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NEW FRONTIERS IN THE COGNITIVE ASSESSMENT OF AMYOTROPHIC LATERAL SCLEROSIS: BRAIN COMPUTER INTERFACE AND EYE TRACKING

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Abstract

Background: Over the last 20 years, cognitive and behavioural alterations in amyotrophic lateral sclerosis (ALS) have been recognized as an integral part of the disease. A proportion of patients present with a full-blown frontotemporal dementia syndrome, while selective cognitive changes are more commonly found, especially regarding frontal-executive abilities. Moreover, recent studies have highlighted a broader cognitive involvement in this population, concerning language and social cognition. Despite the increased awareness of ALS as a multisystem disorder and the importance of an accurate cognitive evaluation of these patients, the traditional neuropsychological 'paper and pencil' tools do not compensate for patients' physical disability and can not be adequately used in the moderate-advanced stages of the disease.

Objective: To investigate the use of P300-based Brain Computer Interface (BCI) and Eye Tracking (ET) technology for the administration of motor-verbal free cognitive measures in ALS.

Materials and Methods: 34 patients diagnosed with ALS and 30 healthy subjects have been recruited. All participants underwent the BCI and ET-based neuropsychological assessment, together with three traditional cognitive screening tools (*Frontal Assessment Battery - FAB*; *Montreal Cognitive Assessment – MoCA*; *Working Memory subtest of the Brief Assessment of Cognition in Schizophrenia*), two psychological questionnaires (*Beck Depression Inventory - BDI*; *State-Trate Anxiety Inventory - STAI-Y*) and a usability questionnaire. For patients, also respiratory examination was performed, and the *Frontal Behavioural Inventory - FBI* was carried out with caregivers.

Results: Significant **c**orrelations were observed between the traditional cognitive measures and the BCI- and ET-based neuropsychological assessment, mainly concerning accuracy and time-related variables in the ALS patients sample. Patients provided comparable rates than controls with regard to the BCI and ET usability.

Conclusions: The developed motor-verbal free neuropsychological battery allows a longitudinal cognitive assessment during the course of the disease, also when traditional measures are not fully administrable, providing relevant information for clinical practice and ethical issues. Further work will be aimed at refining the developed system and enlarging the cognitive spectrum investigated.

Introduction

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease characterized by loss of motor neurons in the spinal cord, brain stem and motor cortex. For many years, cognitive efficiency has been evaluated as not affected in ALS, even if some anecdotic and historical papers in the early 1900s had highlighted the presence of a cognitive involvement. Over the last 20 years, however, evidence has been consistently demonstrated of cognitive and behavioural alterations in patients with ALS, with the most consistently reported changes regarding dysfunctions in the executive system (Phukan, Pender, Hardiman, 2007). Besides, the presence of a spectrum of frontotemporal impairment in ALS has been highlighted (Phukan et al., 2007; Abrahams et al., 2005a), since cognitive-behavioural deficits in ALS may appear along a continuum, from mild-to-moderate impairment to frontotemporal dementia - FTD (Strong et al., 2009). Studies performed on the ALS population have suggested that about 50% of patients present with some degree of cognitive impairment and 10–15% reach criteria for diagnosis of FTD, thus presenting with amyotrophic lateral sclerosis–frontotemporal dementia (ALS-FTD) (Goldstein & Abrahams, 2013). Neuropathological and genetic studies have supported the hypothesis of a link between FTD and ALS. The recently discovered hexanucleotide repeat expansions in the C9ORF72 gene appears to be the most common genetic cause of familial ALS and FTD, and it has also been found in a significant number of apparently sporadic cases (DeJesus-Hernandez et al., 2011).

Besides the consistent agreement about the centrality of executive functions alterations in ALS, growing evidence is emerging, supporting a broader cognitive involvement in such population. As emphasized by Phukan and coll. (2011), cognitively impaired ALS patients represent an heterogeneous population, where the presence of memory, language and visuo-spatial impairments may be underestimated. In accordance with such view, prominent changes in language (Taylor et al., 2013; Abrahams, 2013b) and social cognition (Abrahams, 2012; Cavallo et al., 2011; Girardi, MacPherson, Abrahams, 2011) have been recently reported.

Few longitudinal studies have investigated the progression of cognitive decline in ALS. In particular, a group of studies evaluating ALS patients during a six-month period overall showed a slight progression of cognitive impairment over the course of the diesease (Strong et al., 1999; Abrahams, Leigh, Goldstein, 2005b; Robinson et al., 2006; Gordon et al., 2010).

Other studies performing longitudinal neuropsychological assessment up to 12, 18 and 24 months (Kilani et al., 2004; Schreiber et al., 2005; Elamin et al., 2013) showed heterogeneous performances among ALS patients. To date, the longitudinal assessment of ALS patients cognitive functioning involves some limitations: first of all, the lack of cognitive measures that take into account the presence of progressive verbal and motor disabilities, in order to reduce the drop-out rate and fully capture the spectrum of cognitive alterations in ALS. This aspect may have lead to underestimate the cognitive decline observed in the recruited samples. Similarly, the motor-verbal impairment makes neuropsychological assessment problematic in moderate-severe stages of ALS, due to the limitation of the traditional 'paper and pencil' cognitive tasks. However, the detection and quantification of cognitive alterations throughout the course of the disease have a relevant impact on clinical practice and ethical issues, the latter concerning the capacity of taking decisions about financial circumstances, invasive treatments proposed in the course of the disease and end-of-life decisions.

Recently, Augmentative and Alternative Communication (AAC) devices, such as Eye-Tracking (ET) and Brain Computer Interface (BCI), have been developed and employed as a mean to restore communication abilities in ALS patients. The ET is a device for recording and analyzing eye movements. Furthermore, through eye tracking, the gaze can be used to actively interact with electronic and technological tools. Several ET paradigms (e.g. ocular fixation, anti-saccade, and smooth pursuit) might be used to assess cognitive functions, with particular attention for the spectrum of frontal impairment. ET devices have been used in recent studies with ALS patients, employing antisaccade/prosaccade and smooth pursuit paradigms, which appeared to be sensitive markers of cognitive frontal alterations in this population (Donaghy et al., 2009, 2010). However, ET application requires a preserved ocular-motor ability and ALS patients have been found to show oculomotor alterations, at least in advanced stages of the disease.

BCI is a communication system that does not depend on the brain's normal output pathways of peripheral nerves and muscles (Wolpaw et al., 2000); it is a direct connection between the human brain and a computer that allows communication. BCI devices could be adequately employed also in moderate-severe ALS, since they do not rely on oculomotor abilities, but on a variety of psychophysiological signals as input commands: electric brain activity is manipulated by the user in order to produce signals that can be used to control computers or communication devices. Electroencephalography (EEG) based BCIs have been employed in

most of clinical applications of BCI with patients presenting severe motor disorders (e.g., Kübler et al., 2005; Vaughan et al., 2006; Nijboer et al., 2008). Besides, recent longitudinal studies employing P300-based BCIs on ALS patients suggested that P300-BCI communication is possible also in advanced stages of the disease (Silvoni et al., 2009, 2013). Recently, some attempts have been performed in order to develop a BCI-based cognitive testing (Iversen et al., 2008a, 2008b; Perego et al., 2011); however, such approaches present some limitations, such as an extensive pre-training and relevant adjustments of the original test procedures, possibly producing biased results. To date, a comprehensive motor-verbal free neuropsychological battery, including standardized test administered with a good level of adherence to the original validated measures, is not still available for ALS patients.

Therefore, the present research was primarily aimed at developing a motor-verbal free neuropsychological battery, suitable to perform a longitudinal cognitive assessment even in the moderate-advanced stage of the disease. The designed battery focuses on the best reasonable adherence to the original validated cognitive tests. This neuropsychological battery based on BCI and ET devices was tested on a sample of ALS patients and healthy controls, in order to evaluate its sensitivity and usability.

The first part of this work summarizes ALS clinical manifestations, disease mechanisms, neuroimaging data and approaches to care, giving an overview of the current understanding of the disease, with a focus on cognitive and behavioural features.

A detailed explanation of Brain Computer Interface and Eye Tracking systems is then provided, illustrating the promising role of such instruments in the cognitive assessment of ALS patients.

Finally, the last part focuses on the research study and the interpretation of results obtained by the administration of a BCI- and ET-based neuropsychological battery on a group of ALS patients.

1

Amyotrophic Lateral Sclerosis

1.1. Definition and diagnostic/classification criteria

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterised by progressive muscular paralysis reflecting degeneration of motor neurons in the primary motor cortex, corticospinal tracts, brainstem and spinal cord (Wijesekera & Leigh, 2009). 'Amyotrophic' refers to the atrophy of muscle fibres, which are denervated as their corresponding anterior horn cells degenerate, leading to weakness of affected muscles and visible fasciculations. 'Lateral sclerosis' refers to hardening of the anterior and lateral corticospinal tracts as motor neurons in these areas degenerate and are replaced by gliosis (Kato, Shaw, Wood-Allum, Leigh, Shaw, 2003). Although ALS appears to preferentially affect motor neurons in the cortex, brainstem and spinal cord, many other cell types are involved. They include glia, interneurons, vascular endothelium and cells involved in controlling immune mediated processes. It is likely that these cells interact in complex two-way and three-way interactions (Eisen, 2009).

The wide variety of clinical features which may be present early in the course of ALS makes absolute diagnosis difficult and compromises the certainty of diagnosis for clinical research purposes and therapeutic trials. Thus, the diagnosis of ALS is based on the presence of very characteristic clinical findings in conjunction with investigations to exclude 'ALS-mimic' syndromes. The clinical finding of signs suggestive of combined upper motor neuron (UMN) and lower motor neuron (LMN) that cannot be explained by any other disease process (evident on electrophysiological, imaging, cerebrospinal fluid (CSF) or serological studies), together with progression compatible with a neurodegenerative disorder, is suggestive of ALS.

In 1994 the World Federation of Neurology (WFN) Research Group on Motor Neuron Diseases developed the 'El Escorial' diagnostic criteria (Brooks, 1994) and in 2000 the revised 'Airlie House' criteria (Brooks, Miller, Swash, Munsat, 2000) as a response to the need for internationally agreed diagnostic criteria to diagnose and classify patients for research studies and drug trials. The original criteria required the presence of positive and negative criteria to make a diagnosis of ALS/MND. The required positive features included a progressive history as well as a combination of upper and lower motor neuron signs on

clinical examination. The essential negative criterion was an absence of sensory signs which could not be explained on any other basis e. g. carpal tunnel syndrome or lumbar spondylotic radiculopathy. Following the original Escorial criteria, a diagnosis of proven MND/ALS could only be made after death on the basis of post mortem histology. During life the criteria only accommodated the diagnosis at the definite, probable, possible or suspected levels. The criteria depended on physical signs in four regions: bulbar, cervical, thoracic and lumbosacral. These criteria have been widely accepted, but some years experience of their practical use as well as new insights into correlations between phenotype and genotype in familial ALS/MND suggested the need to revise them in order to increase their sensitivity. Based on the revised Airlie House criteria (Brooks et al., 2000), patients can be classified into 'Clinically definite', 'Clinically probable', 'Clinically probable-Laboratory supported' and 'Clinically possible' categories. This revision takes into account the special case of patients potentially suffering from familial ALS/MND and provides for a lower burden of clinical evidence in patients who have been shown to have a genetic lesion associated with MND/ALS.

1.2. Epidemiology

The incidence of sporadic ALS (SALS) in the 1990's is reported to be between 1.5 and 2.7 per 100,000 population/year (average 1.89 per 100,000/year) in Europe and North America (Worms, 2001), with a uniform incidence across these countries. The point prevalence in the 1990's ranges from 2.7 to 7.4 per 100,000 (average 5.2 per 100,000) in western countries (Worms, 2001). A recent study suggested that in Europe, the median incidence rate (/100,000 population) was 2.08 (1.47–2.43), while the median prevalence (/100,000 population) was 5.40 (4.06–7.89) (Chiò et al., 2013). A consistent finding in studies is that males are affected more than females, with a M:F ratio about 1.5:1, although more recent data suggests that the gender ratio may be approaching equality (Worms, 2001; Abhinav et al., 2007; Zoccolella et al., 2008; Logroscino et al., 2008). Explanations for this male excess have been attributed to possible protective hormonal factors in women and increased likelihood of males being exposed to putative risk factors (Armon, 2003b Nelson, McGuire, Longstreth, Matkin, 2000). A review published in 2001 found the mortality rates of ALS in the 1990's ranged from 1.54 to 2.55 per 100,000/year and a more recent study estimated the figure to be 1.84 per 100,000 persons in the US population (Worms, 2001;

Sejvar, Holman, Bresee, Kochanek, Schonberger, 2005). The mean age of onset for sporadic ALS (SALS) varies between 55–65 years, with a median age of onset of 64 years (Leigh, 2007; Haverkamp, Appel, Appel, 1995). Only 5% of cases have an onset before the age of 30 years (Haverkamp et al., 1995), although juvenile sporadic onset cases are being increasingly recognized (Gouveia & De Carvalho, 2007). Bulbar onset is commoner in women and in older age groups, with 43% of patients over the age of 70 presenting with bulbar symptoms compared to 15% below the age of 30 (Haverkamp et al., 1995; Beghi, Millul, Micheli, Vitelli, Logroscino, 2007; Forbes, Colville, Swingler, 2004). A recent study found that mean ± SD age for ALS diagnosis was 64.4 ± 2.9 years (range 58–68, while the mean ± SD diagnostic delay was 12.6 ± 2.6 months (range 8.6–16.8) (Chiò et al., 2013). Although most cases of ALS are sporadic, about 5% of cases have a family history of ALS (Familial ALS - FALS) (Anderson, 2003). There is an often Mendelian inheritance and high penetrance, with most cases having autosomal dominant pattern of inheritance, although autosomal recessive pedigrees have been reported (Mulder, Kurland, Offord, Beard, 1986; Gros-Louis, Gaspar, Rouleau, 2006). The ages of onset of FALS is about a decade earlier than for sporadic cases, it affects males and female equally, and has a shorter survival (Mulder et al., 1986; Li, Alberman, Swash, 1988; Veltema, Roos, Bruyn, 1990). Age of onset in FALS has a normal Gaussian distribution, whereas SALS has an age dependant incidence (Strong, Hudson, Alvord, 1991). Some Western Pacific foci where the prevalence is 50–100 times higher than elsewhere world have been reported, although the cause of these aggregations remains elusive (Steele & McGeer, 2008). These populations include the Chamorro people of Guam and Marianas island, the Kii peninsula of Honshu Island, and the Auyu and Jakai people of south west New Guinea, in whom ALS is associated with the Parkinsonism and dementia (ALSPD complex) (Shaw, Arechavala-Gomeza, Al-Chalabi, 2007; Armon, 2003b). More recent studies however have shown a decrease in incidence of both ALS and PDC in these areas over the past 40 years, although the incidence of PDC slightly increased during the 1998's and 1990's (Steele & McGeer, 2008; Plato et al., 2003; Waring et al., 2004; Kuzuhara & Kokubo, 2005).

1.3. Clinical features

ALS leads to a progressive degeneration of the motor neurons that supply voluntary muscles. In addition to variable progression rate, UMN and LMN are differentially affected,

onset occurs in different body districts and cognitive and behavioural alterations may also be present. Thus, ALS presents with a variety of clinical deficits. Loss of LMNs causes fasciculation, cramps, muscle atrophy and marked weakness, which is often more disabling for patients than the spasticity, hyperreflexia and modest weakness associated with UMN disease. Babinski and Hoffmann signs, along with emotional lability are also characteristic findings of UMN degeneration.

About two-third of patients have a spinal form of the disease, presenting with symptoms related to focal muscle weakness that may start either distally or proximally in the upper and lower limbs. Generally, the weakness is of insidious onset and although it is usually asymmetrical at onset, the other limbs develop weakness and wasting sooner or later. Early findings include foot drop, difficulty in walking and loss of manual dexterity, gradually leading to spasticity in the weakened atrophic limbs. Most patients go on to develop bulbar symptoms and eventually respiratory symptoms, which are one of the major cause of death. During late stages of the disease patients may develop 'flexor spasms', which are involuntary spasms occurring due to excess activation of the flexor arc in a spastic limb. As the disease progresses, mobility and function of upper and lower limbs undergo a decline and patients become dependent on caregivers.

Bulbar-onset ALS, which is more frequent in older women, carries a worse prognosis (Chiò, Mutani, Mora, 2003); patients usually present with dysarthria of speech, followed by dysphagia. Limbs symptoms can develop almost simultaneously with bulbar symptoms and in the vast majority of cases will occur within 1–2 years. Almost all patients with bulbar symptoms develop sialorrhoea due to difficulty swallowing saliva and mild UMN type bilateral facial weakness which affects the lower part of the face. Patients gradually lose the ability to articulate words and phrases up to the total loss of verbal communication, that is, anarthria. Moreover, since also limbs mobility is impaired, it deprives patients of the ability to use gestural communication (Cipresso et al., 2012). 'Pseudobulbar' symptoms such as emotional lability, with difficulties in controlling episodes of laughing or crying, are seen in a significant number of cases (Gallagher, 1989).

About 5% of cases with ALS present with respiratory weakness without significant limb or bulbar symptoms (de Carvahlo et al., 1996; Chen, Grand'Maison, Strong, Ramsay, Bolton, 1996). These patients show symptoms of respiratory failure or nocturnal hypoventilation such as dyspnoea and orthopnoea, requiring either noninvasive or invasive ventilation,

disturbed sleep, morning headaches, excessive day time somnolence, anorexia, decreased concentration and irritability or mood changes (Polkey, Lyall, Moxham, Leigh, 1999).

As disease progresses, patients develop the characteristic picture of the combination of upper motor neuron and lower motor neuron signs coexisting within the same central nervous system region, affecting the bulbar, cervical, thoracic and lumbar territories. Respiratory failure and other pulmonary complications are the usual cause of death in ALS. However, patients kept alive by tracheostomy assisted ventilation are found to eventually develop a profound state of motor paralysis named 'complete locked-in state' (CLIS), charcacterized by paralysis of all voluntary muscles and varying degrees of oculomotor impairment (Hayashi & Kato, 1989; Sasaki, Tsutsumi, Yamane, Sakuma, Maruyama, 1992).

The variability and overall rapid progression make it difficult to predict survival time or the timing of interventions. In general, limb-onset, younger age, better motor function, higher breathing capacity, stable weight, and longer interval between symptom onset and diagnosis are associated with longer survival (Gordon et al., 2013).

1.4. Aetiology

The cause of ALS/MND is unknown although a large spectrum of aetiological factors has been considered, including genetics, autoimmune responses, environmental factors, oxidative stress, glutamate excitotoxicity, mitochondrial damage, defective axonal transport, glial cell pathology, aberrant DNA and RNA metabolism, toxic effect of heavy metals, viral infection, paraneoplastic syndromes, lymphomas, paraproteinaemias and many others.

Recent reviews on the role of environmental risk factors in the causation of ALS have concluded that there is no consistent association between a single environmental factor and risk of developing ALS, suggesting the hypothesis of complex genetic-environmental interplay as the causal factor for motor neuron degeneration (Shaw, 2005; Cozzolino, Ferri, Carri, 2008). Putative exogenous risk factors associated with development of ALS investigated in case-control studies have been reviewed, for example dietary factors (Morozova et al., 2008), family history of non-ALS neurodegenerative disease (Parkinson's or Alzheimer's disease) (Cruz, Nelson, McGuire, Longstreth, 1999), playing football professionally (Armon, 2007; Chiò, Benzi, Dossena, Mutani, Mora, 2005; Al-Chalabi & Leigh, 2005), smoking (Weisskopf et al., 2004; Fang, Bellocco, Hernan, 2006; Sutedja et al.,

2007; Nelson, McGuire, Longstreth, Matkin, 2000; Kamel, Umbach, Munsat, Shefner, Sandler, 1999), toxin exposure for example to agricultural chemicals or lead (Armon, Kurland, Daube, O'Brien, 1991; Kamel et al., 2005) and trauma (e.g. head injury) (Cruz et al., 1999; Armon, 2007; Chen, Richard, Sandler, Umbach, Kamel, 2007). By applying an evidenced based approach, it was found that only smoking is likely to be associated with developing ALS, while other risk factors were weakly related (Wijesekera & Leigh, 2009).

1.5. Pathogenesis of motor neuron degeneration in ALS

The pathogenic mechanisms that underlie ALS and cause motor neuron degeneration remain largely unclear, but it is likely to be a complex interplay between multiple pathogenic cellular mechanisms which may not be mutually exclusive. These include:

- *Genetic factors*

Approximately 10% of ALS is classified as familial, whereas the remaining 90% of cases are considered sporadic, as they appear to occur randomly throughout the community (Rowland & Schneider, 2001).

~20% of cases with autosomal dominant FALS and 2% of patients with SALS show mutations in the gene producing the suoperoxide dismutase 1 (SOD1) enzyme (Rosen et al., 1993). Mutations in the gene are thought to cause the disease through a toxic gain of function rather than a decreased antioxidant activity of the SOD1 enzyme (Shaw, 2005). Considerable phenotypic heterogeneity occurs across the various SOD1 mutations. For example, the A4V mutation, which is the most frequent variant in North America, gives rise to an aggressive form of ALS that typically leads to death within a year after symptom onset (Cudkowicz et al., 1997). In contrast, the homozygous D90A mutation in the same gene is associated with an indolent course, with patients developing respiratory failure only after 10 years of illness (Andersen et al., 1996).

Mutations in TARDBP gene (which encodes the TAR-DNA binding protein TDP-43) located on chromosome 1p36.22 were later discovered in autosomal dominant ALS, sporadic ALS and frontotemporal (FTD) families (Neumann et al., 2006; Sreedharan et al., 2008; Kabashi et al., 2008). TDP-43 is a DNA/RNA binding protein involved in many cellular functions such as transcription and slicing regulation and microRNA processing (Ayala et al., 2005; Buratti & Baralle, 2010). TDP-43 is primarily a nuclear protein, but

after acute neuronal injury, TDP43 translocates to the cytoplasm and forms stress granules. In the cortex of ALS and FTD patients, TDP-43 is phosphorylated, ubiquitinated and cleaved, forming insoluble fragments that aggregates in the cytoplasm with loss of the normal TDP-43 nuclear staining in a sizable minority of neurons (Neumann et al., 2006). It has been established that mutations in the TDP-43 gene account for 5% of patients with FALS; TDP-43 inclusions have been found in more than 90% of patients with sporadic ALS. Recently, a mutation in another gene for RNA processing protein, fused in sarcoma (FUS) has been reported. The FUS gene is located on chromosome 16 and encodes a multifunctional protein thought to be involved in DNA repair and regulation of transcription from DNA to the related compound of RNA. These observations reinforced the importance of abnormal RNA metabolism in motor neuron degeneration. The FUS pathology has been found in sporadic ALS and in most familial ALS cases (Kwiatkowski et al., 2009; Valdmanis, Daoud, Dion, Rouleau, 2009; Vance et al., 2009; Yan et al., 2010).

Expanded GGGGCC hexanucleotide repeats in the first intron of C9orfF72 are now thought to be the most common cause of ALS and FTD (Renton et al., 2011; DeJesus-Hernandez et al., 2011), accounting for a remarkable percentage of both familial ALS (~ 40%) and familial FTD (~25%) and genetically explaining the majority of the overlap of these two clinical syndromes (Majounie et al., 2012). Moreover, this C9orf72 mutation is also the most frequent cause of apparently sporadic ALS and FTD found to date, accounting for 5–7% of cases in white Americans, Europeans and Australians (Majounie et al., 2012). This gene encodes a protein of unknown function and has provided additional evidence that impaired RNA function is crucial for ALS. Intranuclear RNA foci, containing expanded RNA transcripts, have been described in ALS and FTD tissue. Similar to other repeat expansion diseases, it was recently demonstrated that the toxicity of these RNA foci are length-dependent and sequester specific RNA binding proteins leading to significant dysregulation of RNA processing (Lee et al., 2013).

Other genes causing familial MND include optineurin (OPTN) (Maruyama et al., 2010), valosin-containing protein (VCP) (DeJesus-Hernandez, Desaro et al., 2011; Koppers et al., 2012), ubiquilin 2 (UBQLN2) (Deng et al., 2011) and profilin 1 (PFN1) (Wu et al., 2012). Several other gene mutations have been identified in sporadic cases which may increase susceptibility to ALS, such as mutations in the KSP repeat region in the NEFH gene (encoding neurofilament heavy subunit) (Figlewicz et al., 1994; Tomkins et al., 1998), apolipoprotein E Σ4 genotype (APOE) (Al-Chalabi et al., 1996), decreased expression of

EAAT2 protein (Meyer et al., 1999; Trotti et al., 2001) and alterations in the Vascular endothelial growth factor (VEGF) gene (Lambrechts et al., 2003), to name a few.

- *Excitotoxicity*

Excitotoxicity is one of the most accepted pathogenic mechanism of neural pathology in ALS. Excessive glutamate induced stimulation of the postsynaptic glutamate receptors such as cell surface NMDA receptors and AMPA receptors provokes neuronal injury. This excessive stimulation of glutamate receptors may result in high calcium influx into the neurons, leading to increased nitric oxide formation and thereby neuronal death. This hypothesis is supported by the finding of elevated levels of glutamate in cerebrospinal fluid (CSF) exhibited by some ALS patients (Rothstein et al., 1990; Shaw, Forrest, Ince, Richardson, Wastell, 1995).

- *Oxidative stress*

Oxidative stress has longer been linked to neurodegeneration and it is well known that accumulation of reactive oxygen species (ROS) cause cell death. As mutations in the anti-oxidant enzyme superoxide dismutase 1 (SOD1) gene can cause familial ALS, there is significant interest in this mechanism underlying neurodegenerative process in ALS. The finding of biochemical changes reflecting free radical damage and abnormal free radical metabolism in CSF and post-mortem tissue samples of ALS patients support this hypothesis (Shaw, Ince, Falkous, Mantle, 1995; Ferrante et al., 1997; Smith, Henry, Mattson, Appel, 1998; Tohgi et al., 1999).

- *Mitochondrial dysfunction*

Mitochondrial dysfunction is likely to be an important point of multiple pathways underlying the ALS pathogenesis and course. It is known that mitochondria are an early target in ALS pathology and contribute to disease progression. Morphological and functional abnormalities in mitochondria have been reported in sporadic ALS patients and SOD1 transgenic mice (Atsumi, 1981; Afifi, Aleu, Goodgold, MacKay, 1966; Hirano, Donnenfeld, Sasaki, Nakano, 1984; Siklos et al., 1996; Kong & Xu, 1998; Krasnianski et al., 2005). The SOD1 mutation was found to be preferentially associated with impairment of mitochondrial function. Recent studies suggest that axonal transport of mitochondria along microtubules is disrupted in ALS and that mitochondria from ALS patients show elevated

calcium levels and decreased activity of respiratory chain, implicating defective energy metabolism (Siklos et al., 1996; Wiedemann et al., 1998). Mitochondrial DNA mutations have been also described in ALS patients (Dhaliwal & Grewal, 2000; Wiedemann, Manfredi, Mawrin, Beal, Schon, 2002; Ro, Lai, Chen, Chen, 2003).

- *Impaired axonal transport*

SOD1 transgenic mouse models show evidence of slowed intracellular anterograde and retrograde transport, on which rely motor neuron axons (Williamson & Cleveland, 1999; Borchelt et al., 1998; Murakami et al., 2001; De Vos, Grierson, Ackerley, Miller, 2008). Although no similar findings have been observed in humans with ALS, mutations in the kinesin genes are known to cause some neurodegenerative motor nerve diseases in humans (i.e. hereditary spastic paraplegia and Type 2A Charcot-Marie-Tooth disease (Reid et al., 2002; Zhao et al., 2001).

- *Neurofilament aggregation*

Abnormal assembly with accumulation of neurofilaments are commonly seen in several neurodegenerative conditions including SALS and FALS (Hirano et al., 1984; Carpenter, 1968; Hirano, Nakano et al., 1984). Neurofilament proteins together with peripherin (an intermediate filament protein) are found in the majority of axonal inclusions motor neurons of ALS patients (Corbo & Hays, 1992). A toxic isoform of peripherin (peripherin 61), has been found to be toxic to motor neurons even when expressed at modest levels and is detectable in spinal cords of ALS patients but not controls (Robertson et al., 2003).

- *Protein aggregation*

Intra-cytoplasmic inclusions are a hallmark of both sporadic and familial ALS. However, it is still unclear as to whether aggregate formation directly causes cellular toxicity and has a key role in pathogenesis, if aggregates may be innocent by-products of the neurodegeneration process, or if formation of the aggregates may actually represent a beneficial process by being part of a defense mechanism to reduce intracellular concentrations of toxic proteins (Shaw, 2005; Cozzolino et al., 2008).

- *Inflammatory dysfunction and contribution of non-neuronal cells*

Although ALS is not primarily a disorder of autoimmunity or immune dysregulation, there is considerable evidence that inflammatory processes and non-neuronal cells may play a role in

pathogenesis of ALS. Microglial and dendritic cell activation is a prominent pathology in human ALS and transgenic SOD1 mice (Troost, Oord Van den, de Jong, Swaab, 1989; Troost, Oord Van den, Vianney de Jong, 1990; Kawamata, Akiyama, Yamada, McGeer, 1992; Henkel et al., 2004; Hall, Oostveen, Gurney, 1998). These activated non-neuronal cells produce inflammatory cytokines and evidence of upregulation is found in CSF or spinal cord specimens of ALS patients or in vitro models (Almer et al., 2001; Robertson et al., 2001; Sekizawa et al., 1998; Wilms et al., 2003).

- *Deficits in neurotrophic factors and dysfunction of signaling pathways*

Decreased levels of neurotrophic factors (*e.g.* CTNF, BDNF, GDNF and IGF-1) have been observed in ALS patients post-mortem and in *in vitro* models (Anand et al., 1995; Elliott & Snider, 1996; Oppenheim, 1996). In humans, three mutations in the VEGF gene were found to be associated with increased risk of developing sporadic ALS (Lambrechts et al., 2003), although a recent meta-analysis by the same authors failed to show an association between VEGF haplotypes and increase the risk of ALS in humans (Lambrechts et al., 2008). The final process of cell death in ALS motor neurons is thought to closely resemble a programmed cell death pathway (apoptosis). Biochemical markers of apoptosis are detected in the terminal stages of human and models of ALS (Guegan & Przedborski, 2003; Pasinelli, Borchelt, Houseweart, Cleveland, Brown, 1998; Pasinelli, Houseweart, Brown, Cleveland, 2000; Li et al., 2000; Vukosavic, Dubois-Dauphin, Romero, Przedborski, 1999). Key elements of the normal apoptotic pathway are found to be involved in cell death in ALS (Shaw, 2005; Sathasivam, Ince, Shaw, 2001; Pasinelli & Brown, 2006).

1.6. Neuroimaging

Neurodegenerative diseases including ALS tend to involve more-insidious pathological processes and present with less clear-cut clinical diagnoses and imaging findings with respect to other nervous system pathologies, such as stroke, cancer and demyelinating diseases. However, neuropathological evidence obtained in ALS with both conventional and modern neuroimaging approaches highlighted an extra-motor involvement in such disease.

Voxel-based morphometry (VBM) studies have found that regional gray matter (GM) loss is not confined to motor regions, but is extended to the frontal, temporal, parietal, and limbic

regions (Grosskreutz et al. 2006; Turner, Hammers, Allsop, 2007; Abrahams et al., 2005a). In particular, frontal regions showed the most severe atrophy in patients with ALS and FTD. Early reports suggested that structural changes in ALS could be detected using MRI, including hyperintense T2-weighted signal in the corticospinal tract (CST) (Comi, Rovaris, Leocani, 1999). However, MRI findings can also be seen in healthy controls (Ngai, Tang, Du, Stuckey, 2007). As a result, the main role of conventional MRI in ALS management is to exclude alternative diagnoses - such as an upper cervical cord lesion - that can mimic ALS and present with UMN and LMN signs.

Over the past 25 years, considerable interest has developed in the use of advanced neuroimaging methods - such as structural MRI, diffusion tensor imaging (DTI), proton magnetic resonance spectroscopy (^{1}H-MRS), resting-state functional connectivity MRI (rs-fcMRI) and PET - to study central nervous system changes in ALS. Advanced MRI techniques allow the investigation of the nervous system for atrophy and alterations in microstructure, biochemistry, neural networks, metabolism and receptor distribution and provide a unique opportunity to assess disease pathophysiology early and noninvasively, therefore offering promise for bio-marker development.

DTI has provided evidence of significant reduction of fractional anisotropy (FA) not only in CST but also in extramotor regions, including frontal, temporal, parietal and occipital WM, corpus callosum, the hippocampal formation, and the insula (Sach, Winkler, Glauche, 2004; Sage, Peeters, Görner, Robberecht, Sunaert, 2007; Senda, Ito, Watanabe, 2009; Lulé, Diekmann, Muller, Kassubek, Ludolph, Birbaumer, 2010).

Moreover, functional neuroimaging has supported the clinical findings of frontal cortical involvement not only in patients with an ALS/dementia complex, but also in patients with ALS and subclinical cognitive impairment. Abnormal activations extending beyond the sensorimotor cortex in ALS has been proved in PET and fMRI studies during motor execution tasks and verbal fluency tasks. In particular, a hypoactivation has been measured in dorsolateral prefrontal cortex (DLPFC) in both conditions (Kew et al., 1993; Stanton et al., 2007). Furthermore, hypoperfusion in the frontal cortex in ALS with or without cognitive deficits measured with PET (Ludolph et al., 1992) and fMRI (Tanaka et al., 1993) and association of reduced frontal executive function and reduced activity in fronto-parietal areas measured with PET have been shown (Abrahams et al., 1996). Other functional imaging studies have provided further evidence for extra-motor involvement in ALS (Lulé et al., 2007; Han & Ma, 2010; Mohammadi, Kollewe, Samii, Dengler, Münte, 2011).

In conclusion, results show that neurodegeneration in ALS involve many nervous system regions, including the motor cortex, corticospinal tract, frontal lobes, thalamus, brainstem and cervical spinal cord. Furthermore, neuroimaging findings suggest an involvement of the corpus callosum and basal ganglia, both of which have important connections to the motor system. Neuroimaging in ALS also provides evidence of neuronal loss, white matter tract disruption, alterations in neural networks, γ-aminobutyric acid system dysfunction, and changes in brain metabolism. Advanced neuroimaging also demonstrates similarities and differences between the motor neuron disease phenotypes in terms of brain metabolism, biochemistry and structural alterations, which should contribute to elucidation of disease aetiologies across the spectrum of motor neuron diseases (see Foerster, Welsh, Feldman, 2013 for an extensive review).

1.7. Management and treatment

Despite recent advances in the understanding of the pathophysiology of ALS, no effective cure is yet available for this disease, so the aim of clinical care is to improve quality of life, help maintaining patients' autonomy and prolong life expectancy as long as possible. Management is centred on a combination of a neuroprotective medication, multidisciplinary care and respiratory support. Many therapies can help relieve symptoms, including anxiolytics and analgesics, which bring comfort in the advanced stages.

Riluzole

Riluzole is the only approved drug that has been shown to have a modest effect on reducing mortality (Miller, Mitchell, Moore, 2012) and slowing disease progression (Lacomblez, Bensimon, Leigh, Guillet, Meininger, 1996; Bensimon, Lacomblez, Meininger, 1994). Its mechanism of action is thought to involve interference with N-methyl-D-aspartate (NMDA) receptor mediated responses, inhibition of glutamate release from pre-synaptic terminals and increasing of extracellular glutamate uptake (Distad et al., 2008). However, it can not reverse the damage of motor neuron that has already occurred and only about a three-month life span expansion for ALS patients in the early stages has been reported, and no therapeutic benefit for ALS patients in the late stages has been observed, indicating lack of therapeutic options for the patients (Bensimon et al., 2002).

Over 100 other neuroprotective agents (e.g. Ceftriaxone, Topiramate, Tamoxifen, Coenzyme Q10, Vitamin E) have been studied in animal models and humans, but none of them has got a recommendation for treatment in ALS patients.

Gene therapy for the delivery of supportive neurotrophic factors directly to neurons has been also explored in SOD1 mouse models with some promising results (Kaspar, Llado, Sherkat, Rothstein, Gage, 20003), but human studies have not been undertaken yet.

Recently, stem cell therapy has been of great interest for ALS treatment, particularly because of the potential for multiple mechanisms of action. Mesenchymal stem cells (MSC) stand out as cells capable of protecting motor neurons, differentiating into multiple neural cell types, modulating immune cell roles, and reducing central nervous system inflammation. The success of MSCs in delaying disease onset, improving motor function, and increasing survival in preclinical models of ALS has resulted in multiple clinical trials of MSC therapy in patients with ALS (see for a review Lewis and Suzuki, 2014), even if further work is necessary to maximize the potential of this therapy.

Symptomatic treatments

Other treatments aim to relieve symptoms and improve quality of life. Different drugs are available to help patients with pain, cramps, spasticity, excessive saliva, emotional lability, depression, anxiety and sleep disturbances.

Ventilatory support

During the course of the disease, respiratory muscles become weak and symptoms of dyspnoea, orthopnoea, disturbed sleep, daytime fatigue, morning headaches and poor concentration develop.

Overnight pulse oximetry and measurement of forced vital capacity (FVC) and slow vital capacity (SVC) are the most widely used measure for detecting respiratory decline. However, FVC has serious limitations as a measure of respiratory muscle function in motor neuron disease. First, it is relatively insensitive to significant change in respiratory function (Lyall, Donaldson, Polkey, Leigh, Moxham, 2001). Moreover, patients with bulbar onset disease or pronounced facial weakness cannot perform the test accurately, even using a mask or mouthpiece. Patients with pseudobulbar features often have an apraxia of facial movements and cannot blow effectively despite having good diaphragm function. Sniff nasal inspiration pressure (SNIP), which estimates intrathoracic pressure, is a better measure of

respiratory muscle dysfunction and diaphragmatic strength, combining linear decline, sensitivity in mild disease, and feasibility in severe disease.

Respiratory insufficiency occurs commonly in patients with ALS and it is a major cause of mortality; approximately 60% of patients have a predictable decline in respiratory function. Respiratory support is usually provided by non-invasive ventilation (NIV) or invasive ventilation via tracheotomy. Bi-level positive pressure devices (BiPAP), which are triggered by a patient's inspiratory effort and turn off during exhalation, are the most common form of NIV, facilitating physiological breathing. Several study showed that NIV help to reduce the breathing effort, enhancing gas exchange, improving quality of life, extending survivor and enhancing cognition (Heckmatt, Loh, Dubowitz, 1990; Bourke et al., 2006; Bourke, Bullock, Williams, Shaw, Gibson, 2003; Newsom-Davis, Lyall, Leigh, Moxham, Goldstein, 2001), especially in patients with more preserved bulbar function (Radunovic, Annane, Rafiq, Mustfa, 2013). NIV is initially used for intermittent nocturnal support to alleviate symptoms of nocturnal hypoventilation; as respiratory function worsens, patients require increasing and eventually continuous support during the day. The timing for initiation of respiratory support through NIV differs between countries and centres; however, at a meeting of the European ALS/MND consortium sponsored by the European Neuromuscular Centre, held in May 2002, provisional criteria for initiating NIV were agreed. These were designed to be simple and practical, and requiring no specialized laboratory support (Leigh et al., 2003). Invasive ventilation may extend survival but it requires 24-hours supervision and is quite expensive.

Expiratory muscle weakness leads to difficulty clearing secretions, plugging of bronchi, and increased risk of infection in ALS patients (Mustfa & Moxham, 2001). Besides suction through portable home suction machine, patients and carers should be taught techniques to assist expiratory movement during cough using a manual thrust to the abdomen.

Nutritional management

Dysphagia is a common symptoms in ALS and leads to an increased risk of aspiration, malnutrition, weight loss and dehydration. Malnutrition and weight loss can also occur because of severe upper limb weakness, that slows eating and makes patients dependent on others for an adequate food and liquid intake. Moreover, many ALS patients show a hypermetabolic state, thus requiring an increased calorie intake (Desport et al., 2001; Kasarskis, Berryman, Vanderleest, Schneider, McClain, 1996).

Treatment strategies include modifications of food consistency (blending food, adding thickeners to drinks), and advising on changes in posture or head position. Most guidelines suggest to consider supplementary enteral feeding when body weight falls by more than 10% of pre-diagnostic body weight. The most common available options for enteric feeding include percutaneous endoscopy gastrostomy (PEG) and radiologically inserted gastrostomy (RIG). PEG is the standard procedure for maintaining a good level of nutrition in ALS patients in whom swallowing is impaired and nutritional intake is inadequate. However, PEG procedure requires mild sedation, having therefore implications in patients with poor respiratory function. RIG represents a valid alternative in patients with VC below 50% predicted (Leigh et al., 2003).

1.8. Prognosis

Data from clinic based populations or population registries consistently show that the overall median survival from onset of symptoms for ALS ranges between 2–3 years for bulbar onset cases and 3–5 years for limb onset ALS cases (Chancellor et al., 1993; Norris et al., 1993; Logroscino et al., 2008). Large clinic cohort studies have shown 3 and 5 year survival tares to be around 48% and 24% respectively, with approximately 4% surviving longer than 10 years (Testa, Lovati, Ferrarini, Salmoiraghi, Filippini, 2004; Turner, Parton, Shaw, Leigh, Al-Chalabi, 2003), whereas 5 year survival reported in population based studies is much lower and ranges from 4–30% (Chancellor et al., 1993). Several population based studies and clinical cohort studies have identified important prognostic indicators of survival, which include:

- *clinical phenotype*: patients with incomplete forms of motor neuron disease, such as progressive muscular atrophy (PMA), have a better prognosis than those with typical forms (Preux et al., 1996);

- *site of onset*: bulbar onset disease is associated with a worse prognosis than spinal onset and bulbar function at any stage at the disease seems to play a major role in determining outcome (Zoccolella et al., 2008; Logroscino et al., 2008; Preux et al., 1996; Chiò et al., 2002; del Aguila, Longstreth, McGuire, Koepsell, van Belle, 2003; Testa et al., 2004). On the other hand, respiratory onset disease is a remarkably negative prognostic factor (Bourke et al., 2006; de Carvalho et al., 1996), even if patients with respiratory onset ALS

who use NIV have an increased survival time compared with patients who did not undergo NIV;

- *age of symptom onset*: an increased age of onset is correlated to a decreasing survival time, with patients with symptom onset before 40 years of age having longer survival (Norris et al., 1993; Logroscino et al., 2008; Preux et al., 1996; Chiò et al., 2002; del Aguila et al., 2003; Millul et al., 2005);

- *diagnostic delay*: shorter time from symptom onset to diagnosis carries a worse prognosis, perhaps reflecting a more aggressive disease, which leads to seek medical attention more rapidly and is more easily diagnosed (del Aguila et al., 2003; Testa et al., 2004);

- *rate of disease progression*: several studies found that ALS outcome was significantly related to the decline of several measures of disease progression (i.e., FVC, ALSFRS-R score) (Armon & Moses, 1998; Magnus, Beck, Giess, Naumann, Toyka, 2002; Armon et al., 2000; Kimura et al., 2006; Kollewe et al., 2008; Elamin et al., 2011);

- *respiratory status*: a lower predicted FVC at diagnosis was found to be the single most relevant prognostic factor in ALS (Logroscino et al., 2008; Chiò et al., 2002; Magnus et al., 2002). Although FVC represents a reliable indicator of survivor, this measure is not suitable for patients with bulbar onset, as above mentioned; SNIP has been found to accurately reflect intra-thoracic pressures and to be more significantly associated with respiratory failure than FVC% (Morgan et al., 2005);

- *cognitive functions*: it is now recognized that cognitive impairment may accompany the motor symptoms associated with ALS (Lomen-Hoerth et al., 2003; Abrahams, 2013a; Goldstein & Abrahams, 2013), with suggested roughly 50% of ALS patients having some cognitive impairment, and 10–15% reaching criteria for diagnosis of frontotemporal dementia - FTD (Ringholz et al., 2005; Witgert et al., 2010; Lomen-Hoerth et al., 2003; Gordon et al., 2011). Some studies have found that median survival time was shorter in patients with ALS with comorbid FTD; moreover, in patients without dementia, the presence of executive dysfunction was found to be significantly associated with shorter survival and faster rates of motor functional decline, particularly in bulbar function (Olney et al., 2005; Elamin et al., 2011; Elamin et al., 2013). On the contrary, mild cognitive impairment seems to have little or no effect on ALS outcome (Rippon et al., 2006);

- *El Escorial category at presentation*: a diagnosis of definite ALS at the time of presentation has been found to lead to a poorer prognosis compared to the other diagnostic levels. Since a diagnosis of definite ALS implies muscle weakness and wasting in at least three regions, this findings is likely to indicate that patients with more widespread clinical involvement at the time of diagnosis have a more rapid progression of the disorder (Zooccolella et al., 2008; Millul et al., 2005; Turner et al., 2002; Paillisse et al., 2005; Chiò et al., 2002; Magnus et al., 2002; Kaufmann et al., 2005; Brooks, 1994);

- *Riluzole use*: several studies have highlighted positive independent effect of this medication on ALS patients outcome (Turner et al., 2002; Chiò et al., 2002; Traynor, Alexander, Corr, Frost, Hardiman, 2003; Mitchell, O'Brien, Joshi, 2006). Riluzole has also been found to improve mortality rate (Traynor et al., 2003; Miller, Mitchell, Moore, 2007).

2

Cognitive and behavioural changes in Amyotrophic Lateral Sclerosis

2.1. Cognitive and behavioural alterations

For many years, cognitive efficiency has been thought not to be affected in ALS, which has been traditionally described as a pure motor disease, even if some previous anectodical and historical papers had highlighted the presence of a cognitive involvement in ALS. However, over the last 20 years growing evidence has accumulated that ALS is a multisystem disease that involves a range of cognitive alterations, especially regarding frontal abilities. Frequency, severity and type of cognitive impairment in ALS vary depending to the several methods and tools used to assess cognition in the different studies. Early reports suggested a prevalence of cognitive impairment of about 1–4% (Brownell, Oppenheimer, Hughes, 2970; Jokelainen, 1977; Eisen & Krieger 1993; Strong, Grace, Orange, Leeper, 1996), but one of the largest study so far found a significant cognitive impairment in 36% of non-demented patients (Massman et al., 1996). In more recent studies, the occurrence of cognitive deficits in ALS without dementia has been reported in up to 50% of patients (Abe et al. 1997; Lomen-Hoerth et al. 2003; Phukan et al., 2011). The most consistently reported cognitive changes regard frontal executive functions, that is mental flexibility, attention, working memory, planning, and abstract reasoning.

Besides, the presence of a spectrum of frontotemporal impairment in ALS has been highlighted (Phukan, Pender, Hardiman, 2007; Abrahams et al., 2005), since cognitive-behavioral deficits in ALS may appear along a continuum, from mild-to-moderate impairment to frontotemporal dementia (Strong et al., 2009). In the past 10 years, studies performed on ALS population have suggested that about 50% of patients present with some cognitive impairment, and 10–15% reach criteria for diagnosis of frontotemporal dementia, so presenting with amyotrophic lateral sclerosis–frontotemporal dementia (ALS-FTD) (Goldstein & Abrahams, 2013).

2.2. Patterns of cognitive involvement in ALS

The most consistently reported changes in ALS are related to dysfunction in the executive system; however, growing evidence is emerging, supporting a broader cognitive involvement in such population, especially regarding memory and language abilities.

Executive functions

Executive functions are traditionally described as higher-level mental processes that control and organise other cognitive processes (Shallice, 1988). They represent a heterogeneous set of skills that enable problem solving and responses to novelty. Executive functions are also implicated in behavioural regulation, response initiation, planning, abstraction, motivation, and elements of memory functioning (Cummings & Miller, 2007).

Verbal fluency, which is a sensitive indicator of damage to frontal or striatofrontal areas involved in intrinsic initiation of responses, has been found to be impaired in almost all studies of cognitive impairment in ALS (Ludolph et al., 1992; Massman et al., 1996; Abrahams et al., 1997; Frank, Haas, Heinze, Stark, Munte, 1997). Both letter and category fluency, which require rapid generation of words, seem to be disturbed and this simultaneous effect on both types of fluency implicate dysfunction in components of the executive system. Abrahams et al. (2000) related the impairment on tests of intrinsic response generation (i.e. Written Verbal Fluency Test, Category Fluency Test, and Design Fluency Test), to a higher order dysfunction, implicating deficits in the central executive component of working memory; these deficiencies do not depend on an impairment in primary linguistic ability. Tests of verbal fluency are sensitive, but they depend on verbal or written responses and the results can be confounded by motor impairments in ALS. However, modifications to control for the speed of response can allow patients with upper limb disabilities, and consequent writing disabilities, to be assessed meaningfully. Abrahams and colleagues (20004), for example, developed a verbal fluency index using the time patients took to copy words they had previously written in fluency tests, thus controlling for motor speed.

Although deficits in verbal fluency are commonly believed to result from executive dysfunction, true language dysfunction might also contribute to deficiencies in verbal fluency in ALS. A functional MRI study (Abrahams et al., 2004) has shown abnormal activation of the inferior frontal gyrus and Broca's area during verbal fluency and naming tasks in patients with sporadic ALS without aphasia or dementia. Cerebral structures

involved in language seemed to be affected before naming deficits had become clinically significant.

Deficits in mental flexibility and rule shifting, typically recorded by the Wisconsin card-sorting test (WCST), have also been reported in many studies (David & Gillham, 1986; Massman et al., 1996; Abrahams et al., 1997; Evdokimidis et al., 2002). However, according to Phukan et al. (2007), this test can be less reliable than tests of verbal fluency in patients with ALS. Accordingly, findings of impaired performance on WCST have not been confirmed in several studies (Ludolph et al. 1992; Kew et al. 1993; Talbot et al., 1995).

Impairment in the attentional system is often associated with damage to the frontal lobes and attention deficits have been reported in ALS, especially with concern to selective and divided attention (Massman et al., 1996; Chari, Shaw, Sahgal, 1996). Evaluation of attentional abilities plays an important role, since disinhibited-type patients might have near-normal results in traditional tests of frontal executive function, but show impaired responses in tests of selective attention (Neary, Snowden, Mann, 2000). Rakowicz and Hodges (1998) reported a consistent and significant reduction in reverse digit span in patients with ALS, indicating impaired working memory rather than pure attentional impairments. Problems with concentration and distractibility have been described at the Stroop Test; Evdokimidis et al. (2002) correlated the difficulties in the WCST and Stroop Test with distractibility factor scores for an anti-saccade ocular motor paradigm, which represents another valid test of frontal lobe function, allowing to avoid motor and verbal responses.

Memory

ALS patients show impairment in other cognitive areas than executive functions, but evidence is less consistent. As a matter of fact, there has been no agreement about memory deficits in ALS. Several studies have shown impairment in immediate recall and short-term memory (Gallassi et al., 1989; Kew et al., 1993; Hanagasi et al., 2002), while deficits in delayed recall are highly variable, thus suggesting a disorder in the information encoding process, rather than in the speed of forgetting (Bak & Hodges, 2001). These results are consistent with current theories that encoding is an executive component of memory and involves a neuronal circuit that arises in the left frontal lobe (Tulving, 2000). Mantovan and colleagues (2003) detected a poor primacy effect, that is indicative of a long-term memory deficit, and suggested that poor performance on memory tests may be indicative of a failure to generate stable long memory traces at encoding, rather than a failure in memory retrieval.

However, there is no agreement in the interpretation of memory failures in terms of frontal lobe dysfunction: impairments in delayed recall (of three words and a short story) were assumed by Iwasaki et al. (1990) as an effect of medial temporal lobe pathology, on the basis of the role of the medial temporal lobes in recall of learned information.

More recently, Machts et al. (2014) underlined that memory deficits in ALS are different from those observed in amnestic mild cognitive impairment (aMCI), with the ALS group showing impairment only in recognition, whereas aMCI patients showed short and delayed recall performance deficits and reduced short-term capacity. The authors suggested that these results can be explained only in the context of comorbid-coexisting executive dysfunction, highlighting qualitative differences in temporal lobe dysfunction between ALS and aMCI patients.

Visuoperceptual functions

Visuoperceptual functions represent a heterogeneous set of processes that include attention, object identification and object recognition. Visuoperceptual processes are largely preserved in many ALS patients (Kew et al., 1993; Robinson et al., 2006; Talbot, 1996), even if Strong and colleagues (1999) have underlined some visuoperceptual difficulties in this population.

Language

Language abnormalities have also been described; they included impoverished verbal output (Strong et al. 1999; Bak & Hodges, 2004), deficits in confrontation and objects naming (Massman et al., 1996; Strong et al. 1999; Bak & Hodges, 2004; Abrahams et al., 2005), difficulties in syntactic comprehension (Rakowicz & Hodges, 1998), and paraphasias (Rakowicz & Hodges, 1998; Strong et al. 1999). Moreover, language networks seem to be impaired in MRI and PET studies of ALS patients (Abrahams et al., 1996) which lends support to the aforementioned findings that ALS affects extra-motor pathways. Naming deficits have been reported in several studies of ALS (Massman et al., 1996; Rakowicz & Hodges, 1998; Abrahams, Leigh, Goldstein, 2005), suggesting that a language dysfunction underlies basic word-findings processes. However, in other studies, naming abilities have found to be intact (Kew et al., 1993; Abrahams et al., 2000), so the existence of a true aphasic disorder is still a matter of debate. Moreover, the difficulty in distinguishing articulatory difficulties from linguistic disturbances complicates the interpretation of the aphasic-like symptoms. Most authors ascribed the language impairment to executive deficits rather than to aphasia; poor verbal fluency or errors in sentences comprehension in the

absence of expressive or receptive language impairment, for example, result from a higher order executive deficit rather than primary linguistic abilities (Talbot et al., 1995; Abrahams et al., 2000). On the contrary, other studies interpreted difficulties in comprehension as an evidence of aphasia (Doran, Xuereb, Hodges, 1995). Recently Taylor and coll. (2013), using a broad range of language tests, highlighted the presence of language deficits in ALS patients, which appear, in part, dissociable from executive alterations and suggested that language impairment could be more prevalent than executive dysfunction. Further work in Japanese patients with ALS (with and without dementia) has shown writing errors (Satoh, Takeda, Kuzuhara, 2009; Ichikawa et al., 2010), which were postulated to be predictive of subsequent frontotemporal degeneration (Ichikawa et al., 2011). Such findings show that executive dysfunction alone cannot account for variations in cognitive phenotypes, which implies heterogeneity in ALS. Goldstein and Abrahams (2013) concluded that the exact nature of the language impairment and whether impairment represents subclinical levels of language variants of frontotemporal dementia or a specific feature of amyotrophic lateral sclerosis, or both, has yet to be established (Abrahams, 2012).

These findings raise a relevant issue with regard to the diagnosis and the nosographic classification of ALS patients, since the current Strong's consensus criteria rely on executive dysfunctions measures alone for diagnosing cognitive impairment in ALS and so it should need to be updated to include language assessment (Taylor et al., 2013, Abrahams et al., 2013b).

Social cognition and emotional processing

Social cognition, which is crucial for human interaction, has been reported as impaired in various studies of frontotemporal dementia. Lough and colleagues (2006) showed that patients with bvFTD had impaired recognition of all emotions, and particularly anger and disgust, which might partly explain the difficulty these patients have with identification of social violations. Empathy, as rated by carers, was also abnormal in these patients. Thus, social reasoning seems to be disrupted in various ways in bvFTD. This possibility has also been investigated in patients with ALS. Lulé and colleagues (2005) showed that in early stages of the disease emotional responses of ALS patients tend to be altered toward positive valence and toward a more balanced arousal state: they express more positive verbal emotional judgements and rate exciting pictures as less arousing and exciting than controls; moreover, patients with ALS showed lower responses in the anterior insula and extrastriate

visual areas than other participants did. This suggests that arousal is reduced at the neural and behavioural levels during the course of ALS. The authors also noted a greater response in the right supramarginal area in patients with ALS than in control patients, maybe suggesting an altered sensitivity to social and emotional cues. In addition, Papps et al. (2005) found a failure to show the normative pattern of enhanced recognition memory for emotional words in ALS patients.

Impaired performance on tests of the Theory of Mind - ToM (i.e., the ability to infer mental states in others) has been detected on the basis of tests that use cartoons, story comprehension tests, judgments of whether *faux pas* have been committed or of preference based on eye gaze, and understanding of social contexts (Girardi, MacPherson, Abrahams, 2011; Palmieri et al., 2010; Gibbons et al., 2007). This deficit was over and above the presence of executive dysfunctions, suggesting a profile of cognitive and behavioural dysfunction indicative of a subclinical FTD syndrome.

Recently, van der Hulst and colleagues (2014) delineated ToM deficits, by distinguishing between Affective and Cognitive subcomponents. They found that ALS patients showed a significant impairment in Affective ToM only when compared with healthy controls. A Cognitive ToM deficit was found in 27% of patients. Moreover, patients with either an Affective and/or Cognitive ToM deficit exhibited poor self-awareness of their performance and abnormalities on verbal fluency, while those with an Affective ToM deficit also displayed higher levels of apathy and a naming deficit.

In conclusion, these composite studies show that a significant subgroup of ALS patients exhibit cognitive deficits affecting frontal lobe functioning, specifically in planning, attention, and verbal fluency. Furhtermore, an involvement of memory and language skills, which could be partly due to frontal dysfunction, is now recognised as integral part of ALS cognitive profile. The severity of cognitive abnormalities ranges from a subtle impairment, detected only by accurate neuropsychological testing, to overt dementia, meeting criteria for FTD.

2.3. Behavioural impairment

Behavioural impairment is now recognized as a feature of ALS and cognitive and behavioural impairment seem to co-exist in approximately 25% of ALS patients (Newsom-Davis, Abrahams, Goldstein, Leigh, 1999; Murphy, Henry, Lomen-Hoerth, 2007). Several

rating scales such as the Neuropsychiatry Inventory, Frontal Behaviour Inventory, and Frontal Systems Behaviour Scale have shown that up to 63% of patients with ALS are apathetic, irritable, inflexible, restless and disinhibited (Lomen-Hoerth et al. 2003; Murphy, Henry, Lomen-Hoerth, 2007; Phukan et al., 2007). Emotional lability, i.e. the pathological occurrence of sudden episode of laughing or crying that are not necessarily coherent with patient's emotional state, has been estimated in 10–20% of ALS patients (Newsom-Davis et al., 1999). Apathy, one of the most common findings, should be differentiated from depression, fatigue, and respiratory dysfunction by careful examination of medical history and use of validated scales. The need to interview carers to identify behavioural changes might be heightened by patients' apparent absence of insight into such changes (Palmieri et al., 2010); absence of insight might be more pronounced in people with ALS-FTD than in those with only ALS (Woolley, Moore, Katz, 2010; Terada et al., 2011) and it is not noted in all patients or across all measures (Flaherty-Craig, Brothers, Yang, Svoboda, Simmons, 2011). An association between apathy and absence of insight has been postulated in ALS, in so far as absence of insight into cognitive change is accompanied by little concern about (progressive) cognitive decline (Flaherty-Craig et al., 2011).

Apathy and difficulties with social judgment seem to be more frequent in patients with bulbar onset ALS, than in patients with non-bulbar ALS (Grossman, Woolley-Levine, Bradley, Miller, 2007; Flaherty-Craig, Eslinger, Stephens, Simmons, 2006). This finding is consistent with other reports demonstrating that cognitive dysfunctions are more common in individuals with bulbar-onset ALS (Abrahams et al., 1997; Strong et al., 1999; Lomen-Hoerth et al., 2003; Schreiber et al., 2005; Ogawa, Tanaka, Hirata, 2009). However, other studies have failed in finding a link between bulbar-onset and cognitive decline (Kew et al., 1993; Mantovan et al., 2003; Ringholz et al., 2005; Rippon et al., 2006).

According to current consensus criteria (Strong et al., 2009), a diagnosis of ALS with behavioural impairment (ALSbi) requires that the patient meets at least two non-overlapping supportive diagnostic features from either the Neary criteria (Neary et al., 1998) and/or Hodges' criteria (Hodges & Miller, 2001) for FTD. The prevalence of ALSbi varies depending on methodology and diagnostic criteria and behavioural impairment in ALS can be classified on the basis of presentation of frontal-lobe-type behavioural impairment in two or more areas, as measured from a standardised caregiver interview (Murphy et al., 2007). Even in the absence of full-blown frontotemporal dementia, the interrelations between cognitive and behavioural changes in ALS are complex and have led to classifications of

patients based on whether a cognitive or behavioural impairment, or both, is present (Strong et al., 2009; Murphy et al., 2007b), providing a useful framework to establish the range of behavioural and cognitive problems in people with ALS. Lillo and colleagues (Lillo, Savage, Mioshi, Kiernan, Hodges, 2012) combined neuropsychological, behavioural, and neuropsychiatric measures in order to compare patients with ALS with patients presenting with behavioural variant of FTD (bvFTD) and healthy controls; they found that the cognitive and behavioural profiles of patients with ALS closely mirrored those noted in bvFTD, with a clear overlap between bvFTD and ALS. The authors concluded that the impaired inhibitory control and behavioural changes in ALS patients might result from oribitofrontal dysfunction.

A recent study (Mioshi et al., 2014) investigated patient susceptibility to neuropsychiatric symptoms in the context of progression of more classic motor symptoms, also examining the impact of neuropsychiatric symptoms on survival. Results showed that such symptoms may appear as early features in ALS, even before the development of more classic ALS motor symptoms, although they do not seem to alter survival. Such findings further confirm ALS as a multisystem disorder, involving different brain regions concomitantly, and support the concept of an ALS-FTD continuum, leading to relevant clinical implications regarding disease-modifying therapies and carer management.

2.4. Subphenotypes associated with genotypic variation

Several studies comparing patients with familial disease with those with sporadic disease reported the potential effects of specific gene mutations on cognition and behaviour; in particular, patients with a hexanucleotide repeat expansion in C9orf72 have been found to manifest a distinct phenotype, characterized by a significantly higher frequency of co-morbid frontotemporal dementia, a greater impairment in executive and non-executive cognitive function, and more relevant behavioural changes (Byrne et al., 2012). Moreover, Byrne and colleagues found a distinct pattern of non-motor cortex changes on high-resolution 3T magnetic resonance structural neuroimaging, identifying significant differences in grey-matter atrophy in the cohort with the repeat expansion with regard to the following brain regions: right inferior frontal gyrus, right superior frontal gyrus, left anterior cingulated gyrus, and the right precentral gyrus. These findings strengthen the notion that some subtypes of ALS are aetiologically associated with cognitive or behavioural

impairment and sometimes FTD. This association has previously been reported in rare families with ALS and mutations in SOD1, TARDBP, OPTN, UBQLN2, or FUS, but seems to be more common in families with the C9orf72 mutation. In fact, most genes linked to an ALS motor phenotype might, in some carriers of the gene defect, also give rise to cognitive or behavioural impairment and sometimes FTD, suggesting that the frontal lobes including the motor cortex might be a primary target for the effects of mutations in these genes.

Several studies further characterized the phenotype of ALS with the c9orf72 expansion, demonstrating that this cohort of patients seems to present earlier disease onset, a strong family history of neurodegeneration (ALS or FTD), bulbar-onset, more rapid progression and reduced survival (Irwin et al., 2013; Chiò et al., 2012; Ratti et al., 2012; Mahoney et al., 2012).

With regard to FTD-ALS associated with c9orf72 mutations, distinct neuropsychiatric features were identified, with an increased incidence of psychotic symptoms (particularly delusions, but also visual and auditory hallucinations) in FTD-ALS c9orf72 carriers (Snowden et al., 2012; Takada & Sha, 2012; Boeve & Graff-Radford, 2012).

2.5. Overlapping between ALS and FTD

Although ALS and FTD represent two different entities, it is now recognised that these two disorders are neurodegenerative conditions with overlapping clinical and neuropathological features, that represent a continuum between motor and non-motor cortical degeneration. Such overlap is further confirmed by the presence of TDP-43 inclusions both in FTD patients without tau pathology, and in sporadic and familial case of ALS (Neumann et al., 2006; Kwong, Neumann, Sampathu, Lee, Trojanowski, 2007). Moreover, neuroimaging data demonstrated the involvement of extra-motor cerebral regions in ALS patients, including cortical areas typically involved in FTD (Ludolph et al., 1992; Jeong et al., 2005). Furthermore, a proportion of ALS patients displays cognitive and behavioural changes that can reach criteria for FTD. Strong and colleagues (2006) found a pattern of mental change that was indistinguishable from that of FTD in a group of patients with dementia and ALS. In a large study involving 279 ALS patients, 50% manifest cognitive impairment and 15% met criteria for FTD (Ringholz et al. 2005). Murphy et al. (2007) found a spectrum of frontal lobe dysfunction in half of the patients, with five of them (22%) meeting Neary's criteria for FTD. These studies support the hypothesis of a clinical

continuum between ALS and FTD. The timescale of onset and the pattern of cognitive/behavioural symptoms in this continuum are still not clear, but reports have suggested that FTD may reflect its end stage. However, as above mentioned, several studies have found no meaningful progression of cognitive deficits over time. Moreover, if cognitive deficit exists in ALS, these could affect an effective assessment of cognitive abilities in these patients.

2.6. Cognitive assessment and longitudinal studies in ALS: feasibility and limitations

Few longitudinal studies have investigated the progression of cognitive decline in ALS. In particular, a group of studies evaluating ALS patients during a six-month period overall showed a slight progression of cognitive impairment over the course of the illness (Strong et al., 1999; Abrahams et al., 2005; Robinson et al., 2006; Gordon et al., 2010). In particular, Strong and colleagues (1999) found a progression over time of the cognitive deficits across several domains, including working memory, problem solving, mental flexibility, recognition memory for words and faces, and visual-perceptual skills in five patients with bulbar-onset ALS, while limb-onset ALS patients showed no decline at the six months follow-up. A RM spectroscopy following the neuropsychological testing demonstrated a significant neuronal loss in the anterior cingulate gyrus in bulbar patients that was evident early in the course of cognitive impairment and correlated with the onset of impaired cognition. However, other 12-, 18- and 24-months follow-up studies (Kilani et al., 2004; Schreiber et al., 2005; Elamin et al., 2013) showed heterogeneous cognitive performances among ALS patients. Schreiber and colleagues (2005), for example, noted that cognitive deficits were present at initial testing and, after the early decline, seemed to remain stable over time in contrast to motor decline; in addition, bulbar-onset patients performed worst in many neuropsychological tests than spinal-onset ones and this subgroup difference increased on follow-up. These findings were replicated by another longitudinal study (Abrahams et al. 2005), in which selective deficits in spoken and written verbal fluency did not show deterioration over a six months period in a group of non-demented ALS patients. In a study by Robinson et al. (2006), no significant and meaningful between-group and within-group differences in cognitive function were found over time. Individual analyses, however, showed that seven of 19 ALS patients developed abnormal cognitive performances

especially in tests assessing short-term recognition memory, hypothesis generation, working memory, verbal learning, and verbal long-term recognition memory over a six-month period. Elamin and colleagues' results (2013) supported the variability of cognitive profiles detected at the baseline and at the follow-ups in ALS, associated with genetic factors and motor progression, concluding that cognitive status in ALS may represent a phenotypic marker for distinct subtypes within the disease.

To date, longitudinal studies performed on ALS patients with regard to neuropsychological performances present some limitations; first of all, the lack of cognitive measures that take into account the presence of progressive verbal and motor impairments, in order to reduce the drop-out rate and fully capture the spectrum of cognitive alterations in ALS. This aspect may have lead to underestimate the cognitive decline observed in the recruited samples. Similarly, limb dysfunction and deterioration of speech make standard 'pen and pencil' neuropsychological testing rather complicated or even impossible in moderate-severe stages of ALS. Tests currently available often cannot be completed by a significant number of patients, making it impossible to assess the existence or degree of the cognitive impairment in these patients. However, evidence suggest the need for some task modifications in order to make the assessment suitable for patients with verbal-motor impairment (Osborne, Sekhon, Johnston, Kalra, 2014). Recently, Abrahams et al. (2014) developed the *Edinburgh Cognitive and Behavioural ALS Screen – ECAS*, a multidomain assessment designed to be sensitive to the range of cognitive and behaviour change in ALS, including not only measures of executive function and fluency, but also language (ALS-Specific functions) and ALS Non-specific functions (recall and recognition memory and visuospatial functions), in order to differentiate cognitive change characteristic of ALS from other disorders common in older adults, such as Alzheimer's disease (AD). Nevertheless, also this innovative screening tool could not be performed in moderate-severe stages of the disease.

Recently, as a mean to compensate for progressive loss of verbal and gestural communication in ALS patients, Augmentative and Alternative Communication (AAC) devices have been developed and employed, such as Eye-Tracking (ET) systems and Brain Computer Interface (BCI) devices.

3
Brain Computer Interface

3.1. Definition and essential features

Brain Computer Interface (BCI) is a communication system that does not depend on the brain's normal output pathways of peripheral nerves and muscles (Wolpaw et al., 2000). It enables the generation of a control signal from brain responses such as sensorimotor rhythms and evoked potentials and bypasses motor output to convey messages directly from the brain to a computer. BCI is a technical direct interface between the human brain and a computer that allows communication. Therefore, it provides new augmentative communication options to people with severe motor disabilities, such as ALS patients, since all other augmentative communication technologies require some form of muscle control, and thus may not be useful for this group of patients.

Users explicitly manipulate their brain activity instead of using motor movements to produce signals that can be used to control computers or communication devices: a BCI system sends a message via brain activity to an external device, which performs the desired action. In order to successfully use a BCI, feedback and the following adaptation of brain activity are extremely important.

Existing BCI systems can use a variety of different electrophysiological signals. Brain activity is mainly based on the Electroencephalography (EEG) to measure, for example, event-related brain potential (ERPs), but other techniques are available, such as magnetoencephalography (MEG), positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and functional near-infrared spectroscopy (fNIRS) (Wolpaw et al., 2000). However, PET, fMRI, and fNIRS are technically demanding and very expensive. Besides, they have a slow time resolution that does not allow rapid communication. On the contrary, EEG has a relatively short time constant, can be operated in many environments, and requires inexpensive devices, so it represents the most practical and suitable method for BCI development.

3.2. BCI use: learning a new skill

To successfully use BCIs, with the exception of the P300-based BCI, users have to learn how to intentionally manipulate their brain signals. Two approaches are used for training users to control their brain signals (Curran and Stokes, 2003). In the first, users are given specific cognitive tasks such as motor imagery to generate measurable brain activity; using this technique the user can send a binary signal to the computer. The second approach, called operant conditioning, provides users with continuous feedback as they try to control the interface (Tan & Nijholt, 2010). Over many sessions, users acquire control of the interface without being consciously aware of how they are performing the task. This latter method is based on the need for feedback of the normal neuromuscular output pathways in order to successfully perform operations. Consequently, a BCI that works as a replacement for these normal output channels also depends on feedback and on adaptation of brain activity based on the feedback. Thus, a BCI system must provide feedback and must interact productively with the adaptations the brain makes in response to that feedback. This means that BCI operation depends on the interaction of two adaptive controllers: the user's brain, which produces the signals measured by the BCI; and the BCI itself, which translates these signals into specific commands (Wolpaw et al., 2002). Thus, a successful BCI requires that the user develops a new skill, that is, the control of specific electrophysiological signals, and that the BCI turns this control into an output, which should correspond to the user's intent. As mentioned, a certain level of training is required for this dual adaptation between the computer and the user. One of the main problems related to the use of BCI with ALS patients is the fatigue for the sustained attention that is required to learn how to regulate the brain activity. Several studies of BCI with ALS patients, in fact, showed that they may not be able to learn how to regulate brain activity because of the fatigue of tolerating long-term training with focused attention (Kübler et al., 1999; Hill et al., 2006). Bai, Lin, Huang, Fei, Floeter (2010) developed a user-friendly BCI which requires minimal training and reduced mental effort; with this BCI, ALS patients could achieve a good accuracy in a BCI paradigm associated with human natural motor behaviour.

3.3. Dependent and Independent BCIs

There are two different classes of BCIs: dependent and independent. A dependent BCI does not use the brain's normal output pathways to carry the message, but activity in these pathways is needed to generate the brain activity (Wolpaw et al., 2002). For example, a dependent BCI uses a matrix of letters that the subject selects by looking directly at it, so that by recording the visual evoked potential (VEP) from the scalp over the visual cortex it is possible to determine gaze direction (Sutter, 1992). In this case, the brain's output channel is EEG, but this signal depends on gaze direction and therefore on extraocular muscles and cranial nerves that activate them.

In contrast, an independent BCI does not depend in any way on the brain's normal output pathways, because the message is not conveyed by peripheral nerves and muscles and the activity in these pathways is not needed to generate the brain activity. For example, an independent BCI uses a matrix of letters that the subject selects by producing a P300-evoked potential when certain letters flash (Donchin, Spencer, Wijesinghe, 2000). The brain's output channel is a certain signal in the EEG and the generation of that signal does not depend on the orientation of the eyes, but on the user's intent (Sutton, Braren, Zubin, John, 1965; Donchin, 1981; Fabiani, Gratton, Karis, Donchin, 1987). This second class of BCIs seems to be more useful with ALS patients, who show damage in the output ways of peripheral nerves and muscles.

3.4. Components of a BCI system

The purpose of a BCI is to detect and quantify features of brain signals that indicate the user's intentions and to translate these features in real time into device commands that accomplish the user's intent. To achieve this, a BCI system consists of 4 sequential components: (1) signal acquisition, (2) feature extraction, (3) feature translation, and (4) device output (see Figure 3.1; Cipresso et al., 2012).

These four components are controlled by an operating protocol that defines the onset and timing of operation, the details of signal processing, the nature of the device commands, and the oversight of performance. An effective operating protocol allows a BCI system to be flexible and to serve the specific needs of each user.

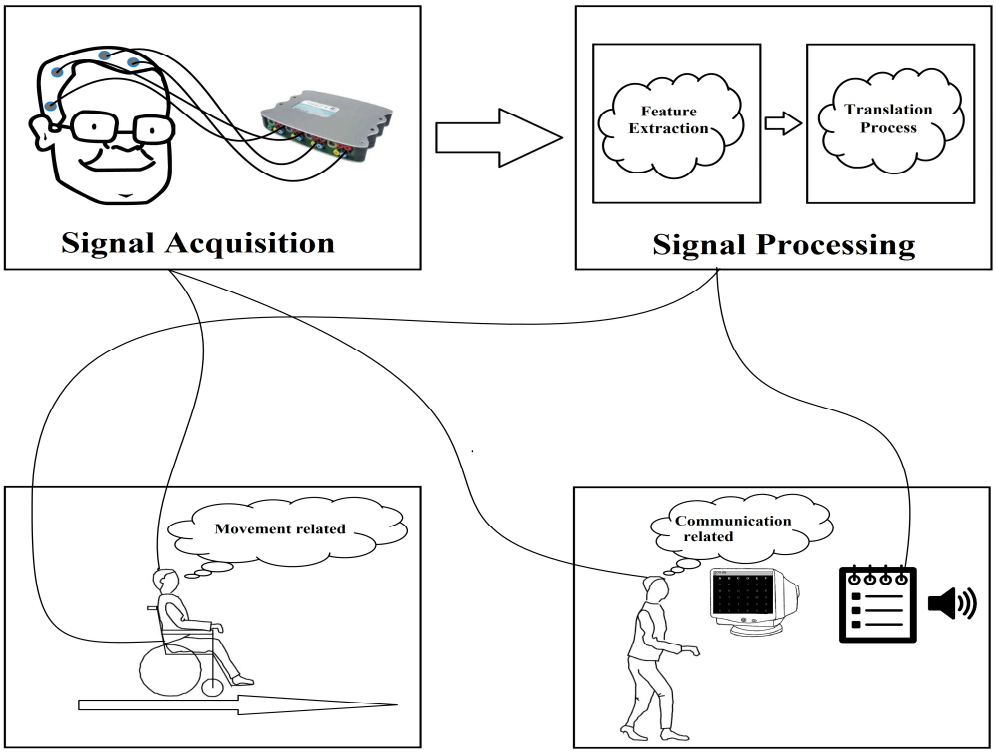

Figure 3.1. Basic design and operation of any BCI systems. Signals from the brain are acquired by electrodes on the scalp and processed to extract specific signal features (e.g. amplitudes of evoked potentials or sensorimotor cortex rhythms, firing rates of cortical neurons) that reflect the user's intent. These features are translated into commands that operate a device (e.g. a simple word processing program or a wheelchair).

1) Signal acquisition

Signal acquisition is the measurement of the electrophysiological activity of the brain. This measurement is usually recorded via electrodes that can be either non-invasive (e.g., EEG) or invasive (i.e., intracortical). Moreover, BCIs can be categorized by whether they use evoked (e.g., EEG signals elicited by flashing letters) or spontaneous (e.g., EEG rhythms over cortex) inputs. Evoked inputs are generated by sensory stimulation provided by the BCI, while spontaneous inputs do not depend on such stimulation. The most common type of input is EEG recorded from the scalp (Vidal, 1977; Farwell & Donchin, 1988; Pfurtscheller et al., 2000; Freeman, Holmes, Burke, Vanhatalo, 2003). In this first part of BCI systems, the input is acquired by the electrodes and amplified to levels suitable for electronic processing. Signals are then digitalized and transmitted to a computer for further analysis.

2) Signal processing: Feature extraction and translation

Future extraction is the process of analyzing the digital signals to distinguish pertinent signal characteristics (ie, signal features related to the person's intent) from extraneous content and representing them in a compact form suitable for translation into output commands. These features should have strong correlations with the user's intent. Environmental artefacts and physiologic artefacts are avoided or removed to ensure accurate measurement of the brain signal features.

The resulting signal features are then passed to the feature translation algorithm, which converts the features into the appropriate commands for the output device (i.e., commands that accomplish the user's intent). The translation algorithm should be dynamic to accommodate and adapt to spontaneous or learned changes in the signal features and to ensure that the user's possible range of feature values covers the full range of device control.

3) Device output

The output device is usually a computer screen and the output is the selection of targets (letters or icons) presented on it, performed by the BCI (see, for example, Farwell & Donchin, 1988; Wolpaw, McFarland, Neat, Forneris, 1991; Perelmouter, Kotchubey, Kübler, Birbaumer, 1999; Pfurtscheller et al., 2000). These targets are flashed or indicated in various ways. Other BCIs output includes moving a cursor on the screen, controlling a robotic arm, or controlling some other physiological process.

4) The operating protocol

The BCI system has an operating protocol that guides its operations. It defines how the system is turned on and off, what kind of feedback is provided to the user, the sequence and the speed of interactions between user and system, and the speed with which the system implements commands (Wolpaw et al. 2002; Leuthardt, Schalk, Roland, Rouse, Moran, 2009). Most research protocols are not completely suitable for BCI applications that serve the needs of people with disabilities, since the investigator sets these parameters and the users do not have on/off control, they just have to achieve very limited goals and tasks. However, in real life the user must be able to do these things by her- or himself and such differences can complicate the shift from research to application.

3.5. Brain activity detection through EEG

Non-invasive EEG-based BCIs are the most widely researched approach owing to the minimal risk involved and the relative convenience of conducting studies and recruiting participants. EEG usually uses small electrodes placed directly on the scalp at standardized positions. When a neuron is activated, a local current flow is produced and weak potential differences (5–100 μV) between electrodes are measured. A large population of active neurons must be involved to generate electrical activity that can be detected with EEG over the scalp (Srinivasan, Nunez, Silberstein, 1998). The electrodes record brain activity that is converted into digital signals and a sequence of steps translate this signals into commands. A limiting issue with EEG recording is its low spatial resolution, ranging between 2 and 3 cm. Moreover, EEG is deduced from apical dendrites of cortical pyramidal cells (Teplan, 2002), thus activity of deeper structures can only be studied indirectly. Because of the fluid, bone, and skin separating the electrodes from the electrical activity, signals tend to be smoothed and noisy. This makes it difficult to locate the exact source of the oscillation. Nevertheless, EEGbased BCI have been shown to support a high performance and most of clinical applications of BCI in patients with severe motor disorders have been demonstrated using EEG (e.g., Kübler et al. 2005; Vaughan et al., 2006; Nijboer et al., 2008). The changes in power of four frequency bands are used as control signals for BCI systems: delta (1–3 Hz), theta (4–7 Hz), alpha (8–12 Hz), and beta (13–30 Hz).

3.6. Present-day BCIs: four groups of electrophysiological signals

Different non-invasive electrophysiological signals can be used as input for BCI systems. Therefore, BCIs can be classified into four groups according to the electrophysiological signals they use.

Visual evoked potentials (VEP)

VEPs are evoked electrophysiological potential that can be recorded from the scalp over visual cortex. VEPs are elicited by visual stimuli such as flashes of light or flickering illumination presented on a screen. Users are presented with a 8 × 8 panel with different items and they have to fix their gaze on the item they want to select. Subgroups of these 64 symbols undergo an equiluminant red/green alternation or a fine red/green check pattern

alternation. Each symbol is included in several subgroups, and the entire set of subgroups is presented several times. BCI detects the VEP elicited by the stimulus where the subject looked at and the waveform of the VEPs depends on stimulus' temporal frequency. However, in patients with neurological disease such as ALS, which causes uncontrollable head and neck muscle activity, communication problems with BCI were described (Sutter, 1992), since scalp EMG can impede reliable VEP measurement and reduce performance. Moreover, the VEP-based BCI requires an intact ability to control gaze direction (Kübler, Kotchoubey, Kaiser, Wolpaw, Birbaumer, 2001b) and this is a problematic issue for patients with completely locked-in syndrome. This kind of communication system is categorized as dependent BCI, because it depends on muscular control of gaze direction.

Slow cortical potentials (SCP)

SCPs are slow voltage changes generated in the cortex, that occur over 0.5-10.0 sec. Negative SCPs are typically associated with movement and other functions involving cortical activation, while positive SCPs are usually associated with reduced cortical activation (Rockstroh, Elbert, Canavan, Lutzenberger, Birbaumer, 1989; Birbaumer, 1997). Users can learn to control SCPs, although it requires a long training. Several studies showed that SCPs originating from central and frontal regions could be brought under voluntary operant control after training (Lutzenberger, Roberts, Birbaumer, 1993) and the importance of the anterior brain systems for the control of these functions has been further confirmed. As a matter of fact, patients with prefrontal dysfunction show extreme difficulties in learning SCP control, even if other cognitive functions are preserved (Lutzenberger et al., 1980; Birbaumer, Elbert, Rockstroh, Lutzenberger, 1986; Schneider et al., 1992). It is suggested that also patient with ALS show difficulties in voluntarily controlling local cortical excitation, because of the involvement of motor and premotor cortical systems in this disease.

Mu rhythm (sensorymotor rhythms - SMR)

Mu rhythm refers to 8–12 Hz EEG activity that can be recorded over primary motor and somatosensory cortex when awake subjects are not engaged in processing sensory input or producing motor output (Niedermeyer, 2004). Mu rhythm activity comprises a variety of different 8–12 Hz rhythms, distinguished from each other by location, frequency, and/or relationship to concurrent sensory input or motor output. These mu rhythms are usually

associated with 18–26 Hz beta rhythms. SMRs are associated with those cortical areas most directly connected to the brain's motor output pathways. Movement or preparation of movement is associated with a decrease in mu and beta rhythms, labelled 'event-related desynchronization' (ERD), while relaxation is accompanied by a rhythm increase or 'event-related synchronization' (ERS) (Pfurtscheller, 1999; Pfurtscheller et al., 2000). Notably, these rhythm changes occur also with motor imagery (i.e., mental representation of a movement) and do not require effective movement (Pfurtscheller & Neuper, 1997; McFarland, Miner, Vaughan, Wolpaw, 2000). Therefore, they may be used in independent BCI systems, which can be successfully adopted by paralyzed patients.

P300 evoked potential

P300 evoked potentials are elicited over parietal cortex at about 300 ms after infrequent or particularly significant auditory, visual or somatosensory stimuli. In the next paragraph, P300-based BCI will be extensively described.

3.7. P300-based BCI systems

3.7.1. P300 event-related potentials

The P300 event-related potential is one possible EEG-based BCI control signal. These signals include both spontaneous electrical activity of the cerebral network and the cortical response to external or internal events. Event-related potentials are defined as brain activity that is elicited in response to events (Donchin et al., 2000). ERPs can be distinguished in exogenous and endogenous. The former are the result of early and automatic processing of stimuli, whereas the latter correspond to later and more conscious processing of stimuli (Kübler et al., 2001b). Conscious processing occurs only after 100 msec, when the visual signal is under way toward extrastriate areas and areas in the parietal and temporal cortex, while unconscious processing starts in the first 100 msec and still occurs after this latency. Since endogenous ERPs depend on subjects' attention to contextual stimuli and intentionality, they seem more suitable to be used in BCIs, with respect to exogenous ones.

As above mentioned, infrequent or significant auditory, visual, or somatosensory stimuli, when interspersed with frequent or routine stimuli, typically evoke in the EEG over parietal cortex a positive peak at about 300 msec (Walter, Cooper, Aldridge, McCallum, Winter,

1964; Sutton et al., 1965; Donchin & Smith, 1970; Fabiani et al., 1987). Donchin and colleagues have used this 'P300', or 'oddball' response to implement a BCI device that can communicate letters to a computer – the 'P300 Speller'. (Farwell & Donchin, 1988; Donchin et al., 2000). The P300 Speller presents a 6 × 6 matrix of letters, numbers, and/or other symbols or commands. Every 125 ms, a single row or column flashes; and, in a complete trial of 12 flashes, each row or column flashes twice. The user makes a selection by counting how many times the row or column containing the desired choice flashes. EEG over parietal cortex is digitized, the average response to each row and column is computed, and P300 amplitude for each possible choice is computed.

Besides, a modification of the oddball task is the three-stimulus paradigm, in which infrequent distractor stimuli are inserted into the sequence of target and standard stimuli. In this case, a novelty P300 can be produced, named P3a, which is an early peak over the frontal and central areas and is thought to reflect frontal lobe function. P3a can be elicited also for typical, rather than novel, stimuli, when the perceptual distinctiveness between the target and the standard stimulus is quite difficult and the distractor stimulus is not novel, but highly discrepant. On the contrary, P300 arising from the target stimulus detection is a later peak with a large parietal amplitude, and has been called P3b, which is synonymous with P300 (Polich, 2004). While P3a is produced when a demanding stimulus automatically drives frontal lobe-mediated attention, P3b is produced when attentional resources are intentionally allocated for stimulus classification. Neuroanatomically, the P3a is thought to reflect activity of the anterior cingulate gyrus when new stimuli are processed into working memory, while the P3b is thought to reflect subsequent activation of the hippocampal formation when frontal lobe mechanisms interact with the temporal/parietal lobe connection (Polich, 2007; Verleger, 2008). High task difficulty increases focal attention and enhances P3a amplitude by constraining other memory operations that reduce P3b amplitude and increase P3b latency (Hagen, Gatherwright, Lopez, Polich, 2006).

The P300 is a slow wave oscillation associated with behavioural and attention processes; it can be considered as an index of the amount of central nervous system (CNS) activity related to incoming information processing. Besides, it is thought to detect brain activity associated to working memory when the neural representation of the stimulus environment changes with new sensory input. For these reasons, it has been used as a measure of cognitive functions in both healthy subjects and patients.

The P300 component is measured by assessing its amplitude (size) and latency (timing). While the amplitude consists of the voltage difference between a prestimulus baseline and the largest positive-going peak of the ERP wave form within a latency window, latency is defined as the time from stimulus onset to the point of maximum positive amplitude within the latency window (Polich, 2004). In particular, P300 latency reflects stimulus classification speed, with shorter latency associated to better cognitive performances in attentional and immediate memory tasks. It follows that P300 measures are affected in neurological and psychiatric disease. Moreover, latency is not dependent on overt behavioural response and reaction times, so that it can be used as a motor-free measure of cognitive function. In order to distinguish the P300 from the background activity, generally dozens or hundreds ERPs are generated and averaged, so that the noise influence can be cancelled. The amplitude of P300 is influenced by several factors: the target probability of appearance, the amount of time between the presentation of two stimuli, habituation effects, attentional and motivational issues, and the task difficulty (Gonsalvez & Polich, 2002; Hoffmann, Vesin, Ebrahimi, Diserens, 2008; Kleih, Nijboer, Halder, Kübler, 2010). In particular, the amplitude of P300 can be decreased in presence of highly probable events, with the probability for the target stimulus set to values around 10%. Besides, the shorter is the amount of time between two stimuli, the lower becomes the P300 amplitude. The P300 amplitude can also be decreased by habituation effects, which can appear when repeatedly presenting the same item, but only when short interblock intervals and many trial blocks are used (Polich & McIsaac, 1994). Finally, fatigue effects, with a reduction in attentional capabilities, and a high task complexity can cause a reduction in P300 amplitude. Also biological factors influence P300 amplitude and latency. Polich (2004) distinguished among natural factors (such as circadian, i.e., body temperature and heart rate, ultradian, seasonal, and menstrual cycles), environmentally induced factors (exercise, fatigue, drugs, and alcohol assumption), together with constitutional (age, gender, handedness), and genetic components. Biologic ERP effects can be reduced by ensuring that subjects are assessed similarly with respect to most of these variables.

3.7.2. Auditory, visual, and tactile P300-based BCIs

P300-based BCIs can involve visual, auditory, or tactile stimuli presentation. In the field of BCI systems development, an important issue is to determine if a BCI device can work effectively using different presentation modalities, since possible users may present

auditory or visual deficits. Previous research has shown that both auditory and visual oddball tasks elicit large P300 responses (Squires, Donchin, Squires, Grossberg, 1977; Duncan-Johnson & Donchin, 1982; Fabiani et al., 1987). In addition, several studies reported higher accuracy and larger ERP amplitude when auditory and visual stimuli were presented simultaneously, than when either modality was presented by itself (McDonald, Teder-Salejarvi, Hillyard, 2000; Teder-Salejarvi, McDonald, Di Russo, Hillyard, 2002).

As described above, Farewell & Donchin (1988) first used P300 to select items displayed on a computer monitor. A problematic issue of visual P300 is its application with subjects suffering from visual impairments. In fact, users are required to fixate the matrix cell on the screen and to concentrate on it. For such reason, a preserved visual attention is supposed to be necessary in order to use P300 BCI. Treder and Blankertz (2010) investigated if a good performance at BCI depends on eye movements control (i.e., overt attention) or whether it is also possible with targets in the visual periphery (covert attention). They found that ERP-based BCI can be driven in both modes of attention, but the performance was significantly better for overt attention. The authors suggest the importance of developing innovative spellers that are reliably based on peripheral vision, since most of ALS patients show impaired eye movements.

Also Brunner and colleagues (2010) explored this issue and found that the accuracy of P300 speller is affected by gaze direction, so its clinical applicability in ALS patients with impaired gaze may be limited. In such cases, auditory stimuli could be more suitable.

The auditory version of the oddball task uses two different tones and an interstimuli interval of a few seconds, with the target stimulus occurring less frequently than the standard one. As in the classic visual paradigm, the subject is required to distinguish between the two tones by responding to the target with a covert or overt response. Only few studies have employed auditory oddball to elicit particular event-related potentials with P300 BCIs systems (see, for example, Hill et al. 2006; Sellers & Donchin, 2006; Furdea et al., 2009; Klobassa et al., 2009). Hill et al., (2006) used a paradigm of two simultaneous pure tone streams, in which subjects focused their attention on one of the streams and ignored the other to make a binary decision. Sellers and Donchin (2006) tested healthy volunteers and ALS patients with a P300-based BCI. The words were presented visually, auditorily, or in both modalities. The authors showed that although the visual and visual plus auditory modality reached higher accuracy levels, a P300-based BCI using the auditory modality is feasible for both healthy

and disabled subjects. However, the speed of the system is reduced, since spoken words were used.

The major limitation of some of these paradigms is that they provide no more than two to four alternative choices per trial. An auditory spelling system was presented by Furdea et al., (2009), which realized a multichoice auditory BCI by a 5 × 5 matrix of spoken numbers. Each character's position in the matrix was coded by two auditorily presented number words: one corresponding to the row and one corresponding to the column. To select a particular target, the participant had to attend to the two target stimuli representing the coordinates of the character in the matrix. The subjects were instructed to first select the row number and then the column number containing the target letter. The authors found lower accuracy in the auditory modality than in the visual modality. Klobassa et al., (2009) designed a paradigm that employed auditory stimuli to operate a 6 × 6 P300 speller, thereby increasing the number of choices per trial to 36. Even if they found a higher accuracy with respect to previous studies using auditory BCIs, however, the speed and accuracy of the auditory speller was still lower than that of the visual version. In fact, average accuracy for the 6 × 6 36-item matrix for the visual P300 speller is typically 80–90% (e.g., Krusienski et al., 2006; Sellers, Kübler, Donchin, 2006), whereas in this study the mean online accuracy of the auditory P300 speller for the last sessions was about 66%. BCI based on EEG responses to vibrotactile stimuli has the advantage of not requiring the presence of preserved visual or auditory system and of being potentially unnoticeable to other people. Moreover, they can be used in navigational applications, since a correspondence between the tactile stimulation and the spatial information is present. Brower and van Erp (2010) investigated the feasibility of a tactile P300-BCI. Participants were asked to attend to the vibrations of a target, embedded within a stream of distracters. The number of targets was two, four, or six. The authors did not find a difference in Step-Wise Linear Discriminate Analysis (SWLDA) classification performance between the different numbers of tactors. They demonstrated the feasibility of a tactile P300 BCI and also proved that the stimulus onset asynchrony (SOA) for an optimum performance was close to the conventional SOA of visual P300 BCIs.

3.7.3. P300-based BCIs: advantages, current changes and emerging trends

P300-based BCIs are independent BCI systems, since the generation of P300 does not depend on the exact orientation of the eyes and on the activity of peripheral nerves and muscles, but it mainly depends on user's intent to pay attention to one stimulus. Thus, with

the limitations described below, its use is possible with patients suffering from oculomotor dysfunctions, such as ALS and locked-in patients.

Besides, one of the greatest advantages of the P300 BCI is that it does not require intensive user training, as the P300component results from endogenous attention-based brain function. In a recent study, Guger et al. (2009) proved that the P300-based BCI can achieve high accuracy after only 5 min of training. In such study, 72.8% of the subjects reached 100% accuracy with a row-column paradigm speller. Interestingly, the authors found that the system was more accurate for people who slept less the previous night, while no significant differences were observed with regard to gender, level of education, working duration, and cigarette and coffee consumption. These results overcome those obtained in a previous study by Guger, Edlinger, Harkam, Niedermayer, Pfurtscheller (2003), where they tested a motor imagery-based BCI system and found that, after 20–30 min (two sessions) of training, about 93% of the subjects were able to achieve classification accuracy above 60%.

These findings highlights that P300-based BCIs are a far more practical choice than SMR-based BCIs. In P300-BCIs, P300 ERPs from several trials are averaged, in order to improve accuracy and reduce noise. The classifier discriminates which stimuli elicit a robust P300. If none of the stimuli provoke an ERP different from other ERPs, it means that it is not possible to use P300 for communicating. Such phenomenon has been observed across different BCI approaches, with 20% of subjects being not proficient in using BCI, and it has been called ‘BCI illiteracy’ (Kübler & Muller, 2007). The main explanation of such phenomenon is that not every person can generate the brain activity necessary to control a specific BCI. In fact, even if people’s brain shares (more or less) the same functional properties and subdivisions, some differences in brain structure can be present. For example, some users produce P300 evoked-related potential not detectable at the level of the scalp, so that EEG cannot be effectively performed. In particular, it has been observed that 10% of healthy subjects do not produce a robust P300.

However, P300 detection for real-time applications presents several challenges.

Firstly, two important criteria for evaluating the feasibility of a BCI system are the speed and the accuracy (Kübler et al., 2001a). The former is strictly related to the fact that the more rapidly a BCI can be controlled, the more amount of information can be produced by the user, and the faster communication is possible. Obviously, compared to speech, the BCI communication rate is reduced, but a limit presumably exists, below which the communication rate of a BCI should not fall.

Accuracy is the percentage of correct selections per time intervals. It represents a relevant issue, since a wrong selection could turn into an error in communication, with both practical and psychological consequences for the user. In order to avoid it, the BCI system must be equipped with options that allow a user to correct wrong selections. A balance between speed and accuracy should be identified. Besides, accuracy is diminished also by the close temporal proximity of multiple target stimulus presentation (Martens, Hill, Farquhar, Schölkopf, 2009; Salvaris & Sepulveda, 2009; Citi, Poli R, Cinel, 2010). Although this phenomenon may have been detected early on (Serby, Yom-Tov E, Inbar, 2005), recently a more concerted effort has been observed in the literature in order to try to overcome such limitation.

Moreover, technical challenges are related to the recording quality in environment different from the laboratory setting, such as the user home, when different sources of noise can disturb the EEG recording (Sellers, Kübler, Donchin, 2006). Besides, the patient respirators may introduce electrical or mechanical artefacts.

Finally, perceptual and cognitive skills, in particular the ability to pay selective and sustained attention to the target stimuli must be considered when employing P300 with neurological patients. It is necessary to determine whether or not a user is able not only to detect item on the computer display, but also to focus on a particular stimulus on the display. Furthermore, since using a P300-BCI requires attention and concentration and interference may occur between counting the number of flashes of the matrix cell and simultaneously concentrating on the characters to be selected, the user should not be distracted. Therefore, it may be difficult to use P300 in everyday life. Some pilot studies have shown that some patients may not be able to learn the necessary skills for proper and effective use of the P300-based BCI, due to an excessive distractibility and an incapacity to tolerate a long-term training (Kübler et al., 1999; Hill et al., 2006).

Other issues that may be problematic, especially during the initial stages of training with the BCI, are boredom and frustration sometimes reported by patients themselves. However, a recent study showed that motivational aspects are positively correlated with performance related to the BCI, by suggesting that highly motivated patients can get good results from the use of BCI as a communication tool; besides, some benefits with regard to patients psychological well-being seem to arise from satisfaction for the obtained results (Martens et al., 2009; Kleih, Nijboer, Halder, Kübler , 2010).

When planning to use P300 as an AAC device, it should be taken into account the common need of a training, which should involve both patients and their family, since such technology is not always easy to understand.

Besides, the AAC system should be developed according to the communicative needs of the specific person, and the tools provided must be flexible and adapt to the changes which may occur in the person needs (psychological, emotional, and social) and abilities. In fact, most of the patients who use BCI devices show some degree of cognitive impairment, which may has negative effects on the performances. Thus, it is compelling to extensively assess the presence of cognitive deficits and this is particularly relevant for ALS patients according to the most recent findings.

Recently, new paradigms have been introduced to improve P300 BCI performance. Among these, the most promising include:

▪ *single character (SC) speller*: SC randomly flashes one character at a time with a brief delay between flashes. The SC speller has a longer delay between flashes than the raw and column (RC) and character classification can be made with fewer flashes per character: the RC flasher is about two times faster than the SC flasher. However, the SC speller (15 flashes) results in larger P300 amplitudes compared to the RC speller with 15 flashes per column and row (Guger et al., 2009);

▪ *checkerboard (CB)*: CB paradigm was designed to overcome two specific problems with the standard RC presentation method. Firstly, the CB eliminates instances when the same character flashes twice in succession (also called a double target item flash). Moreover, the CB paradigm reduces the amount of distraction and/or inherent noise (i.e., non-target items receiving apparent target responses) to the RC paradigm. CB was found to produce significant improvements in accuracy, as compared to the RC paradigm: this novel paradigm disassociates the rows and columns of the matrix, thereby eliminating double flashes and significantly reducing distraction (Townsend et al., 2010);

▪ *region based (RB)*: this paradigm (Fazel-Rezai & Abhari, 2009) presents flashes of several regions instead of rows and columns. The character recognition is performed in two levels (Fazel-Rezai & Ahmad, 2011). In the first level, the characters are placed into seven groups located at different regions of the screen. Similar to the Farwell and Donchin paradigm, the user is instructed to attend a specific character in one of the seven groups while each group

of seven characters randomly flashes. After several flashes of each group the desired group is identified. In the second level, individual characters of the selected group are singly distributed into the seven regions. Similarly to the first level, different regions are flashed while the subject attends to one region (i.e., character). The desired character is selected by identifying one of the seven regions. The RB paradigm has been shown to decrease the near-target effect and human error and adjacency problem significantly (Fazel-Rezai & Abhari, 2009; Fazel-Rezai & Ahmad, 2011; Fazel-Rezai, 2007). Moreover, it was found that the overall spelling accuracies averaged for the same set of subjects, trials, and characters for RC, SC, and two variations of RB paradigms were 85%, 72.2%, 90.6%, and 86.1%, respectively, (Fazel-Rezai, Gavett, Ahmad, Rabbi, Schneider, 2011);

▪ *Moving and alternative stimuli*: this emerging approach involves motion rather than flashing stimuli. Guo et al. (2008), for example, introduced a new paradigm with five possible targets that represented left, right, up, down, and enter commands for a virtual keyboard. In this task, instead of flashing, a vertical bar below each of the five stimuli appeared and moved leftward at random intervals for 140ms. Results from offline analyses suggested that this approach yielded visual evoked potentials that could be useful in an offline BCI. Moreover, familiar faces, which are known to elicit the N170 and N400f (“f ” for faces) components of the ERP (Eimer, 2000), have also been used in a P300 BCI (Kaufmann, Schulz, Grünzinger, Kübler, 2011). Letters of the P300 matrix were superimposed on the flashed behind the letters. Taking into account the additional ERPs, the number of sequences necessary to achieve 100% accuracy could be significantly decreased and thus, bitrate increased.

3.8. BCI application in ALS: from augmentative and alternative communication (AAC) to cognitive assessment tool

During the past three decades, AAC technologies have been developed to compensate for natural communication loss, offering tools that allow to communicate to patients with severe disabilities, such as ALS (Beukelman, Fager, Ball, Dietz, 2007). As above described, as ALS progresses and dysarthria becomes severe, profound weakness resulting in reduced movement of the speech musculature and severely reduced phonation become increasingly common, making verbal communication progressively difficult and

ultimately impossible for patients. The choice of appropriate timing of referral for AAC assessment and intervention, as well as of an appropriate alternative communication methods is crucial, ranging from pen and paper and alphabet board, as long as the patient is still able to use upper limbs, to electronic communication devices.

One of the most critical needs regarding quality of life (QoL) for people with severe motor disabilities is restoring the ability to communicate. As a matter of fact, QoL of patients with complete locked-in state (LIS) has been reported to be quite good if they can communicate with caregivers and family (Perelmouter & Birbaumer, 2000). Over the past 20 years, several patients with ALS and LIS have been trained with BCI not only in research laboratories, but also in their domestic environments. Birbaumer et al. (1999) first developed a communication system for completely paralyzed patients with advanced ALS that employed SCP to drive an electronic spelling device. Two patients were trained to voluntarily produce positive and negative changes and were provided by visual feedback. After achieving at least 75% control, the patients began to work with the spelling device, in which letters were presented on a screen. Patients selected a letter by progressively reducing letter strings containing the desired letter by creating SCPs after its appearance. Thus, the authors demonstrated that patients who lack muscular control could learn to control variations in their SCPs sufficiently accurately to operate an electronic spelling device. Although writing sentences was time consuming (a rate of about 2 characters per minute), it was reliable and precise enough to allow patients to operate an electronic spelling device. Many other studies described BCI systems based on self-regulation of SCPs in patients with physical impairment, thus supporting Birbaumer and colleagues' findings (Kübler et al., 2001a, 2001b; Wolpaw et al. 2002; Birbaumer, Hinterberger, Kübler, Neumann, 2003). Other researchers did extensive experiments using BCI based on SMR rather than on SCP. Wolpaw and McFarland (2004), for example, demonstrated that subjects with spinal cord injuries can learn to use scalp-recorded EEG rhythms to control rapid and accurate movement of a cursor in two dimensions on a computer screen, learning such ability in a few sessions of training. Moreover, the speed, accuracy, and learning performance were comparable to those of invasive BCIs. Kübler et al. (2005) showed that four patients with ALS learned to operate a BCI with rhythms recorded over sensorimotor cortex and suggested that a sensorimotor rhythm–based BCI could help ALS patients to maintain an acceptable QoL despite severe disability, for example enabling them to operate an environmental control system or a simple word-processing system more rapidly than usually

possible with an SCP-based BCI. To confirm these encouraging results, other studies have confronted patients with various types of BCI, in order to provide them with a system that could fit and work best for each individual subject. In a study investigating the effects of psychological state and motivation on BCI performance in ALS, Nijboer et al. (2010) made a comparison between SMR- and P300-based BCI paradigms in order to assess which one could be used by most patients. A higher information transfer rate with the P300 BCI, than with the SMR BCI was found. Moreover, this information transfer rate could be achieved by patients after only one session with P300-based BCI, whereas the use of SMR-BCI required more extensive training (10 training session). Therefore, P300-based BCI seems to be superior to the SMR one for rapid and reliable communication. Other studies evaluated the effectiveness of BCI systems that operate by detecting a P300 signals. Sellers and Donchin (2006) assessed BCI effects across three different stimulation modes (auditory, visual, and both), using a paradigm based on a four-choice oddball. Participant's task was to attend to one of four stimulus (i.e. the words 'yes', 'no', 'pass', and 'end') and disregard the other three. The authors demonstrated that these stimuli can be used as a P300-BCI control signal and they supported the effectiveness of P300-BCI with a population of ALS patient, although the small sample size ($N = 3$). To extend these initial findings, Nijboer et al. (2008) evaluated the ability to use a P300-based matrix speller to communicate spontaneous words and phrases in a larger group of individuals with ALS. They also tested the stability of their BCI performance in repeated sessions over a prolonged period of time. In a two-phase study, participants completed two calibration sessions followed by 10 copy-spelling sessions (Phase I) and then 10 free-spelling sessions (Phase II). Results showed that severely disabled patients were able to use a P300-based BCI for both cued and spontaneous text production with a satisfying level of accuracy; moreover, performance was found not to degrade over weeks and months, with P300 amplitude and latency remaining stable for up to 40 weeks. More recently, Silvoni et al. (2009) described results of training and one-year follow-up of brain communication in early and middle stage ALS patients using a P300-BCI. In addition, they investigated the relationship between acquired BCI-skill and the clinical status, including cognition and the degree of physical impairment. A four choice visual paradigm was employed and subjects were asked to reach with a cursor one of four icons on a screen, representing basic needs (i.e., 'I'm hungry', 'I'm sleepy', 'I need a doctor', 'I would like something to drink', etc.). The comparison between BCI-skill of the training and follow-up protocols did not reveal any difference, corroborating the hypothesis that patients maintain

preserved BCI skills even after a long period and in spite of physical impairment progression, although the small sample size (N = 5) limits this conclusion. No significant relationship was found between BCI skills and clinical status, including cognitive abilities. The positive correlation founded between patients' age and some BCI-skill parameters demonstrated a possible positive influence of age and other individual factors in the acquisition of the BCI-skills, in terms of higher attribution of the BCI communication tool's utility and higher motivation of older patients to achieve control over a BCI. Similar results were found by Kübler and Birbaumer (2008) in a meta-analysis of all reviewed publications, in which the authors concluded that there was no relationship between severity of the disease, physical impairment and BCI performance, except for completely locked-in (CLIS) patients, who were unable to learn to use a BCI. Therefore, this study supports the idea that a BCI system can be usefully adopted by ALS patients at early-intermediate stage of the disease, before entering the LIS phase. The usefulness of BCI in CLIS still remains a matter of debate. LIS and CLIS patients are conscious and alert, but the paralysis of all voluntary muscles prevent them to communicate either vocally or by writing (Kübler et al. 2001b). In LIS, vertical eye movements and eye blinks are spared, while in the complete LIS patient lose any control of eye muscular response. Kübler and Birbaumer (2008) stated that BCI technology has been unable '*to restore basic communication (yes/no) in patients who were in the complete locked-in state at the beginning of the training*'. However, CLIS patients showed ERP responses to one or more complex cognitive task, thus indicating partially intact processing stages, despite a reduced general arousal (Hinterberger et al., 2005). Assuming intact processing modules and possible transfer of already learned BCI communication from basic eye movement control to LIS and CLIS, the question why patients who enter the CLIS before learning BCI use do not acquire control of their brain signals remains to be determined. However, in order to prevent failure in BCI use, Kübler and Birbaumer (2008) suggest that users should be entered in BCI training before the beginning of complete locked-in phase. As mentioned above, another available technology for communication purposes is the Eye Tracking (ET) system. However, a main limitation of this system is the need of a preserved ocular motor ability, in order to point with the gaze toward the target (letter or pictures) to be selected. Even if visual P300 requires the patient to perform ocular movements and fixation to some extent, several studies show that it can be employed also with ALS patients in the late stage of the disease; in fact, no continuous

decrement has been observed in BCI performance with physical decline (Kübler & Birbaumer, 2008).

Thus, BCIs have been studied with the primary motivation of providing assistive technologies for those patients with severe motor disabilities, particularly locked-in syndrome (LIS) caused by ALS. As above reported, nowadays the cognitive evaluation of ALS patients in advanced stages of paralysis still represents a challenge, due to the fact that all standard assessment tools for both verbal and non-verbal cognitive abilities involve a motor response. Besides, even tests relying on some form of rudimentary motor function such as blinking, nodding, or pointing (Anastasia & Urbina, 1997), are not administrable to complete locked-in patients. However, recently some attempts have been performed in order to develop a BCI-based cognitive testing. Iversen and coll. (2008b) aimed at assessing cognitive abilities in completely paralyzed ALS patients. Based on previous results showing that some late-stage ALS patients can learn to communicate reliably,at high accuracy using only their EEG (Kotchoubey et al., 1997; Pfurtscheller & Neuper, 1997; Kübler et al., 2001b), the authors developed a BCI methodology based on self-regulation of slow-cortical potentials (SCPs) of the EEG. In a first study (Iversen et al. 2008a), two late-stage ALS patients have been trained to control certain EEG components in order to direct a cursor to one visual target on a monitor. Following, a series of two-choice cognitive task were administered, where patients were asked to differentiate odd/even numbers, consonants/vowels, nouns/verbs, large/small numbers, and to perform simple computations. In a successive study, Iversen et al. (2008b) employed the same SCP-EEG control in order to administrate a conditional-associative learning task to a late-stage ALS patient, testing the ability to learn arbitrary associations among visual stimuli. Results of both studies showed a good level of accuracy in detecting patients performances, according to a within-subjects experimental design. Moreover, patients were found to be able to understand verbal instructions and to respond accordingly in the successive tasks. However, this approach presents some important limitation: first, it requires an extensive pre training in order to learn to control EEG; second, the method can not be used for tasks based on recall or where patient's choice is among more than two stimuli.

Besides, Perego and coll. (2011) adapted a widely used cognitive test (i.e., Raven Coloured Progressive Matrix) to a Steady State Visually Evoked Potentials (SSVEP) BCI paradigm in a healthy subjects sample. Overall, also this approach presents some limitations, such as a significant re-arrangement of the original test procedures, possibly producing biased results.

Differently from the other existing BCIs, P300-based BCIs do not require learning of self-regulation of brain response and feedback. Moreover, the short latency of the P300 allows faster selection of letters than any other BCI system and the possibility to reduce the amount of time required for the users training represents a very important chance in order to extend the use of AAC also for cognitive assessment purposes. On the other side, an intact visual system is needed as a precondition to using P300, at least for the visual modality, which has been proved to be more reliable than the auditory one; similarly, a preserved ability to pay sustained attention is required, and it may represent a problem in some ALS patients. Despite the several advantages of P300-based BCIs, to date a comprehensive motor-verbal free neuropsychological battery, including standardized test administered with a good level of adherence to the original validated measures, is not still available for ALS patients.

4
Eye Tracking

4.1. Definition and essential features

Eye Tracker (ET) is a device for recording and analyzing eye movements. It allows to track the position of the pupil and to trace the point observed by the user. Furthermore, through eye tracking, the gaze can be used to actively interact with electronic and technological tools. Thus, ET represent one of the most advanced devices for communication in patients with motor/verbal deficits, which prevent normal oral or written language. In addition to the vocal synthesis, ET allows internet connection, webmail and domotics.

Early ETs were developed for scientific exploration in controlled environments or laboratories. Eye gaze data have been used in ophthalmology, neurology, psychology, and related areas to study oculomotor characteristics and abnormalities, and their relation to cognition and mental states (Morimoto & Mimica, 2005).

4.2. Eye gaze trackers

4.2.1. Intrusive eye trackers

Intrusive eye tracking techniques require some equipment to be put in physical contact with the user and they are in general more accurate than remote ones. One of the most traditional methods is based on contact lenses. Robinson (1963) used a small coil (called search coil) embedded into a contact lens that is tightly fit over the sclera with a slight suction to avoid drift during fast eye movements. The user's gaze is estimated from measuring the voltage induced in the search coil by an external electro-magnetic field. Although very intrusive, the system is very accurate (approximately 0.08°). A less expensive technique is based on measuring skin potentials, as described by Kaufman et al. (1993). The electro-oculogram (EOG) is a very common technique for recording eye movements in clinical applications due to its technical simplicity. By placing electrodes around the eye, small differences in the skin potential corresponding to eye movement are measured.

However, this technique is not appropriate for everyday use, and its reported accuracy is about 2°.

Cameras or other optical devices can be used to measure eye position without direct contact with the user. Some camera-based methods, however, require the eye to be very close to the optical device, and therefore, must be head mounted, or the motion of the head must be restricted with the use of a chin rest or bite bar.

4.2.2. Camera-based eye trackers

Camera-based eye tracking techniques rely on some eye's properties and characteristics that can be detected and tracked by a camera or other optical or photosensitive device. Most of these techniques have the potential to be implemented in a non-intrusive way.

The limbus and the pupil are common features used for tracking. Limbus is the boundary between the sclera and the iris. Due to the contrast of these two regions, it can be easily tracked horizontally, but because the eyelids in general cover part of the iris, limbus tracking techniques have low vertical accuracy. Pupils are harder to detect and track because of the lower contrast between the pupil–iris boundary, but pupil tracking techniques have better accuracy since they are not covered by the eyelids (except during blinking). To enhance the contrast between the pupil and the iris, many eye trackers use an infrared (IR) light source. These kinds of trackers usually consist of a standard desktop computer with an IR camera mounted beneath (or next to) a display monitor, with image processing software to locate and identify the features of the eye used for tracking. In operation, IR light from a LED embedded in the infrared camera is first directed into the eye to create strong reflections in target eye features to make them easier to track. The light enters the retina and a large proportion is reflected back, making the pupil appear as a bright, well defined disc (i.e., the 'bright pupil' effect). The corneal reflection (or first Purkinje image) is also generated by the IR light, appearing as a small but sharp glint. Once the image processing software has identified the centre of the pupil and the location of the corneal reflection, the vector between them is measured, and, with further trigonometric calculations, point-of-regard can be found. Although it is possible to determine approximate point-of-regard by the corneal reflection alone, by tracking both features eye movements can critically be disassociated from head movements (Duchowski, 2003; Jacob & Karn, 2003). Sometimes, the IR source is placed near the optical axis of the camera. Because the camera now is able to 'see' the

light reflected from the back of the eye, the camera sees a bright pupil, instead of a regular dark pupil. The light source also generates a corneal reflection (CR) or glint on the cornea surface, near the pupil. This glint is used as a reference point in the pupil–corneal reflection technique.

A calibration procedure is required to compute the mapping from the pupil-glint vector to monitor screen coordinates. In general, the user is asked to look at several points on the computer screen, one point at a time, and press a button. However, this simple model presents some limitations. First of all, the calibration mapping decays as the head moves away from its original position; moreover, as mentioned by Schnipke and Todd (2000), the calibration is one of the worst problems in current remote ETs because it requires the operator to adjust several system parameters such as illumination conditions and relative position of the user, monitor, and camera. The use of the differential lighting scheme may facilitate the system setup and make it more robust to illumination changes, even if calibration still represents a problematic issue.

Another very accurate eye tracker uses the first and fourth Purkinje images (dual Purkinje image – DPI eye tracker) (Cornsweet & Crane, 1973). The Purkinje images are reflections created at different layers of the eye structure. The first Purkinje image corresponds to the reflection from the external surface of the cornea. This is the brightest and easiest reflection to detect and track. Detecting the other Purkinje images require special hardware, but allow the estimation of the 3D point of regard from the third and fourth Purkinje images that correspond to the relaxation of the eye lens, as described by Crane and Steele (1978).

Instead of using explicit geometric features such as the contours of the limbus or the pupil, an alternative approach to object pose estimation is to treat an image as a point in a high-dimensional space. Techniques using this representation are often referred to as being appearance-based or view-based and can achieve an accuracy of about 0.5° (Tan, Kriegman, Ahuja, 2002).

Baluja and Pomerleau (1994) also does not use explicit geometric features. They describe an eye gaze tracker based on artificial neural networks (ANN). Once the eye is detected, the image of the eyes is cropped and used as input to a ANN. Training images are taken when the user is looking at a specific point on a computer monitor. This prototype tracker runs at 15 Hz, with an accuracy of about 2°, also allowing for some head movements.

4.2.3. Calibration and head motion

The above described measurements, such as pupil and limbus position, skin potentials, relative position of Purkinje images, and so on, must be translated to eye orientation. A calibration procedure is required to compute the mapping between measurements and eye orientation. Besides the eye orientation, the accommodation of the lens could be measured for 3D eye tracking.

A typical calibration procedure presents the user a set of visual targets that he/she has to look at while the corresponding measurement is taken. From these correspondences, a mapping or calibration function can be computed.

For the traditional pupil and limbus tracking techniques, the position of the center of the pupil or iris must be mapped to the visual targets. Since the eye position varies with the head position, the head should remain still during and after the calibration. One way to compensate for small head motion, is to consider the pupil/iris position relative to the eye socket, or some reliable fixed point on the user's face. Therefore the mapping is computed using the vector from the reference point to the pupil/iris center.

For the pupil–corneal reflection technique, the CR is used as the reference point, allowing for small head motion since the CR follows the head motion, and the calibration nicely handles the offset due to the difference of the line of gaze and line of sight, imperfections of the cornea, position of the camera relative to the computer screen, etc.

The appearance-based and ANN techniques acquire the calibration from a large set of images, and generalize this mapping to other users, i.e., they have the advantage of not requiring a per user calibration once they are trained; however, since the image of the eye also changes with head positions (and illumination conditions), these techniques are also sensitive to head motion.

4.3. Eye Tracking paradigms

Several ET paradigms might be used to assess cognitive functions; in particular ocular fixation, anti-saccade, and smooth pursuit paradigms have been employed to evaluate the spectrum of frontal impairment, which typically occurs in association with psychiatric and neurological disorders such as schizophrenia, pervasive developmental disorders and neurodegenerative diseases (see for example: Keedy, Ebens, Keshavan, Sweeney, 2006;

Takarae, Minshew, Luna, Krisky, Sweeney, 2004; Amador, Hood, Schiess, Izor , Sereno, 2006).

▪ *Fixations* are moments when the eyes are relatively stationary and focused on a particular target.

▪ *Saccades* are defined as rapid, involuntary, small movements that allow the eyes to move from one fixation point to another while scanning and processing information. Saccades are ballistic, i.e. the target of a saccade cannot be changed during the movement. During saccades, vision is suppressed to allow stable perception of surroundings. These eye movements are used to move the fovea to the object/region of interest.

- *The pro–saccade task*: a target appears to the left or right of a fixation point presented at midline on a screen. The subject is asked to make saccade in the target direction and then move back the eyes to the fixation point in the middle of the screen.
- *The anti-saccade task*: in this task, the subjects is instructed to inhibit a reflexive, visually-guided saccade towards a target stimulus and to perform an eye movement in the opposite direction. Correct performance on this task requires two steps: the subject must first suppress the automatic response to look at the target (pro-saccade) and then transform the location of the stimulus into a voluntary motor command to look away from the target (anti-saccade) (Munoz & Everling, 2004).

Brain imaging studies have shown that a widely distributed cortical and subcortical network is active during the generation of antisaccades (Figure 4.1; Everling & Fischer, 1998). Because of the dependency on frontal and basal ganglia structures, this task is now recognized as an important tool to investigate not only normal brain function, but also cognitive dysfunction in several neurological and psychiatric disease conditions.

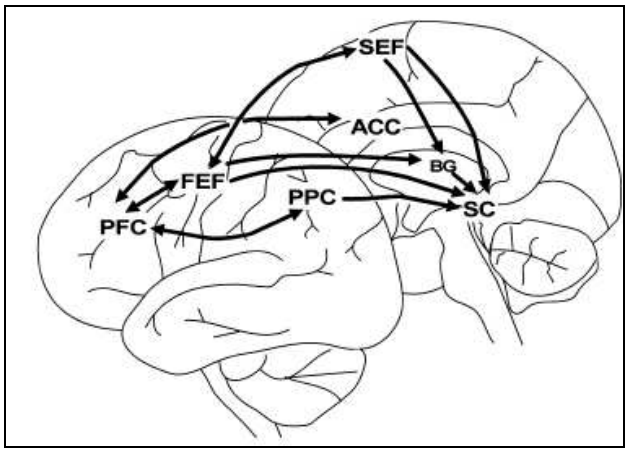

Figure 4.1. Cortical and subcortical structures believed to be involved in the antisaccade generation.
ACC: anterior cingulate cortex; BG: basal ganglia; FEF: frontal eye feld; PFC: prefrontal cortex; PPC: posterior parietal cortex; SC: superior colliculus; SEF: supplementary eye feld.

▪ *Smooth Pursuit* are smooth movement of the eyes for visually tracking a moving object, which cannot be performed in static scenes.

- *Smooth Pursuit task*: a stimulus moved across a screen from one side to the other before jumping back to its start point and starting again. The same sequence of trials is then replayed in the opposite direction. The subject is instructed to follow the stimulus as closely as possible.

▪ *Go/No-Go paradigm* requires the subjects to continuously monitoring series of stimuli presented individually in the center of a computer screen. The participants have to respond as rapidly as possible by pressing a button whenever a target (Go) stimulus is present, but not respond to an infrequently presented not target (No-go) stimulus. Go/No-go tests are used to measure subject's sustained attention and response control abilities. There are several different versions of this paradigm:

- *No-Go*: subject is instructed to ignore the target stimulus and to maintain fixation in the middle of the screen for the duration of the trial.
- *Go-Right/No-Go-Left*: subjects are instructed to look at the targets presented in the right visual field, but to suppress eye movements to all targets in the left field.
- *Go-Left/No-Go-Right*: subjects are instructed to look at the targets presented in the left visual field, but to suppress eye movements to all targets in the right field.

4.4. Oculomotor abnormalities in neurological and psychiatrical disorders

Eye movement abnormalities represent sensitive markers of psychiatric and neurological disease and are useful in the differential diagnosis of a variety of clinical neurological syndromes.

Alterations of eye coordination and decreased saccade velocity may be objectified in Parkinson's disease (PD), multiple system atrophy (MSA), progressive supranuclear palsy (PSP) or Wilson's disease (Vidal, Turner, Bulling, Gellersen, 2012; Gorges, Pinkhardt, Kassubek, 2014). Gaze holding deficit, slowing and impairment of saccades and impaired smooth pursuit eye movement have been reported in patients with Huntington's disease (HD) (Lasker & Zee, 1997), also in presymptomatic gene carriers (Blekher et al., 2004; Turner et al., 2011). FTD is characterized by impaired reflexive saccade inibition, with the exception of semantic dementia population (Meyniel, Rivaud-Pechoux, Damier, Gaymard,

2005; Boxer et al., 2006). Hypometric saccades, prolonged saccade latencies (Hershey et al., 1983; Fletcher & Sharpe, 1983; Moser, Kompf, Olschinka, 1995; Lueck, Mendez, Perryman, 2000; Schewe, Uebelhack, Vohs, 1999), reduced peak velocities (Fletcher & Sharpe, 1983) and disorganized visual scanning (Lueck et al., 2000; Mosimann et al., 2005) have been noted in Alzheimer's disease. Cerebellar disorders (Veneri, Federico, Rufa, 2014; Gorges et al., 2014) show nystagmus, dysmetric and interrupted saccades, as well as a disturbed pursuit, as highlighted by ET analysis. The demyelinating lesions of multiple sclerosis in brainstem and cerebellar pathways may be followed by internuclear ophthalmoplegia, ocular motor palsy, pathologic nystagmus, saccadic intrusions or impaired pursuit. In this case, an ET evaluation may prove useful during the process of monitoring disease progression and treatment efficiency (Vidal et al., 2012; Prasad & Galetta, 2010).

In psychiatric field, hypometric saccades and reduced saccade velocity were measured in autism. More intrusive saccades were reported in attention deficit hyperactivity disorder and in dyslexia. Moreover, a high number of antisaccade errors were registered in autistic spectrum and obsessive compulsive disorders, anxiety and depression. A decrease in smooth pursuit eye movements and an increase in anticipatory and intrusive saccades were observed in schizophrenia (Vidal et al., 2012; Rommelse, van der Stigchel, Sergeant, 2008).

4.5. Eye movement abnormalities in ALS

Eye movements have been traditionally regarded as spared from involvement in ALS; however, early studies reported a range of oculographic abnormalities, including reduced saccadic eye movement velocity and low smooth eye pursuit gain (Fabregas JM & Roig C, 1993; Jacobs, Bozian, Heffner, Barron, 1981; Leveille, Kiernan, Goodwin, Antel, 1982) that were not evident in the clinical evaluation of the patients.

ET devices have been used in recent studies with ALS patients, in particular employing antisaccade/prosaccade and smooth pursuit paradigms, which appeared to be sensitive in the early detection of cognitive frontal alteration in this population (Donaghy et al., 2009, 2010). Following Sharma and colleagues (2011) and Donaghy et al. (2011), the most frequent oculomotor dysfunctions in ALS include:

▪ *Ophthalmoplegia*

Oculomotor dysfunction was described as early as 1925 in a case of ALS said to have occurred with convergent strabismus that progressed to complete ophthalmoplegia (Van Bogaert, 1925). An early postmortem case series (Lawyer & Netsky, 1953) observed abnormal oculomotor nuclei in only 4 of 54 cases of ALS with no clinical ophthalmoparesis. More recently, Hayashi & Kato (1989) postulated that the impairment of cranial motor functions in ALS might follow the reverse order of their developmental sequence, with the preserved ocular motor function reflecting the fact that this network is relatively old ontogenetically.

▪ *Abnormal Pursuit*

Defective pursuit movements have been commonly described in ALS, although the pursuit system becomes progressively more inefficient with advancing age, with increasing discrepancies between target and eye velocities leading to more frequent catch-up saccades in older individuals (Spooner, Sakala, Baloh, 1980). In a study of 18 patients using electrooculography (Jacobs et al., 1981), 11 were found to have defective pursuit movements, correcting for the age effect, with the conclusion that the selective pursuit defect in ALS was probably due to nonnuclear involvement (supratentorial or infratentorial) of extrapyramidal or corticobulbar components of the oculomotor system. Donaghy et al. (2010) found a reduced velocity gain in 44 ALS patients compared with controls, as well as a reduced proportion of time spent in smooth pursuit.

▪ *Saccadic Impairments*

Slowing of saccades, especially vertical saccades, has been a widely reported abnormality, with one study (Averbuch-Heller, Helmchen, Horn, Leigh, Büttner-Ennerver, 1998) suggesting that ALS with early such involvement represented a distinct clinicopathological entity. Slow vertical saccades were noted to be common in ALS-FTD (Moon, Lee, Seo, Kang, Na, 2008); moreover, Gizzi, DiRocco, Sivak, Cohen (1992) suggested that oculomotor dysfunction (including saccadic impairment) in ALS might reflect the incidence of secondary abnormalities, such as parkinsonism. Patients with ALS have been shown to have significantly elevated error rates (distractability) and latency in the antisaccade and memory-guided (remembered) saccade paradigms, but no abnormality of reflexive saccades (the most widely used but least sensitive saccadic paradigm) (Shaunak et al., 1995). With the advent of sophisticated equipment, such as ET, detailed examination of saccades and their

abnormalities in various disease processes has been made easier. Donaghy et al. (2009) noted that saccadic intrusion amplitude was greater in patients than in controls and, in particular, in patients with spinal onset. Saccadic intrusion amplitude in patients also correlated with neuropsychological measures sensitive to frontal lobe lesions. The authors postulated that progressive deterioration in saccadic intrusion amplitude could be a quantifiable objective marker of disease progression and that ocular fixation instabilities can be considered a marker of the sub-clinical frontal lobe dysfunction in ALS. In a later study of Donaghy and colleagues (2010), reflexive saccades were found to be slower in bulbar-onset compared with limb-onset patients, suggesting that slow saccades may be due to increased brainstem pathology in bulbar-onset disease that involves burst cell neurons and highlighting the potential for overlap between bulbar-onset MND and PSP, as both can have a bulbar palsy and slowed saccades.

- *Nystagmus*

An early case report (Boisseau & Brissard, 1932) described horizontal gaze–evoked nystagmus in one case and rotatory horizontal gaze–evoked nystagmus in another. In another case with rotatory nystagmus and primary position nystagmus, with reversal of direction of the slow phase, the researchers concluded that it could be due to dysfunction of vestibulocerebellar connections with an intact peripheral vestibular apparatus (Kushner et al., 1984).

- *Bell Phenomenon*

Other less frequently reported oculomotor abnormalities include an abnormal Bell phenomenon, the physiologic upward rotation of the globe in response to attempted eyelid closure. Esteban, De Andrés, Giménez-Roldán (1978) reported a Bell phenomenon alteration in 15 of 24 ALS patients. They all had preserved oculocephalic reflexes, with the conclusion that the lesions were supranuclear as a result of corticogeniculate tract involvement. These oculomotor alterations were not directly related to the type of ALS at onset or to its duration; however, they were correlated with the relative degree of the clinical bilateral pyramidal tract signs at the supraspinal level.

Thus, ocular mobility assessment in ALS patients by means of ET devices has led to the identification of a range of ocular motor disorders in such population. Shaunak et al., (1995) concluded that the specific pattern of eye movement impairment found in ALS patients (i.e.,

significantly elevated error rates and latency in antisaccade and remembered saccade paradigms, with relative preservation of reflexive saccades and a significantly increased frequency of small saccades intruding on fixation) suggest a prefrontal lobe dysfunction in ALS and provide an independent support for the exsistance of a pathology invading frontal lobe cortex in this condition. Neuropsychological and neuroradiological findings further supported the specific association between eye movement disorders (with particular reference to performance at the antisaccade task) and frontal lobe dysfunctions (Evdokimidis et al., 2002; Donagy et al., 2009).

4.6. ET application in ALS: from communication device to cognitive assessment tool

Thanks to their characteristics, ET devices may be used as Augmentative/Alternative Communication (AAC) tools in order to translate eye movements into effective communication by those patients presenting with severe verbal/motor disabilities, such as ALS patients. As previously mentioned, patients with late-stage ALS or in locked-in status (LIS) present a complete paralysis of any voluntary muscles, except those controlling blinking and eye movements, which represent the only motor communication pathway with the surrounding environment.

By coupling ET with a computer, the patient can select words or images from a menu in order to entertain a conversation or to write an email, as well as to control household equipment and systems. Thus, ET represents a potentially useful method for AAC, and it has been previously successfully adopted by ALS patients when close to a LIS stage (Gibbons & Beneteau, 2010; Ball et al., 2010). Recent studies also suggested that the device has a relatively good rate of acceptance in this disease (Ball et al., 2010; Calvo et al., 2008). Furthermore, other studies demonstrated that this high-technology communication instrument improves quality of life, since it ensures a greater degree of independence and autonomy, increases self-respect and facilitates familial- and social-life reinsertion of persons with disabilities (Calvo et al., 2008; Caligari, Godi, Guglielmetti, Franchignoni, Nardone, 2013). The ET assistive devices have also been found to reduce caregivers' burden (Hwang, Hwang, Weng, Wang, Tsai, Chang, 2014).

However, as highlighted by Spataro, Ciriacono, Manno, La Bella (2014), the use of ET presents some limitations: first, ET computer systems require a highly efficient oculomotor

activity in order to correctly point to each letter/number of the screen keyboard. In addition, the head must be held still; therefore, building up sentences and commands on the keyboard might be very fatiguing for patients. The gaze tiredness or oculomotor impairment, which are sometimes reported by patients when communicating through the device, may be due to the presence of oculomotor dysfunctions (i.e., progressive ophthalmoparesis, abnormal pursuit, oculomotor apraxia, and abnormal saccades) which have been described in ALS patients, as the disease progresses to advanced stages.

Although such limitations, several ET paradigms might be used not only as AAC systems, but also as innovative tools for the assessment of cognitive functions in ALS. As a matter of fact, '*by trascending the requirement to write or speak, loss of which precludes standard neuropsychological testing in some patients with advanced ALS, cognitive tests performed using only oculomotor functions offer additional potential, allowing the study of patients much later in their disease course*', as stated by Sharma and colleagues (2011). As above outlined, ocular fixation, antisaccade, and smooth pursuit paradigms allow the assessment of frontal involvement characterizing the ALS cognitive pattern. Unfortunately, the previously described poor or lack of eye-motor control, frequently observed especially in the advanced stages of the disease when patients present with the LIS, make this device unfeasible. However, in early to mid-stage, ET still seems to be proficiently used.

To date, however, ET systems have never been used to administer cognitive tasks to ALS patients, although it may have many interesting application in this field. In fact, because of the variable presence of cognitive impairment in this population, an accurate and extensive assessment of frontal executive functions, as well as language, memory and social cognition abilities, is required. Traditional neuropsychological measures show several limitations when employed with ALS patients. Therefore, ET represents a highly innovative tool in this field, because it allows the administration of cognitive tests that do not require movement or language components. Specifically created computerized versions of the most commonly used neuropsychological tests could be administered to patients in order to reduce fatigue and stress and to address physical impairment limitations. However, some critical aspects are directly linked directly to the use of ET systems: in addition to the aforementioned eye movement deficits in ALS patients, other problems concern economical and technical issues. In fact, these technologies are much more expensive compared to traditional 'paper and pencil' tests; moreover, the use of ET requires technical set up and expertise.

Nevertheless, promising perspectives arise from a recent study of Hicks and colleagues

(2013), who developed an oculomotor-driven version of the Trail Making Test (TMT) and compared it to the traditional written version in a goup of healthy subjects. A good correlation between total time taken to complete the written vs. oculomotor version of Part B (but not of Part A) was found, thus demonstrating that an ET-computerized version of the TMT, in which Part B performs comparabily to the standard witten version, can be reliably applied to healthy volunteers. Notwithstanding the need for validation testing on the ALS population, this study enphasizes the potential to explore dysexecutive functioning in those patients who are unable to write/speak, expanding the possible ET application to perform cognitive tests in ALS. This first encouraging results demonstrate that, provided oculomotor functions are mechanically preserved, such paradigms could be usefully adopted to test other cognitive domains in subjects presenting with severe motor and verbal disabilities that prevent them oral or written communication. Moreover, the increased sophistication of eyetracking equipment also allows to delineate the nature of errors made by patients while scanning targets, such as perseverative errors, errors due to disinhibition or sequencing errors.

5

Cognitive assessment in Amyotrophic Lateral Sclerosis by means of Eye tracking and Brain Computer Interface devices

5.1. Introduction

The detailed analysis carried above about ALS and its clinical, cognitive and behavioural features clearly points out the importance of a careful evaluation of patients' cognitive status in clinical settings. Several studies found that the presence of cognitive deficits represents a negative prognostic indicator in ALS (Olney et al., 2005; Elamin et al., 2011; Elamin et al., 2013). Moreover, the detection and quantification of cognitive alterations throughout the course of the disease have a relevant impact not only on clinical practice, but also on ethical issues, these concerning the capacity of taking decisions about financial circumstances, invasive treatments proposed in the course of the disease and end-of-life decisions.

However, to date, the cognitive assessment of ALS patients involve some restrictions; first of all, the lack of cognitive measures that take into account the presence of progressive verbal and motor impairments, in order to fully capture the spectrum of cognitive alterations in ALS Similarly, motor-verbal impairment makes neuropsychological assessment problematic in moderate-severe stages of ALS: patients may not be able to perform traditional 'paper and pencil' tasks, all involving a motor or verbal response, because of their muscle paralysis and marked dysarthria/anarthria.

Recently, some attempts have been performed in order to develop a BCI-based cognitive testing (Iversen et al., 2008a; Perego et al., 2011). Overall, such approaches present some limitations, such as extensive pre-training in order to learn to control EEG and important adjustments of the original tests, which could produce biased results. As above asserted, to date a motor-verbal free neuropsychological battery is not still available for ALS patients.

5.2. Research aims

The aim of the present study was to investigate the use of P300-based Brain Computer Interface (BCI) and Eye Tracking (ET) technologies for the administration of

cognitive measures in ALS and to develop a motor-verbal free neuropsychological battery, suitable to perform a longitudinal assessment of cognitive functions even in the moderate-advanced stage of the disease. The implemented battery focuses on the best reasonable adherence to the original validated neuropsychological tests. This cognitive battery based on BCI and ET devices was tested on a sample of ALS patients and healthy controls, in order to evaluate its sensitivity and usability.

5.3. Materials and Methods

5.3.1. Participants

Thirty-four consecutive ALS patients within two years from the onset were recruited at the Department of Neurology, IRCCS Istituto Auxologico Italiano, Milan. The diagnosis of ALS was made by neurologists experienced in the field of neuromuscular diseases, according to the revised El Escorial criteria for clinically definite, probable or possible ALS (Brooks et al., 2000).

Disease severity was assessed by the Revised ALS Functional Rating Scale (ALSFRS-R; Cedarbaum et al., 1999) and respiratory function was also evaluated (Spirometry). Furthermore, genomic DNA was extracted from peripheral blood according to standard protocols. The samples have been amplified by repeat-primed PCR using fluorescent oligonucleotides previously reported (DeJesus-Hernandez et al., 2011). The determination of the number of C9orf72 hexanucleotide GGGGCC repetitions was performed by electrophoretic separation on agarose gels and by capillary electrophoresis on a 3500 ABI Prism Genetic Analyzer and subsequent analysis with GeneScan software. The size of C9orf72 extension was not quantified.

Thirty healthy subjects were also recruited. Exclusion criteria for both groups were the presence of major co-morbid cardiovascular, neurological or psychiatric conditions that could affect cognition, together with severe sensory loss (auditory or visual) and use of high-dose psychoactive medications. For patients, structural brain MRI 1.5 Tesla excluded major causes of cerebrovascular disease and white matter lesions. Participants were required to avoid drinking caffeine or alcohol and smoking prior to the experimental test, in order to prevent any effects of these substances on the central and autonomic nervous system.

The study protocol was reviewed and approved by the Ethics Committee of the Institution and all eligible subjects received verbal and written information about the study. All participants signed an informed consent, according to the Declaration of Helsinki.

5.3.2. Standard cognitive and psychological/behavioural assessment

Neuropsychological assessment

A 'paper and pencil' cognitive screening was administered to both patients and healthy participants. In particular, it included:

- the *Frontal Assessment Battery – FAB* (Dubois, , Slachevsky, Litvan, Pillon, 2000), a bedside battery to assess the presence and severity of dysexecutive syndrome affecting both cognition and motor aspects;

- the *Montreal Cognitive Assessment – MoCA* (Pirani, Nasreddine, Tulipani, Neri, 2007), a brief screening tool evaluating global cognitive efficiency. For the MoCA, a cut-off score of 24 was considered, as suggested by recent studies (Luis, Keegan, Mullan, 2009; Osborne et al., 2014);

- the *Working Memory subtest (Digit Sequencing Task)* - *WM* of the *Brief Assessment of Cognition in Schizophrenia – BACS* (Anselmetti et al., 2008), a task assessing working memory abilities.

Psychological assessment

Psychological measures of depression and anxiety were administered. In particular, *Beck Depression Inventory – BDI* (Beck, Ward, Mendelson, Mock, Erbaugh, 1961) and *State-Trait Anxiety Inventory-Y – STAI-Y* (Spielberger, Gorsuch, Lushene, 1970) were used.

Behavioral assessment

In order to identify possible behaviour and personality changes of ALS patients, the *Frontal Behavioural Inventory – FBI* (Alberici et al, 2007) was employed. The FBI consists of a 24 items quantifiable questionnaire administered to the caregiver; 12 items assess deficit (negative) behaviours (i.e., apathy, aspontaneity, indifference, inflexibility, personal neglect, disorganization, inattention, loss of insight, logopenia, verbal apraxia, loss of comprehension and alien hand), while the remaining 12 items assess disinhibition (positive) behaviours (i.e.,

perseverations/obsessions, irritability, excessive jocularity, social inappropriateness, impulsivity, restlessness, aggression, hyperorality, hypersexuality, utilization behavior, and incontinence).

5.3.3. The BCI and ET systems setting

Test architecture (Figure 5.1) was composed of an ET system and a BCI device, both controlled by a laptop PC, connected to an external monitor, where stimuli are presented (Display PC). Figure 5.1 shows the adopted test setup. The BCI device module was based on the g.USBAmp (7) biosignal amplifier, connected to an active electrode head cuff (5). The biosignal amplifier was connected to a portable laptop (HP DV3-4101SL, Hewlett Packard, USA), running Windows 7 64 bit (6). This laptop was connected to an external monitor (2), where the stimuli were presented to participants. The ET consisted of a high-speed infrared camera and the related illuminator (3), positioned just below the Display Monitor. The ET host computer (1) acquired eye-head information via the camera and processed them in real time. The two computers were connected by Ethernet cross-cable, for fast communications in order to synchronize the different acquisitions performed by the BCI and the ET. This allowed to extract interesting features from the combined use of both technologies, e.g. the screen eye-gaze patterns during the BCI tests.

On the Display PC, a suitable custom software provided information of the general management of the tests and the sequence of the stimuli for the ET tests, while for the BCI, a customized version of the widespread used BCI2000 software was employed.

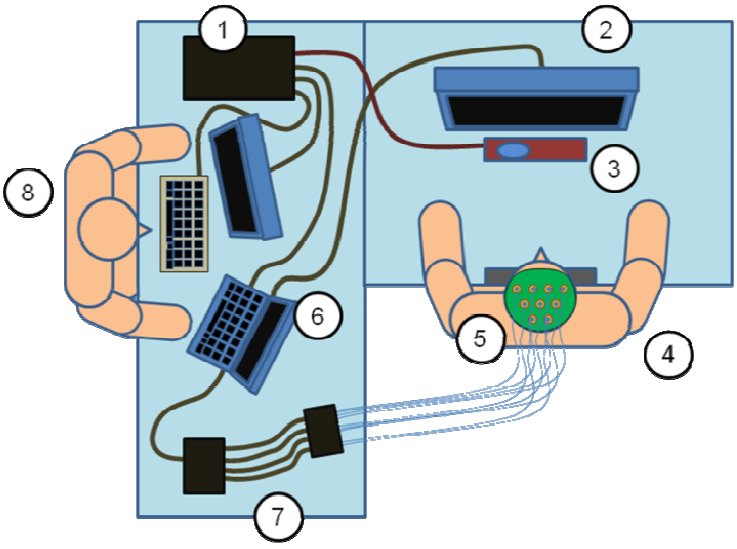

Figure 5.1. Setup Example: (1) Eye Tracker Host Computer, (2) Eye Tracker Display Monitor, (3) Eye Tracker sensing device, (4) User, (5) EEG Head Cuff, (6) Display PC, (7) EEG Amplifier, (8) Operator.

5.3.4. Signal processing and translation algorithms

The neuropsychological protocol was administered through a customized version of BCI2000 and a suitable custom software. A new software suite, named '*eBrain Test Engine*' (ETE), was developed for satisfying the study purposes concerning the joint use of BCI and ET devices. ETE is an Open Source application (http://ebrainengine.codeplex.com/), developed in C#, using .NET Framework 2.0 to maximize the compatibility also with old hardware. In addition to the possibility to deal with both devices, ETE provides an high flexible framework to develop new neuropsychological test batteries in a very simple manner.

5.3.5. Brain Computer Interface

ERP data recording

The BCI module consisted of a g.USBAmp biosignal amplifier (Guger Technologies, Graz, Austria), connected to an active electrodes head cuff (g.GammaCap, Guger Technologies). It was enabled with 16 simultaneously sampled biosignal channels (FZ, C3, C4, CZ, CPZ, P3, P4, PZ, PO3, PO4, POZ, PO7, PO8, O1, O2, OZ) with 24-bit resolution and simultaneous sampling of all channels with up to 38.4 kHz, and digital signal filtering and preprocessing with active electrode. Ground was placed in FPZ, and reference was located on the left ear lobe. Input voltage ranged +/- 250 mV with a resolution of less than 30 nV. Moreover, a floating point DSP performed oversampling and real-time filtering of the signal data (between 0 Hz and 2.400 Hz).

The systems came as CE-certified and FDA-approved medical device, safety class: II, conformity class: IIa, type of applied part: CF.

The BCI paradigm and calibration phase

Each participant sat in a comfortable chair or in his/her own wheelchair about 0.7–1.5 m away from a computer screen that displayed a 7 × 4 matrix (Figure 5.2). The 28 rectangle of the matrix contains the English alphabet, an underscore and a leftward arrow. Characters were arranged from left to right and top to bottom. The text-to-copy (e.g., the word 'VOLPE') appeared on the first horizontal line (i.e., the text-to-copy line). The character to be selected, (e.g., the letter 'O' into 'VOLPE'), appeared in parentheses at the end of the

presented text. The task consisted in paying attention to the character-to-select in the flashing matrix and mentally counting how many times it flashed. The flashes were presented in random order. For each character-to-select, 20 sequences of flashes were presented. Each stimulus flashed for 100 ms and then the screen was static for 75 ms. Thus, flashes occurred every 175 ms. Accordingly, the duration of each sequence was 2.1 s and each character selection totaled 42 s. One and one half seconds after the matrix stopped flashing, the selection was displayed on the second horizontal line. Simultaneously, the next character-to-select appeared in parentheses at the end of the text-to-copy. Participants were then given an additional 3.5 s to identify the matrix location of the next character-to-select. In addition to this short rest, an auditory signal was provided, in order to make easier for participants to recognize the end of a selection and the start of a new one. Thus, the period of time between the end of one character selection and the beginning of the next total was 5 s. In this calibration phase, the 8 words of the sentence 'MA LA VOLPE COL SUO BALZO HA RAGGIUNTO' were divided into three runs, composed by three separated text-to-copy runs ('MA', 'MA LA VOLPE COL', 'SUO BALZO HA RAGGIUNTO'). During these three runs, data were gathered for system calibration and participants received no feedback. In the subsequent three words copy session ('IL QUIETO FIDO'), online feedback was presented.

Figure 5.2. The 7 × 4 matrix of the P300 BCI Speller.

Brain Computer Interface: neuropsychological tests administered

- *Verbal Fluency test:* A single-letter (M) phonemic fluency test was administered. The time (seconds) needed by participants to generate five words starting with the indicated letter was recorded, and the mean total time was then computed. After participants have communicated the mental generation of the word, they were asked to use the P300 speller system in order to select each correct letter and finally visualize the complete word on the computer display. The '*percentage of accuracy in letters production*' with the BCI system was recorded.

Adaptations of four widely used traditional cognitive tests were developed in order to be administered by means of BCI (Figure 5.3):

- *Token test:* The Token test represents a verbal comprehension measure, based on 36 verbal instructions with increasing level of syntactic complexity (De Renzi & Vignolo, 1962). A short version has been developed from the original test for the study purposes, made by 13 items chosen from each of the six sections of the test. Items were excluded when requiring a multiple-token selection or moving tokens from one location to another. Tokens were displayed on the PC monitor, arranged in the same configuration of the original test. Verbal instructions concerning tokens to be selected have been recorded and auditory presented to participants with a standardized procedure.

- *d2 test:* The d2 Test of Attention is a timed test of selective attention and it represents a standardized refinement of a visual cancellation test (Brickenkamp & Zillmer, 1998). The computerized version of the original d2 test has been adapted for the BCI administration. A 3 × 3 matrix of 'd' letters with one to four dashes arranged either above or below the letter was presented to participants on the desktop monitor. The task of the subject was to select, for each trial, the only item corresponding to the 'd' letter with two dashes. The test was composed by 20 trials.

- *Raven's Colored Progressive Matrices (RCPM)*: The original Raven's Colored Progressive Matrices consists of a measure of non-verbal logical reasoning (Raven, 1947). For the study purposes, a reduced version have been developed from the original test, made by 18 items. These were selected from the three sections of the RCPM test (A, AB, B), by maintaining the original hierarchical structure. The first two items of the reduced version were used as practice trials.

- *Modified Card Sorting Test (MCST):* This simplified and shortened version of the Wisconsin Card Sorting Test was chosen as a test of abstract reasoning and mental flexibility (Caffarra, Vezzadini, Dieci, Zonato, Venneri, 2004). The original test has been adapted to a computerized administration, by maintaining the original 48-cards structure and the same instructions. The performance on the MCST was scored on the number of categories achieved and number of cards employed.

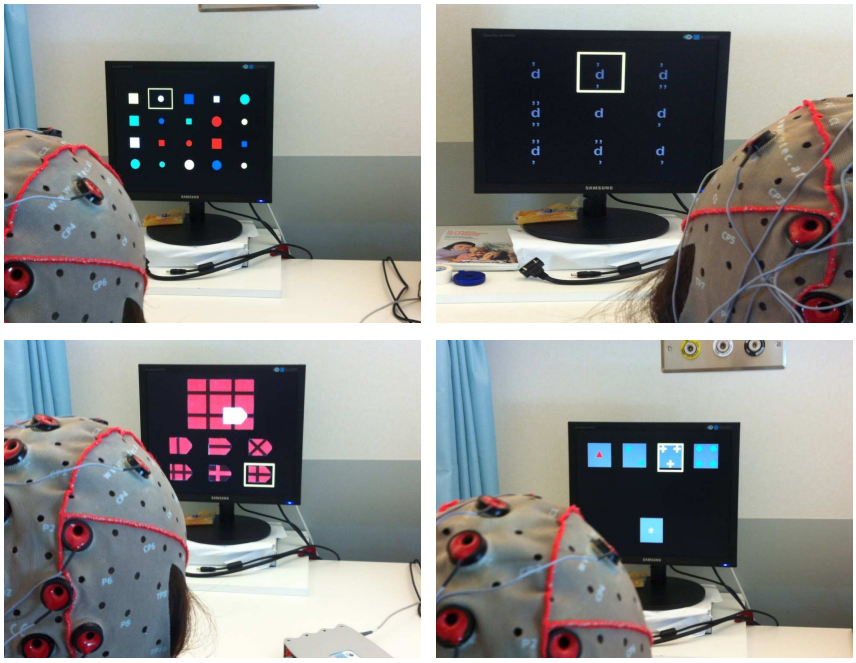

Figure 5.3. The BCI version of the neuropsychological tests (from above: Token test; d2; Raven's Colored Progressive Matrices; Modified Card Sorting Test)

5.3.6. Eye Tracking

Eye movement recording

The ET was an Eyelink-1000 (SR Research Ltd., Mississauga, Ontario, Canada), consisting of a high-speed infrared camera and the related illuminator, positioned just below the Display Monitor. Eye-movement data consisted of moment-to-moment measures of eyes displacements along the vertical and horizontal axes (in millimeters) within the spatial working area of the monitor screen. The pupil dilation and gazes were acquired, based on the pupil position and the corneal reflection on the frontal surface of participants' eyes (caused by an infrared light source), at 500 Hz (one record data per 2 milliseconds) by means of a EyeLink 1000 using custom software programmed in C#. After the experiment, the signals

were extracted and processed with custom software developed using MATLAB 7.2 (The Mathworks, Inc.; Natick, MA).

Eye Tracking: neuropsychological tests administered

- *Verbal Fluency test:* Participants were instructed about the ET mode of operation and then asked to copy a list of words ('FAX WHISKEY ZAINO FUNGO DATO BIC'), in order to provide them with a training phase. The execution time and the percentage of correct characters typed were recorded, as a measure of participants' confidence with the instrument. Then, a three letters (F-P-L) and three categories (animals-fruits-car brands) verbal fluency test was administered, according to the original validated test procedure (Novelli et al., 1986). Participants were required to produce as more words as possible in one minute for each letter or category, by typing the letters with the ET device. The percentage of correct characters typed was recorded. Then, subjects were asked to copy the generated words, and the time needed for the copy task was measured, together with the percentage of correct characters.

A parallel version of the tests adapted to the BCI system were developed, in order to include the following measures also into the ET protocol:

- *Token Test*
- *d2 test*
- *Raven's Colored Progressive Matrices test* (*RCPM*)
- *Modified Card Sorting Test* (*MCST*)

Besides, different neuropsychological tasks were adapted to the ET system (Figures 5.4, 5.5 and 5.6):

- *Reading the Mind in the Eyes Test (RME):* The original test (Baron-Cohen et al., 2001), evaluating a subcomponent of theory of mind abilities, i.e. facial emotion recognition, was adapted to a computerized administration. The RME test consisted in the presentation of 36 black and white photographs of the eye region of both male and female human faces. Participants were required to choose which of four words best describes what the person in the photograph is thinking or feeling. Before starting, one practice picture was presented. Moreover, a control task, designed to investigate participants' ability to correctly identify human physical attributes such as gender, was administered.

- *Iowa Gambling Task (IGT):* The computerized version of the 100-card version IGT (Bechara, Tranel, Damasio, 2000), assessing affective decision-making abilities, was employed with the ET system. In the task, the participant selects a card from four separate decks of cards (A, B, C, D). Following selection, the participant wins and sometimes loses a certain amount of money. Selection from decks A and B results not only in high wins but also high losses and overall accumulative loss, and hence selection from these decks is disadvantageous, while selection from decks C and D results in lower wins and lower losses and overall accumulative gain, and hence selection from these decks is advantageous.

- *Ocular movements recording:* Starting from previous studies of Donaghy and colleagues (2009, 2010), adapted versions of smooth pursuit test and saccade/antisaccade test was developed, aimed at evaluating ocular movements abnormalities.

  - S*mooth pursuit test*: Participants were presented with a yellow stimulus moving across the screen along the sides and diagonals of an imaginary rectangle, covering about 80% of the screen, at a variable speed (from 5° to 20° per second); they were requested to follow the movement of the stimulus with their eyes. Velocity gain was measured.
  - *Saccade/Antisaccade test:* Participants were asked to fixate a central point on the screen; when aware of a red peripheral stimulus, they have to look at it, while when aware of a green peripheral stimulus, the were asked to look in the opposite direction. This test consisted of 48 stimuli, preceded by a 6-stimuli practice. The target stimuli appeared at variable and randomized time intervals, in order to reduce the anticipatory saccades. For each saccade and antisaccade, errors, latency, amplitude, speed and duration were recorded.

- *Arrow-Colour Test (ACT)*: It consists of a newly developed neuropsychological test, measuring frontal abilities, in particular cognitive flexibility and response inhibition. The test is composed of four trials, made by 12 items each, where participants are required to select the appropriate arrow according to a written instruction. A two-items practice has been introduced before each trial. Two instructions are presented. In the first and third trial: “Note the arrow in the upper center. In the following exercise you will be asked to choose, between the arrows below, the one with the different color, but the same direction of the target arrow”. In the second and fourth trial: “Note the arrow in the upper center. In the following exercise you will be asked to choose, between the arrows below, the one with the same color, but the different direction of the target arrow”. Upper arrows are equally distributed

on the four directions (3 for each direction). Target arrows are equally distributed on right, left and central positions (4 for each position in 3-arrows trials, 3 for each position in 4-arrows trials). For each task, two or three distracters are present.

Figure 5.4. The ET version of the RME Test and control task

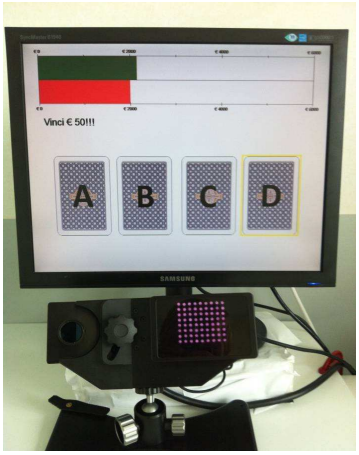

Fig. 5.5. The ET version of the IGT

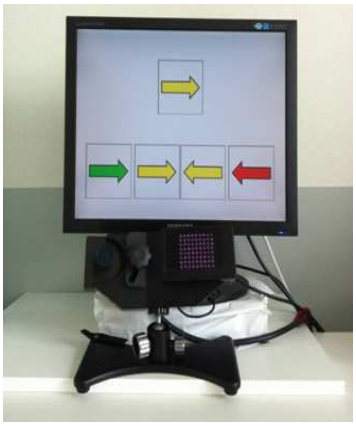

Fig. 5.6. The ET-based Arrow-Colours Test

5.3.7. Usability questionnaire

Since no validated usability measures were available for ET and BCI systems, an *ad hoc* questionnaire composed of 18 items on a 7-point Likert scale has been developed, aimed at evaluating the instruments' general usability and some specific variables such as fatigue, emotional aspects and perceived usefulness. The questionnaire has been divided into 'positive' and 'negative' items, in order to highlight perceived advantages and disadvantages of the two devices, and two different scores have been so generated.

5.3.8. Procedure

In Figure 5.7, the structure of the overall assessment protocol is presented. Clinical data concerning disease severity (ALSFRS-R scale) and respiratory parameters (spirometry) were available from neurological examination preceding the experiment start.

In a first session, traditional cognitive/behavioural assessment has been performed, based on the standard neuropsychological, psychological and behavioural tests administration. Besides, calibration phases with the two devices (BCI and ET) and Verbal Fluency tests have been carried out.

During the second session, the BCI and ET-based neuropsychological assessment has been performed, ending with the administration of two usability questionnaires concerning the two instruments.

The administration order of BCI and ET procedures has been randomized, in order to ensure that half of the sample first performed the BCI calibration and verbal fluency test, while the other half first executed the ET ones. Besides, the administration order of each neuropsychological test within the specific modality of administration (BCI and ET) has been randomized, in order to avoid learning and fatigue effects.

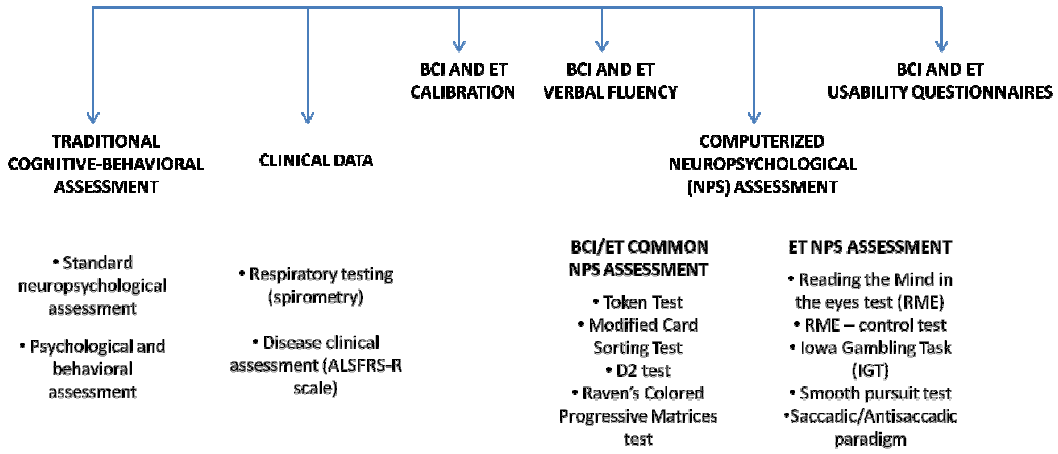

Figure 5.7. Structure of the overall assessment protocol.

5.3.9. Statistical analysis

Descriptive statistics (mean±standard deviations for continuous variables and frequencies for discrete variables) were used to describe the main characteristics and tests scores. Distribution of the variables in terms of proximity to normal and the homogeneity of variances were detected by Shapiro-Wilk test and Bartlett test, respectively. To compare the mean scores between groups, a two-sided t-test was used with pooled estimates of the sample variance or the Welch approximation when the continuous variables were normally distributed, homoscedastic or heteroscedastic, respectively. Otherwise we performed a two-sided Wilcoxon's signed-rank test. Finally, to assess the degree of association between scores we used the Pearson's correlation coefficient. An α level of 0.05 was used for all hypothesis tests. All data analyses were performed using R Core Team (2014), Vienna, Austria.

5.4. Results

Characteristics of patients and healthy subjects are presented in Table 1.

	ALS patients	Healthy controls
N	34	30
Gender [N]	M: 28; F: 6	M: 15; F: 15
Age	62.1. ± 11.7	56.2 ± 13.1
Education [years]	10.2 ± 3.6	13.8 ± 4.2
Duration of illness [months]	23.2 ± 15.9	---
ALS-FRS/R	36.9 ± 8.2	---
Type of onset [N]		
Bulbar	8	---
Spinal	26	---
NIV	1	---
PEG	1	---
ALS-FTD	0	---
Familial cases [N]	0	---
Sporadic cases [N]	34	---
Genetic Screening		
C9orf72 [N]	5	---
SOD1 [N]	0	---
TDP43 [N]	0	---
FAB	15.6 ± 1.7	16.5 ± 1.3
MoCA	24.2 ± 3.1	26.9 ± 2.0
WM subtest	19.5 ± 3.5	22.2 ± 2.7

Table 5.1. Demographic, clinical and cognitive characteristics of ALS patients and healthy subjects. Data are expressed as means ± standard deviations or absolute numbers.

5.4.1. Patients data

Of the thirty-four patients, twenty-one managed to complete the overall assessment protocol, while partial data have been collected from the remaining thirteen patients. Reasons for incomplete data can be grouped into '*technical*' issues (i.e., presence of unstable EEG waves, interference in eye movements recording due to eyeglasses), '*clinical*' issues (i.e., fluctuations in the level of vigilance, poor collaboration) and '*hospital setting*' issues (i.e., patients' discharge from the clinic before the entire protocol completion, with patients

residing far from the hospital). Overall, about 45% of incomplete data (6/13 cases) were due to '*technical*' issues, which need to be further investigated in future studies.

Standard neuropsychological assessment

ALS patients obtained significantly lower scores than controls at the three traditional neuropsychological tools (FAB, MoCA and Working Memory subtest) (Table 5.1). In particular, an higher level of significance was observed with regard to the MoCA assessment (p<0.001), followed by the WM test (p<0.01) and by the FAB assessment (p<0.05) (Figure 5.8).

Twenty-six patients completed the entire MoCA and eleven of them (42,3%) were classified as impaired. Twenty-seven patients managed to perform the entire FAB assessment, with three patients (11%) classified as impaired and one patient obtaining a score equivalent to the cut-off.

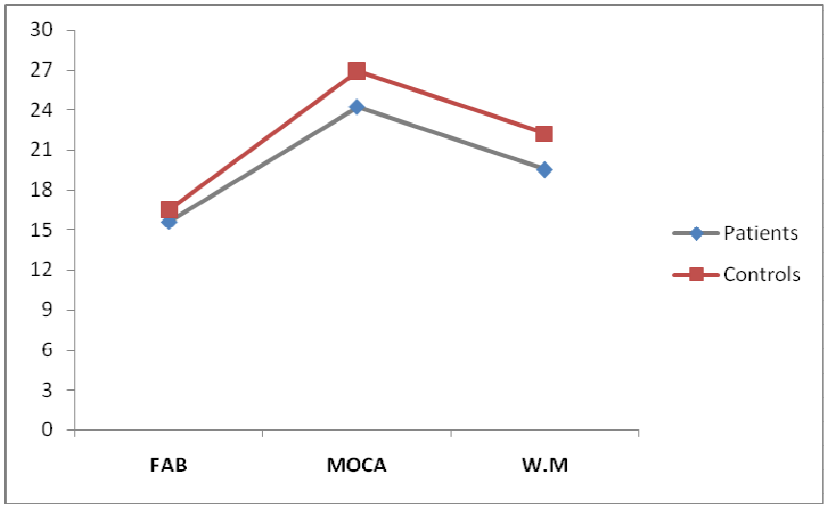

Figure 5.8. Comparison of patients and controls on the three traditional NPS measures

Twenty-five caregivers completed the FBI. Seven patients showed no behavioural changes (FBI=0), while none of the other eighteen patients reached the FBI cut-off for the presence of FTD behavioural changes, obtaining scores ranging from 1 to 13.

Psychological parameters

No significant differences were found between patients and controls with regard to trait anxiety (i.e., the anxiety level as a personal characteristic) assessment (STAY-Y2: ALS patients: 48.3±10.7, healthy subjects: 44.7±8.2, p=0.22), while the two groups significantly

differed at the assessment of state anxiety (i.e., the anxiety about a specific event or context) (STAI-Y1: ALS patients: 46.2±8.5, healthy subjects: 41.2±7.4, $p<0.05$), with patients reporting higher levels of anxiety than controls. Moreover, significant differences emerged at the evaluation of depressive symptoms, with patients obtaining significantly higher scores at the somatic-performance subscale (BDI Somatic-Performance score: ALS patients: 6.3±4.0; healthy controls: 3.4±2.7, $p<0.01$) and at the global index (BDI Total score: ALS patients: 11.7±7.3; healthy controls: 7.3±6.5, $p<0.05$) of the BDI questionnaire, while no significant differences were highlighted concerning the cognitive-affective subscale (BDI Cognitive-Affective score: ALS patients: 5.4±4.1; healthy controls: 3.9±4.7, $p=0.1$).

Brain Computer Interface – Calibration and Verbal Fluency

No significant differences were observed between patients and healthy subjects with regard to the percentage of accuracy at the BCI calibration for both groups ($p=0.50$) (Table 2), nor for the percentage of correct characters generated at the Copy test with the three words ('IL QUIETO FIDO') (ALS patients: 86.5±16.4; healthy controls: 84.7±16.2, $p=0.57$) (Figure 5.9).

At the phonemic Verbal Fluency test ('M' letter), patients were found to need significantly more time than controls to generate 5 words (Latency (sec): ALS patients: 5.1±2.1; healthy controls: 4.2±3.3, $p<0.05$), while there was no significant difference concerning the percentage of characters correctly produced with the BCI system (ALS patients: 88.0±16.6; healthy controls: 91.8±7.6, $p=.80$).

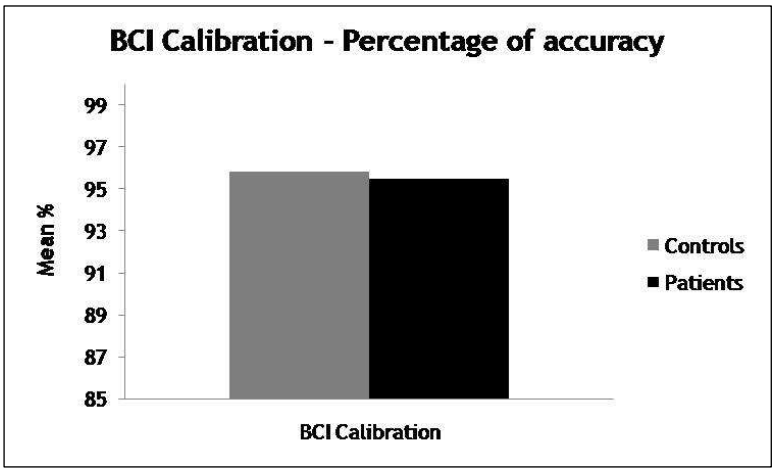

Figure 5.9. Comparison between patients and controls performance on the BCI calibration.

Brain Computer Interface – Neuropsychological assessment

For the BCI-neuropsychological assessment, the following variables were overall extracted from participants' performance: '*length of the session*' (computed starting from the test presentation to participants to the last answer given at the final item), '*number of correct responses*' (total number of correct answers), '*reasoning*' (time needed by participants to decide the correct answer and to give signal for starting the flashes) and '*selection*' (total time including reasoning time and BCI item selection). Performance obtained at the four NPS test were analyzed (Table 2).

At the d2 Test of Attention, significant differences were observed concerning the '*reasoning*' (p<0.01) and '*selection*' (p<0.05) variables, with higher times needed by patients in comparison to healthy subjects. The two groups did not scored differently with regard to the accuracy in detecting the correct answer (p=0.96) e in the '*length of the session*' (p=0.17).

In the MCST, in addition to the described outcomes, two further variables were considered: '*number of cards*' employed and '*number of categories*' completed. Patients performed poorer than healthy controls at all the time-related outcomes (i.e., '*length of session*', '*reasoning*' and '*selection*', p<0.001) and in the '*number of cards*' employed to complete the categories (p<0.05); no significant differences were observed about the '*number of correct responses*' (p=0.56) and the '*number of categories*' completed (p=0.37).

At the RCPM test, the two groups performance were significantly different at the '*number of correct responses*', '*reasoning*' and '*selection*' outcome variables (p<0.01), while no different values were observed with regard to the '*length of the session*' one (p=0.10).

Finally, at the Token test, comparable performance were obtained at the '*length of the session*' (p=0.42) and '*number of correct responses*' (p=1) variables, whilst ALS patients took more time to generate the answer ('*reasoning*': p<0.05), thus needing more time to complete the items selection ('*selection*': p<0.01).

Test	ALS patients	Healthy controls	*p-value*
BCI-Calibration (%)	95.5 ± 7.5	95.8 ± 4.9	0.508
BCI-d2	*Length sess.*: 498.5 ± 120.2	*Length sess.*: 464.1 ± 79.7	0.170
	Correct: 20 ± 0.2	*Correct*: 20 ± 0.2	0.96
	Reasoning: 5125.3 ± 1498	*Reasoning*: 4067.4 ± 1000	**0.003**
	Selection: 20791 ± 1961.3	*Selection*: 19675.2 ± 2123.6	**0.020**
BCI-MCST	*Length sess.*: 1213.6 ± 255.4	*Length sess.*: 938.3 ± 139.5	**0.000**
	Correct: 34.9 ± 2.6	*Correct*: 34.7 ± 3.4	0.569
	Reasoning: 8578.4 ± 3879	*Reasoning*: 5108.3 ± 3286.9	**0.000**
	Selection: 26393.1 ± 4182.2	*Selection*: 22147.1 ± 3831.6	**0.000**
	Cards: 43.1 ±4.1	*Cards:*40.8 ± 3.8	**0.036**
	Categories: 5.7 ± 0.5	*Categories:* 5.8 ± 0.6	0.371
BCI-RCPM	*Length sess.*: 524.8 ± 106.4	*Length sess.*: 488.4 ± 125.8	0.108
	Correct: 12.8 ± 3.1	*Correct*: 14.8 ± 1.4	**0.009**
	Reasoning: 9324.3 ± 3503.1	*Reasoning*: 6488.3 ± 2958.2	**0.001**
	Selection: 25525.3 ± 4986.5	*Selection*: 22059.9 ± 3555.3	**0.007**
BCI-Token Test	*Length sess.*: 417.9 ± 69.9	*Length sess.*: 417.8 ± 79.4	0.428
	Correct: 12.6 ± 0.9	*Correct*: 12.7 ± 0.5	1
	Reasoning: 2791.4 ± 1942.3	*Reasoning*: 1970.8 ± 1983.6	**0.016**
	Selection: 24481.9 ± 3862.4	*Selection*: 22710.2 ± 3561.1	**0.009**

Table 5.2. Performance at neuropsychological tests administered by means of BCI.
Data are expressed as raw scores means ± standard deviation.

Brain Computer Interface assessment – Rate of error in detecting participants' intentions

An index was computed, in order to investigate the reliability of the BCI system in correctly detecting participants' intentions. For each test, the number of time the experimenter corrected the BCI selection, when different from those intentioned by the participants, was automatically recorded and the percentage of errors on the total number of responses was computed. The comparison between the four BCI tests showed that the more reliable test was the D2 Test of Attention, with the 13.6% of errors. With regard to the other cognitive tests, 16.2% of errors was recorded for Token test, 23.10% for MCST and 27.80% for the RCPM test. When comparing the two experimental groups, the percentage of errors was significantly different only with regard to the MCST (p<0.05), with higher values for the

patients' group; a comparable rate of BCI errors among the two groups in detecting participants real intentions was observed for the other tests.

Eye Tracking – Verbal Fluency

The percentage of correct characters generated at the initial copy test ('FAX WHISKEY ZAINO FUNGO DATO BIC') was significantly higher for controls than for patients (ALS patients: 57.9±32.5; healthy controls: 80.8±25.5, $p<0.01$) (Figure 5.10). Besides, patients needed more time to copy the words sequence (ALS patients: 134.6±59.2; healthy controls: 89.2±32.5 sec, $p<0.001$) (Figure 5.11).

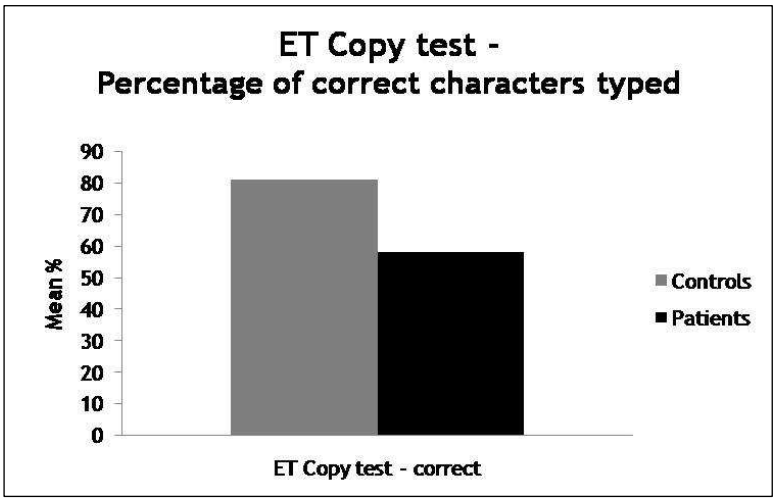

Figure 5.10. Comparison between patients and controls performance on the training ET copy test.

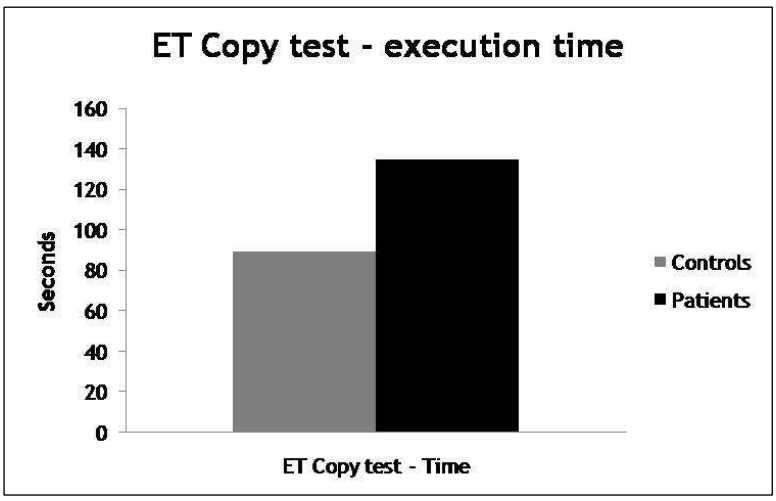

Figure 5.11. ET copy test execution times of patients and controls.

At the Phonemic Verbal Fluency test (F-P-L), the percentage of correct characters typed with the ET was significantly higher for controls than for patients with regard to letter F (ALS patients: 68.6±23.9; healthy controls: 92.6±10.4; $p<0.0001$), letter P (ALS patients: 76.4±20.6; healthy controls: 90.5±11.6, $p<0.01$) and letter L (ALS patients: 76.6±23.2; healthy controls: 92.8±9.2, $p<0.01$), as well as the total number of generated characters (F and P letters: $p<0.01$; L letter: $p<0.001$).

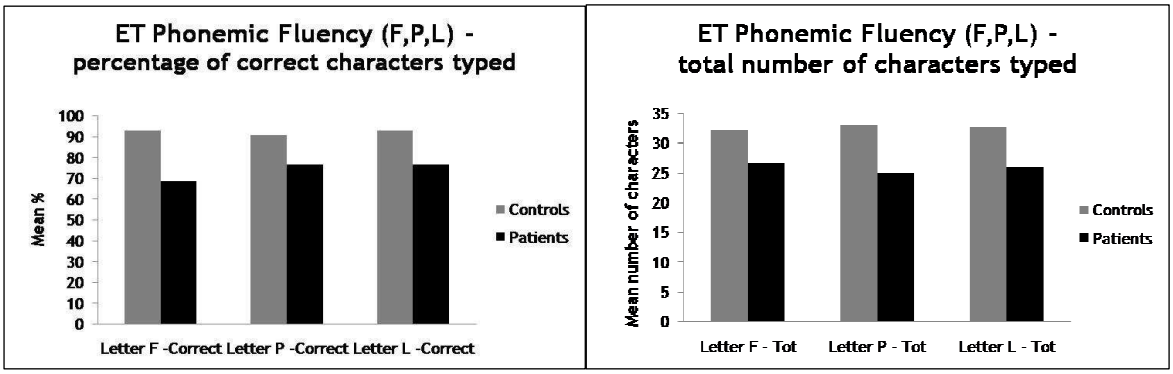

Figure 5.12. Comparison between patients and controls performance at the ET Phonemic Verbal Fluency test.

Similarly, at the Semantic Verbal Fluency test, healthy subjects performed better than patients regarding both the percentage of correct characters typed (animals - ALS patients: 82.4±21.0; healthy controls: 97.5±5.0, $p<0.001$; fruits - ALS patients: 84.7±14.3; healthy controls: 96.0±5.6, $p<0.001$; car brands - ALS patients: 84.6±17.7; healthy controls: 94.0±8.2, $p<0.01$) and the total number of generated characters (animals, fruits and car brands: $p<0.01$).

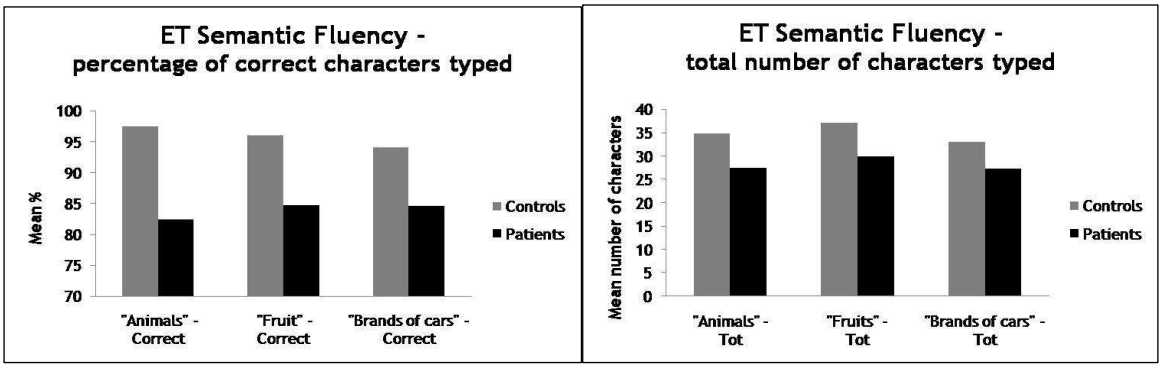

Figure 5.13. Comparison between patients and controls performance at the ET Semantic Verbal Fluency test.

Eye Tracking – Neuropsychological Assessment

For the ET-neuropsychological assessment, the following variables were overall extracted from the participants' performance: '*length of the session*', '*number of correct responses*' and '*selection*' (Table 5.3). The '*reasoning*' outcome variable was not computed with the ET, since the time interval between item presentation and response generation was not meaningful with this device.

For the D2 Test of Attention, patients performed significantly worse than controls in all the considered variables: '*length of the session*' ($p<0.05$), '*number of correct responses*' ($p<0.01$) and '*selection*' ($p<0.01$).

Similar results were detected for the MCST, for all the considered outcome variables: '*number of correct responses*' ($p<0.001$), '*number of categories*' achieved ($p<0.001$), '*number of cards*' used ($p<0.01$), '*selection*' ($p<0.001$) and '*length of the session*' ($p<0.05$).

For the RCPM test, healthy subjects performed better than patients in '*number of correct responses*' ($p<0.01$), '*selection*'($p<0.001$) and '*length of the session*' ($p<0.05$).

On the contrary, no significant differences were found where comparing patients' and control's performance at the Token test ('*length of the session*': $p=0.90$; '*selection*': $p=0.67$; '*number of correct responses*': $p=0.51$).

Regarding to the RME test, a significant higher number of correct responses was given by healthy subjects compared to patients ($p<0.01$), while no differences were observed concerning time-related variables ('*selection*', $p=0.09$; '*length of the session*': $p=0.21$). Conversely, at the control condition of the RME test, significant differences were found with regard to time-related measures, with patients taking longer time than controls to perform the test ('*selection*' and '*length of the session*': $p<0.01$); at this task, patients and controls were comparable when considering accuracy in the test execution ('*number of correct responses*': $p=.93$).

With regard to the Iowa Gambling Task, the performed analysis did not reveal any significant differences among the two groups, with only a tendency to engage in more disadvantageous choice (decks A and B) in the patients' group.

The ACT task was divided into four subtest (Table 5.4); each of them was considered with regard to the '*number of correct responses*' and '*selection*' variables, while '*length of the session*' was computed considering the first and the last subtests. Significant differences between the two groups were observed in the '*number of correct responses*' at ACT2 and ACT4 subtests, with patients performing worse than controls ($p<0.05$); '*selection*' variable

results were significantly different in all the four conditions (ACT1: $p<0.001$; ACT2: $p<0.001$; ACT3: $p<0.01$; ACT4: $p<0.001$), with higher execution times needed by patients. The '*length of the session*' was longer for the latter group ($p<0.05$).

To date, results from the two measures based on ocular movements recording, i.e. the Smooth Pursuit test and the Saccade/Antisaccade test, have not been fully analyzed and require more time to be formally presented.

Test	ALS patients	Healthy controls	*p-value*
ET-d2	*Length sess.*: 186 ± 53.2	*Length sess.*: 165.4 ± 54.2	**0.025**
	Correct: 18.5 ± 2.2	*Correct*: 19.7 ± 1	**0.001**
	Selection: 7057.8 ± 11329.1	*Selection*: 4350.2 ± 1069.1	**0.001**
ET-MCST	*Length sess.*: 385.1 ± 99.3	*Length sess.*: 330.9 ± 121.1	**0.027**
	Correct: 28.7 ± 7.6	*Correct*: 34 ± 4.3	**0.000**
	Selection: 5956.7 ± 1569.3	*Selection*: 4378.7 ± 1054.2	**0.000**
	Cards: 46 ± 3.9	*Cards:* 43.7 ± 4	**0.005**
	Categories: 4.4 ± 1.4	*Categories:* 5.6 ± 0.9	**0.000**
ET-RCPM	*Length sess.*: 269.9 ± 133.4	*Length sess.*: 217 ± 50.5	**0.044**
	Correct: 12.6 ± 3.1	*Correct*: 14.7 ± 2.5	**0.005**
	Selection: 8979.5 ± 3119.1	*Selection*: 6404.1 ± 1647.5	**0.000**
ET-Token Test	*Length sess.*: 188 ± 47.3	*Length sess.*: 191.1 ± 42.9	0.904
	Correct: 11.7 ± 0.7	*Correct*: 11.9 ± 0.4	0.514
	Selection: 2626.7 ± 620.2	*Selection*: 3070 ± 1418.7	0.673
ET-RME test	*Length sess.*: 410.3 ± 103.3	*Length sess.*: 368.2 ± 122.6	0.212
	Correct: 21.9 ± 4.7	*Correct*: 25.9 ± 3.8	**0.003**
	Selection: 8734.2 ± 2826.5	*Selection*:11934.9 ± 17542.2	0.090
ET-RME control test	*Length sess.:*255.6 ± 242.9	*Length sess.:* 179.8 ± 26.2	**0.001**
	Correct: 34.1 ± 1.7	*Correct:* 33.9 ± 2.2	0.936
	Selection: 4431.7 ± 2638.7	*Selection:* 3331.4 ± 590.2	**0.001**
ET-IGT	*Length sess.*: 748.7 ± 138.1	*Length sess.*: 718.4 ± 186.1	0.144
	Selection: 2644.1 ± 1408.4	*Selection*: 2076.2 ± 602.8	0.059
	Netscore-Global: -14.2 ± 23.8	*Netscore-Global:* -12.3 ± 21.4	0.833
	Netscore-Learning:-8.4 ± 14	*Netscore-Learning:*-7.6 ± 13.2	0.662
	Netscore-Performance:-5.8 ±12.5	*Netscore-Performance.*-4.7 ±13.8	0.634

Table 5.3. Performance at neuropsychological tests administered by means of ET. Data are expressed as raw scores means ± standard deviation.

Test	ALS patients	Healthy controls	*p-value*
ET-ACT	*Length sess.*: 471.6 ± 102.7	*Length sess.*: 402.2 ± 78	**0.012**
ET-ACT1	*Correct*: 13.4 ± 0.8 *Selection*: 4389.1 ± 1114.7	*Correct*: 13.7 ± 0.6 *Selection*: 3344.9 ± 461.3	0.098 **0.000**
ET-ACT2	*Correct*: 12 ± 2.7 *Selection*: 5095.8 ± 1468.6	*Correct*: 13.4 ± 0.7 *Selection*: 3661.9 ± 648.3	**0.013** **0.000**
ET-ACT3	*Correct*: 12.5 ± 2.2 *Selection*: 4884.7 ± 1329.6	*Correct*: 13.2 ± 1.3 *Selection*: 3966.4 ± 760.4	0.060 **0.001**
ET-ACT4	*Correct*: 12.5 ± 2.6 *Selection*: 4924.2 ± 1463	*Correct*: 13.5 ± 0.9 *Selection*: 3647.5 ± 623.4	**0.034** **0.000**

Table 5.4. Performance at the Arrow-Colours test administered by means of ET. Data are expressed as raw scores means ± standard deviation.

Correlations between traditional measures and BCI-ET neuropsychological assessment

Significant correlations have been highlighted between the BCI- and ET-based neuropsychological measures and traditional 'paper and pencil' tools. In particular, scores obtained at the MoCA correlated with both accuracy index ('*number of correct responses*': r = 0.57, $p<0.01$) and time-related variables ('*selection*': r = -0.63, $p<0.01$) of the BCI-RCPM test and with the '*reasoning*' variable of the D2 test ('*reasoning*': r = -0.55, $p<0.01$). Moreover, MoCA also correlated with the ET- RCPM test ('*number of correct responses*': r = 0.61, $p<0.01$) and ET-ACT1 test ('*selection*': r = -0.71, $p<0.01$).

With regard to the WM test, a correlation was found with the BCI-D2 test ('*reasoning*': r = -0.61, $p<0.01$) and the BCI-MCST ('*length of the session*': r = -0.66, $p<0.01$). WM scores also correlated with the following ET test: ACT1 ('*selection*': r = -0.6, $p<0.01$, and '*length of the session*': r = -0.61, $p<0.01$); ACT2 ('*selection*': r= -0.53, $p<0.01$); MCST ('*selection*': r = -0.55, $p<0.01$); RCPM ('*number of correct responses*': r = 0.65, $p<0.01$, '*selection*': r = -0.38, $p<0.05$, '*length of the session*': r = - 0.58, $p<0.01$); RME test ('*number of correct responses*': r = 0.61, $p<0.01$) and RME control test ('*selection*': r = -0.52, $p<0.01$).

No significant correlations were observed between the FAB test and the BCI- and ET-based neuropsychological measures.

When considering the relationship between clinical measures and BCI and ET testing, no significant correlations were detected with regard to both disease progression, evaluated by means of the ALSFRS-R Scale global score, and time from onset (months). However, a positive correlation was observed between the bulbar subscale of the ALSFRS-R, which evaluates the degree of bulbar impairment according to the first three items of the global scale, and the total number of characters correctly computed at the ET-Phonemic Verbal Fluency test with the 'F' letter ($r = 0.53$, $p<0.05$). Respiratory parameters (spirometry) did not significantly correlated with cognitive performance; the only significant correlation was observed between the FEV1 parameter and the time needed to copy the previously written words at the ET Verbal Fluency test with the 'P' letter ($r = -0.71$, $p<0.01$).

Usability questionnaire

The subjective perception of BCI and ET usability was evaluated comparing healthy controls and patients' scores on both positive and negative statements included in the questionnaire. No significant differences were observed between the two samples. However, a tendency to evaluate as more positive the ET device (ET-mean positive score: 64.8), compared to the BCI one (BCI-mean positive score: 60.7), was highlighted in the controls group. ALS patients did not showed a preference towards one of the two tools, with comparable positive and negative statements about BCI and ET (ET-mean positive score: 61.6; BCI-mean positive score: 61.5; ET-mean negative score: 17.6; BCI-mean negative score: 17.4).

5.5 Discussion

Overall considered, both the BCI- and the ET-based neuropsychological assessment batteries were useful in detecting also slight cognitive changes in ALS patients. In addition to performance accuracy (errors), execution times have proved to be a sensitive index in both BCI and ET neuropsychological tasks. When comparing the two approaches as cognitive assessment tools, the ET battery seems to better discriminate ALS patients from controls, than BCI does. However, the high calibration accuracy obtained by ALS patients with the P300 Speller and the absence of correlations between the BCI outcome and the disease progression (ALFRS-R) support the feasibility of such tool, in particular in those

patients presenting with ocular-motor impairments. Furthermore, the positive ratings given by patients, that judged BCI as usable as the ET device, provide further promising evidence concerning the possibility to use the BCI-based cognitive testing when the eye-movement recording is not adequately practicable.

Some considerations can be drawn from patients' performances at the neuropsychological tests administered by means of BCI and ET devices. In this study, patients performed significantly worse than controls at the test of affective Theory of Mind, i.e. the Reading the Mind in the Eyes test. A previous study employing the RME test with ALS patients did not revealed a significant impairment in such ability when comparing patients to healthy subjects (Cavallo et al., 2011). Only a trend towards significantly lower RME accuracy scores in ALS patients compared to healthy controls was observed in a second study of Girardi and coll. (2011). Since time-related variables did not differ among the two groups, the worst performance of patients cannot be explained according to the presence of impulsivity in ALS patients.

Moreover, the RME test seems to discriminate patients from controls better than the Iowa Gambling test does, suggesting a selective impairment in ToM abilities and not in affective decision making.

With regard to the Token Test, the absence of even slight differences between patients and controls performance (accuracy) suggests an integrity of language comprehension abilities in the clinical group. However, the adopted version, made up by a selection of item from the original test, is probably simpler than the traditional Token Test and this aspect may have lead to underestimate the possible presence of comprehension deficits in the ALS patients sample. Nevertheless, the higher time needed by patients to identify the correct answer ('*reasoning*' variable) may suggest the possible presence of a subclinical pattern of cognitive impairment in ALS patients, primarily affecting processing speed. This slight alteration in ALS patients can be revealed only if including time-related variables into the traditional assessment. Such hypothesis is in contrast with results provided by a recent study that support the absence of impairments on tests of processing speed in a sample of ALS patients (Pettit et al., 2013). Therefore, the assumption of a possible cognitive slowing in ALS patients should be more deeply investigated.

Finally, the *ad hoc* developed measures of cognitive flexibility and response inhibition, the Arrow-Colour Test, seems useful to highlight slight frontal alterations in ALS patients.

Correlations have been found between the BCI and ET measures and two traditional ‘paper and pencil’ tests, i.e. the MOCA and the Working Memory subtask. These two tests have been shown more sensitive in discriminating ALS patients from healthy subjects, when compared to the FAB. Taken as a whole, these results suggest a good concurrent validity of the developed computerized assessment and a better match with those traditional measures that have been shown to be particularly useful in detecting mild cognitive alterations observed in our non-demented ALS patients sample.

The absence of correlations between respiratory parameters and performances at the cognitive tests may be due to the small proportion of ALS patients presenting with respiratory problems in our sample.

Even if the ET seems to have higher level of usability than the BCI, patients were inclined to rate more positively the latter approach than controls did. Besides, patients easily managed to learn and communicate with the P300-BCI paradigm. These aspects, together with the unfeasibility of the ET devices in presence of oculor-motor impairments, suggest the complementary use of both approaches during the entire course of the disease.

Conclusions

An accurate quantitative and qualitative assessment of cognitive functioning in ALS is now recognized as an integral part of patients clinical and care management. In order to satisfy both clinical and research purposes, highly sensitive diagnostic tools and adequate screening tests are needed, in order to better characterize the cognitive spectrum phenotypes in ALS.

In light of such considerations, the present study is characterized by several innovative elements. First, the development and the availability of a motor-verbal free neuropsychological battery allows a longitudinal cognitive assessment during the entire course of the disease, also in moderate-severe stage when traditional measures are not fully administrable due to severe motor and verbal disabilities presented by patients.

Moreover, these results have an impact on clinical practice and regarding ethical issues in ALS, with particular concern for the capacity of taking decisions about financial circumstances, invasive treatments proposed in the course of the disease and end-of-life decisions.

Although these positive and promising aspects, BCI and ET devices also present some critical issues, such as the high economical costs of these instuments and the need for specialized professions specifically trained to develope new neuropsychological tests and to administered them by means of BCI and ET systems. Moreover, BCI 2000 open source mangement and data extrapolation require a high level of expertise.

Further work will be aimed at refining the developed system, particularly improving the BCI accuracy in detecting the patients' willing choices during the tests administration, and at enlarging the cognitive spectrum investigated.

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