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COGNITIVE PROCESSING AND BRAIN COMMUNICATION IN AMYOTROPHIC LATERAL SCLEROSIS

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Coordinatore: Ch.mo Prof. Gaetano Thiene

Supervisore: Ch.ma Prof.ssa Elena Pegoraro

Co-Supervisore: Ch.mo Prof. Niels Birbaumer

Valutatori: Prof. Christoph Braun and Prof. Boris Kleber

Dottoranda : María Ángeles Prats Sedano

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Glossary

AAT	Aphasia Aachener Test
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	ALS functional rating scale-revised
BCI	Brain Computer Interface
C9ORF72	Chromosome 9 open reading frame 72
CLIS	Completely Locked-in State
EEG	Electroencephalography
EOG	Electrooculography
ERPs	Event-related potentials
fALS	Familial Amyotrophic Lateral Sclerosis
fNIRS	Functional Near Infrared Spectroscopy
FTD	Fronto-temporal Dementia
LIS	Locked-in State
LMN	Lower Motor Neuron
M-WCST	Modified Wisconsin Card Sorting test
MCI	Mild cognitive impairment
MND	Motor Neuron Disease
PSD	Power spectrum density
QoL	Quality of life
RCPM	Raven coloured progressive matrices
RON	Re-orienting negativity
RVMD	Rivermead memory test
SOD1	Superoxide dismutase 1
SSEPs	Somatosensory evoked potentials
SVM	Support Vector Machine
ToM	Theory of Mind
UMN	Upper Motor Neuron

ABSTRACT

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive paralysis of limbs and bulbar musculature. This severe physical impairment makes cognitive evaluation a big challenge, thus there is a great need for an assessment that does not require overt motor responses. Moreover, we need of augmentative communication strategies because the disease generally leads to complete paralysis and, therefore, patients are unable to communicate with the external world by any means. For this purpose, Brain Computer Interfaces (BCIs) seem a promising approach to facilitate communication with these patients.

The aim of this thesis is twofold. First, assessing cognitive processing in ALS by means of a novel evaluation tool. Second, allowing brain communication in completely paralyzed ALS patients who had lost their vision in order to eliminate the unbearable loss of communication in paralysis ("unlocking the locked-in").

The first study introduces a novel approach for assessing cognitive functions in ALS. This approach uses neuropsychological tests that require minimal overt motor or verbal responses; together with vibro-tactile P300s. Results indicate mild cognitive impairment in oral language comprehension tasks and reduced vibro-tactile P300 amplitudes in patients compared to healthy controls. Importantly, correlations between the vibro-tactile P300 latency and psychometric test results suggest that the former measure could serve as a neurophysiological marker of cognitive decline in ALS patients.

The second study introduces a distraction paradigm based in auditory event-related potentials (ERPs) to evaluate the ability of change detection, focusing, and re-orientation of attention in ALS. The results revealed a modification of the amplitude and the latency of the N200, the P300 and the re-orienting negativity (RON) components. This could suggest an alteration of the endogenous mechanism that controls the detection of change, thus resulting in a reduction of the allocation and the re-orientation of attentional resources.

The third study aimed at testing the feasibility of a Near Infrared Spectroscopy (NIRS) - based BCI communication approach for patients in the Completely Locked-in Stage (CLIS) due to ALS. For this purpose two CLIS patients were trained to control their cerebral-cortex´s functional-activations in response to auditory processing of correct or incorrect statements assessed with NIRS. The results of the study are very promising, showing that both CLIS patients communicated with fronto-cortical oxygenation based BCI at an average correct response rate of 70% over a period of several weeks. We conclude that this novel approach of brain-communication is safe and, reliable, representing, so far, the best communication possible for patients in completely locked-in state.

In conclusion we propose a) the novel combination of vibro-tactile or acoustic ERPs and motor-independent neuropsychological tests as an alternative and easily implementable way for assessing cognitive functions in ALS and b) we confirm the usefulness and effectiveness of above mentioned electrophysiological approaches in the late stage of ALS either to assess cognitive processing or to establish communication with a BCI system.

TABLE OF CONTENTS

1. AN INTRODUCTION TO AMYOTROPHIC LATERAL SCLEROSIS

1.1. Terminology and clinical features

Amyotrophic lateral sclerosis (ALS) is a fatal motor neurodegenerative disease (MND) characterized by a progressive degeneration of motor neurons in the primary motor cortex, brain stem and spinal cord, which results into total paralysis. The term ALS was first described by Jean-Martin Charcot (a french neurologist) in the 19th century. In the United States of America the disease is also known as “Lou Gehrig” because of a famous baseball player who suffered from ALS.

ALS disease is the most common form of motor neuron disease (MND) accounting for approximately 85% of cases. The disease is characterized by lower and upper neuron motor dysfunction. Lower motor neuron dysfunction includes signs and symptoms as fatigue, muscular weakness, cramps, muscle atrophy, fasciculations, hyporreflexia and hypotonia, while upper motor neuron dysfunction includes: slowing of distal movements, stiffness, spasticity, pathological hyperreflexia, tonic-flexor spams and pseudo-bulbar affect.

The most common region of symptom’s onset is in the limbs, where distal or proximal weakness of the upper or lower limb muscles may appear. Patients might notice symptoms as focal muscle wasting, slowness in the execution of fine motor skills, inco-ordination and foot drop (1). Bulbar onset arises in approximately 25% of cases and manifests in the form of dysarthria (motor speech disorder), dysphagia (swallowing difficulty), sialorrhea (excessive drooling), laringospam and pseudo-bulbar palsy. ALS patients with bulbar involvement may have prominent emotional liability showing uncontrollable crying or laughing. The bulbar onset is associated to a worst disease prognosis. Approximately 5% of patients show

respiratory onset, displaying signs of diaphragmatic weakness in the absence of significant bulbar or limb symptoms.

About 30 to 50% of the patients with ALS showed mild cognitive deficits or/and behavioural disturbances, whom 10-15% meet criteria for frontotemporal degeneration.

During the course of ALS disease the anal sphincter muscle and the striated urinary are relatively preserved until the late stage of the disease. Ocular muscles also seem to remain intact until the last stage of the disease being one of the only channels for patients to communicate with the external world.

1.2. Epidemiology

The worldwide incidence of ALS is approximately 1.2-1.8 per 100.000 (2) The worldwide mortality rate of the disease is around 1.89 -1.91 per 100.00/year. The mean age of onset for sporadic ALS is about 55-65 years. Only 5% of cases have an onset before the age of 30 years (3). Overall, male prevalence is higher than women (M:F ratio 1.5:1). The survival mean is around 36 months. ALS causes progressive paralysis and leads to death within 2–3 years for bulbar onset cases and 3–5 years for limb onset ALS cases (4).

In the European population Logroscino et al. (5) found that the crude annual incidence rate of ALS in the general European population was 2.16 per 100.000 person-years. Their study was based on data from six prospective, population-based ALS registers from different countries: Ireland, Scotland, England and Italy. A vast number of ALS patients were collected in their study (n = 1,028) and allowed the epidemiology of ALS to be precisely quantified (Table 1). The incidence was higher among men (3.0 per 100,000 person-years); than among women (2.4 per 100,000 person-years).

	Males				Females				Total			
Age[a]	Cases[b]	Person-year[c]	Incidence[d]	95% CI	Cases	Person-year	Incidence	95% CI	Cases	Person-year	Incidence	95% CI
18-19	2	596,328	0.3	0.0-0.8	0	571,361	-	-	2	1,167,688	0.2	0.0-0.4
20-24	1	1,563,778	0.1	0.0-0.2	1	1,525,677	0.1	0.0-0.2	2	3,089,455	0.1	0.0-0.2
25-29	6	1,720,225	0.3	0.1-0.6	6	1,706,432	0.4	0.1-0.6	12	3,426,657	0.4	0.2-0.5
30-34	5	1,856,398	0.3	0.0-0.5	2	1,859,155	0.1	0.0-0.3	7	3,715,553	0.2	0.0-0.3
35-39	11	1,869,135	0.6	0.2-0.9	3	1,885,445	0.2	0.0-0.3	14	3,754,580	0.4	0.2-0.6
40-44	24	1,693,896	1.4	0.8-2.0	14	1,710,534	0.8	0.4-1.2	38	3,404,430	1.1	0.8-1.5
45-49	35	1,565,127	2.2	1.5-3.0	23	1,582,262	1.5	0.9-2.0	58	3,147,389	1.8	1.4-2.3
50-54	38	1,606,043	2.4	1.6-3.1	37	1,615,772	2.3	1.6-3.0	75	3,221,815	2.3	1.8-2.9
55-59	77	1,348,148	5.7	4.4-7.0	39	1,385,152	2.8	1.9-3.7	116	2,733,300	4.2	3.5-5.0
60-64	72	1,280,496	5.6	4.3-6.9	79	1,367,302	5.8	4.5-7.1	151	2,647,798	5.7	4.8-6.6
65-69	95	1,098,073	8.7	6.9-10.4	84	1,261,865	6.7	5.2-8.1	179	2,359,938	7.6	6.5-8.7
70-74	96	900,352	10.7	8.5-12.8	76	1,170,278	6.5	5.0-8.0	172	2,070,630	8.3	7.1-9.5
75-79	63	649,547	9.7	7.3-12.1	69	990,265	7.0	5.3-8.6	132	1,639,811	8.0	6.7-9.4
80-84	24	324,676	7.4	4.4-10.3	24	602,654	4.0	2.4-5.6	48	927,330	5.2	3.7-6.6
>85	5	245,499	2.0	0.3-3.8	17	643,615	2.6	1.4-3.9	22	889,113	2.5	1.4-3.5
Total	554	18,317,722	3.0	2.8-3.3	474	19,877,768	2.4	2.2-2.6	1028	38,195,487	2.7	2.5-2.9

[a]Age at diagnosis
[b]Number of cases of ALS diagnosed in the six European ALS registers for the two year period, 1998-1999
[c]combined person-years for the six European for the two year period, 1998-1999
[d]per 100,000 person-years

Table 1. Incidence of ALS per age (>18) and gender, per 100,000 person-years, according to Logroscino et al. (3).

In Italy the ALS disease has an incidence of 2.96 per 100.000 and a prevalence of 7.89 per 100.000 (6). Chiò et al., (6) described the incidence of ALS in Piemonte and Valle d'Aosta, Italy, in the 10-year period 1995 through 2004 and they reported no changes in the incidence over the 10-year period of the study. Table 2 describes the demographics and clinical features of ALS patients, in 1995–1999 and 2000–2004 cohorts, and of the prevalent cohort.

	1995-1999	2000-2004	Prevalent cohort
No. of cases	618	642	343
Mean age at onset, y (SD)	64.2 (11.2)	65.4 (11.1)	60.9 (11.9)
Mean age at diagnosis, y (SD)	65.1 (11.3)	66.1 (10.8)	61.9 (11.7)
Median age at onset, y (SD)	65.1	65.8	61.1
Median age at diagnosis, y (SD)	66.8	67.7	62.4
Men:women	329:289	358:284	193/150
Spinal:bulbar	395:223	392:250	261/82
Mean diagnostic delay, mo (SD)*	11.0 (11.5)	9.6 (7.6)	12.1 (14.3)
Incidence/100,000 (95% CI)†	2.65 (2.44-2.87)	2.64 (2.44-2.86)	—
Men, incidence/100,000 (95% CI)‡	2.93 (2.67-3.26)	3.00 (2.70-3.34)	—
Women, incidence/100,000 (95% CI)‡	2.39 (2.13-2.68)	2.30 (2.05-2.57)	—
Men (spinal onset), incidence/100,000 (95% CI)‡	2.04 (1.80-2.31)	2.01 (1.77-2.27)	—
Men (bulbar onset), incidence/100,000 (95% CI)‡	0.89 (0.73-1.09)	0.99 (0.83-1.19)	—
Women (spinal onset), incidence/100,000 (95% CI)‡	1.45 (1.24-1.69)	1.29 (1.10-1.51)	—
Women (bulbar onset), incidence/100,000 (95% CI)‡	0.94 (0.78-1.13)	1.01 (0.84-1.20)	—
Men to women rate ratio	1.2:1	1.3:1	1.3:1§
Men to women rate ratio, spinal onset	1.4:1	1.6:1	1.6:1
Men to women rate ratio, bulbar onset	0.9:1	1:1	1:1
Incidence/100,000, >15 y (95% CI)*	3.09 (2.84-3.34)	3.05 (2.82-3.29)	—

*1995-1999 vs 2000-2004 cohorts, $p < 0.0001$.
†Age- and gender-adjusted to the 2001 Italian population census.
‡Age-adjusted to the 2001 Italian population census.
§Men to women ratio.
ALS = amyotrophic lateral sclerosis; CI = confidence interval.

Table 2. Demographics and clinical features of ALS patients, in 1995–1999 and 2000–2004 cohorts, and of the prevalent cohort. Image from (6).

1.3. Pathology and Pathogenesis

ALS is a heterogeneous disease with diverse genetic causes and complex pathology. The most predominant pathological features of ALS are degeneration of the corticospinal tracts and extensive loss of lower motor neurons (LMNs) or anterior horn cells (7), degeneration and loss of Betz cells and other pyramidal cells in the primary motor cortex (8,9) and reactive gliosis in the motor cortex and spinal cord (10,11).

In the last decade, huge progress was made in unravelling the aetiology of the disease, with the identification of ALS-causing mutations in new genes, as well as significant molecular causes involved in the origin or progression of ALS.

While the vast of ALS cases are classed as sporadic (around 90%), the 10% of cases are familial (fALS); with a Mendelian pattern of inheritance. fALS is caused by mutations in different genes that trigger pathogenesis. The most important genes and proteins that are linked to ALS are: SOD1; TP-43, FUS and C9ORF72.

Superoxide dismutase 1 (*SOD1*) is the first ALS-linked gene that was identified (12). Nowadays, more than 170 mutations in the SOD1 gene have been linked to the pathogenesis of ALS. The role of these SOD1 mutations in fALS is clear; however the presence of misfolded SOD1 in sporadic ALS remains controversial.

The discovery of ALS-linked mutations in the DNA/RNA-binding proteins as TDP-43 (13) and FUS (14) contribute to the understanding of the ALS pathogenesis. In the case of TDP-43 more than 35 mutations in the gene which encodes TDP-43 are the causes of ALS; being the responsible of around the 5% of fALS and less the 1% of sporadic ALS (*s*ALS). More recently mutations in FUS gene have been described as a cause of around 4% of fALS and rare sALS (14,15).

In 2011, the most common genetic cause of ALS was identified to be a gene called *C9ORF72* (16). C9ORF72 is the most prevalent genetic change identified in ALS patients. These C9ORF72 expansions are specifically found in patients with FTD. Expansions are found in around 39% of fALS cases and around the 7% of s ALS. However significant differences are not found between world populations.

In sALS, there is no indication of genetic inheritance. The cause of most sALS is not well-characterized, but recently studies have shown that fALS-linked mutations may also trigger disease in sporadic cases. Certainly a small percentage of sALS patients revealed to carry *de novo* mutations in known ALS-causing genes. *C9ORF72* repeat expansions, which were also found in a considerable part (~7%) of *s*ALS patients, probably are not occurring *de novo*, but rather represent cases with insufficient family history. Taken together, approximately around the 10% of apparently sporadic ALS cases are caused by known genetic mutations, while the aetiology of the rest ~90% sALS remains enigmatic (17). Figure 1 illustrates the gene mutations in familial and sporadic ALS.

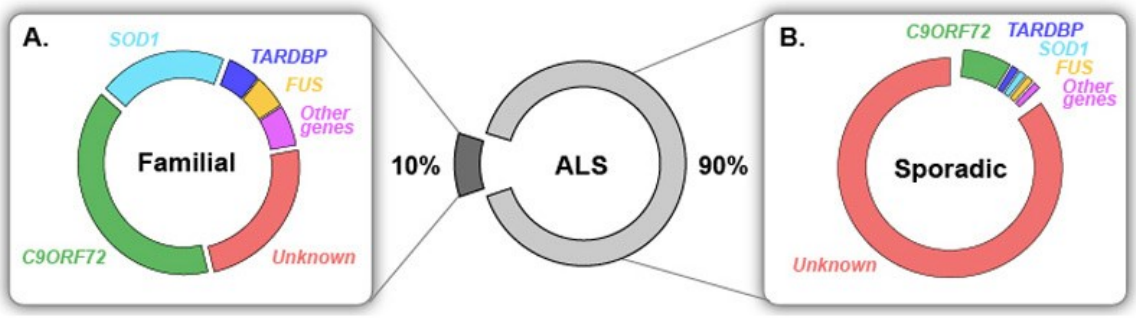

Figure 1. Gene mutations in familial and sporadic ALS. Image from (17)

1.4. Diagnosis

Nowadays the diagnostic of ALS is based on the presence of very characteristic clinical features. A suggestion of a diagnose of ALS disease can be made after finding signs of combined upper motor neuron and lower motor neuron involvement, that cannot be explained by other disease process, together with progression of symptoms or signs within a region or to other regions.

The only two available and validated diagnostic criteria are “El Escorial Diagnostic Criteria”, which was defined in Spain in 1994 (18); and the revised Airlie House criteria (19).

According to the Revised El Escorial Research Diagnostic Criteria for ALS (18) the diagnosis of ALS requires:

1) Evidence of LMN degeneration by clinical, electrophysiological or neuropathological examination.
2) Evidence of UMN degeneration by clinical examination, and 3 Progressive spread of symptoms or signs within a region or to other regions, as determined by history or examination.

Together with the absence of:

1) Electrophysiological and pathological evidence of other disease that might explain the signs of LMN and/or UMN degeneration.
2) Neuroimaging evidence of other disease processes that might explain the observed clinical and electrophysiological signs.

El Escorial Research Diagnostic Criteria for ALS revised use of clinical, electrophysiological, genetic and neuroimaging modalities to apply a level of certainty to the diagnosis of ALS. The diagnostic categories of ALS include clinically definite, probable, possible and suspected and are described in the Table 3.

Table II. Diagnostic categories for ALS

Clinically definite ALS	Clinical evidence of the presence of LMN as well as UMN signs in the bulbar region and at least two spinal regions, or the presence of UMN and LMN signs in at least three spinal regions
Clinically definite familial ALS – laboratory supported	May be applied when ALS presents with progressive UMN and/or LMN signs in at least one region (in the absence of another cause for the abnormal neurological signs)
Clinically probable ALS	Clinical evidence alone by UMN and LMN signs in at least two regions with some UMN signs necessarily rostral to the LMN signs
Clinically probable ALS – laboratory supported	Clinical signs of UMN and LMN dysfunction alone are present in one region, and LMN signs defined by EMG criteria are present in at least two regions, with proper application of neuroimaging and clinical laboratory protocols to exclude other causes
Clinically possible ALS	Clinical signs of UMN and LMN dysfunction are found together in only one region, or UMN signs are found alone in two or more regions; or LMN signs are found rostral to UMN signs and the diagnosis of clinically probable ALS – laboratory supported cannot be proven
Clinically suspected ALS	Where the diagnosis could not be regarded as sufficiently certain to include the patient in a research study

Abbreviations: LMN: lower motor neuron; UMN: upper motor neuron.

Table 3. Diagnostic categories for ALS. Image from (20)

1.5. Quality of life

Quality of life (QoL) is an important issue for patients with ALS and their families. Although early thoughts frequently are that quality of life in ALS will be poor, there are strong data to support a moderately good quality of life despite the unavoidable deterioration of physical function with disease progression (20–25).

Quality of life of ALS patients uses to be underestimated. Caretakers and relatives of severely paralyzed patients usually tend to think that the emotional status of the patient is worse than it really is. Kübler et al.,(22) evaluated 76 ALS patients and assess the level of QoL. According to the previous hypothesis, they found that the vast majority of the ALS sample (80%) rated their QoL as satisfactory or good (see Figure 2). Contrarily, partners or caregivers were also asked to rate patients' quality of life and the outcome was significantly lower (see Figure 3).

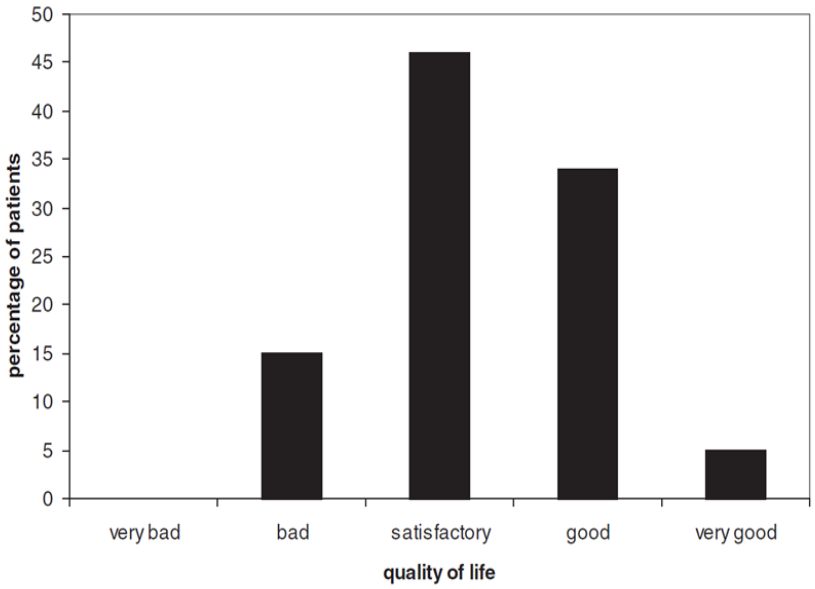

Figure 2. Bar chart showing the QoL rating of 61 ALS patients. The 80% of the patients rated they QoL as satisfactory/good. Image from (22)

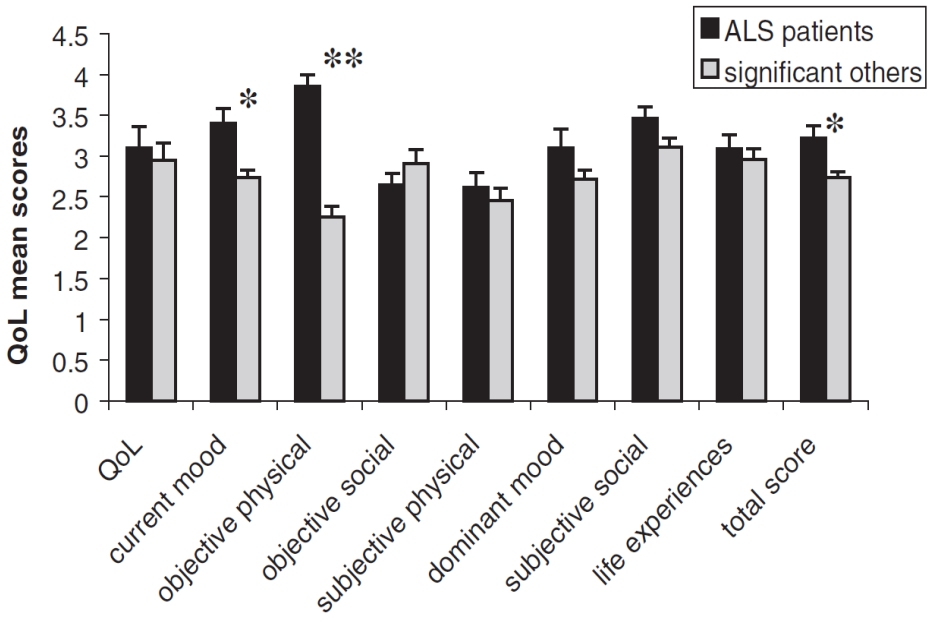

Figure 3. Comparison of patient's rating of QoL and the rating of their most significant other. Image from (21)

According to some authors (26) a possible reason for the underestimation of patients' QoL by their relatives/caregivers could be the inability of healthy people to imagine experiences and feelings of a severely paralyzed person.

Moreover, depression symptoms have to be taken into account when studying QoL in neurodegenerative diseases. Some studies have focused in investigating QoL and the emotional status of ALS patients (21,27) . Despite what is commonly thought that physical deterioration affect patient's mood, the link between depression and physical impairment in ALS is not clear. Some studies support a relationship between depressive mood and disease stage (22,28,29) while others do not (21,30,31). Lulé et al., (21) carried out a longitudinal study within the ALS patients and a comparative study between ALS group and healthy participants. The results of these studies highlighted that physical disability do not correlate with depression in ALS patients (see Figure 4). More importantly, they found that the emotional status in ALS is similar to healthy controls. Those results provide evidence that ALS patients can experience a pleasing QoL without depressive mood even if they are severely physically impaired.

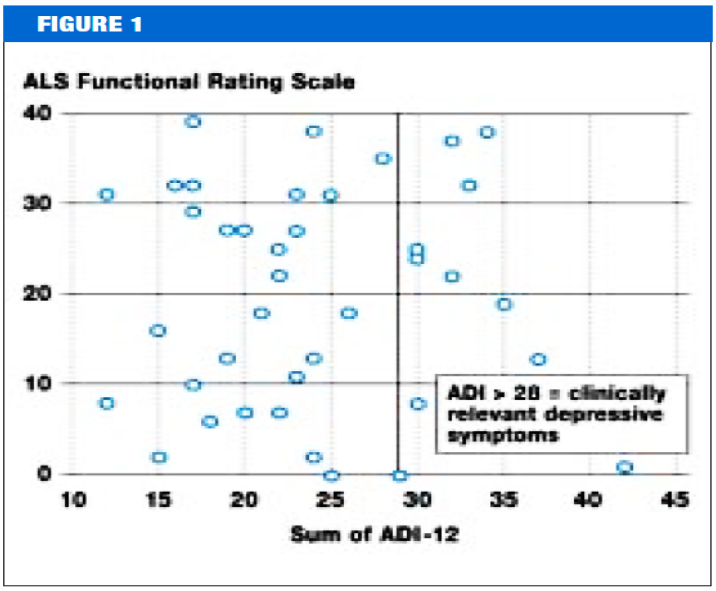

Figure 4. Scattered Plot of depressive symptoms and physical impairment (measured with the ALS Functional Rating Scale, ALSFRS) in ALS patients. Image from (21).

However, QoL of life in ALS remains a controversial issue. For instance, contrary to those previous studies, Körner et al., (27) reported QoL was reduced in ALS specially based the

positive correlation found between the disease progression and physical impairment. However, there was no significant correlation between depression and disease duration. This finding is in line with a previous literature (22) and suggest that patients can develop strategies to cope with the diagnosis and the physical impairment; therefore depressive mood is less frequent or at least not progressive with longer disease duration despite increasing disability.

In conclusion, even if depression occurs among ALS patients, it can be prevented. Patients can maintain a good QoL and remain free of depression. Thus, factors that contribute to patient's QoL, depression, and approaches toward treatment options need to be regularly examined throughout the course of the disease. Depression should be treated, and patients have to be provided with unbiased information, including their medical and palliative care options.

For this purpose, some recommendations can be taken into account to prevent depression and increase QoL in ALS.

- Assistive devices to improve mobility and autonomy.
- Pharmacological treatment of depressive symptoms.
- Psychotherapeutic support for patients and their relatives.
- Use of augmentative and alternative communication strategies in the late stage of the disease.

Regardless of depression and physical disability, other areas such as social functioning and spirituality/religiousness need to be considered when assessing QoL.

1.6. Late stage of ALS and end of life management:

ALS has a fatal prognosis and death results usually from respiratory failure within 3 to 5 years of symptom onset. Other causes of death include cardiac arrest, coronary disease, asphyxia and pulmonary embolism (32).

Different areas need to be considered in the end-of life management of ALS patients; these areas include respiration, nutrition, therapy and exercise, pain, depression and suicide, spirituality and religion, cognitive changes, the development of advance directives, and care at the end of life (33). From the pharmacological point of view, the use of riluzole can prolong survival only by about two to three months in ALS patients (34). Also non-pharmacological treatments including ventilation and gastrostomy can improve the survival and quality of life, respectively.

When a patient can no longer tolerate non-invasive ventilation or it stops being effective, invasive mechanical ventilation with tracheostomy can be used (when available) or patients can be managed with supportive symptomatic care together with palliative care facilities (32). If patients accepted to be artificially mechanical ventilated and fed, their life expectancy can be increased.

ALS disease may lead to severe or complete motor paralysis rendering communication impossible. The late stage of ALS is divided in two phases: the state of severely paralyzed patients with residual voluntary control of particular muscles (e.g. eye muscles, lips, fingers) is known as locked-in state (LIS) (35,36). Patients with several remaining functional motor channels (as those in LIS) can benefit from assistive communication that uses a range of augmentative and alternative communication (AAC) strategies, such as eye trackers with speech-generating devices. However, there are also patients who lose all motor control

resulting in the completely locked-in state (CLIS) (37). Thus, these patients have the greatest need to reestablish communication and interaction with the social environment.

The total number of LIS/CLIS cases is not known for sure, because a high percentage of patients do not receive assistance in hospitals or in specialized rehabilitations institutes and instead are treated at home. The lack of prevalence of LIS/CLIS is also explained by the high misdiagnosis among these patients as being in a vegetative state. The clinical population in the late stage of the ALS has not been thoroughly investigated, so exhaustive investigations of this clinical population are required.

2. COGNITIVE AND BEHAVIORAL IMPAIRMENT IN ALS

The impairment due to ALS is traditionally known to be physical only, but there is a growing body of literature that suggests the presence of cognitive impairment in ALS patients. Although the degeneration in ALS disease predominantly affects the motor system, cognitive and behavioural deficits have been also reported indicating an extra-motor cerebral involvement in motor neuron disease. The conception of ALS of a heterogeneous disease is based on the phenotypic variability together with the presence or absence of cognitive deficits, and the diverse ranges in survivorship (3).

2.1. Spectrum of ALS and dementia

The link between ALS and fronto-temporal dementia was postulated in 1932 (38) and since then several studies have reported strong clinical, pathological, radiological and genetic evidence of this association. In addition, several studies suggested that ALS disease and frontotemporal dementia form a clinical spectrum based in neuropsychological and genetic tests.

Recently, this continuum between FTD and motor neuron disease has gain support after the discovery of the C9ORF72 mutation as one of the most frequent genetic linkage in familial variants of FTD and ALS (39–42). Moreover, few clinical features of cognitive impairment in ALS as irritability, personality changes, obsessions and deficits in tests that are related to the executive system (verbal fluency, working memory, attention) are consistent with the features of frontotemporal dementia.

The study performed by Lillo et al. (41), aimed at investigating the continuum between bvFTD and ALS based on the performance on neuropsychological and behavioral tests. They assessed a group of 20 ALS patients and 20 bvFTD patients and a similar cognitive profile was

found in both groups in tests related to behaviour, inhibitory control and working memory. Thus, this result together with the Rasch analysis that was performed exposed an overlap between bvFTD and ALS.

From a neuroanatomic point of view, neuroimaging investigations have showed frontal and temporal lobe atrophy (anterior cingular gyrus and frontotemporal areas) in ALS patients who present cognitive deficits. Those neuroimaging investigations found similar patterns of atrophy including neuronal loss, superficial linear spongiosis and ubiquitinated taunegative and synuclein-negative intraneuronal inclusions. These studies revealed an involvement of some areas of the brain other than the motor cortex and provide some evidence of that possible continuum between FTD and ALS.

However, this continuum nowadays still remains controversial and needs of a further investigation.

2.2. Cognitive deficits in ALS

A total of 30 to 50% of ALS patients suffer from cognitive disorders, whom 10-15% meet criteria for frontotemporal degeneration (43). The current section will provide an overview of the literature concerning cognitive and behavioural changes in non–demented ALS patients.

2.2.1. Executive dysfunctions

Executive functions are usually known as high mental processes that control cognitive processes such as attention, working memory, abstraction, cognitive flexibility, problem solving and multitasking.

Traditionally, executive dysfunctions have represented a prominent feature of ALS. ALS patients have shown deficits related to different components of the executive system such as verbal fluency, selective attention and mental flexibility (44–49).

Verbal fluency is one of the most salient impairment in the executive domain and have been reported in the majority of the studies about cognitive impairment in ALS (39,44,48–51). The most well know neuropsychological tests to assess verbal fluency are Controlled Oral Word Association Test (COWA) (52) and the Thurstone's Word Fluency Test (TWF) (52). However, as those verbal tests require speech motor skills, the frequency of impaired cognition might have been misrepresented. Thus, Abrahams designed a modified version of the TWF to control the impact of verbal disabilities in the outcome of the test. In this modified version the patient is asked to copy the words that were previously generated. After that, a verbal fluency index was calculated by the

$$Verbal\ Fluency\ Index: \frac{(time\ for\ generation\ condition) - (time\ for\ copy\ condition)}{Total\ number\ of\ words\ generated}$$

The robustness of this methodology to detect verbal impairments in ALS has been proved in some studies (44,46,53,54) and the findings of verbal impairments remained after the application of this procedure. Moreover, verbal fluency deficits seem to be correlated with neural markers of frontal or striato-frontal lobe dysfunction, so it could be a good marker to detect frontal impairments (53,55).

Deficits in cognitive flexibility have been reported in some ALS studies. One of the most popular tests to assess cognitive flexibility is the Wisconsin Card Sorting Test (WCST). Several studies have described that MND patients showed lower performance in the

WCST compared to age-matched healthy participants (50,54,56). However, those results seem not to be consistent with the ones found in further investigations.

Attention deficits have been also found in different studies (57,58). The assessment of those deficits seem to be extremely important as some disinhibited patients (a clinical feature of executive dysfunction) might have normal results in usual neuropsychological tests of executive system but dysfunctions in selective attention tests. Abnormal dysfunctions in the attentional network in ALS have been also associated with lesions on the frontal lobes.

2.2.2. Memory

There is some controversy regarding memory impairment in ALS. Previously, studies revealing impairments in the memory domain in ALS were less prevalent in comparison with those reporting executive dysfunctions. Memory impairment in ALS has been previously ignored or considered as a failure in the memory encoding process specific of the executive system. However, there are several studies describing memory deficits in ALS, tested using picture recall, word list learning, pair association learning or story recall (40,46,56,59–62). These studies have exposed that memory deficits are specially related to short immediate recall and encoding processing (48,63). Deficits in delay recall are highly variable and are considered as an abnormality in the encoding rather than in the speed of forgetting (64). Recent studies in ALS have displayed the involvement of the temporal lobe reporting significant volume reduction in some medial-temporal lobe related areas as the basal ganglia (65) and hippocampal TPD-43 pathology (66). As temporal lobe atrophy is a key feature of Alzheimer disease, Macths and colleagues (67) aimed at investigating the relationship between memory dysfunctions in ALS and Mild cognitive impairment (MCI) patients and they found a different pattern in temporal lobe

dysfunction in between both pathologies. Moreover, memory deficits beyond executive dysfunctions were found in ALS.

The implications of memory impairment in ALS in clinical management (as medication or treatment) have been little studied; urging for a great need of further evaluation of amnesic deficits in ALS which are equally important as executive dysfunctions.

2.2.3. Language

There is some neuro-imaging (PET and MRI studies) evidence that language areas seem to be damaged in ALS (55), which give support to the fact that ALS disease affects extra-motor networks of the brain. Recently several studies have highlighted that language deficits in ALS are more common than previously thought. The most described language impairments in ALS are associated to word generation (measured by verbal fluency tasks) (46,59), sentence comprehension and verb processing (59,68) .

The possibility that those language deficits are related to aphasia-like impairment or to executive dysfunctions is not clear. Primarily language impairments were observed in association with executive dysfunctions (48,69). However, recently some large population-based studies showed some evidence that non-demented ALS patients without executive dysfunctions can have language deficits. Taylor and colleagues (70) performed a comprehensive language assessment and found mild language impairments in about the 43% of a sample of 51 non-demented ALS patients. Moreover they found that the performance in executive and language domain shared only 44% of variance, and more than the 40% of the patients who had impaired language composite did not have executive dysfunctions. Taken all the results together, they concluded that language impairment in ALS could be more common than deficits in the executive system

and that those language dysfunctions could also occur in absence of executive deficits. Abrahams in an editorial commentary (71) suggested that the area of language impairment in ALS could have been neglected during the past years and propose that further researches in this area should clarify the nature of language deficits in ALS and specially determine whether those language deficits are seen as subclinical language feature of FTD or a specific pattern of impairment in ALS disease.

Former cognitive studies assessing language deficits in ALS performed typical neuropsychological test batteries that require of physical and verbal abilities, and did not take in account that speech dysfunctions may interfere with the performance of the psychometric tests and therefore could pose a problem of masking or over-emphasized the results. Different authors started to take this problem into account and the tests they used were designed to control for motor/speech speed and to accommodate for the range of disabilities that are characteristic of ALS patients (60) or they used neuropsychological tests that not require verbalizations (72,73). Recently, Abrahams and colleagues (51) designed a questionnaire to assess cognitive functions in ALS called "the Edinburgh Cognitive and Behavioural ALS Screen ECAS", developed for ALS patients with physical and speech disability. The ECAS was validated in different countries and it seems to be a sensitive screening tool with high sensitivity and specificity ALS impairments. Either in the UK and the German-Swing version of the ECAS language was one of the most impaired domains.

2.2.4. Visuo-perceptual functions

Visuo-perceptual functions are a set of skills we use to gather visual information from the environment and integrate them with our other senses. These visuo-perceptual functions include visual discrimination, figure perception and attention.

Visuo-perceptual dysfunctions seem not to be very frequent in ALS. However, Strong and colleagues (62) have reported unexpected difficulties in ALS patients on some non-verbal tests including verbal-perceptual ability.

2.3. Social cognition and emotional processing

There is a growing body of literature suggesting deficits in social cognition and emotional processing in ALS. Deficits in emotional perception have also been reported in other neurological disorders such as Alzheimer (74–76), Parkinson (77–79) and mild cognitive impairment.

Social cognition refers to cognitive processes that facilitate the encoding and decoding of socially salient information, such as the emotions and intentions of others (80). Previous studies of social cognition in ALS were basically focused on basic emotion recognition and Theory of Mind (ToM). ToM is the capacity to attribute and infer to oneself and other emotional and mental states (thoughts, feelings, desires, intentions) in order to understand and predict their behaviour (81). Mostly all the studies regarding theory of mind in ALS were focused in emotional perception. Some authors reported changes in emotional perception (i.e emotional faces recognition) in both in ALS and FTD patients (82–84). Papps et al., (85) revealed a deficit for emotional material in ALS, more concretely, they found a selective failure to show the normative pattern of enhanced recognition memory for emotional words in comparison to neutral words.

In other hand, the study of Lulé et al, (86) investigating the emotional response to visual socio-emotional stimuli with different valence and arousal concluded that emotional processing in ALS tends to be altered showing a more positive response to neural stimuli and a neutralizing response to extreme stimuli. These results minimize the global impact

of the disease in the emotional processing in ALS and suggest a compensatory mechanism leading to cognitive and/or changes in neuroplasticity of the brain.

In conclusion, the potential clinical implications of dysfunctions in emotional processing in ALS patients might be a crucial issue because this impairment may aggravate mood and behavioural disturbance in ALS, as well as increasing the caregiver's burden. Importantly, emotional dysfunctions have to be taken into account when planning the patient's care or experimental trials, specifically, focusing on the patient's ability to evaluate emotional consequences and implications of therapeutic approaches, for them and their families.

2.4. Behaviour

Behavioural impairment is recognized as a feature of ALS, occurring in up to the 67% of that clinical population. There is a close link between behavioural changes and FTD reported by some numerous studies suggesting that ALS patients suffering from FTD have a propensity to manifest behavioural abnormalities as executive dysfunctions and disinhibition. Different questionnaires as the Neuropsychiatry inventory, Frontal Behaviour Inventory and Frontal System Behaviour Scale (87,88) have been used to assess behavioural changes in ALS, and have identified apathy as the most prominent behavioral trait in these patients (41).

An important contribution to the field was done by Murphy et al. (89) that assessed a sample of 274 ALS patients using behavioural validated interviews with accompanying caregivers and they reported that 27% to 66% of the patients presented higher levels of apathy, irritability, emotional indifference and poor frustration tolerance. Those behavioural changes increased the emotional burden in their caregivers. Moreover a

relationship between cognitive impairment and emotional indifference, aphasia/apraxia and logopenia was found, showing that ALS patients that present these behavioural traits are more likely to show cognitive problems. So, the presence of behavioural traits could alert the clinicians about the occurrence of cognitive decline and thus could signal the need of an exhaustive neuropsychological assessment and treatment strategies.

Abrahams et al (51) performed a behavioral screening test battery and found behavioral changes in 40% of a sample of 20 ALS patients. The most common behavioral change was apathy, followed by loss of empathy and changes in eating behaviour. In contrast to previous studies, they took into account ALS motor deficits and for this purpose they used an assessment that was specifically design to this pathology. The result was in line with the previous literature indicating that apathy is the most prevalent behavioural trait in ALS (89–91) .

However, apathy could be confused and swap with depression, fatigue and respiratory function so it is important to distinguish between them by using validated scales and an exhaustive examination of the medical history of the patient. In addition to apathy, changes as irritability and lack of inhibition are also found (62) and these behavioural traits might not be related to cognitive impairment (measured by standardized neuropsychological testing).

3. EVENT-RELATED POTENTIALS AND BRAIN COMMUNICATION IN ALS

3.1. Event-related potentials in ALS

3.1.1. Event-related potentials

Event-related potentials (ERPs) are electrophysiological responses recorded over the scalp that are related to an internal cognitive event. ERPs can provide significant information about how the human brain processes information and has been demonstrated that abnormalities in this processing can give us some information about neurological or psychiatric disorders.

It is commonly well-known that ERPs reflect synchronous changes of slow postsynaptic potential occurring within a large number of similarly oriented cortical pyramidal neurons in a certain area of the cortex. ERPs are short monophasic deflections that are shown in the background EEG. These deflections are characterized by their polarity (positive or negative), peak latency (relative to the onset of the event), peak amplitude (relative to a baseline or peak-to-peak) and scalp distribution (92).

The main advantages of using ERPs to assess cognitive functions/dysfunctions in ALS are its high temporal resolution and its applicability in cases of severely paralyzed patients unable to performed neuropsychological tests (72,93,94).

The most studied ERPs are the following ones:

P100: It is a positive deflection in the EEG that it shows up around 50ms/100ms after the stimulus onset. P100 is not usually easy to identified/visualized. P100 component is a neurophysiological marker of attentional processing to the sensorial stimulus and it provides information about the integrity of the sensorial channel used for the

stimulation. The auditory P100 usually appears before 100ms after the stimulus and it has its biggest amplitude on the frontal/central areas of the cortex. Contrarily the visual P100 usually has biggest amplitude in the occipital part of the brain and it varies regarding the degree of attentional demand to the task.

N100: The N100 is an early ERP component whose characteristics are associated with cognitive/endogenous processing of the stimuli. N1 is large negative deflection at central sites around 100 ms after stimulus onset. The N1 is evoked by a relatively unexpected change that violates a sensorial pattern (95). N100 classically appears together with the P200 wave and it is usually called "N100-P200 complex". The N100 in heathy adults, peaks between 80 and 120 ms after the stimulus onset and it is predominantly distributed over the fronto-central region of the scalp.

P200: P200 is a positive deflection in the EEG around 200ms after a target stimulus appears. P200 could be obtained by auditory stimuli or visual stimuli. Auditory P200 is usually merged with the N100 (N100-P200 complex) and its higher amplitudes are usually found in the frontal regions of the cortex. Visual P200 is predominantly seen in the frontal regions and its amplitudes vary when increasing the complexity of the visual stimulus or of the cognitive task.

The P200 wave of the ERP is assumed to represent a later stage of stimulus processing and is viewed as an index of both sensory processing and cognitive demands as the stimulus classification process (96).

Mismatch negativity (MMN): The MMN is a negative component of the ERPs elicited by any discriminable change in the auditory system, concretely, to a violation of pattern regularity. The MMN is a negative deflection over the fronto-central and central scalp electrodes in the difference wave obtained by subtracting the event related potential

(ERP) to frequent, ‘‘target’’, stimuli from that to deviant stimuli (97). The MMN frequently appears around 150–250 ms after the frequent stimulus onset, with this peak latency getting shorter with the increasing magnitude of stimulus change. MMN represents a pre-perceptual electrophysiological measure of the accuracy of the central sound representation in the human brain (98).

In many neurological disorders, MMN amplitude to sound distractor has been revealed to be smaller or the peak-latency of the component delayed compared to controls.

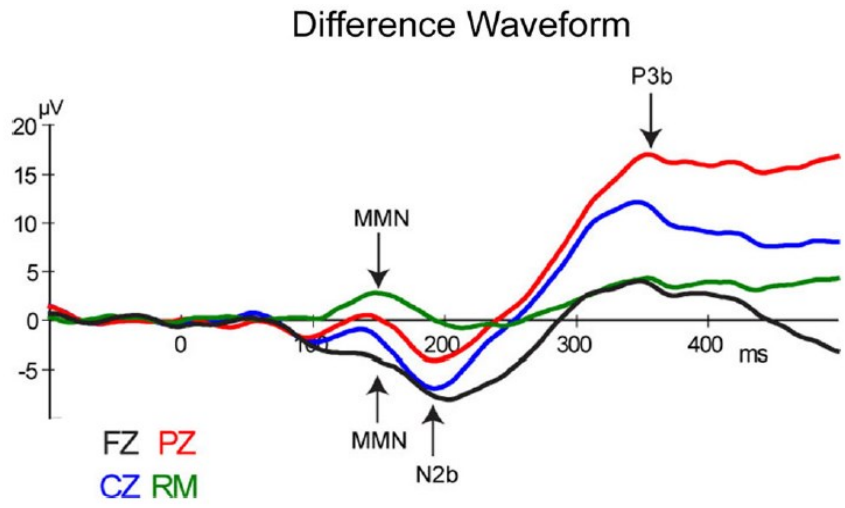

Figure 5. Mismatch negativity (MMN) evoked during active detection of a deviant stimulus is elicited along with attention-related ERP components N200 and P300. Image extracted from Nääatänen et at., 2014 (99).

N200: N200 is a negative wave peaking between 180 and 350 ms after stimulus onset in and oddball task. It is the second negative peak in the averaged ERP waveform and it is usually observed as a prominent frontocentral negative peak at around 100 ms in the auditory modality or a prominent temporo-occipital negative peak at around 180 ms in the visual modality (100). N200 typically appears together with the P300 wave and it is often referred as the “N2-P3 complex”. N200 reflects cognitive mechanisms that are

related to the discrimination of the characteristics of the relevant stimulus of the oddball task. The N200 displays the automatic detection of the novel stimuli in and oddball task (101). The N200 is a neurophysiological marker of cognitive control and it is associated to inhibition responses, conflict responses and error monitoring.

P300: One of the most studied ERP components is the P300 wave, a large, broad, positive deflection of the EEG that typically appears about 250 to 700 ms after the perception of a "meaningful" target stimulus in an odd-ball task (101,102) (See Figure 6).

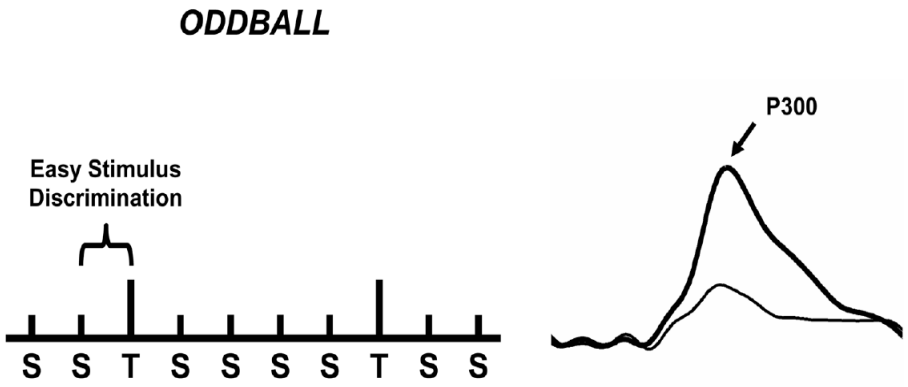

Figure 6. Example of an oddball task: two different stimuli are presented in a random sequence, with one occurring less frequently than the other does (target=T, standard=S). Image from Polich 2007 (103).

After initial sensory processing, attention-driven comparison process evaluates the representation of the previous event in working memory. In an oddball paradigm if there is no identification of the stimulus as the novel one, the current mental schema of the stimulus context is well-maintained, and only sensory evoked potentials are recorded (N100, P200, N200). By contrast, if a new stimulus is detected, attentional processes lead into a change of the stimulus representation that is associated with P300 (See Figure 7).

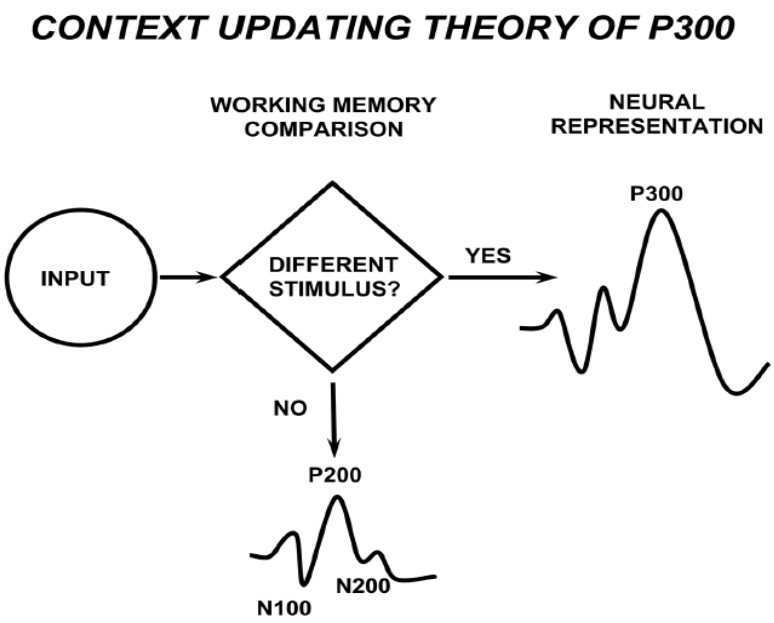

Figure 7. Schema of the context updating theory of P300. Image from Polich 2003 (78)

P300 scalp distribution is defined as the amplitude change over the midline electrodes (Fz, Cz, Pz), which typically increases in magnitude from the frontal to parietal electrode sites (104).

There have been discovered two different peaks that characterized the P300:

- P3a that is originated from stimulus driven frontal attention mechanisms during task processing.
- P3b originates from temporal parietal activity associated with attention and appears related to subsequent memory processing.

  The P300 wave it have been demonstrated to be a sensitive neurophysiological marker of cognitive processes including attentional demand and cognitive workload (54,103,105).

N400: The N400 is a negative deflection in the EEG that occurs approximately 400 ms after a meaningful stimulus onset. It has been linked to the semantic integration of a given stimulus into a previous context (106).

This component was first discovered in 1980 by Kutas and Hillyard (106) as a response to semantic anomalous sentence endings in linguistic paradigms (see figure 8). However, similar effects were lately observed related to non-linguistic material involving meaningful actions (107). N400 could be elicited by different kind of stimulus including written, spoken, and signed (pseudo)words, drawings, photos, and videos of faces, objects and actions, sounds, and mathematical symbols.

In the linguistic domain, the N400 is a robust neurophysiological marker of semantic processing. Studies investigating the N400 have reported that its latency remains remarkably constant. Contrarily its amplitude seems to be very variable and it is sensitive not only to the degree of semantic incongruity *per se* but also to several other factors. Thus, the N400 it is not just a marker of a violation of a pattern; it gives us information about the processing of the meaning. There is some growing evidence that the meaningful/non-meaningful dimension might be more important than the linguistic/nonlinguistic dimension (108).

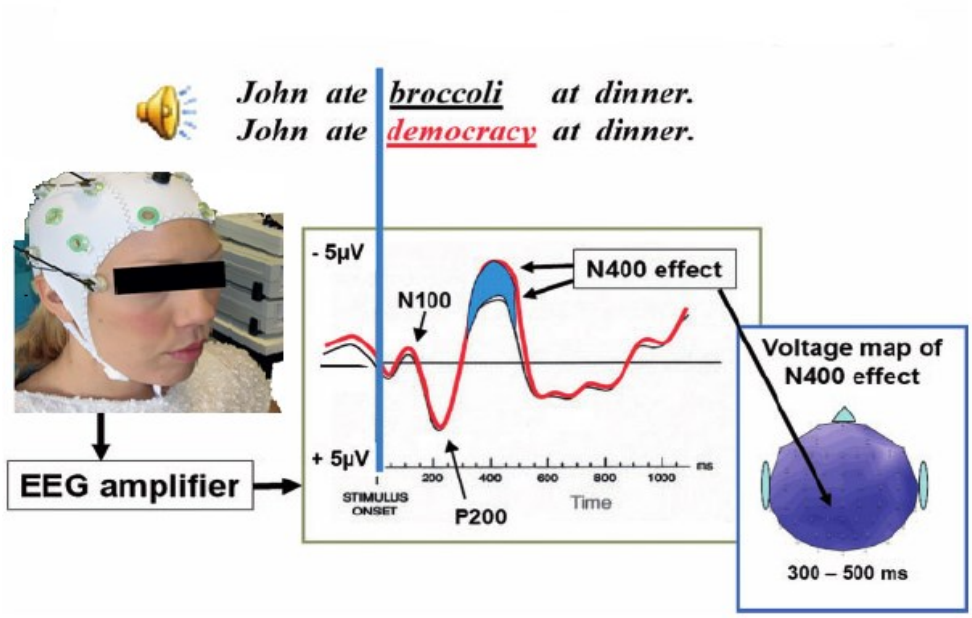

Figure 8. Image of the N400 effect. Image modified from Steinhauer 2014 (84)

3.1.2. Event-related potentials studies in ALS:

Event-related potentials (ERPs) have been used to assess cognitive and clinical status of ALS patients whose cognitive functions cannot be easily expressed in their behavior (109).

Different ERP components have been studied in ALS disease using different paradigms (93). Studies concerned with differences in ERPs between ALS patients and healthy controls have been controversially discussed. Literature reporting abnormalities in the ERPs in ALS is mixed: there are several studies reporting significantly longer latencies in the ERPs components (110–112), smaller amplitudes (93,113), and both latencies and amplitudes (58,61).

In contrast, other studies reported comparable ERPs amplitudes and latencies between ALS patients and healthy participants (114).

Table 4 summarized the main studies regarding ERPs abnormalities in ALS.

Authors	ALS Patients/Controls	ERPs paradigm used	ERPs abnormalities in ALS patients
Gil et al., 1995 (110)	20/20	Auditory oddball paradigm.	Delayed latencies in the N200 and P300.
Münte et al., 1998 (113)	8/8	Visual recognition memory test: immediate and delay recall.	Reduced amplitudes of the N400 and enhancement of a late positive component (LPC) for repeated items.
Vieregge et al., 1999 (115)	8/8	Auditory oddball. Processing negativity (PN) was recorded.	PN was smaller in ALS patients.
Hanagasi et al., 2002 (61)	20/13	P3b classical auditory oddball paradigm, P3a in a novelty paradigm, CNV and MMN.	Smaller P3a and P3b amplitudes. Delayed P3a latencies. Mean CNV were higher.
Paulus et al., 2002 (111)	16/30	Visual and auditory oddball.	Delayed latencies in the P300 in both paradigms.
Kotchoubey et al., 2003 (116)	3 ALS patients in CLIS	Passive auditory oddball, emotional oddball, word-pairs, learning paradigm, movement intention.	Patient 1: P300 wave was observed in the auditory and word-pair tasks. Patient 2: N300 observed in the auditory and emotional oddball. Movement intention showed adequate preparation of the motor areas. Patient 3: ERPs responses were not found.
Raggi et al., 2008 (93)	10/10	Passive auditory three stimuli oddball.	Lower amplitudes in the N100, P3a and MMN.
Pinkhardt et al., 2008 (117)	20/20	Auditory oddball using four types of tones. N100, Nd, MMN and P300 were recorded.	Decrease of the fronto-precentral negative difference wave (Nd). Analysis of the P300 showed increased processing of non-relevant stimuli.
Ogawa et al., 2009 (112)	19/19	Active auditory oddball.	Prolonged N1/N2/P3 GFP latencies.
Volpato et al., 2010 (54)	24/17	Active auditory oddball.	Delayed N1/P2/N2 latencies.
Silvoni et al., 2015 (118)	14/10	Vibro-tactile oddball.	Delayed N2
Volpato et al., 2016 (58)	15/15	Active auditory oddball with four types of tones. N200, P300 and re-orienting negativity (RON) components were analyzed.	Reduced amplitudes and delayed latencies of N200, P300 and RON:

Table 4. Main ERPs studies in non-demented ALS patients.

The results of the aforementioned studies demonstrate significant alterations in the different stages of the information processing in ALS. Those results suggest that ERPs evaluation could be of central importance to assess the clinical and cognitive profile of ALS patients. Moreover, some studies (54,116) proposed that ERPs could serve as a strategy for the evaluation of cognitive impairment in cases of severely paralyzed patients (such as ALS patients) unable to perform cognitive test that requires overt motor/verbal responses.

3.2. Brain communication in Amyotrophic Lateral Sclerosis

3.2.1. Introduction to Brain Computer Interfaces

Brain Computer Interfaces (BCI) or brain–machine interfaces (BMI) are systems that decode brain activity and transform it into commands to control external devices. Some of the applications of BCIs are to achieve direct brain communication in completely paralyzed patients (such as those suffering from ALS) and restoration of movement in paralyzed limbs (i.e in patients with severe stroke) through the transmission of brain signals to the muscles or to external prosthetic devices (119).

From an historical perspective, Hans Berger, who discovered the EEG, was the first to talk about the possibility of reading thoughts from the EEG signal by using refined mathematical analyses. About 40 years ago, Jacques Vidal coined the term "Brain-Computer Interface" when presented a system capable of decoding EEG signals and transform it into computer control commands. He predicted in this visionary paper (120): *"As the reader undoubtedly realizes, direct brain-computer communication still lies somewhat in the future. Even the relatively modest experimental program outlined in this paper may take several years to reach maturity, at which time new directions probably*

will have emerged. In summary, it can be said that the feasibility of the communication concept rests on three basic assumptions. The first assumption is that mental decisions and reactions can be probed, in a dimension that both transcends and complements overt behavior, from the array of observable bioelectric signals and, in particular, from the electroencephalographic potential fluctuations as measured on the human scalp. A second assumption is that all meaningful EEG phenomena should be viewed as a complex structure of elementary wavelets, similar in nature to components of evoked responses that sequentially reflect individual cortical events and create a continuous flow of neuroelectric messages. The third assumption is that operant conditioning procedures can increase the reliability and stability of these time signatures and patterns. Admittedly the validity and implications of these assumptions are far from universally accepted".

Types of BCIs

- *Invasive BCIs:* Invasive BCIs use activity recorded by brain implanted micro or macro-electrodes. Different types of brain activity could be measured with invasive BCIs: local field potentials (LFPs) (121,122), single-unit activity (SUA) (123–125), multi-unit activity (MUA)(121), electrocorticographic activity (ECoG) (126,127), and calcium channel permeability (128).
- *Non-invasive BCIs:* Non-invasive BCIs use brain signals recorded using sensors over the scalp. Different brain signals are used to control a BCI as: Slow cortical potentials (129,130), sensory motor rhythms (SMR) and motor-related beta rhythms (131), event-related potentials (ERPs) (i.e the well-known P300 (132)), steady-state visual or auditory-evoked potential (133,134), blood oxygenation level-dependent (BOLD) imaging using functional magnetic resonance imaging

(fMRI) (135,136), concentration changes of oxy/deoxy hemoglobin using near-infrared spectroscopy (NIRS)) (137).

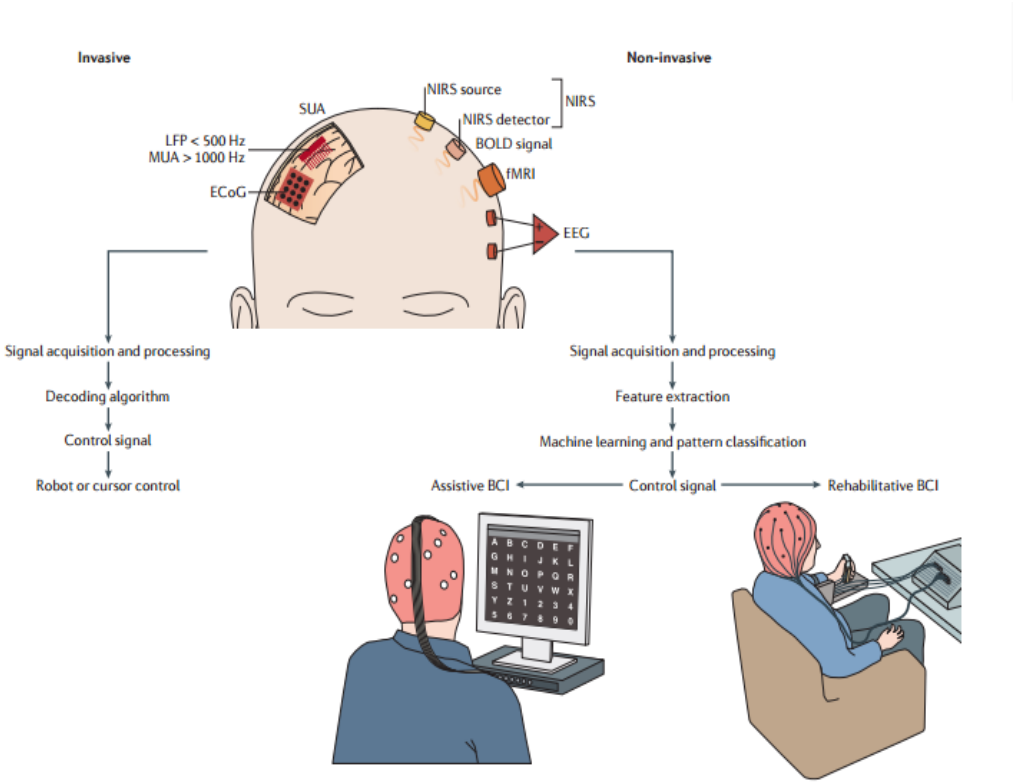

Figure 9. General framework of brain–computer interface (BCI) systems. Invasive BCI approaches (left) include the measurement of local field potentials (LFPs), single-unit activity (SUA), multi-unit activity (MUA), and electrocorticography (ECoG). Noninvasive BCI approaches (right) include EEG, near-infrared spectroscopy (NIRS) and blood oxygenation level-dependent (BOLD) functional MRI. Brain signals are processed to extract features relevant to the aim of the BCI (for example, communication) and then classified using a translational algorithm to construct a control signal that drives the BCI. BCIs can be classified as assistive to help patients with communication or movement, or as rehabilitative to help recover neural function. Image and legend from (138).

Additionally, a BCI system can be characterized according to the control task required to the user, which is the mental task that the participant is asked to perform in order to generate the brain signal used to drive the BCI. We can differentiate between two types of brain signal to control a BCI:

- *Exogenous (evoked) brain signals to drive BCIs:* consists of brain responses generated in response to specific stimuli. For instance, external stimuli might

trigger evoked potentials (EP) in the EEG which can be identified by the BCI system. For instance, in the P300 paradigm different external stimuli can be employed both visual (114,132,139), auditory (140,141) or tactile (142). During the BCI experiment the user is asked to pay attention to a particular stimulus.

- *Endogenous (self-generated) brain signals:* consists of brain activation pattern occurring during normal brain function and initiated by the participant. In this case BCIs require of a training period in which the user has to learn how to produce a certain brain oscillatory pattern that is associate with the control of the system. In this type of BCI the feedback plays an essential role since it produces a change in the brain signals required by the paradigm. On the other hand, this type of BCI is more sensitive to physiological and psychological state of the participant, i.e. motivation, fatigue, etc. The most employed endogenous EEG signals for BCIs are characterized by slow cortical potentials (SCPs) (129) and brain oscillations associated with sensory processing and motor behavior.

The parts of a BCI:

BCIs requires of an input (e.g. electrophysiological activity from the user), output (i.e. device commands), components that decode inputs into outputs, and a procedure that determines the onset, offset, and timing of operation (131). A functional BCI system is divided in the following parts:

- Signal acquisition: In the signal-acquisition part, the chosen input is acquired (by the recording sensors), amplified, and digitized.
- Signal processing and feature extraction: After digitalizing the signals and pre-processing the data, feature extraction procedures are required (e.g spatial filtering,

voltage amplitude measurements, spectral analyses, or single-neuron separation). This analysis extracts the signal features that encode the participants' commands. BCIs can use signal features that are in the time domain, in the frequency domain or both. In general, the signal features used in present-day BCIs reflect distinguishable brain events.

- Pattern Classification: After extracting the signal features, a translation algorithm is required to translate these signal features into device commands that carry out the user's orders. This algorithm might use linear models (e.g. linear discriminant analysis (LDA) or support vector machine (SVM)) or nonlinear methods (e.g. neural networks).
- The output device: This output is the feedback that serves to the participant to learn how to modulate his/her brain activity and to improve the accuracy in the performance.

A schematic view of the BCIs parts and its functionality is shown in Figure 10.

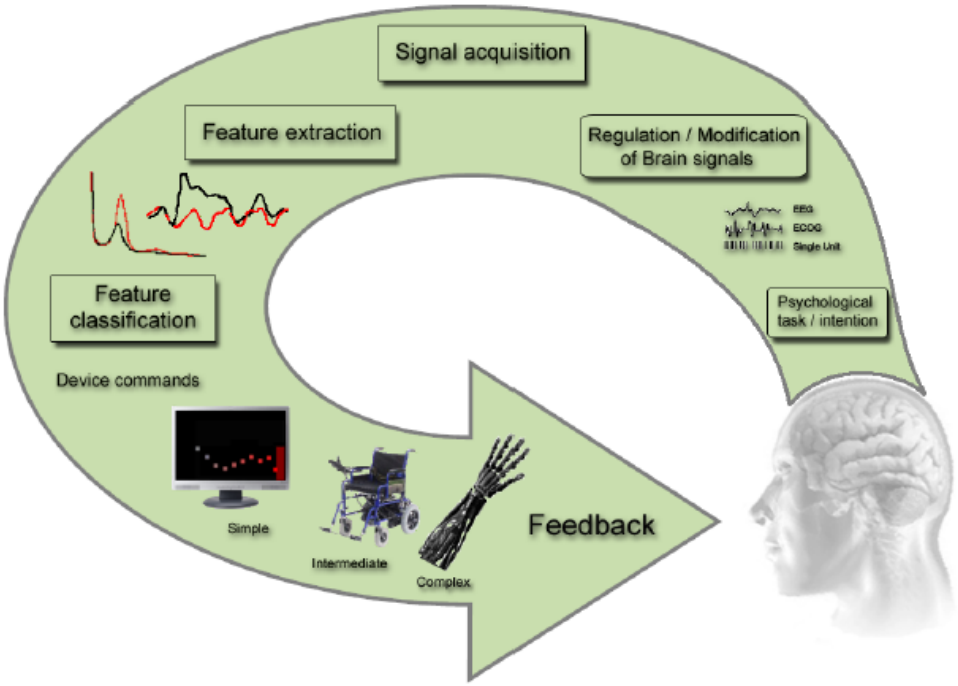

Figure 10. Schematic diagram representing the information stream in a BCI, image from (143).

3.2.2. Brain computer interfaces for communication in the late stage of ALS

BCI-based communication involves generation of brain signals by the patient to drive alphanumeric grids, binary cursers, and/or web browsing tools to formulate sentence or express feelings or desires to the caregivers/family members (138).

In the past several BCI applications for communication (invasive and non-invasive) had been used in severely paralyzed patients as those with ALS (144–148) or with severe brain damage, such as stroke or spinal cord injury (139,149,150). Nowadays, the target population to use a BCI for communication is represented mainly by patients in the late stage of ALS who suffer the completely locked-in syndrome.

Since the first application of BCIs using non-invasive EEG-recordings in the LIS patients published in “Nature” in 1999 by Prof. Birbaumer and co-workers (129), several groups have shown that different types of non-invasive brain-computer interfaces allow communication with locked-in patients. However, in a meta-analysis it have been shown

that while brain-computer interfaces allow communication with locked-in patients, all attempts to communicate with a patient in a completely locked-in state (CLIS) failed (37,119,147). Kübler and Birbaumer in 2008 (36) exposed that CLIS patients do not achieve adequate control of their brain activity to enable communication using EEG signals. Specific cognitive problems and abnormal neurophysiological signatures in the EEG might be, at least in part, responsible for the failure in CLIS. In this meta-analysis of all completely locked-in patients at that point in time, they hypothesized that complete paralysis with no output-channel available leads to extinction of goal-directed thinking and extinction of intentional communication and thought. If no attempt to express desires and wishes is followed by the anticipated consequences, extinction of this specific type of behavior and thinking should follow.

However, De Massari and co-workers in 2013 provide evidence that communication could be achieved in the CLIS (151). In their study, a novel paradigm was introduced using Pavlovian semantic conditioning for online classification of EEG signals to discriminate between covert (cognitive) 'yes' and 'no' responses. The paradigm included the presentation of true and false statements used as conditioned stimuli, and unconditioned stimulus consisted of skin electrical stimulation paired with affirmative statements. The results suggested that a reliable level of accuracy in the BCI performance in the CLIS patient was not achieved uniformly throughout the 37 sessions (despite intact cognitive processing capacity), but in some sessions communication accuracies up to 70% were reached.

In order to improve levels of accuracy of the aforementioned BCI study, other methodologies using different neurophysiological approaches were tested by Tübingen

research group. The first prototype introducing fundamental strategy changes was proposed by Gallegos-Ayala et al. in 2014 (137).

3.2.3. fNIRS-EEG based BCIs for communication in the late stage of ALS

Functional near-infrared spectroscopy (fNIRS) is a non-invasive technique used to measure brain activation on the basis of cerebral hemodynamic response. It measures regional cerebral oxygen saturation and higher temporal changes in hemoglobin concentration in brain tissues using trans-illumination spectroscopy. Measurement of tissue oxygen saturation and tissue hemoglobin content is determined by the difference in intensity between a transmitted and received light delivered at specific wavelengths. Some advantages of fNIRS compared to other neurophysiological techniques are its applicability at bedside, its reasonably low cost as neuroimaging technique and the short preparation times that it requires.

Recently, fNIRS based BCI was developed in Prof. Birbaumer lab in the University of Tübingen, based on classical semantic conditioning, for communication in CLIS. Gallegos-Ayala et al. (2014) (137) for the first time described a case of a CLIS patient with ALS that could establish “yes/no” communication to simple questions with known answers using a fNIRS-based BCI system. fNIRS classified cortical oxygenation and deoxygenation in brain tissues during following the questions. The BCI methodology used in this study contrast with the previous BCI approaches (151) and propose a more “reflexive” mode based on learning principles of classical conditioning that makes easier to differentiate between yes and no answers responses. The classifier was trained separating brain responses to patient’s yes and no answers. While EEG-BCI study of de Massari (151) resulted in significant (above 70%) classification of “yes” and “no” responses in only 7 of 37 sessions and no discriminatory answer in brain oscillations to the images at frequencies between

3 and 30 Hz, fNIRS-BCI study of Gallegos-Ayala obtained significantly above chance-level answers with an overall performance of 76.30% in the last training period and the 100% correct performance across 14 consecutive sessions. Therefore, we can propose fNIRS-BCI as a viable approach to enable communication in CLIS.

Hence, to validate these preliminary findings of Gallegos-Ayala et al. (137), and refine the FNIRS-BCI technology for communication in the CLIS some studies were performed by our BCI group combining the use of on four ALS patients in CLIS using combined NIRS-EEG-based BCIs (submitted for publication). fNIRS-EEG-based BCI was used to train four CLIS patients to regulate their fronto-central brain activity to answer yes/no questions. These successful preliminary results could indicate a next step towards to unlock the CLIS.

4. ASSESSING COGNITIVE FUNCTIONING IN AMYOTROPHIC LATERAL SCLEROSIS WITH A NOVEL COMBINATION OF VIBRO-TACTILE P300 AND NEUROPSYCHOLOGICAL TESTING

4.1. Introduction

Amyotrophic Lateral Sclerosis (ALS) is a motor neurodegenerative disease that is characterized by a progressive degeneration of the upper and lower motor neurons, causing progressive muscular paralysis of limbs and bulbar musculature. The disease generally progresses to total paralysis and has a fatal prognosis unless the patient is artificially ventilated and fed.

In addition to motor impairment, a widespread fronto-temporal degeneration with corresponding cognitive deficits has been reported in half of the ALS patient population (44,45,50), Ringholz and colleagues (40) have investigated the nature of cognitive changes associated with sporadic ALS in a large sample of 279 patients at different stages of the disease. This study has reported that about 51% of the patients with ALS showed various degrees of cognitive impairment. That is, 30% displayed mild cognitive impairment and approximately 20% had severe deficits in multiple cognitive domains that met the criteria for dementia.

The most often reported cognitive impairments in ALS involve components of the executive system (e.g., verbal fluency, selective attention and mental flexibility) (44–49). Deficits in verbal fluency have been reported in almost all studies about cognitive impairment in ALS. Verbal fluency deficits are considered as a sensitive indicator of damage in frontal and striato-frontal areas of the brain (46,48,50). However, as the majority of verbal fluency tests

require speech motor skills, the frequency of impaired cognition might have been overestimated (94). With respect to executive functions, the attentional network is most commonly affected in ALS (54,117). With respect to language functions, language impairment in ALS is an area that has been partially neglected. However, some studies have contribute to the fact that language deficits in ALS are at least as common as executive dysfunctions (48,70,152). For instance, Taylor and colleagues (70) in their study they highlighted the occurrence of language deficits in non-demented ALS. They reported that the 43% of the patients of a sample of 51 ALS patients showed language dysfunctions (in contrast to the 31% of patients having deficits in the executive domain). They suggested that even if these two domains are strongly coupled, executive dysfunction does not fully account for the cognitive profile of language impairments observed.

Other studies have also found cognitive deficits to be related to the memory (67,153,154) yet at lower prevalence rates.

The assessment of cognitive functions in late stages of the disease is one of the most challenging problems. Due to motor paralysis, patients have a limited ability to communicate. This might either mask or over-emphasize the detection of cognitive deficits (72,154). As patients are facing important decisions, such as choosing their clinical treatment (medication, artificial ventilation and nutrition) as well as expressing competently their will to live (155), the assessment of cognitive impairment in ALS remains a crucial issue.

Event-related potentials (ERPs) have been used to provide information about the clinical and cognitive status of ALS patients, complementary to neuropsychological testing (54,58,61,72). One of the most studied ERP components is the P300 wave, a positive deflection of the EEG that appears about 250 to 700 ms after the perception of a “meaningful” target stimulus in

an odd-ball task (101). The P300 wave is a sensitive neurophysiological marker of cognitive processes including attentional demand and cognitive workload (54,103,105). In ALS, studies concerned with differences in P300 waves between patients and healthy participants have been controversially discussed. Some ERPs studies revealed a significantly longer P300 latency in the in ALS group than in the control group (110,111) as well as reduced P300 amplitudes (69). In contrast, other studies reported comparable P300 amplitudes and latencies in both groups (54,114). These irregularities in the P300 features in ALS could reflect a dysfunction in the cognitive evaluation of the significance of a stimulus and might therefore serve as a physiological marker for cognitive impairment in ALS (61,110,112). Abnormal P300 features have been also reported in other neurodegenerative diseases, such as Alzheimer's (156–158) and Parkinson's disease (159,160) or Multiple Sclerosis (161,162). These studies found a relationship between P300 features and cognitive decline, such as a delayed and/or a reduced P300 response which could indicate a globally impaired cognition and attention.

Interestingly, most ERPs paradigms concerning cognitive function in ALS used visual and auditory stimuli (54,111,112), whereas somatosensory stimuli have been less frequently used. However, somatosensory ERPs might be particularly advantageous in late stages of ALS disease, when visual stimulus presentation fails to elicit reliable ERPs due to the often severely compromised vision due to reduced oculo-motor control and eyelid weakness (151,155,163). The somatosensory pathway might therefore represent a promising alternative or adjunctive pathway to elicit eye-gaze independent ERPs, as it has been demonstrated with healthy subjects using electrical and mechanical stimulation of hands and wrists (164,165). Brouwer and Van Erp (142) established that vibro-tactile stimuli elicit robust P300 waves suitable for BCI-based communication in healthy participants. The same

technology was also used to restore communication in severely paralyzed patients following brain injury (166). In a recent case study with a Locked-in syndrome (LIS) patient, tactile stimuli presentation modality has shown to be highly efficient in terms of BCI-accuracy. This type of tactile presentation was also superior compared to visual and auditory modalities (167). In our previous work, we have already demonstrated that vibro-tactile event-related potentials can be reliably triggered in ALS patients (118).

In the present study, we aimed to investigate cognitive functioning by neuropsychological and neurophysiological testing, using the somatosensory system to elicit ERPs in a group of mild to moderately progressed ALS patients compared to a healthy control group. Based on prior evidence, suggesting fronto-temporal dementia and cognitive deficits in ALS, we anticipated a significant difference in psychometric and neurophysiological measures between the two groups. Moreover, we expected the neuropsychological and neurophysiological test outcome to be significantly correlated with the patients' clinical status. We also hypothesized that declined neuropsychological performance would correlate with reduced amplitudes and delayed latencies of the vibro-tactile P300s in the ALS group. As the primary somatosensory system is reciprocally connected with the primary motor cortex (severely affected in ALS) and as fronto-parietal degeneration might alter processing in secondary and posterior insula cortices (50,168), these systems should also reflect changes in both the cognitive and the somatosensory domain.

4.2. Materials and Methods

4.2.1. Participants

Fifteen ALS patients were recruited from the Department of Neurorehabilitation of the San Camillo Hospital (Venice, Italy) for participation in this study. ALS was diagnosed according to

the revised El-Escorial criteria (169). All patients were in a mild or moderate stage of the disease according to the ALS functional rating-scale (ALSFRS-R) (170) with a mean score of 28.6, where 0 represents total dependency and 48 complete functionality. All patients were under riluzole treatment.

The control group consisted of thirteen healthy participants. Healthy participants with a history of neurological and psychiatric disorders were excluded.

Demographic and clinical data are given in Table 1. Patients with a diagnosis of 'ALS-dementia' were excluded from the current study. Depression was excluded by psychological assessment according to DSM-V criteria (171).

All participants (or their authorized representatives where applicable) gave written informed consent according to the Declaration of Helsinki prior to their participation in the study. The San Camillo Hospital Ethical Committee approved the study. Clinical and demographic data are shown in Table 5.

Parameter	ALS patients (n=15)	CTRL subjects (n=13)
	Range; Mean (SD)	Range; Mean (SD)
Age (years)	33-76; 59.1 (14.7)	28-86; 58.2 (15.6)
Gender (M/F)	9/6	9/4
Education (years)	5-18; 9.6 (5.7)	5-18; 11.8 (5.2)
Disease duration (months)	17-154; 65.7 (43.5)	---
Diagnosis (Spinal/Bulbar)	14/1	---
ALSFRS-R score (min-max: 0-48)	15-41; 28.6 (8.0)	---

Table 5. Demographic and clinical data. Standard Deviation (SD).

4.2.2. Neuropsychological Assessment

Neuropsychological assessments were conducted in both groups using standardized tests that requires minimal overt motor or verbal responses and could also be administered to patients with severe motor impairment. This battery contains four tests to assess intelligence, language, memory and executive functions respectively. The following tests were included:

Non-verbal Intelligence:

- Raven's Colored Progressive Matrices (RCPM) (172): this test highlights analytical skills independently from the patient's previous knowledge. The test consists of 36 items, grouped in three different sets (A, Ab, B). For each item of the test, the subject has to identify a missing piece (by pointing or looking at it) that completes a larger pattern in a multiple-choice format (6 different choices possible) (see Figure 11). The number of correctly matched items was used as the dependent variable.

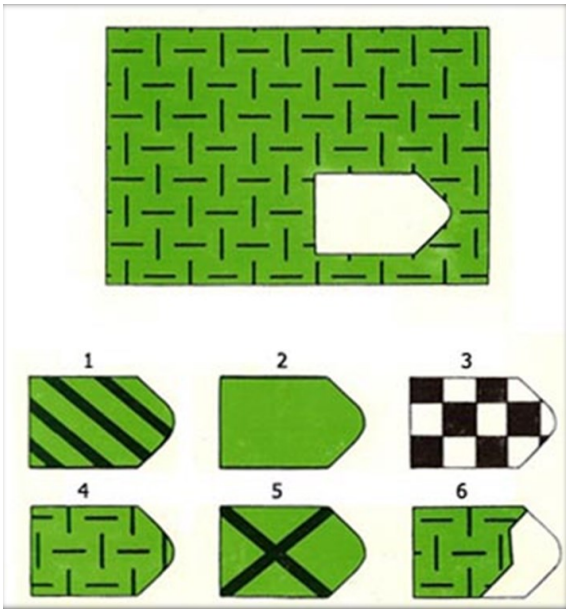

Figure 11. First item of the first set of the Raven's Colored Progressive Matrices (RCPM) (172)

Executive functions:

- Modified Wisconsin Card Sorting Test (M-WCST) (173): this test is used to assess executive functions, such as abstraction and cognitive flexibility. During the test, patients are asked to match cards to one of four key cards according to a changing criterion (form, color or number) (see Figure 12). During the task the participant has to learn a sorting strategy based on the feedback given by the examiner. After the subject achieved the last of the three sorting criteria, the three categories are repeated in the original order. Two patients aborted the test prematurely due to fatigue.

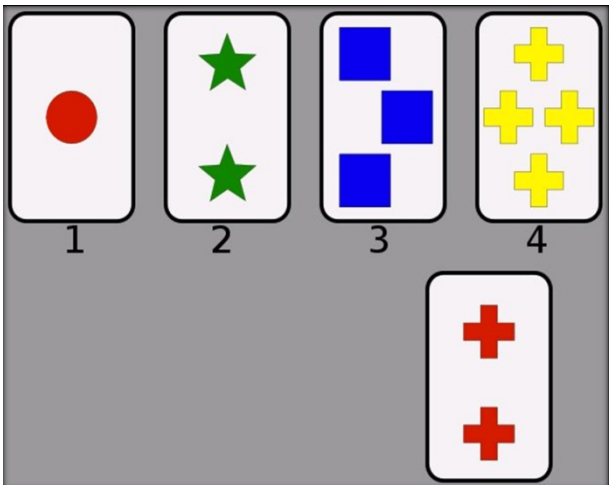

Figure 12. Example of the configuration of the Modified Wisconsin card sorting test (MCST).

Memory:

- Rivermead recognition of figures and faces (174): as part of a larger memory test battery, this test is used to detect impairments of everyday memory functioning to assess "ecological" memory. On the "Rivermead recognition of figures" subtest, 10 cards of different pictures of figures are presented. While presenting the cards, the subject has to name the figures. About ten minutes later the subject is asked to recognize the 10 figures out of a set of 20 figures. On the "Rivermead recognition of faces" subtest, 5 pictures of

different faces were presented to the subject. While the cards were presented, the subject has to distinguish if the person in the picture is a man or a woman, and younger or older than 40 years old. Ten minutes later the subject has to recognize the 5 out of 10 faces.

Language:

- Oral and written comprehension of words and sentences from the Aachener Aphasie Test –AAT (175): this subtest is used to assess language comprehension. During the test, participants are instructed to match a verbal or written stimulus to one out of four images. In the first part, the examiner has to read aloud the target stimulus (words and sentences) while the participant has to match it with one out of four images. In the second part, the participant recites mentally and matches the target stimulus with one out of four images. Each part of the test consisted of 10 items. The four pictures of each item are chosen in such a way that inappropriate pictures have phonetic or semantic similarity to the stimulus word or a thematic and syntactic-morphological similarity to the stimulus sentences.

The tests described above were chosen because they were less vulnerable to the effects of physical disability (i.e., the inability to talk or move the upper limbs) and because reaction time was not an essential factor. For one patient with severe motor impairment and speech difficulties, after common agreement, we changed the response type to a residual motor signal. We especially used an eye blink to specify a yes/no response for target stimulus selection. This procedure had previously been successfully employed to assess cognitive functions in severely disabled individuals (72). As the neuropsychological tests required more time in this particular patient, we performed the assessment across two different days. For all patients, we included shorts breaks of 5 minutes between tests in order to avoid fatigue.

4.2.3. ERPs assessment and data acquisition and processing of the EEG

Participants were seated in a chair with their arms resting on the table and grasping a vibrating stimulator with their left hand (see figure 13). In case patients experienced difficulties with holding the device, an elastic band was used to attach the hand to the effector. Furthermore, ear-plugs were used to mask the sound of the vibrating unit. The vibro-tactile "oddball" paradigm consisted of a non-target stimulus (probability 70%, frequency 20Hz) and a target stimulus (probability 30%, frequency of 100Hz). Tactile stimuli were applied with a Phantom® Premium 3.0 6 DOF end-effector (Sensable Technologies, Inc. Canada). A stimulus consisted of a sinusoidal force field along the x-axis of the end-effector lasting for 600ms with the stimulation magnitude set to 1.5N in both cases. A total of 160 randomized stimuli were delivered (divided on four blocks of 40 stimuli) with an inter-stimulus interval of 2-sec.

During the test, participants were asked to close their eyes and to mentally count target stimuli. At the end of each session, they reported their target count.

Electroencephalogram (EEG) was recorded during the oddball task using a gUSBAmp amplifier (g.tec OG, Graz, Austria) and 4 Ag/AgCl scalp electrodes (Cz, Pz, P3, P4) positioned according to the International 10–20 System. All electrodes were referenced to the left ear lobe and grounded at FPz. The electro-oculogram (EOG) was recorded from electrodes placed on the right outer epicanthus and upper medial rim. Inter-electrodes impedances were kept below 5KΩ.

EEG was sampled at 512Hz by the BCI2000 platform (176). EEG data were segmented in epochs of 1250ms synchronized with each stimulus, including 250ms before and 1000ms after stimulus onset (synchronization error smaller than 5ms (177,178)).

Epochs with signal amplitudes exceeding ±70μV (141,179,180) or containing ocular artifacts were rejected (identified by the second order statistics blind source separation algorithm implemented in the Automatic Artifact Removal toolbox for EEGLAB v10.2.5.8b). An average of 95 non-target and 41 target epochs were included in the following analysis for each participant.

After artifact rejection, non-target and target epochs were low-pass filtered at 20 Hz, baseline corrected using pre-epoch interval from -250ms up to stimulus onset and averaged for targets and non-targets respectively.

P300 features were analyzed in each participant and each channel. A P300 wave was defined as the maximum positive peak between 250 and 700 ms (103). In case of ambiguous identification of the P300 peak, two expert neurologists were involved and their judgment about the P300 peak latency was taken into account. The P300 amplitude was identified by means of peak-to-baseline measurement (104). This part of the methodology was also described in a previous paper (118).

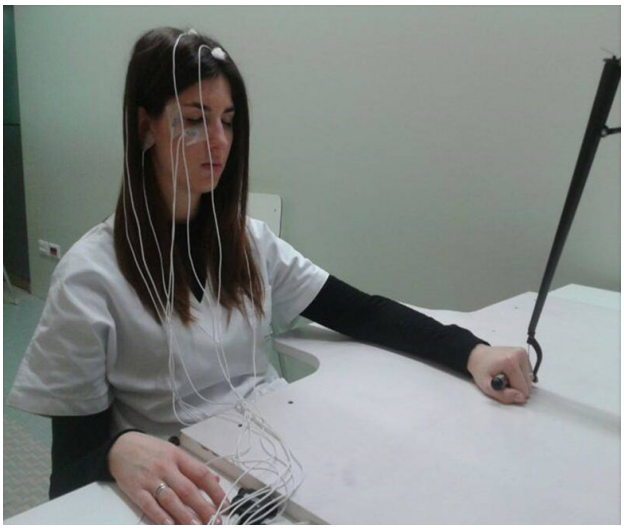

Figure 13. Healthy participant performing the ERPs experiment.

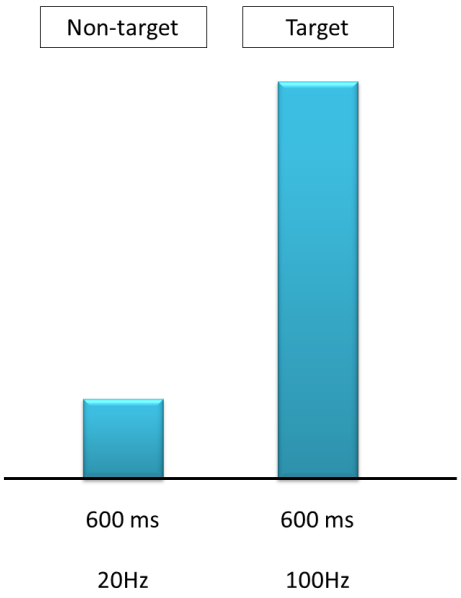

Figure 14. In an oddball paradigm two types of stimulus are presented. Each stimulus differs in it frequency: the non-target stimulus had a probability of 70% and a frequency of 20Hz, while the target stimulus had a probability of 30% and a frequency of 100Hz.

4.2.4 Median-nerve somato-sensory evoked potentials (SSEPs)

To assess the transmission integrity of the afferent pathway, we performed median-nerve SSEPs of the upper limb. Median nerve stimulation at wrist was performed with a stimulus intensity that elicited sustained thumb twitching at a frequency of 3 Hz. Electrical stimulation was delivered and recorded by Keypoint® 4 (Medtronic A/S, Tonsbakken, Denmark). Cortical waveforms were recorded using two Ag/AgCl scalp electrodes placed on C3'-C4' (according to the international 10-20 system) and referenced to Fpz.

N20 was recorded over the somatosensory cortex contra-lateral to the stimulated wrist. We measured the latency of the N20 and the amplitude between N20 and P25 peaks.

4.2.5 Statistical analysis

Statistical analyses of the results were carried out using SPSS (IBM SPSS Statistics 22, 2013, USA) and Matlab (The Mathworks Inc., USA). The normal distribution of the data was

assessed using the Kolmogorov-Smirnov test. The assumption of normality was not violated in any of the data. Parametric t-tests for independent samples were used to compare the ALS and the control group concerning demographic and neuropsychological data.

The neurophysiological data was analyzed using a repeated measures analysis of variance (ANOVA) with "group" (ALS vs Controls) as between-subjects factor and "electrode position" (Cz, Pz, P3 and P4 for ERPs; C3' and C4' for SSEPs) as within-subjects factor. Statistically significant differences were further assessed with post-hoc t-tests. The relationship between clinical, neuropsychological and neurophysiological data in the patient's and the control group was analyzed using Pearson´s rank correlation coefficient.

In order to control for a possible confounding effect of age on P300 latencies in the ALS group, we first split the ALS sample in two subgroups with (a) severe cognitive impairment (in at least one neuropsychological test/sub-test) and (b) non-severe cognitive impairment in none of the neuropsychological tests/subtests. We then performed an analysis of covariance (ANCOVA) with "sub-group" as between-subjects factors and "P300 latency electrode position" (Cz, Pz, P3 and P4) as within-subjects factor, while controlling for "age" as a covariate. Differences between sub-groups were calculated with a parametric post-hoc t-test for independent samples. The results of this test are used to determine whether potential differences in P300 features in ALS are due to cognitive decline independent from age-related effects.

Based on the exploratory nature of this study, we decided against correction for multiple comparisons in correlations analyses. The significance level for all statistics was set to 0.05.

4.3. Results

4.3.1. Demographic and clinical data

No statistically significant differences were found between groups (i.e., ALS patients and control participants) with respect to sex, age and education. For the comparison of ALS sub-groups (i.e., severe and non-severe cognitive impairment), age was not significantly different. Demographic and clinical data is shown in Table 6.

Parameter	ALS patients (n=15)	CTRL subjects (n=13)
	Range; Mean (SD)	Range; Mean (SD)
Age (years)	33-76; 59.1 (14.7)	28-86; 58.2 (15.6)
Gender (M/F)	9/6	9/4
Education (years)	5-18; 9.6 (5.7)	5-18; 11.8 (5.2)
Disease duration (months)	17-154; 65.7 (43.5)	---
Diagnosis (Spinal/Bulbar)	14/1	---
ALSFRS-R score (min-max: 0-48)	15-41; 28.6 (8.0)	---

Table 6. Demographic and clinical data. Standard Deviation (SD).

4.3.2. Neuropsychological performance

A significant group difference was found in AAT oral comprehension, revealing a lower performance in ALS patients compared to controls (t (26)= 1.51, p=0.011). No differences were observed between these two groups in other tests. Group means for non-verbal intelligence, memory, executive function, and language tests were within the normal range according to normative data. A summary of the neuropsychological data is given in Table 7.

Test/Subtest	ALS patients (n=15)	Controls (n=13)	*p-value*
	Range; Mean (SD)	Range; Mean (SD)	
RCPM	22-35; 29.7 (4.2)	26-36; 32.2 (2.91)	0.940
M-WCST categories	2-6; 4.85 (1.46)	2-6; 5.08 (1.26)	0.670
M-WCST perseverative errors	0-16; 4.15 (4.57)	0-9; 2.77 (2.89)	0.366
RBMT recognition of objects	9-10; 9.7 (0.49)	9-10; 9.85 (0.38)	0.372
RBMT recognition of faces	3-5; 4.46 (0.66)	4-5; 4.85 (0.38)	0.080
AAT Oral comprehension	49-60; 54.4 (4.32)	48-60; 58.2 (2.38)	**0.011**
AAT Writing comprehension	50-60; 54.7 (3.71)	52-60; 56.9 (3.86)	0.142

Table 7. Neuropsychological assessment of patients and healthy participants. Raven's Coloured Progressive Matrices (RCPM). Modified Wisconsin Card Sorting Test (M-WCST). Rivermead Behavioural Memory test (RBMT). Aphasie Aachener Test (AAT). Standard Deviation (SD). Significant *p*-values in bold.

4.3.3. P300 components

P300 wave components were obtained in all participants and no significant differences were found in the silent target count between groups. However, ANOVA revealed a significant interaction between "group" and "electrode position" for the P300 amplitudes ($F_{2,52} = 11.32$, $p < 0.01$). Paired t-tests showed that significantly diminished P300 amplitudes could only be found for the Cz electrode ($t(26) = 2.92$, $p = 0.007$); in the ALS group. No differences were found for the P300s latencies.

In order to investigate whether differences in patient's age could be a confound in our results, we performed an ANCOVA analysis with the factors: ALS "sub-group" (severe vs non-severe cognitive impairment according to Italian normative data (173,181) in at least one neuropsychological test/sub-test) and "electrode position for the P300 latencies" while controlling for the effect of "age" as a covariate. Results showed an effect of cognitive impairment severity in ALS on P300s latencies ($F_{4,9} = 22.76$, $p < 0.01$), independent of age.

Paired t-tests revealed significant differences between ALS sub-groups (Cz: t (13)= -2.70, p=0.018; Pz: t (13)= -3.4, p< 0.01; P3: t (13)= -2.92, p= 0.012, ; P4: t (13)= -2.95, p= 0.011), indicating that the ALS subgroup with low cognitive abilities (i.e., severe impairment) showed longer P300 latency delays compared to ALS patients with non-severe cognitive impairment.

Figure 15 illustrates the average ERPs response in ALS patients and healthy participants. Table 8 shows the data for P300 amplitudes and latencies from Cz, Pz, P3 and P4 in both groups.

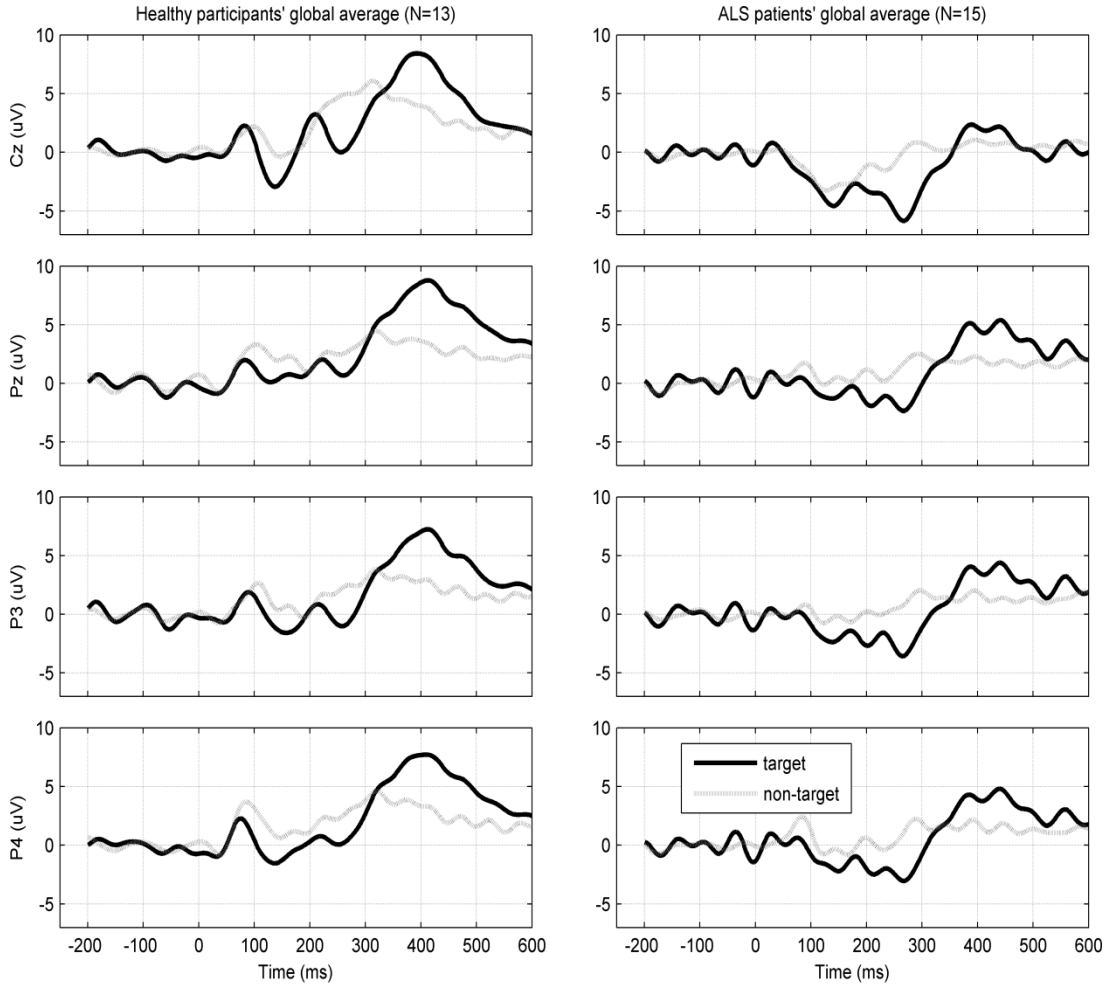

Figure 15. Global average of target and non-target ERP response in healthy participants and ALS patients at channels Cz, PZ, P3 and P4.

	Mean Latency (ms) ± SD			Mean Amplitude (mV) ± SD		
	ALS patients	**Controls**	***p-value***	**ALS patients**	**Controls**	***p-value***
P300 (CZ)	454.80 ± 59.20	427.00 ± 51.59	0.200	3.32 ± 3.19	7.77 ± 4.81	**0.007**
P300 (PZ)	454.00 ± 58.70	432.78 ± 48.84	0.297	7.53 ± 3.50	8.62 ± 3.01	0.386
P300 (P3)	453.6 ± 55.42	432.46 ± 47.13	0.472	5.97 ± 3.23	6.98 ± 2.66	0.379
P300 (P4)	454.00 ± 67.68	430.77 ± 46.48	0.218	6.12 ± 2.85	7.55 ± 2.50	0.171

Table 8. P300 features. Mean latencies and amplitudes of vibro-tactile P300 at channels Cz, Pz, P3 and P4. Standard deviation (SD). Significant p-values in bold.

4.3.4. Median nerve somato-sensory evoked potentials data

Repeated measures analysis of variance did not reveal any significant main effect of "group" factor for N20 features. The "group" X "electrode position" for N20 features was not significant. Table 9 shows the N20 data comparing both groups.

	Mean Latency (ms)			Mean Amplitude (µV)		
	ALS patients	Controls	*p-value*	ALS patients	Controls	*p-value*
N20 (C4)	20.8 ± 1.99	20.1 ± 1.29	0.262	3.59 ± 2.66	4.47 ± 2.51	0.365
N20 (C3)	20.6 ± 1.46	20.2 ± 1.53	0.548	4.09 ± 3.31	6.01 ± 4.39	0.202

Table 9. Median nerve somatosensory evoked potentials (SSEPs). Mean amplitudes and latencies ± standard deviation of N20 median nerve evoked potentials at channels C3 and C4.

4.3.5. Relationship between patient´s clinical characteristics and neuropsychological performance

No significant correlations were found between disease duration and severity of the disease (measured by ALSFRS-R) and neuropsychological data.

4.3.6 Relationship between demographic/clinical data and P300 components

Although significant correlations were found between the age and P300 latency in the ALS group (Cz: r= 0.822, p< 0.001; Pz: r= 0.820, p< 0.001 P3: r= 0.835, p < 0.001; P4: r= 0.791, p< 0.001) and in healthy participants (Cz: r= 0.568, p=0.043), no significant correlations were found between P300 features with ALS disease duration and disease severity based on the ALS-FRS-R results.

4.3.7. Relationship between clinical data and median-nerve somatosensory evoked potentials

No significant correlation emerged between N20 features (latency and amplitude), age, and disease severity.

4.3.8. Relationship between P300 and neuropsychological performance

Significant correlations in the ALS group were found between P300 components and the following tests: Rivermead-Faces recognition and P300 latency (Cz: r= -0.722, p= 0.005; Pz: r= -0.631, p= 0.021; P3: r= -0.763, p= 0.002; P4: r= -0.583, p= 0.037); Raven Color Progressive Matrices and P300 latency indicating with decreased latency test performance increased (Pz: r= -0.554, p= 0.040; P3: r= -0.551, p= 0.041; P4: r= -0.550, p= 0.042); Modified card sorting test (i.e., number of achieved categories) and P300 latency (Cz: r= -0.648, p= 0.017; Pz: r= -0.707. p= 0.007; P3: r= -0.661, p= 0.014; P4: r= -0.649, p= 0.016); Modified card sorting test (i.e., number of perseverative errors) and P300 latency (Cz: r= 0.623, p= 0.023; Pz: r= 0.624, p= 0.023; P3: r= 0.628, p= 0.021; P4: r= 0.600, p= 0.030). No significant correlations were found in the healthy group between P300 features and neuropsychological performance.

4.4. Discussion

4.4.1. Neuropsychological differences in ALS

The significant difference we found in a sub-test of the language test AAT showing that ALS patients performed lower than healthy participants in the recognition of spoken words suggests mild impairment of oral language comprehension in moderate ALS. This result contradicts old studies reporting an absence of language dysfunctions in ALS but is in line with novel studies reporting that language deficits in ALS are more common than previously thought (48,70). Abrahams (71) discussed in an editorial commentary the study of Taylor and colleagues (70) that reports language dysfunctions in ALS in a high prevalent rate. She suggests that the above mentioned study (13) might have changed the view of the nature of cognitive impairment in ALS; considering language impairments as of central importance of this field. Moreover, it demonstrates heterogeneity of the cognitive profile in ALS disease.

The difference in language comprehension found in our study cannot be attributed to motor deficits because the tests we have chosen are less vulnerable to the effects of physical disability and have no reaction time limitation (73). Although previous studies reported impairments in both executive and memory domains (40,46,47,49,50,55), we did not find any significant differences in executive or memory tasks between groups, perhaps because individual differences in cognitive impairment might have masked differences at the group level.

With an individual analysis for each participant separately comparing normative standardized values of the neuropsychological tests (181), we found that 73% of our ALS patients showed impaired performance in at least one test related to a cognitive domain. In particular, only 33% of the patients showed slightly lower performance while 40% showed severe cognitive impairment in language comprehension, executive functions, and/or

memory. The mild performance deficits of some patients related to recognition memory could be interpreted as alterations in both the encoding and the retrieval of information. Alternatively, they could be related to deficits in long-term memory (182).

Altogether, these data indicate a tendency towards mild cognitive impairment in ALS patients, although results did not reach statistical significance at the group level. The heterogeneity and complexity of the ALS disease might affect cognitive functions at various degrees in individual patients but only to a very moderate degree across patients; otherwise significant group differences would have been detected.

4.4.2. Neurophysiological differences in ALS

A significant between-groups difference was found for the P300 amplitude in Cz, indicating smaller amplitudes in the ALS group in that channel. This is line with previous studies (93,112)and might indicate a dysfunction in the attentional network. However, this difference appears only in one out of four channels (Cz), thus suggesting a mild dysfunction in circumscribed local networks only. In addition, the absence of between-group differences in the P300 latencies is in accordance with the literature (114).

An important question related to our approach refers to the integrity of somatosensory pathways in ALS. The fact that we did not find any significant correlations between clinical features (age, ALS-FRS; months of disease onset) and N20 data supports the notion that the somatosensory response is not affected by the ALS disease. The somatosensory modality thus seems to be a valid communication channel that may still be intact even at late stages of the disease (together with auditory modality).

4.4.3. Relationship between test outcome and clinical data

The correlation analysis showed a significant positive correlation between the age of healthy participants and the P300 latency (54,93). This relationship also held true for patients with

ALS, indicating that the vibro-tactile P300 may be affected by age in both groups (93). However, when controlling for age, we found a significant difference between ALS subgroups with and without severe cognitive impairment in at least one neuropsychological test. This result suggests that prolonged P300 latencies could serve as a marker for cognitive decline in ALS. Therefore, abnormal P300 latencies are unlikely to be only a consequence of aging. Nevertheless, we must take such inferences with caution considering the small sub-sample size.

In contrast to Raggi et al., (93), we found no correlation between ALS-FRS-R scores and P300 features. Thus, no correlation was found between disease duration and P300 parameters (26). This fact lends support to the notion that the P300 wave is not generally affected by the severity/duration of the disease (111). We therefore propose that the vibro-tactile P300 could also be used in the last stages of the disease, either as a marker of cognitive impairment or for BCI communication (118,166).

Other significant correlations in ALS patients were found between neuropsychological data and P300 features, which were not present in the healthy group. We found that in ALS patients, P300 latencies correlated significantly with the performance in most of the psychometric tests (i.e., Rivermead-faces recognition, Raven Color progressive matrices, Modified card sorting test-number of achieved categories, Modified card sorting test-number of perseverative errors). This significant correlation between the somatosensory P300 latency and tests involving working memory components (e.g., MCST-categories and MCST-perseverative errors, RVMD-faces) supports the idea that a delay in P300 latency could be a useful indicator for deficits in the executive component of working memory, such as selective attention and inhibition, abstract thinking, planning, and decision making. In this

context, may also consider the Raven Color Progressive Matrices as a test of the central executive of working memory.

However, this relationship does not necessarily reflect significant differences at the group level as it is also present in many other neurodegenerative diseases (157,158), schizophrenia (183), and advanced aging. It may therefore rather be described and used as a sensitive indicator of cognitive decline in non-communicative patients who are unable to perform neuropsychological testing. In addition, these results indicate that the primary and secondary cortical projection areas processing somatosensory information are tightly coupled with higher cortical areas that are responsible for attentional processing, memory, and executive functions. The mutual interaction between somatosensory and cognitive systems may be masked by the rich and compensatory neural processes in the healthy brain, as reflected in smaller correlations in the healthy group compared to augmented correlations due to neurodegenerative changes in ALS afflicted brains.

In conclusion, our findings based on both neuropsychological and neurophysiological data suggest that ALS patients in a mild to moderate stage of the disease suffer from a dysfunction in the attentional network, which leads to a circumscribed reduction in P300 amplitudes and a mild impairment in language processing as reflected by the performance in oral comprehension tests. Moreover, our data indicate that the vibro-tactile P300 may be a useful tool in the assessment of cognitive and attentional processes in severely paralyzed ALS patients, as the integrity of the somatosensory pathway is not affected by the severity of the disease. Significant correlations between the vibro-tactile P300 features and neuropsychological tests in the ALS group support this claim. Future studies are warranted to replicate and verify the efficacy of the vibro-tactile P300 in combination with neuropsychological tests in late stage of the ALS disease.

5. SELECTIVE ATTENTION IMPAIRMENT IN AMYOTROPHIC LATERAL SCLEROSIS

5.1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by a progressive degeneration of motor neurons in the spinal cord, brainstem and neocortex causing progressive paralysis (184). There is substantial clinical and experimental evidence indicating the presence of cognitive dysfunction at least in a subpopulation of ALS patients and cognitive impairments are currently part of the diagnostic criteria for ALS (94,185). However, from the early stage of the disease motor deficits may affect the results of neuropsychological tests, slowing and/or altering patients' performance. An important contribution to the evaluation of cognitive dysfunction and related neural mechanisms in ALS can be provided by event-related potentials (ERPs). ERPs, not requiring overt verbal or motor response, can be administered also to patients with severe motor disability. Many different paradigms and several ERPs resulted altered in ALS (186). A modification of the Negative Difference, Processing Negativity and N200 was reported suggesting impairment in novelty/change detection (54,115,117). The analysis of the P300 component shows a more elaborated processing of the irrelevant stimuli, especially in the frontal areas, indicating a reduction in the capacity to focus attention (117,187). Concerning the Contingent Negative Variation the results are heterogeneous suggesting that this component may change with the progress and phenotype of the disease (61,188,189). In summary, the neurophysiological changes in ALS patients seem to reflect an involvement of neural networks responsible for mechanisms of cognitive control, including selective attention. The neural basis of selective attention can be studied using the ERPs auditory oddball paradigm (190). The main ERPs associated with different stages of selective attention processing are the N200, P300 and Re-Orientation Negativity (RON). During an oddball paradigm the presentation of a rare

stimulus following a string of standard stimuli elicits the N200, a negative wave typically evoked between 180 and 320 ms after the stimulus presentation, indicating the detection of a deviation from a mentally-stored expectation of the prevailing stimulus (standard) (191). The P300 is a positive deflection following the N200, detectable between 300 and 400ms after the deviant stimulus. The P300 is believed to index a distributed network of brain processes, mainly involving focused attention and working memory mechanisms (103,192). The RON is a negative component detectable 400–600 ms after a distracting stimulus (i.e. deviant) representing the re-orientation of attention following a distracting event. The RON might reflect a central attentional process that maintains information relevant to the task (193). This study aims to evaluate auditory selective attention in a group of patients with ALS using a modified version of the auditory oddball paradigm. Participants were presented standard and deviant tones, short and long, with the instruction to respond only to long deviant tones. This paradigm allowed us to specifically assess selective attention processing and, in particular: (a) the detection of change, represented by the N200 component; (b) the orientation of the focus of attention towards the target stimulus, represented by the P300 component; (c) the re-orientation of attentional resources following a distracting event, represented by the RON component. Based on the data reported in the literature, we could hypothesize that ALS patients show an alteration of one or more stages of the selective attention processing reflected by the N200, P300 and RON component.

5.2 Methods

5.2.1. Participants

Fifteen patients diagnosed with ALS according to the El Escorial criteria (169) and 15 healthy participants, comparable for sociodemographic characteristics, as controls were recruited at the IRCCS San Camillo Hospital Foundation in Venice to participate in this study (Table I).The

patients resulted in mild to moderate stage of the disease according to the ALS Functional Rating Scale-Revised (ALSFRS-R) (170). No patient required assisted ventilation. All ALS patients were taking riluzole (Table I). The exclusion criteria were: (a) presence of dementia according to Diagnostic and Statistical Manual of Mental Disorders (171); (b) presence of comorbid psychiatric or neurological illness. The study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee of the IRCCS San Camillo Hospital Foundation. All patients gave informed consent to participate in the study.

5.2.2. Neuropsychological assessment

The neuropsychological test battery consisted in the administration of the Montreal Cognitive Assessment (MoCA) (194) screening tests and the tests described in the section 4.2.2 that could be even in patients with severe motor disability by opening and closing their eyes in agreement with the examiner.

5.2.3. ERPs paradigm

Four hundred tones were randomly presented with an inter-stimulus interval of 1200 ms. Standard stimuli (80% probability, 200 Hz) and deviant stimuli (20% probability, 1000 Hz) were used. Half of both standard and deviant stimuli lasted 200ms (short) and the other half 500 ms (long) (195,196). Participants were instructed to listen to the binaurally presented tones delivered by a PC through the software E-prime (PST, Sharpsburg, USA) and to press the spacebar when the long deviant stimulus occurred. Computerized responses were used because they provide reliable results giving information on any false-positives as well as omissions. However, the patients with severe motor deficit (n=4) were required to mentally count the target stimuli. A short training session was performed to ascertain that the participants perceived correctly all the sounds and understood the task. The experiment lasted about 1 hour.

5.2.4. Acquisition and analysis of the electroencephalogram (EEG)

During task performance the EEG was recorded from a 29-electrodes cap with a standard 10–10 position connected to an amplifier (BrainAmp, BrainProducts, Gilching, Germany). The signal was continuously recorded by applying a bandpass filter from 0.15 Hz to 70 Hz and with a sampling frequency of 500 Hz. The reference electrodes were positioned on the two mastoids and the ground electrode was placed at POz. The vertical and horizontal electro-oculogram was recorded from electrodes placed below and on the outer canthi of the right eye. Electrode impedances were kept below 10 kΩ. EEG signal was processed using a MATLAB toolbox (EEGLAB v10.2.5.8b) (197). The signal was low-pass filtered at 30 Hz and the ocular artefacts were detected and removed by Independent Component Analysis (ICA). The bad components were identified by visual inspection. The signal was segmented into epochs lasting 1500 ms with 500 ms pre- and 1000 ms post-stimulus. Distinct stimulus-locked epochs were extracted for short standard, long standard, short deviant and long deviant stimuli. The epochs were baseline corrected relative to 100 ms pre-stimulus interval. With respect to the stimulus onset the N200 component was defined as the maximum negative peak between 200 and 350 ms, the P300 as the maximum positive peak between 300 and 450ms, and the RON as the maximum negative peak between 400 and 600 ms. The amplitude was measured with respect to the baseline (104). The ERPs at Fz, Cz and Pz were evaluated because these components appeared most strongly at the midline.

5.2.5. Statistical analysis

Statistical analyses were performed with SPSS (Statistical Package for Social Science) software. The Kolmogorov-Smirnov test confirmed the assumptions of normality of all the variables, thus parametric tests were used. Comparison between groups of demographic and neuropsychological data was performed with Student's t-test for independent samples. The

ERPs data were analyzed with repeated measures ANOVA with a between-subjects factor, 'group' (ALS vs. controls), and three within-group factors: 'electrode' (Fz, Cz and Pz), 'frequency' (standard vs. deviant) and 'length' (short vs. long). Pairwise comparisons were performed with Bonferroni correction.

5.3 Results

Statistical analysis showed no significant difference between the two groups with respect to age, education and MoCA (194).

5.3.1. Neuropsychological test

Analysis showed that the patients commit a significantly higher number of errors than controls in the WCST-M (T=2.292, p=.030), objects (T=2.514, p=0.029) and faces (T=2.086, p=0.046) recognition task and RCPM (T=2.600, p=0.014) (Table 10).

Test	ALS	Controls	SIG.
M-WCST Category	5.20 (1.65)	5.86 (1.65)	0.295
M-WCST Errors	8.73 (9.37)	2.71 (2.99)	0.030*
Object recognition	9.53 (0.75)	10.00 (0.00)	0.029*
Face recognition	5.57 (0.51)	5.81 (0.50)	0.048*
Coloured Progressive Matrices	26.50 (5.52)	30.88 (3.85)	0.015*
Verbal Comprehension	55.29 (3.85)	56.21 (3.52)	0.158

Table 10. Mean (standard deviation) of neuropsychological data in ALS patients and controls. M-WCST: Modified-Wisconsin Card Sorting Test. *p=0.05.

5.3.2 ERPs paradigm

No significant difference in the detection of the target stimulus between healthy participants (correct answers: mean=92%, SD=12%) and ALS patients (correct answers: mean=91%, SD=7%) was found.

5.3.3. N200

N200 amplitude analysis showed a significant effect of 'electrode' (F (2,56)=2.898, p=0.035), indicating a larger amplitude at fronto-central than posterior sites, and 'frequency' (F (1,28)=21.139, p50.000), indicating a larger amplitude for deviant than frequent stimuli. Moreover, a significant 'frequency' x 'group' interaction was found (F (1,28)=5.24, p=0.030) revealing a larger amplitude for deviant than standard stimuli in the control group (p50.000). The significant 'electrode' x 'frequency' x 'length' x 'group' interaction (F (2,56)=6.122, p=0.004) showed that at Pz short deviant stimuli elicited a larger N200 amplitude in the healthy than ALS group (p=0.003). Moreover, in the control group the deviant stimuli elicited a larger N200 amplitude than the standard stimuli for both short and long tones, at all the electrodes (Fz, long: p=0.039; Fz, short: p=0.002; Cz, long: p=0.004; Cz, short: p=0.000; Pz, long: p=0.000; Pz, short: p=0.001). Finally, in ALS patients, the amplitude was larger for short deviant than short standard tones at Cz (p=0.041). Concerning N200 latency, the analysis of variance showed an effect of 'electrode' (F (2,56)=3.355, p=0.042) indicating a larger latency at fronto-central than the posterior sites, and an effect of 'electrode' x 'frequency' x 'group' interaction (F (2,56)=4.294, p=0.018) showing in ALS a prolonged latency for the standard stimuli at Pz (p=0.016) and in the control group a longer latency for deviant than standard stimuli at Pz (p=0.014).

5.3.4. P300

Amplitude analysis showed a significant effect of 'electrode' (F (2,56)=12,381, p50.000), indicating a larger amplitude at Pz and Cz than Fz, 'frequency' (F (1,28)=15,694, p50.000) showing a larger amplitude for deviant than standard stimuli, and 'length' (F (1,28)=30.509, p50.000), indicating a larger amplitude for short than long stimuli. The 'group' factor also resulted significant (F (1,28)=6.751, p=0.015), indicating a larger P300 in healthy participants

than patients with ALS. The significant 'length' x 'group' interaction (F (1,28)=6.222, p=0.019) revealed that in healthy controls the amplitude of P300 for short stimuli was larger than in ALS patients (p=0.004). The analysis of the latency of P300 showed an effect of 'electrode' (F (2,56)=6.064, p=0.004) indicating a longer latency at Cz and Pz than Fz, 'frequency' (F=(1,28)=4.446, p=0.044) indicating a longer latency for deviant than frequent stimuli, and 'length' (F (1,28)=6.995, p=0.013) indicating a longer latency for short than long stimuli. The significant 'group' factor (F (1,28)=6.128, p=0.020) highlighted a longer delay in ALS patients compared to controls. Moreover, the 'electrode' x 'length' x 'group' interaction (F (2,56)=3.331, p=0.043) showed that the latency of the P300 was prolonged for short compared to long stimuli at Cz in the control group (p=0.036) and at Fz in the ALS group (p=0.013).

5.3.5. RON

Amplitude analysis demonstrated an effect of 'electrode' (F (2,56)=49.976, p50.000) indicating a larger amplitude at Fz than Cz and Pz, and 'length' (F (1,28)=30.045, p50.000) showing a larger amplitude for long than short stimuli, and 'group' (F (1,28)=4.554, p=0.042) revealing a larger amplitude in healthy participants than ALS patients. The 'length' x 'group' interaction (F (1,28)=4.789, p=0.037) showed a larger amplitude in the control group compared to patients for long stimuli (p=0.005). The significant 'electrode' x 'frequency' interaction (F (2,56)=13.202, p50.000) revealed that the amplitude of the RON was larger for deviant than frequent stimuli at Fz (p=0.011) and Cz (p=0.007). The analysis of RON latency showed a significant 'frequency' x 'length' interaction (F (1,28) p=0.002) indicating a longer latency for long standard compared to short standard stimuli (p=0.002) and a significant 'electrode' x 'frequency' x 'length' interaction (F (2,56)=3.750, p=0.029) demonstrating that at Fz the RON latency was more prolonged for short deviant than short frequent stimuli

(p=0.008). Moreover, at Fz the latency was longer for the long than the short tones both for deviant (p=0.005) and standard (p=0.008) stimuli. Finally, at Pz the RON had a longer latency for long tones than short tones for standard stimuli (p=0.012). Statistical results are reported in Table 11. Correlation between neuropsychological test and ERPs Correlation analysis (Spearman's rank correlation coefficient) between the scores of neuropsychological tests and ERPs latencies and amplitudes was performed.

ANOVA (factors and interactions)	Bonferroni pairwise comparisons	F-value	*p*-value
N200 amplitude			
Electrode		F (2,56)= 2.898	$p=0.035$
	Cz > Pz		$p=0.003$
	Fz > Cz		$p<0.001$
Frequency		F (1,28)= 21.139	$p<0.001$
Frequency × group		F (1,28)= 5.244	$p=0.030$
	deviant > standard		*controls:* $p<0.001$
Electrode × frequency		F (2,56) = 9.670	$p<0.001$
	deviant > standard		*Fz:* $p=0.003$
			Cz: $p<0.001$
			Pz: $p<0.001$
Frequency × length		F (1,28)= 4.883	$p=0.035$
	deviant > standard		*long:* $p=0.005$
			short $p<0.001$
Electrode × frequency × length		F (2,56)= 4.532	$p=0.015$
	Fz > Cz		$p<0.001$
	Fz > Pz		$p=0.043$
	Cz > Pz		$p=0.001$
	deviant > standard		*short, Fz:* $p=0.001$
	short > long		*long, Cz:* $p=0.003$
			short, Cz: $p<0.001$
			long, Pz: $p=0.001$
			short, Pz: $p=0.001$
			deviant, Fz: $p=0.044$
			deviant, Cz: $p=0.041$
Electrode × frequency ×		F (2,56)= 6.122	$p=0.004$
length × group	*controls > patients*		*deviant, short:* $p=0.003$
	Fz > Cz		*controls, deviant, short:* $p=0.024$;
	Cz > Pz		*controls, deviant, short:* $p=0.002$
	Fz > Cz		*controls, standard, short:* $p<0.001$
	deviant > standard		*controls, long, Fz:* $p=0.039$;
	long > short		*controls, short, Fz:* $p=0.002$;
	deviant > standard		*controls, long, Cz:* $p=0.004$;
			controls, short, Cz: $p<0.001$;
			controls, long, Pz: $p<0.001$;
			controls, short, Pz: $p=0.001$
			controls, standard, Cz: $p=0.016$
			ALS, short, Cz: $p=0.041$
N200 latency			
electrode		F (2,56) = 3.355	$p=0.042$
electrode × frequency × group		F (2,56)= 4.294	$p=0.018$
	ALS > controls		*standard, Pz:* $p=0.016$
	Deviant > standard		*controls, Pz:* $p=0.014$
P300 amplitude			
Electrode		F (2,56)= 12.381	$p<0.001$
	Fz < Pz		$p=0.009$
	Cz < Pz		$p<0.001$
Frequency		F (1,28)= 15.694	$p<0.001$
Length		F (1,28)= 30.509	$p<0.001$
Group		F (1,28)= 6.751	$p=0.015$
Length × group		F (1,28)= 6.222	$p=0.019$
	controls > ALS		*short:* $p=0.004$
	long > short		*controls:* $p<0.001$
			ALS: $p=0.041$
Electrode × frequency		F (2,56)= 5.247	$p=0.008$
	Pz > Fz		*deviant:* $p=0.001$
	Pz > Cz		*deviant:* $p<0.001$
	deviant > standard		*Cz:* $p=0.024$
			Pz: $p<0.001$
Electrode × frequency × length		F (2,56)= 4.460	$p=0.016$
	Pz > Cz		*deviant, long:* $p=0.002$
	Pz > Fz		*deviants, short:* $p<0.001$
	Cz > Fz		*deviant, short:* $p<0.001$

ANOVA (factors and interactions)	Bonferroni pairwise comparisons	F-value	*p*-value
	deviant > standard		*long, Pz: p < 0.001*
	short > long		*short, Pz: p < 0.001*
			standard, Fz: p < 0.001
			standard, Cz: p < 0.001
			standard, Pz: p = 0.002
			deviant, Pz: p = 0.002
P300 latency			
Electrode		F (2,56)= 6.064	*p = 0.004*
	Fz > Cz		*p < 0.001*
	Fz < Pz		*p = 0.016*
	Cz < Pz		*p < 0.001*
Frequency		F (1,28)= 4.446	*p* = 0.044
Length		F (1,28)= 6.995	*p* = 0.013
Group		F (1,28)= 6.128	*p* = 0.020
Frequency × length		F (2,56)= 7.382	*p* = 0.011
	deviant > standard		*long: p = 0.006*
	long > short		*standard: p = 0.002*
Electrode × length × group		F (2,56)= 6.128	*p* = 0.020
	Cz > Fz		*ALS, long: p = 0.014*
	short > long		*controls, Cz: p = 0.036*
			ALS, Fz: p = 0.013
RON amplitude			
Electrode		F (2,56) = 49.976	*p* = 0.001
	Fz > Cz		*p < 0.001*
			p < 0.001
Length		F (1,28)= 30.045	*p* < 0.001
Group		F (1,28)= 4.554	*p* = 0.042
Electrode × frequency		F (2,56)= 13.202	*p* < 0.001
	Fz > Cz		*deviant, p = 0.004*
	Fz > Pz		*deviant, p < 0.001*
	Cz > Pz		*deviant, p < 0.000*
	deviant > frequent		*Fz: p = 0.011*
			Cz: p = 0.007
Length × group		F (2,56)= 13.202	*p* < 0.001
	Controls > ALS		*long: p = 0.005*
RON latency			
Frequency x length		F (2,56)= 11.184	*p* = 0.002
	long > short		*standard, p < 0.002*
Electrode x frequency x length		F (2,56)= 3.750	*p* = 0.029
	deviant > frequent		*short, Fz: p = 0.008*
	long > short		*deviant, Fz: p = 0.005*
	long > short		*standard, Fz: p = 0.008*
	long > short		*standard, Pz: p = 0.012*

Table 11. Significant results of ERPs statistical analysis (ANOVA and pairwise comparisons).

Many significant correlations were found. However, the majority of the correlations concerned amplitudes and latencies of deviant stimuli (both long and short). Significant correlations are reported in Table 12.

Figure 16 illustrates the grand average ERPs of ALS patients and controls.

Neuropsychological test	ERPs	Spearman's correlation coefficients, *p* value
Coloured Progressive Matrices	N2 long deviant amplitude at Fz	r = −0.584, *p* = 0.028
	P300 short deviant amplitude at Fz	r = −0.567, r = 0.034
	P300 long deviant latency at Cz	r = −0.784, *p* = 0.001
	RON short deviant amplitude at Fz	r = −0.611, *p* = 0.020
	RON long deviant amplitude at Cz	r = −0.833, *p* < 0.000
	RON short deviant amplitude at Cz	r = −0.551, *p* = 0.041
M-WCST category	N200 short deviant latency at Fz	r = −0.756, *p* = 0.002
	N200 long frequent latency at Pz	r = −0.549, *p* = 0.042
M-WCST errors	N200 short deviant amplitude at Fz	r = 0.633, *p* = 0.015
	N200 short deviant amplitude at Cz	r = 0.624, r = 0.017
	N200 long deviant latency at Pz	r = 0.686, *p* = 0.007
	P300 short frequent latency at Cz	r = −0.583, *p* = 0.029
	RON long deviant latency at Pz	r = 0.533, *p* = 0.050
Face Recognition	N200 short deviant latency at Fz	r = 0.681, *p* = 0.010
	N200 short deviant latency at Fz	r = 0.681, *p* = 0.010
	N200 short frequent latency at Cz	r = −0.643, *p* = 0.018
	RON long deviant latency at Cz	r = −0.557, *p* = 0.048
Object Recognition	P300 short deviant latency at Fz	r = −0.765, *p* = 0.002
	P300 short deviant amplitude at Pz	r = −0.665, *p* = 0.013
Verbal Comprehension	RON long deviant amplitude at Cz	r = −0.599, *p* = 0.018

Table 12. Significant correlations between neuropsychological scores and ERPs parameters.

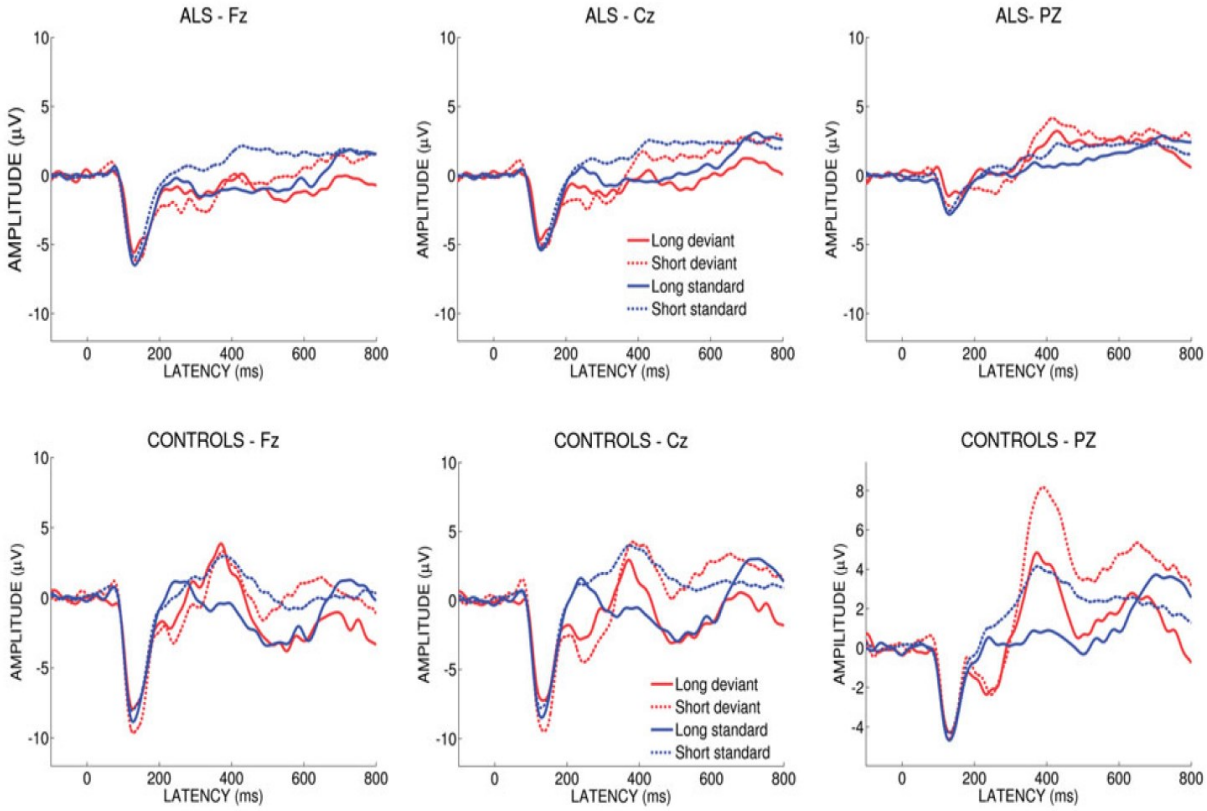

Figure 16. Grand average ERPs recorded in response to short deviant, long deviant, short standard and long standard tones of ALS patients and controls at Fz, Cz and Pz.

5.4. Discussion

Neuropsychological results highlighted that ALS patients were compromised in tasks that require executive (M-WCST and Raven Colored Progressive Matrices) and amnesic (figures and faces recognition) resources. These results are in line with those reported in the previous studies, corroborating the hypothesis of an extra-motor frontotemporal involvement in ALS (50,55,94,198). Moreover, the poor performance in neuropsychological test cannot be attributable to motor deficit because tests used in this study were not time limited and did not require overt responses (except for the eyes movement). However, some caution is necessary: the cognitive deficits are significant but not dramatic.

The electrophysiological evaluation revealed also some significant data consistent with cognitive changes in ALS. The ERPs paradigm implied that the participants responded only to the long deviant tone ('target') and ignore all the other stimuli, including the short deviant tone ('distractor'). This modified version of the 'oddball' paradigm allowed us to specifically assess selective attention, because the participant had to focus attention on the 'target' stimulus and inhibit the response to the 'distractor' stimulus.

The N200 analysis showed that in healthy participants the N200 was larger for deviant than standard stimuli, while in ALS patients' amplitude was not sensitive to deviance. In ALS, thus, the amplitude of the N200 does not seem to differ between the standard and deviant stimuli, indicating a defective or incomplete analysis of auditory stimulus features in sensory memory. The N200 latency showed a delay in ALS patients suggesting a slowing in change/deviation detection probably attributable to a dysfunction of the responsible prefrontal network (54,110,111). Furthermore, the comparison between groups showed that in ALS the N200 latency was delayed for standard stimuli while in the control group the N200 was longer for the deviant stimuli, as expected. These data suggest an anomalous

processing time of standard vs. deviant stimuli, indicating a defective detection of perceptual novelty or attentional deviation in ALS patients. In summary, the N200 parameters in ALS patients could indicate a similar processing for deviant and standard tones, suggesting an alteration of the central mechanism of change detection. The N200 should be slower and larger to deviant stimuli that require deeper processing both in terms of amount of resources dedicated and prolonged processing time compared to standard stimuli (100). Moreover, the analysis of N200 did not reveal any effect of stimulus length. This finding could be consistent with the interpretations discussed above. If the electrophysiological response of ALS patients could not distinguish significantly between deviant and frequent stimuli, they could hardly discriminate between long and short deviant, since this operation requires a higher level of attentional control. A measure of deviance/change detection is given also by the Mismatch Negativity (MMN), a component that is believed to reflect the mismatch between the representations of the current stimulus with the predictive model (191). Probably, the MMN expresses an implicit and automatic bottom-up process whereas the N200 reflects a voluntary and conscious processing when the participants selectively attend to deviations, as in this case (199).

The analysis of the P300 component highlighted a reduction of the amplitude in ALS patients compared to healthy controls, in agreement with previous authors (61,93). Moreover, an interesting effect of the stimulus length was found. In ALS patients the amplitude of the P300 for short deviant stimuli was significantly reduced compared to the control group, suggesting that in ALS patients the distracting effect of the stimulus distractor is reduced or absent. The latency of the P300 was also delayed in ALS patients compared to healthy controls, especially at the central-parietal areas, indicating a substantial slowing of distractor stimulus processing (61,110). Thus, data concerning the P300 in ALS also seem to indicate an

impairment of the mechanisms of detection of novel stimuli associated with prefrontal functioning. In particular, the alteration of P300 parameters in ALS could suggest a reduced amount of attentional resources available and a slowing of processing speed. ALS patients showed reduced amplitude of the RON to all types of stimuli compared to healthy controls, suggesting an alteration in the prefrontal mechanism of re-orientation of the attentional resources to the relevant information. It could be hypothesized that ALS patients had difficulty in maintaining controlled attentional processes for an extended period (200). The RON elicited by long stimuli was larger in the control group compared to the patients group. Although it did not reveal a specific effect of the distractor stimulus (long deviant), this finding may suggest that in controls the long stimuli were more distracting than in ALS patients and required a stronger re-orientation of the attentional resources.

The RON, moreover, was larger for deviant than frequent stimuli and larger for long tones compared to short tones, in both groups. In the same way, the RON latency was prolonged for the long standard compared to short standard stimuli and for short deviant than short standard stimuli. Since this component should reflect the process of re-allocating of attentional resources to the relevant characteristics of the task, we could hypothesize that the significant effect of 'length' and 'deviance' on both RON amplitude and latency may reflect the disengagement of increased attentional resources allocated toward the long and deviant stimuli (169). Significant correlations between neuropsychological test and ERPs mainly concerned amplitudes and latencies of deviant stimuli. This result could indicate that the processing of deviant stimuli required a greater recruiting of cognitive resources than frequent stimuli, with a probable involvement of the prefrontal area. Thus, the correlations between deviant stimulus-locked ERPs and neuropsychological scores could be interpreted as the expression of a common involvement of the prefrontal network. Significant

correlations between neuropsychological and ERPs data in ALS were already reported by previous studies (54,186). In summary, the electrophysiological results in ALS patients suggest alterations at different stages of selective attention processing: a) detection of change (N200); b) focusing (P300); and c) reorientation of attention (RON). These findings may originate from an alteration of central mechanisms of change detection and a consequent reduction of the allocation and re-orientation of attentional resources in ALS. An alternative interpretation is that the changes of ERPs could arise from a general slowing or reduction of neural processing efficiency in the same system.

However, because the electrophysiological data were measured and analyzed only at the three midline electrodes, these results could not reflect the complex processing mechanisms involved in performing the task. The modifications of ERPs in ALS could be the expression of a lower processing efficiency that takes a longer time to work and shows reduced neural activity signatures.

Moreover, it could be argued that, as the study includes a motor reaction, changes of motor networks in ALS could affect cognitive ERPs in ALS patients. Although a study of Gu et al. (2013) (201) demonstrated that there was no significant difference in movement related cortical potential between healthy participants and ALS patients, we cannot definitely conclude that the results obtained in this study are exclusively attributable to a cognitive impairment (201).

Another issue is that the long tones (500 ms) effects of stimulus length on the MMN, finding a modulation of the ERP reflecting sound duration (202). In this study no effect of stimulus duration was found for the N200. Concerning the P300, the latency was prolonged and the amplitude was larger for short than long stimuli, indicating a possible modulatory effect of stimulus length. In contrast, we found significant differences between groups in the ERPs

elicited by long and short stimuli. Thus, although the present procedure does not allow complete separation of the physical (duration) and attentional (pitch) factors, we suggest that the electrophysiological responses reported here in ALS are the expression of altered psychophysiological functioning.

In conclusion, altogether the data obtained in this study support the hypothesis that ALS is a multisystem disease with extra-motor involvement of cognitive functions and provides further evidence that the ERPs represent an effective tool for assessing cognitive functions of ALS patients requiring only minimal motor responses (186).

6. BRAIN COMPUTER INTERFACE FOR COMMUNICATION IN THE LATE STAGE OF AMYOTROPHIC LATERAL SCLEROSIS

6.1. Introduction

Communication with patients suffering from the completely locked-in syndrome and other forms of paralysis is an enormous challenge. Brain computer interfaces (BCIs) have been seen as a promising solution to overcome these challenges. More concretely, BCIs represent a promising strategy to establish communication with severely paralyzed ALS patients and it seems optimal for these patients, as it does not require of muscle control. BCI research currently uses invasive (implanted electrodes on or in the neocortex) and noninvasive strategies (including electroencephalography (EEG), magnetoencephalography (MEG), functional magnetic resonance imaging (fMRI), and functional near-infrared spectroscopy (fNIRS)) to record brain activity and decode user's intention and transform it into commands to control simple word processing programs. Non-invasive methods are currently used in for people with disabilities (such as those with ALS) (37,203).

Brain-Computer-Interface for patients in the LIS and CLIS of ALS were developed and tested extensively since the first publication of Birbaumer et al., (1999) (129) in Nature in which they reported two cases of LIS patients suffering from ALS that learned how to control their brain activity (more concretely variations in their SCPs) in a way accurate enough to control an electronic spelling device and allow the patient to communicate with his/her environment.

BCIs have been proved successfully for communication purposes. While healthy participants and ALS patients up to the LIS, showed accurate BCI control and communication (151), completely paralyzed ALS patients in CLIS so far did not learn sufficient BCI control for brain communication (147).

However, a single case report by Gallegos-Ayala et al., 2014 (137) reported that a CLIS patient with ALS could achieve BCI-control and "yes" - "no" communication to simple questions with known positive or negative answers. In their study they used Functional Near Infrared Spectroscopy (*f*NIRS) to measure and classify cortical oxygenation and deoxygenation changes following the known questions. The BCI methodology used in this report was completely new from the previous BCI-procedures: a more "reflexive" mode based on learning principles of classical conditioning to simple questions was used to train the classifier separating "yes" and "no" brain answers silently imagined by the patient using the neuroimaging technique fNIRS instead of the classically used EEG.

The aim of this project was to test the feasibility of an fNIRS-based brain-computer-interface (BCI)-based communication approach. We conclude that this approach is safe, reliable and allowed communication for patients in the completely locked-in state.

6.2. Materials and Methods

The authorized representatives of the patients gave written informed consent according to the Declaration of Helsinki prior to their participation in the study. The study was approved by the Internal Review Board of the Medical Faculty of the University of Tubingen.

6.2.1. Patients

Patient F, female 68

Patient 1 was diagnosed with bulbar sporadic ALS in May 2007, as locked-in in 2009, and as completely locked-in May 2010. The patient scored 0/48 in the ALS-FRS-R (170). The diagnostic of the disease was based on the criteria of experienced neurologists. The patient was under riluzole treatment. She was tracheostomized and under artificial ventilation since September 2007 and fed through a percutaneous endoscopic gastrostomy tube since October 2007. Her cognitive functions were assessed in 2011 by de Massari and colleagues

(204) using an extensive neurophysiological examination based on ERPs as described in Neuman and Kotchoubey (72) and suggested partially intact cognitive functioning. Patient F receives home assistant. She lost all the communication channels in 2010 (eyes and muscles movements) and she was unable to control any assistive communication device (137).

Patient B, male 61

Patient B was diagnosed with spinal and non-familiar ALS in May 2011. He has been artificially ventilated since August 2011 and fed through a percutaneous endoscopic gastrostomy tube since October 2011. The patient was under riluzole treatment. The patient received home assistance. He started communicating with a speech device in his throat from December 2011 which finally failed and he started using an eye-tracking system (MyTobii) in April 2012. He was able to communicate with the eye-tracking device until December 2013. After that, any attempt to communicate with their relatives was only based in eye movements (movement to the right to answer "yes" and to left to answer "no") but the response was not always accurate. Since August 2014 no communications were possible. Patient died in November 2015 after almost one year participating regularly in our study.

6.2.2. Instrumentation and data acquisition

We used a continuous wave (CW) based fNIRS system, NIRSPORT (NIRX), which performs dual-wavelength (760 nm & 850 nm). fNIRS was sampled at 6.25 Hz. The NIRS optodes were placed on the fronto- central regions of the scalp (see Figure 17).

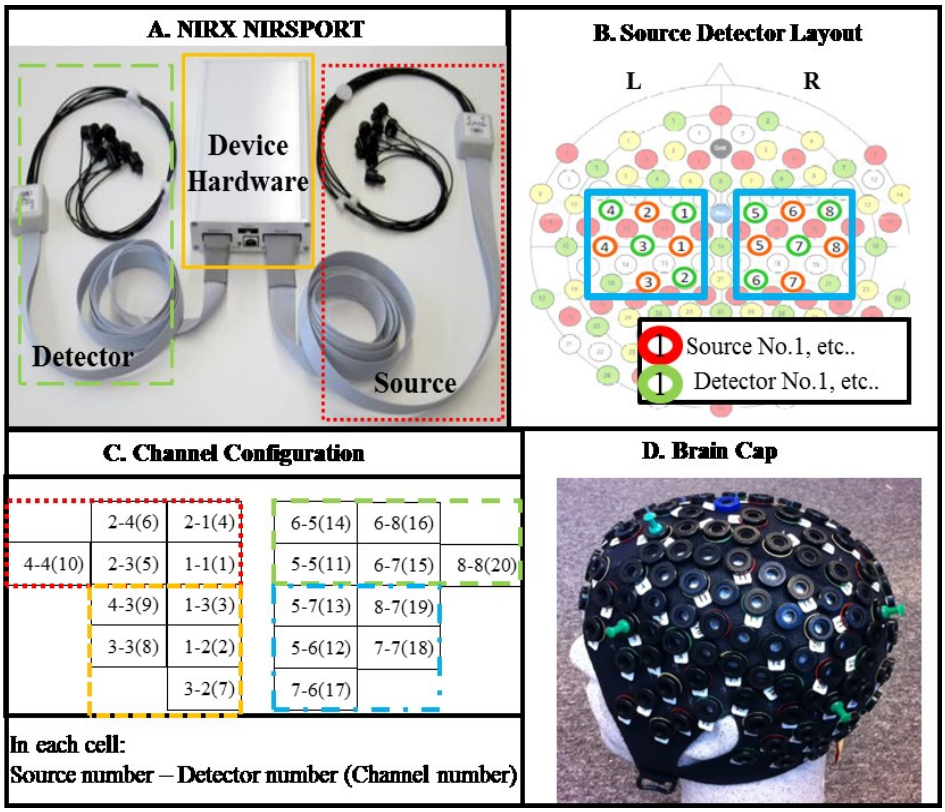

Figure 17. 16 A: The continuous wave based portable NIRX NIRSport Instrument used for brain computer interface. The device consists of 8 near infrared light sources, highlighted in red, and 8 detectors, highlighted in green and the NIRS data acquisition hardware is highlighted in yellow. 16 B: Image of the placement of sources and detectors (optodes) on the fronto-central region of the scalp (blue). 4 sources, highlighted in red, and 4 detectors, highlighted in green, were placed on each hemisphere to form channel. 16 C: Depicts the translation of sources and detectors placed on the motor region of the brain into channels, each cell contains a source number and detector number which combines to form a channel. The channels placed on the motor region were divided into 4 different regions; namely LF – Left front, RF – Right front, LB – Left back, and RB – Right back each consisting of 5 channels. 16D: The cap to place optodes and EEG electrodes on the scalp.

During the BCI sessions EEG was recorded with a multi-channel EEG amplifier (Brain Amp DC, Brain Products GmbH, Germany) from ten active electrodes (actiCAP, Brain Products GmbH, Germany) mounted on the same cap shown in Figure 16D. Electroencephalogram (EEG) was recorded using scalp electrodes (FC5, FC1, FC6, CP5, CP1 and CP6) positioned according to the International 10–10 System. All electrodes were referenced to the left mastoid and grounded at FPz. Vertical and horizontal electro-oculogram (EOG) was recorded from four electrodes placed below and above the left eye and laterally to and above the outer canthus of both eyes. Electrode impedances were kept below 10 kΩ. EEG was band-pass filtered

from 0.1 Hz up to 30 Hz and sampled at 500 Hz. The EOG was filtered with different filters (3.5 Hz, 10 Hz, and 30 Hz) but none of these filters led to significant differences of neurophysiological patterns related neither to the ocular activity nor to significant differences of their Support Vector Machine (SVM) classification. No eye movements were visually detected in any patient at any time. During all BCI sessions the spontaneous EEG was visually controlled by one of the authors to avoid longer periods of slow wave sleep and the BCI was just initiated if it the EEG was free of high amplitude slow activity below 3.5Hz.

6.2.3. Experimental Procedures

An auditory paradigm was employed to: a) train patients on questions with known answers, termed as “training sessions” b) give feedback on questions with known answers, termed as “feedback sessions" and c) answer open questions, termed as “open question sessions”. Known questions were personal questions based on patient’s life (i.e., “your mother’s name is Chiara”). Open questions are general questions related to quality of life of the patient or questions that the caretakers wanted to know (i.e., “do you have any pain?”). For every known question with “yes” response a semantically related question with a “no” response was made (i.e., “you are a woman” and “you are a man”). Each session consisted in 20 randomized personal questions (with known answers) consisting of 10 true and 10 false sentences. Patients were instructed to answer the questions by thinking “yes, yes, …” or “no, no, …” for all the duration of the inter stimulus interval (ISI), until they heard the next sentence. The experimental procedure is shown in Figure 18 and Figure 19.

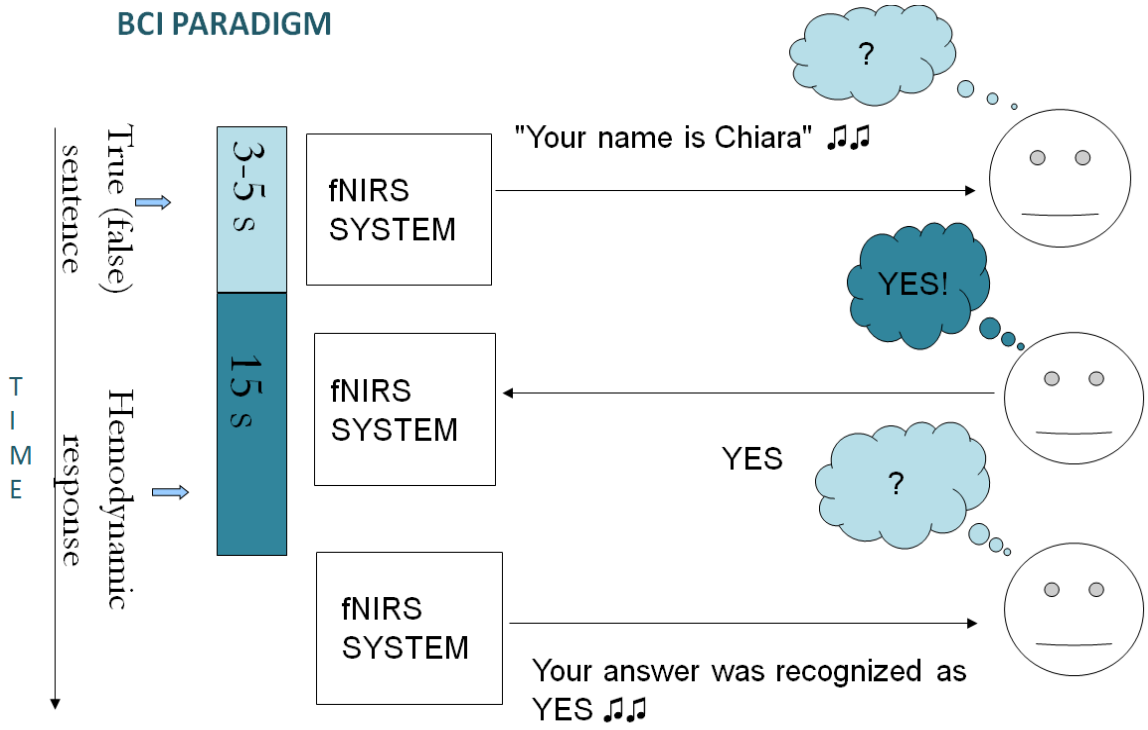

Figure 18. The auditory brain computer interface paradigm used in our ALS patients during the feedback session.

After the end of each training session the fNIRS feature necessary to differentiate between "yes" and "no" answers was extracted and classified during the ISI. During the online feedback sessions the patients were presented the same sentences as described above but now auditory feedback was provided to the patients at the end of the 15 sec answering period saying whether their answer was recognized as "yes" or as a "no" (see Figure 18). The validity of answers to open questions can only be assessed by the stability over time and the internal validity between questions (i.e. the concordance between the answer to "I love to live" with the answer to "I rarely feel sad"). Table 13 shows the details of the experimental training performed by each of our patients. Figure 19 shows a diagram of the BCI system used in this experiment.

Patient / Sessions	Training Sessions	Feedback Sessions	Open Questions Sessions
Patient F	51	7	2
Patient B	40	4	2

Table 13. Lists the total number of training, feedback, open question sessions performed by each patient.

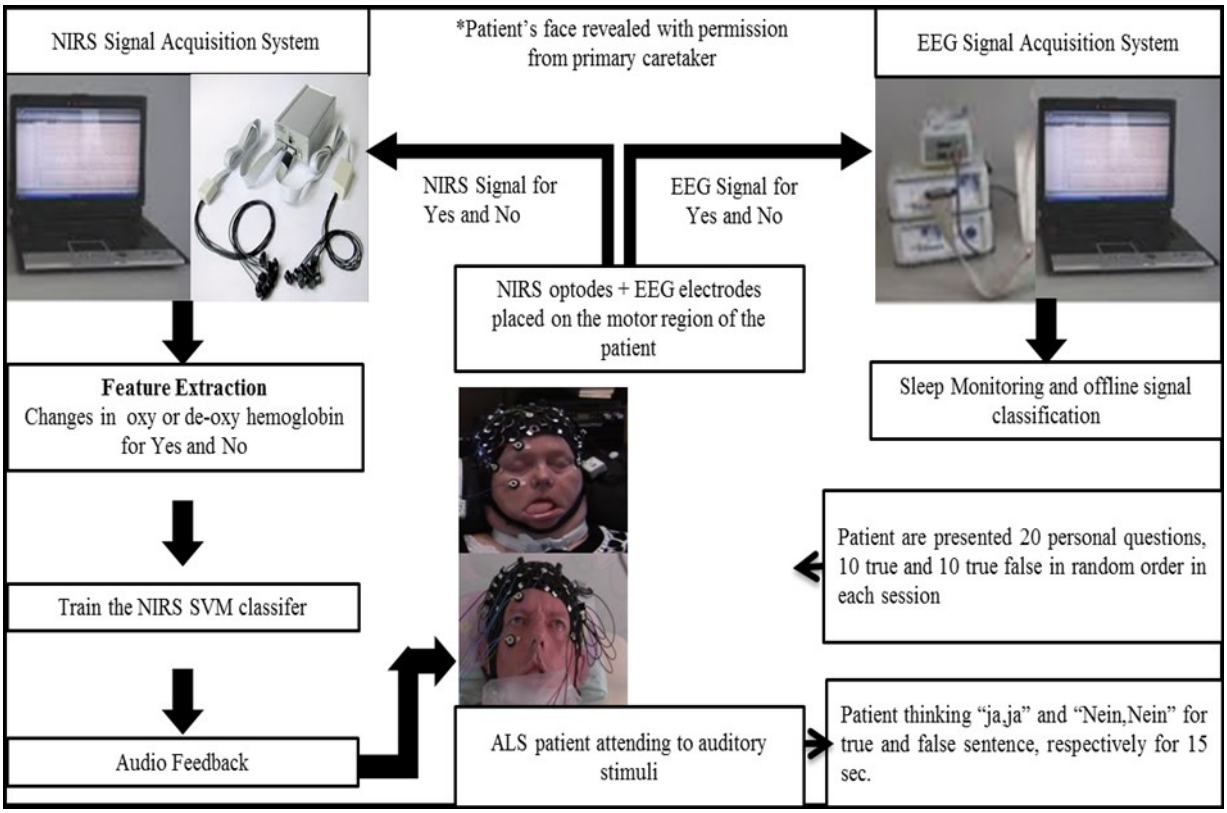

Figure 19. The setup and flow diagram of the brain computer interface for communication in ALS patients.

6.2.4 Data analysis

The fNIRS data acquired online throughout all the sessions was normalized. NIRS was filtered using a bandpass filter of 0.0016 Hz – 0.3 Hz and processed using Modified Beer Lambert's law, as described in Cope et al., (205) and Chaudhary et al., (206), to calculate the relative change in concentration of oxy (HbO2) and deoxy hemoglobin (HbR).

The relative change in HbO2 (with respect to the baseline), that was calculated online during each training session was used to train a support vector machine (SVM) classifier model to check the cross validation classification accuracy. In this study only the relative change in HbO2 was used as because after the end of training sessions it was observed that O2Hb provided stable and higher cross validation classification accuracy.

The offline classification procedure used the mean of relative change in HbO2 across each channel as input feature to train a 5-fold linear support vector machine (SVM) classifier. The SVM model interpolates the data corresponding to true and false sentences' ISI in a two-dimensional space such that the two categories are divided by a hyperplane and the gap between them is as wide as possible. Firstly a model space is determined and the input feature, extracted from the recorded and processed fNIRS signal, i.e., the relative change in HbO2, is mapped onto the model space to determine the side of the hyperplane the input feature falls on. For the fNIRS signal the mean of the relative change in HbO2 across all the channels were used as input feature to map onto model space, while for EEG and EOG signals common spatial pattern was used for feature extraction. The relative change in HbO2, EEG and EOG data acquired during BCI sessions from our patients were processed off-line separately for each patient to determine:

1) The statistical difference in the particular physiological signal (HbO2, EEG and EOG) during the ISI of true (yes) and false (no) sentences.

2) Classification accuracy, using SVM as described above, of HbO2, EEG and EOG signals across each session between the true and false sentences' ISI.

Statistical analyses of the results were carried out using Matlab (The Mathworks Inc., USA). The normal distribution of the data was assessed using the Kolmogorov-Smirnov test. The assumption of normality was not violated in any of the data. Parametric t-tests for

independent samples were used to compare true and false sentences (using the averaged ISI). The t-test analysis was performed across all the channels in a session and for all the sessions of acquired HbO2, EEG and EOG signals. Furthermore, t-test was also performed between the ISI of all the 10 true sentences and all the 10 false sentences across different channels in a session averaged over many sessions varying slightly between patients. The significance level for all statistics was set to 0.05.

6.3. Results

Results of the t-test ($p < 0.05$) performed between the average of all the true and false sentences' ISI using the relative change in HbO2, EEG and EOG from all the training sessions, are summarized in Table 14. A significant difference was found between the true and false sentences' ISI across all patients. However, no significant differences were found in the EEG and EOG data across all the training sessions showed no significant between the true and false sentences' ISI across each patient.

			Patient F	Patient B
NIRS	Number of Sessions averaged		51	40
	Number of Channels averaged		20	20
	T value		4.01	3.67
	P value		0.0001	0.0004
EEG	Number of Sessions averaged		51	40
	Number of Channels averaged		6	6
	T value		0.97	0.83
	P value		0.33	0.40
EOG	Number of Sessions averaged		51	40
	T value	Horizontal EOG	.61	1.01
		Vertical EOG	.59	1.47
	P value	Horizontal EOG	0.54	0.31
		Vertical EOG	0.55	0.14

Table 14. Lists the total number of sessions averaged and degree of freedom used to perform t test analysis between the true and false sentence ISI corresponding to NIRS, EEG and EOG signal.

The relative change in HbO2 in five channels over the fronto-central brain region of our patients during the true and false sentence ISI is shown in Figure 20. The figure illustrates that the shape of the change in HbO2 during true sentence ISI is significantly different from false sentence ISI. Figure 20 also illustrates that the shape of the change in HbO2 during a true or a false sentence ISI is not consistent between the patients even though within each patient the shape of the change in HbO2 is stable.

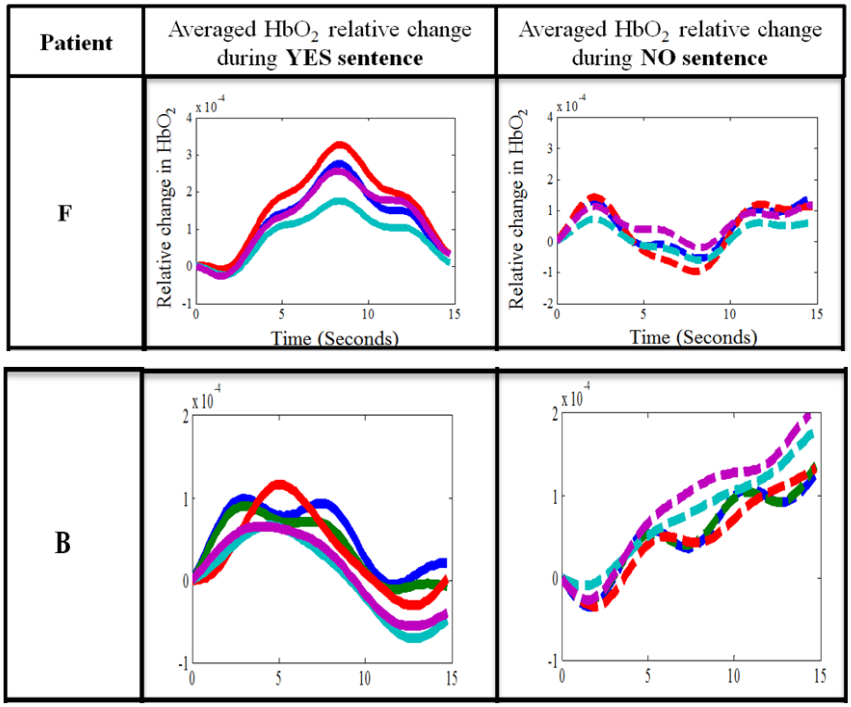

Figure 20. The averaged relative change in HbO2 across 5 out of 20 channels corresponding to YES and NO sentence interstimuli interval (ISI) in Patient F and B. In each subplot; five different colored trace corresponds to relative change in HbO2 across five different channel, x-axis is time range in seconds and y-axis is the relative change in the HbO2.

Figure 21 illustrates the power spectrum density (PSD) of EEG oscillations, in the frequency band 0 to 10 Hz, during the true and false sentence ISI from both patients. Only frequencies between 0 and 10 Hz were used for classification and statistical testing because in ALS patients in CLIS frequencies above 10 Hz are extremely rare10. The PSD of EEG signal shows that there was no significant difference between the true and false sentences ISI across all

patients. The shapes of the relative change in HbO2 and EOG during ISI corresponding to true and false sentences from all the sessions are also plotted in Figure 21. It illustrates that there was no significant difference in the eye movements between the true and false sentences ISI for all patients, confirmed by the t-test.

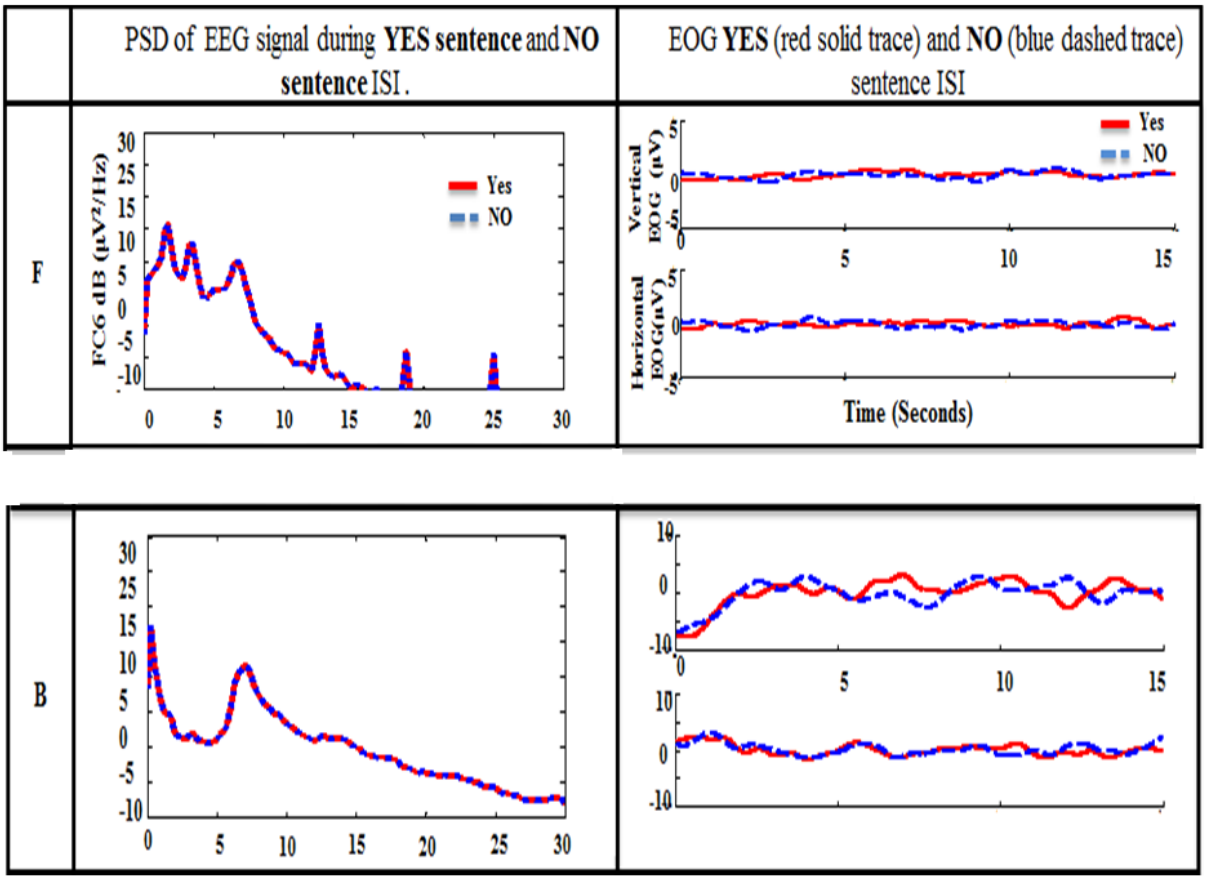

Figure 21. Power spectrum of the EEG and the EOG signal.

Figures 22 and 23 depict the SVM classification across all the sessions using the change in (a) HbO2, (b) EEG and (c) EOG in Patient F and B respectively. A 65% cut off was used to define whether the classification accuracy was above or below the acceptable level (chance level is 50%). The SVM results illustrate that highest classification accuracy was achieved using the change in HbO2 for which more than 75% of the sessions yielded greater than 65% classification accuracy for all the patients with an average classification accuracy of 70%.

While the SVM classification accuracy obtained using EEG and EOG data only few sessions yielded greater than 65% classification accuracy across all patients.

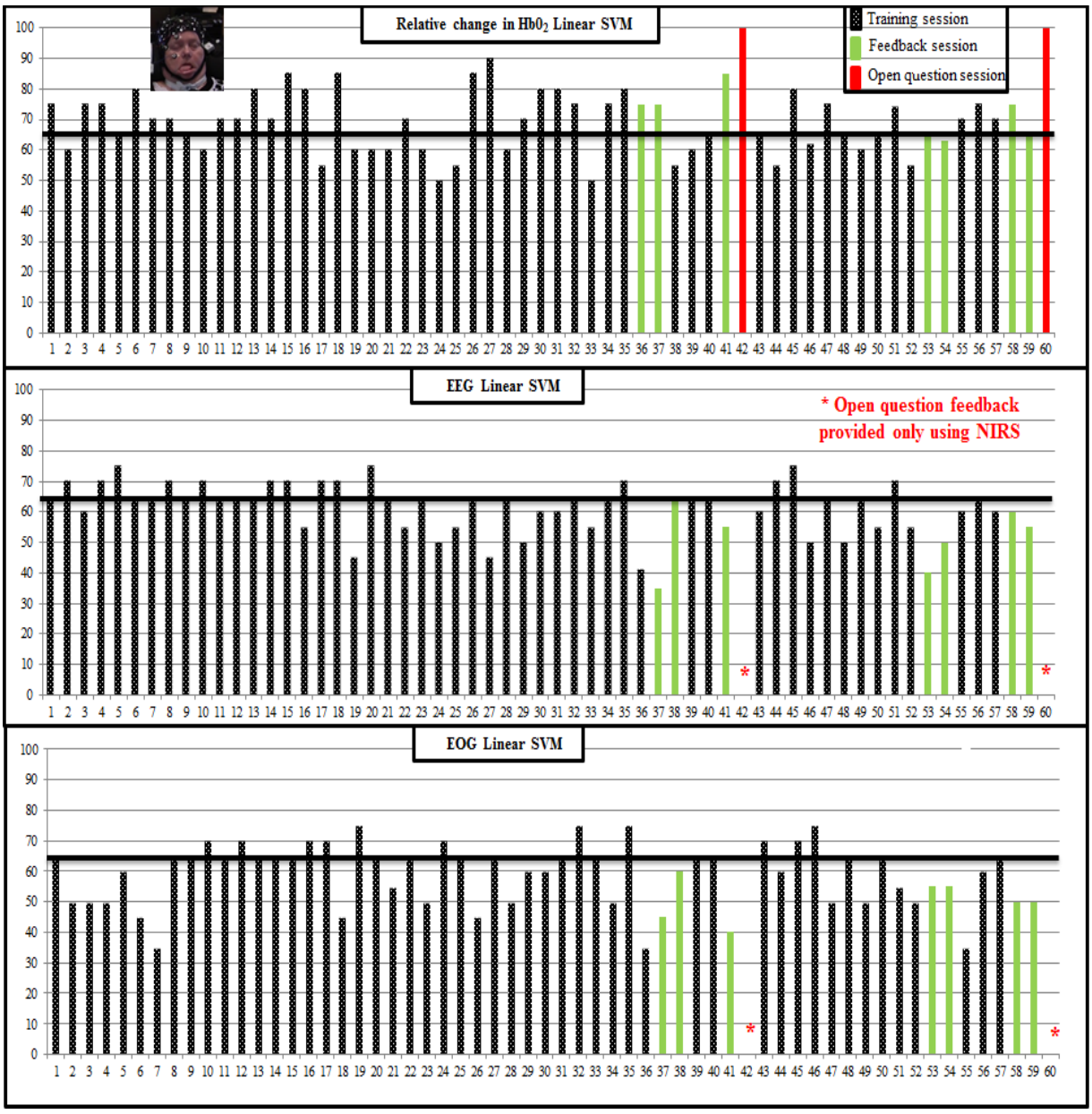

Figure 22. *Patient F*: Linear support vector machine (SVM) classification accuracy across all sessions 1) Training (bar plot in spotted black) 2) Feedback (bar plot in solid green) and 3) Open question (bar plot in solid red) obtained using a) Relative change in HbO2 b) EEG and c) EOG data. In each histogram plot x-axis is the number of sessions and y-axis is classification accuracy. The black horizontal line represent the 65% classification accuracy.

Figure 23. *Patient B*: The description of Figure is same as described in Figure 15.

Classification results using fNIRS for open question in patients F and B using the criteria for correctness described above in the Methods section ranged between 80-90% “correct”.

6.4. Discussion

All our 2 patients in the CLIS communicated with fronto-cortical oxygenation based BCI with an average correct response rate of 70% over a period of several weeks. Correct response rate for open questions as estimated exceeded even 80%. Patient F and B answered open questions containing quality of life estimation (See Supplementary Material) with a yes/no answers indicate a positive attitude towards the present situation and life in general as found by other groups (22) in larger samples of ALS patients. Correct classification of mentally “yes” and “no” responses through fNIRS surpassed classification of EEG oscillations from 0-10 Hz (EEG frequencies in advanced ALS rarely show high frequencies) and vertical and horizontal EOG classification. However, despite the absence of reliable eye communication in all patients as the inclusion criteria in the study, EOG classification was at some sessions above chance despite the inability of the social environment to perceive them.

The results on two CLIS patients reported here allow the following conclusions:

a) Even after extended CLIS in ALS across months and years, reliable communication is possible with NIRS-BCI using questions requiring a covert (cognitive) affirmative (“yes”) or negative (“no”) answer. Moreover, we can conclude that fNIRS seems to provide better classification of patients’ answers compared to oscillatory EEG responses. We can propose that metabolic (vascular) brain changes permit superior brain-self-control because of an existing feedback pathway between the vascular system in the brain and the central nervous system, as it was previously proposed (207).

b) Co-recording of NIRS and EEG oscillations from multiple sites might be important in CLIS in order to monitor and eventually modify non-favorable vigilance changes to improve NIRS classification.

Our results demonstrate that this novel approach of brain -communication is safe, reliable and allowed so far the best communication possible for patients in completely locked-in state.

DISCUSSION AND CONCLUSIONS

This thesis focused on the assessment of cognitive processing and brain communication in patients suffering from ALS. Two main lines have been addressed along the thesis. Firstly, the application of two novel approaches to assess cognitive functions in ALS (study one and two). Secondly, the implementation of a functioning BCI-system for the completely paralyzed locked-in patients due to ALS (CLIS), allowing brain communication based on classical conditioning and the combined use of Functional Near-Infrared-Spectroscopy (fNIRS) and EEG.

The first study has shown the feasibility of a novel approach, combining vibro-tactile P300s and motor-independent neuropsychological tests for assessing cognitive functions in ALS. The results of the neuropsychological and P300 assessment showed a mild impairment in the attentional network with reduced P300 amplitudes in ALS group, and mild dysfunctions in language processing reflected in the performance in oral comprehension tests.

The second study reported lower performance in neuropsychological tests related to the executive system. Moreover, reduced amplitudes and delayed latencies of N200, P300, and RON were found in ALS compared to controls. These results could be attributable to both an alteration in change detection resulting in a reduction of the allocation and re-orientation of attentional resources or a general slowing or reduction of neural processing efficiency in the same system.

Taken together the results of the two first studies we can conclude that ALS is a heterogeneous and complex disease, which affects extra-motor involvement of cognitive functions and cognitive processing at different levels. Moreover these two studies provided further evidence that (vibro-tactile and auditory) ERPs could represent a new and effective tool for assessing cognitive and attentional processing in severely paralyzed ALS patients.

In the third study, we showed the feasibility of an fNIRS-based BCI system for communication in the CLIS, which was reflected by higher accuracies in BCI performance of our two CLIS patients (an average correct response rate of 70% over a period of several weeks). We determined that even after extended CLIS in ALS across months and years, reliable communication using questions that require a mental (imagined) affirmative ("yes") or a negative-rejecting ("no") answer is possible with fNIRS-BCI. Moreover we observed that fNIRS technology seems to provide better classification of patients' answers than oscillatory EEG responses. In conclusion, we strongly support the use of an fNIRS-based BCI to establish communication with the CLIS.

The novelty of studies one and two is that we propose a neuropsychological paradigm that does not require overt motor/verbal skills to assess cognitive functions in severely paralyzed patients. This paradigm has first been described by Neumann and Kotchoubey (72). However, they assessed a wide range of severely paralyzed patients, including individuals with significant speech disabilities following brain damage (vegetative state, minimally conscious state, aphasic patients etc.). In contrast, we decided to implement this innovative paradigm to assess cognitive processing in patients with ALS. The rationale for this choice was that even though some previous studies attempted to integrate neuropsychological tests to assess the effects of ALS (152), these tests require minimal motor and/or speech skills and are therefore not useful in severely paralyzed patients. Thus, our approach responds to the need for cognitive evaluation of ALS patients also in later stages of the disease (except CLIS).

The outcome of the neuropsychological assessment of the two first studies is in line with the previous literature, reporting deficits in language and executive systems as the most affected areas in ALS. From a classical point of view, executive dysfunctions have been seen as the

key feature of cognitive impairment in ALS. However, a growing body of evidence suggests that language deficits in ALS are more common than previously thought. They may, in fact, be just as frequent as executive dysfunctions (70,71).

Another novelty refers to the modalities that we used to assess cognitive and attentional functioning in ALS using ERPs. The first one consisted of a vibro-tactile oddball paradigm that aims at assessing attentional and cognitive processing in ALS. Previous studies suggest that the somato-sensory system elicits robust ERPs (142,166). However, they were tested in different populations (i.e., healthy subjects and spinal cord injured patients). In our study, we used a novel technology consisting of a mechanical arm that was designed and implemented by our group (118). This arm provided a vibration stimulus in the end-effector of the device, which the patients had to grasp. The reason for using the somato-sensory pathway to elicit ERPs was based on the observation that the somato-sensory pathways seems to be intact until the terminal end of the disease. We further proposed that the somato-sensory P300 could reflect cognitive changes in ALS.

The second study used an ERP approach using a distraction paradigm to investigate the neural basis of selective attention in ALS. We introduced a novel modality compared to classical ERPs oddball paradigms (54,93,112) by using 4 types of stimuli (long deviant, short deviant, long frequent and short frequent). The implementation of this modified oddball allowed us to assess selective attention more accurately, as this modified version required more demanding attentional mechanisms due to the complexity of the task.

However, the most important contribution of this work lies in the combination of the aforementioned motor-independent neuropsychological tests with the novel ERP assessments, which allows for an exhaustive assessment of cognitive processing in ALS.

In contrast to the assessment of cognitive functions in the first two studies, the great impact of our third study lies on the development of first reliable BCI system to reestablish communication in CLIS patients following ALS. Previous attempts have been shown that while brain-computer interfaces enabled LIS patients to communicate successfully (129), all attempts to communicate with CLIS patients have failed regardless of the method that was employed (e.g., invasive and non-invasive BCIs or any other assistive technology). This lack of communication in CLIS left these patients in an unbearable "silence". Prof. Birbaumer's group at the University of Tübingen has been working on the development of different BCI systems during the past years with the intention to allow communication in CLIS. The first successful attempts to reestablish communication with a CLIS patient have been reported in a study by de Massari et al. (151), using an EEG-based BCI approach. Eventhough an acceptable level of BCI performance accuracy was found in their CLIS patient in single sessions, this accuracy was not be maintained across sessions. Gallegos et al. (137) introduced a substantial change in the BCI strategy by introducing the hemodynamic brain response (recorded by fNIRS) in combination with the semantical conditioning to 'yes' and 'no' questions. With this novel methodology they achieved an overall performance of 76.30% in one CLIS patient.

A further innovation was to combine the recording of hemodynamic and electrocortical activity (using fNIRS and EEG respectively) in a sample of two CLIS patients using the "yes" and "no" classical conditioning to known questions. Both patients achieved a higher accuracy than the 70% that was found before using only fNIRS. However, the off-line classification of the EEG-based BCI data did not show any good performance of our CLIS patients. This suggests a greater efficacy of using the hemodynamic response to communicate in the late stage of the disease. However, further investigation needs to be carried out to better

understand whether the low performance in the EEG based BCI approach is a consequence of lower sensitivity of the EEG signal, whether EEG doesn't differentiate sufficiently between 'yes' or 'no' answers, or whether the complexity of the signal demands better classifiers.

We can conclude that assessment of cognitive functions in the course of the ALS disease is extremely important, as the patients are constantly facing decisions related to the choice of clinical treatment and end-of-life management (i.e., nutritional and respiratory support, including percutaneous endoscopic gastrostomy insertion, non-invasive ventilation, and invasive mechanical ventilation). This makes cognitive impairment in ALS a sensitive topic, because even though cognitive deficits may be significant, the may neither be dramatic. Therefore, the reliable detection of cognitive deficits in ALS could provide important information for health professionals in order to apply adaptive strategies that could maximize the quality of life of both the patient and the caregiver. Likewise, we can enormously improve the quality of life of patients in late stage of the disease (CLIS) by reestablishing communication with them, thus breaking the "unbearable" silence.

SUPPLEMENTARY MATERIAL

Offene Fragen,Beispiele von Kategorien (German):

1. Gesundheit:

- Hast du Schmerzen?
- Sollen wir deine Augen befeuchten?
- Drückt dich etwas?
- Schläfst du viel?
- Kannst du mich/uns immer hören?

2. Familie/Freunde:

- Willst du, dass ... dich besuchen kommt?
- Sollen wir (Eltern etc) mehr Zeit mit dir verbringen?
- Willst du etwas mit deinen Freunden unternehmen?
- Willst du ... selber besuchen?
- Stört es dich, wenn wir da sind?

3. Laune/Zustand:

- Bist du gut gelaunt?
- Freust du dich auf ... (Person) ?
- Bist du öfters müde?
- Denkst du viel nach?
- Bist du traurig?

4. Wünsche:

- Willst du draußen sitzen?
- Willst du TV anschauen?
- Willst du Musik/Radio hören?

- Willst du in die Stadt/Park gehen?
- Sollen wir dir etwas vorlesen?

Open Questions, examples of categories (English Translation):

1. Health

- Do you have any pain?
- Should we humidify your eyes?
- Does anything squeezing you?
- Do you sleep a lot?
- Can you hear me/us?

2. Family/Friends:

- Do you wish that … will visit you?
- Should we spend more time with you?
- Do you want to organize … with your friends?
- Do you want to visit …?
- Is it disturbing you if we are near you?

3. State/Mood

- Are you in a good mood (now)?
- Are you looking forward to see …?
- Do you feel often tired?
- Do you think a lot?
- Are you sad?

4. Wishes

- Do you want to sit outside?

- Do you want to watch TV?
- Do you want to listen to music/radio?
- Do you want to go to the park?
- Should we read a book to you?

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