for intelligibility by three of the authors (UC, IV, and JZ). Three categories were used: unintelligible, ambiguous, and intelligible. Ambiguous speller output includes grammatically correct words that could not be interpreted in the context as well as strings of letters that could give rise to uncertain interpretations. Intelligible phrases may contain words with spelling mistakes or incomplete words, but the family or experimenter identified and agreed upon their meaning.

Results

One day after the implantation, attempts were initiated to establish communication. The patient was asked to use his previous communication strategy employing eye movements and later to attempt hand, tongue, or foot movements to elicit neural responses. No qualitative differential neural responses could be measured. Subsequently, the communication strategy was changed on day 86, and neurofeedback-based paradigms (described in the Online Methods section) were employed, as shown in Figure 1. The patient was provided auditory feedback of neural activity by mapping a spike rate metric (SRM) for one or more channels to the frequency of an auditory feedback tone, as displayed in Figure 1 (described in the "Neurofeedback communication" section of Online Methods). The patient was able to modulate the sound tone immediately on day 86 and subsequently was able to successfully modulate the neural firing rate and match the frequency of the feedback to the target for the first time 98 days after implantation. Employing the neurofeedback strategy, the patient was able to modulate the neural firing rate and was able to use this method to select letters and to free spell from day 106 onwards. Data reported here include data from days 106–247 after implantation. During this period, the patient was visited on 47 days. Each day, we performed several neurofeedback blocks, each typically consisting of 10 high-frequency target tones and 10 low-frequency target tones presented in pseudo-random order to tune and validate the classifier. If the patient was able to match the frequency of the feedback to the target in 80% of the trials, we proceeded with the speller. Figure 2 shows the amount of time the patient was exposed to different paradigms.

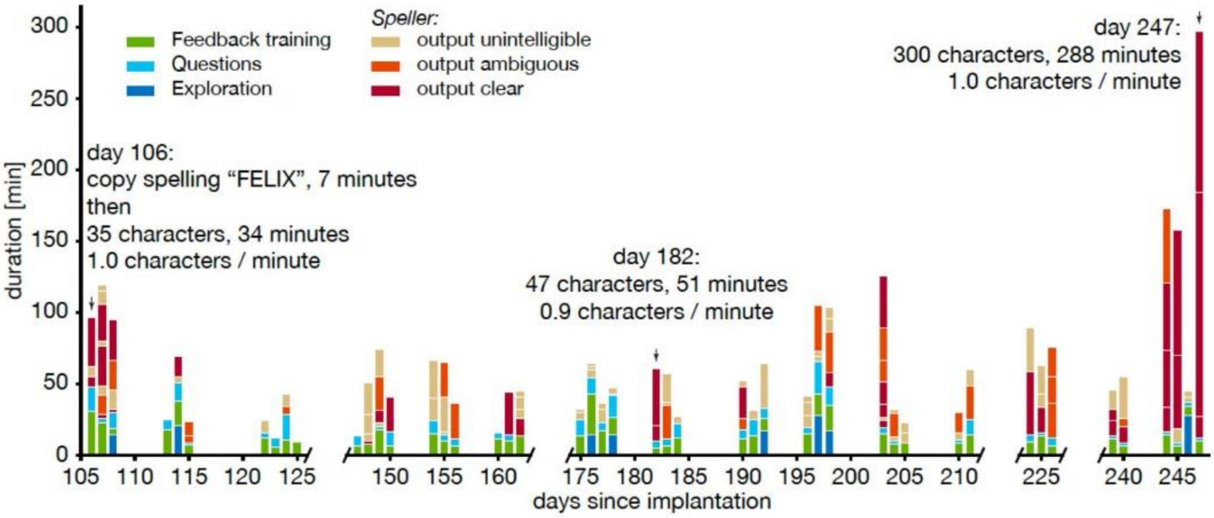

Figure 2: Overview of BCI use – *Time the patient spent in BCI paradigms. Individual speller blocks are shown in their respective order, times for other kinds of blocks are summed per day. The x-axis represents the number of days after implantation and the y-axis the duration of the respective paradigm in minutes. Different colors represent different paradigms, as described in the legend on the top left corner of the figure. The patient was visited 3 to 4 days per week, as shown by 3 to 4 consecutive bars. The patient was hospitalised due to unrelated adverse events between days 120 and 145, 163 and 172, and 212 and 223. Question and exploration tasks are not further discussed in this paper.*

Figure 3A shows individual neurofeedback trials, including an error trial, of one representative block. In the 85 neurofeedback blocks preceding the speller blocks, 1507 of 1700 trials (89%) were correct (Figure 3B), i.e., for target tone up (higher frequency) the decision was up (a "yes" answer), and for target down (low frequency) the decision was down (a "no" answer). The difference in error rates between 'up' and 'down' trials, i.e., the fraction of trials in which the modulated tone did not match the target tone, (13% and 8%, respectively), was significant (Pearson's χ^2 test: $p<0.01$). The patient maintained a high level of accuracy in the neurofeedback condition throughout the reported period: on 74% of the days, the accuracy was at least 90% during at least one of the feedback trial blocks, i.e., the patient was able to match the frequency of the feedback to the target 18 out of 20 times. We observed considerable within-day variability of neural firing rates and hence performance of the neurofeedback classifier, necessitating manual recalibration throughout the day (see Supplementary Figure S1).

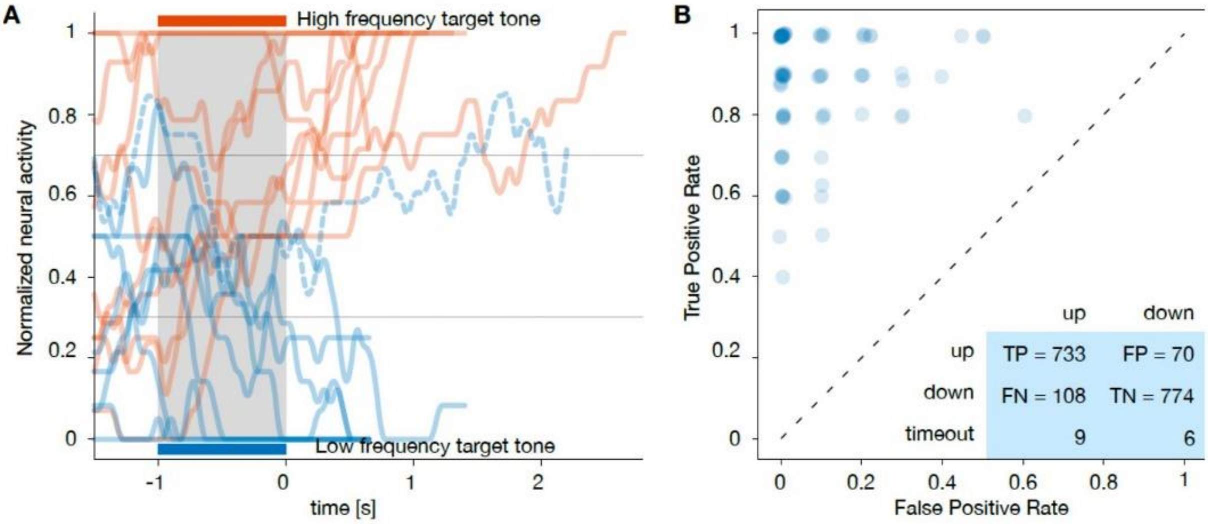

Figure 3: Neurofeedback task and classification – *A) Representative example for normalized and mixed firing rate during ten high (red) and ten low (blue) frequency trials. The patient was asked to match the target tone by modulating the normalized and mixed firing rate, and he succeeded in all, but one trials. Trials were completed as soon as the firing rate was held above (below) the upper (lower) threshold. These blocks were used for parameter tuning, training, and validation, and performed during every day of recordings. B) True positive rate vs. false positive rate of the trials in auditory neurofeedback blocks directly preceding speller blocks on days 123–247. Each circle represents one neurofeedback block; circles are jittered for better visibility. The insert shows a contingency table of all trials in these blocks. Blocks on days 106–122 were excluded from this analysis because time-out and error trials were treated differently in these blocks.*

We continued with the speller paradigm when the patient's performance in a neurofeedback block exceeded an acceptance threshold (usually 80%). To verify that good performance in the neurofeedback task translated to volitional speller control (based on correct word spelling), we asked the patient to copy words before allowing free spelling. On the first three days of speller use, the patient correctly spelled the specified words. After an unrelated stay at the hospital, we again attempted the speller using the same strategy on day 148.

Afterward, we relied on a good performance in the neurofeedback task, i.e., the patient's ability to match the frequency of the feedback to the target in 80% of trials, to advance to free spelling. The selection of two letters from a speller block on day 108 is shown in Figure 4.

The patient produced comprehensible output, as rated independently by three observers, on 20 of 42 days when the speller was used (Figure 2). The median length of these communications was 26.5 characters per day, and the patient's intelligible messages comprised 919 characters produced over 994 minutes, corresponding to an average rate of 0.92 characters per minute. This rate varied across blocks (min/median/max: 0.4/1.1/4.3 characters per minute). Over the reported period, there was no apparent trend in spelling speed.

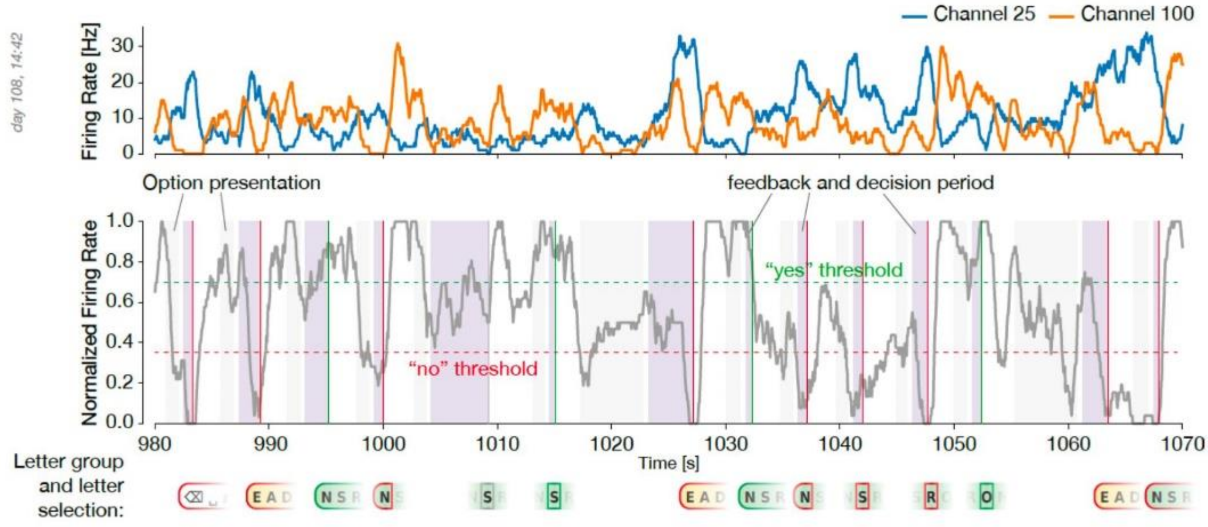

Figure 4: Example of letter selection during a free spelling block – *A) Firing rate of the channels used for "yes"/"no" classification and B) normalized firing rate during the same 90 second period of a speller block. "Yes" / "no" / timeout decisions are marked by vertical lines. Below these lines, the corresponding selection of letter groups/letters is shown. This example is part of the phrase "DEKUBITUS PO ER* ***SO*** *LL ARME MAXIMAL", referring to bedsore and instructing the aide to change arm position.*

Many of the patient's communications concerned his care (e.g. 'KOP?F IMMERLQZ GERAD' – 'head always straight', day 161; 'KEIN SHIRT ABER SOCKEN' – 'no shirts but socks [for the night]', day 244). The patient also participated in social interactions and asked for entertainment ('COME TONIGHT [to continue with the speller]', day 203, 'WILI CH TOOL BALBUM MAL LAUT HOERENZN' – 'I would like to listen to the album by Tool [a band] loud', day 245, 'UND JETWZT EIN BIER' – 'and now a beer', day 247). He even gave suggestions to improve his speller performance by spelling 'TURN ON WORD RECOGNITION' on day 183 in English as the experimenter UC mostly spoke in English with the patient. While the speller software offered the option to delete misspelled characters, the patient often chose to continue spelling when the intended meaning was apparent, possibly because error correction is slow and time-consuming.

Discussion and Conclusion

We demonstrate that a paralyzed patient, according to the presently available physiological and clinical criteria in the completely locked-in state (CLIS), could volitionally select letters to form words and phrases to express his desires and experiences using a neurally-based neurofeedback system. The patient used this intracortical BCI based on voluntarily modulated neural spiking from the motor cortex to spell semantically correct and personally useful phrases. In all blocks, measurable spike rate differentiation between "yes" and "no" during the neurofeedback trials and "select" and "no selection" during speller blocks appeared in few (one to five) channels in SMA (supplementary motor area) out of all active channels, which varied between 40 and 60 channels in total over the sessions. Consequently, typically only one or two channels were used for control. Spelling speed was highly variable, ranging from a few minutes to an hour to spell a short phrase. Free voluntary spelling mainly concerned requests related to body position, health status, pain, and social activities.

It is worth mentioning that no universally accepted clinical definition exists to distinguish LIS from CLIS; the current standard criteria to differentiate LIS from CLIS is the presence or absence of means of communication. During the transition from LIS to CLIS, patients are first left with limited, and finally, no means of communication. The time course of this transition process is patient and disease-specific. In theory, other voluntary muscles than eye-movements could have been used for Electromyography (EMG)-based communication attempts. Particularly face muscles outside the extraocular muscles may remain under voluntary control in some cases even after the loss of eye-muscle control. In the case of this patient, extensive EOG recordings were performed to demonstrate that no other neuromuscular output existed. The patient employed an EOG-based BCI for communication successfully for the last time in February 2019.

Nevertheless, extensive post-hoc EOG analysis showed a significant difference in the maximum, mean, and variance feature of the eye movement corresponding to "yes" and "no" even after the patient's inability to employ the EOG-based system for communication. This failure to communicate despite the presence of a significant difference in some of the features may be due to the limitations of the EOG-based BCI system. However, such limitations cannot be answered with certainty with the post-hoc analysis used here. In this study, caretakers and family members denied and lamented any reliable communication possibility from February 2019 onwards. Thus, we conclude based on our reported measurements that the patient described was in a CLIS a few weeks before and as well as after implantation. This statement does not exclude the possibility that more sensitive measurements of somatic-motor control are possible, revealing some form of volitional control, which would render the diagnostic statement of CLIS at least for this case inaccurate.

We conclude that this BCI communication demonstration broke a "wall" of communication silence encircling this CLIS individual. This experience also highlights that individuals with extremely severe and incapacitating ailments for extended periods are capable of meaningful communication. The current BCI system has several limitations, as several software and hardware modifications would need to be implemented before the system could be used independently by the family or caretakers without the presence of a technically skilled team. The BCI-software is presently being modified to improve communication outcome and self-reliance of the family.

To conclude, this case study has demonstrated that a patient without any stable and reliable means of eye-movement control or identifiable communication route employed a neurofeedback strategy to modulate the firing rates of neurons in a paradigm allowing him to select letters to form words and sentences to express his desires and experiences.

Dataset reported in this article

The dataset here spans days 106 to 247 after implantation. For the analysis of neurofeedback trials in Figure 3 and the corresponding main text, only blocks after day 123 were used because of a change in paradigm (before day 123, incorrect trials and time-outs were not differentiated). For Supplementary Figure 1, all neurofeedback blocks were used, as time-out trials were counted as 'incorrect' as well.

All speller sessions performed between days 106 and 247 were included in the analysis.

Other experimental paradigms performed, such as motor attempt exploration or yes/no question are mentioned in summary (Figure 2), but not analysed.

Acknowledgements:

This research was supported by the Wyss Center for Bio and Neuroengineering, Geneva, Deutsche Forschungsgemeinschaft (DFG BI 195/77-1); German Ministry of Education and Research (BMBF) 16SV7701; CoMiCon, LUMINOUS-H2020-FETOPEN-2014-2015-RIA (686764), Bogenhausen Staedtische Klinik, Munich. The authors thank Andrew Jackson and Nick Ramsey for their comments on an earlier version of the manuscript. We thank the patient and his family.

Financial Disclosures:

All the authors report no disclosures.

Conflict of Interests:

All authors declare no conflict of interests.

Author's contribution:

Ujwal Chaudhary – Initiation; Conceptualization; Ethics Approval; Performed 100% of the sessions with the patient before and after implantation; Neurofeedback paradigm; Manuscript writing.

Ioannis Vlachos – Software development, integration and testing; Data analysis; Neurofeedback paradigm implementation; Performed 10% of the sessions after the implantation.

Jonas B. Zimmermann – Neurofeedback paradigm implementation; Data analysis; Figures; Manuscript writing.

Arnau Espinosa – Software testing; EEG/EOG analysis; Figures.

Alessandro Tonin – Speller software development; Performed 30% of sessions before implantation and 15% of the sessions after implantation; EEG/EOG analysis; Figures.

Andres Jaramillo-Gonzalez – EEG/EOG analysis; Figures.

Majid Khalili-Ardali – Graphical user interface development.

Helge R. Topka – Ethics Approval; Medical patient care; Clinical and diagnostic neurological procedures.

Jens Lehmberg – Ethics approval; Neurosurgery; Clinical care.

Gerhard M. Friehs – Neurosurgical training.

Alain Woodtli – Ethics approval; BfArM approval; Neurosurgical training.

John P. Donoghue – Initiation; Conceptualization, writing, review, editing.

Niels Birbaumer – Initiation; Conceptualization; Coordination, Clinical-psycholgical procedures and care; Neurofeedback paradigm; performed 30% of the sessions with UC; Ethics approval; BfArM approval; Manuscript writing.

Data Availability

Firing rate data recorded in this period as well as log files are available upon request and freely after peer-reviewed publication.

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List of Supplementary Figures

Supplementary Figure S1: Audio-Neurofeedback Accuracy – Accuracy in all neurofeedback blocks from day 106 (first time neurofeedback speller was attempted) to day 247 (minimum/median/maximum number of blocks per day: 3/7/16). A) Each feedback block is represented as a dot. Daily maximum accuracy connected by black line. B) and C) feedback trials on two different blocks.

Supplementary Figure S2: Electrooculogram (EOG) features – evolution of the A) maximum, B) mean, C) variance, D) power in the band 0-2 Hz , and E) power in the band 2-4 Hz of the EOG signal for yes/no questions answered by the patient during the period March 2018 to November 2019. For each day, the vertical and horizontal EOG raw signal was filtered between 0.05Hz and 40Hz. Each yes and no trial was extracted and corrected by subtracting the mean of the trial's baseline. Then the range of the amplitude of the EOG signal was calculated separately for each trial as the difference between the maximum and minimum of the signal. The figure shows the mean and the standard error of the mean of the extracted range of the amplitude of the vertical and horizontal EOG signal across each day for yes and no trials. For each day, a Mann-Whitney U-test was performed between yes and no results separately for the horizontal and vertical EOG represented by the significant p-values shown on the top of the bars. The table in the middle of the figure lists the date and the number of yes and no trials used for vertical and horizontal EOG. The x-axis represents the date of the sessions, and the y-axis represents the amplitude in microvolts. The vertical red line indicates the date of the implantation: 19 March 2019. The month of all the non-invasive sessions performed after implantation are highlighted in red on the x-axis.