INSTITUTE OF NEUROSCIENCE OF CASTILLA Y LEÓN

Laboratory of Audiomotor disorders

ENGLISH SUMMARY

Sensorimotor Alterations in Neurodegenerative Disease.

Lymarie Millian Morell

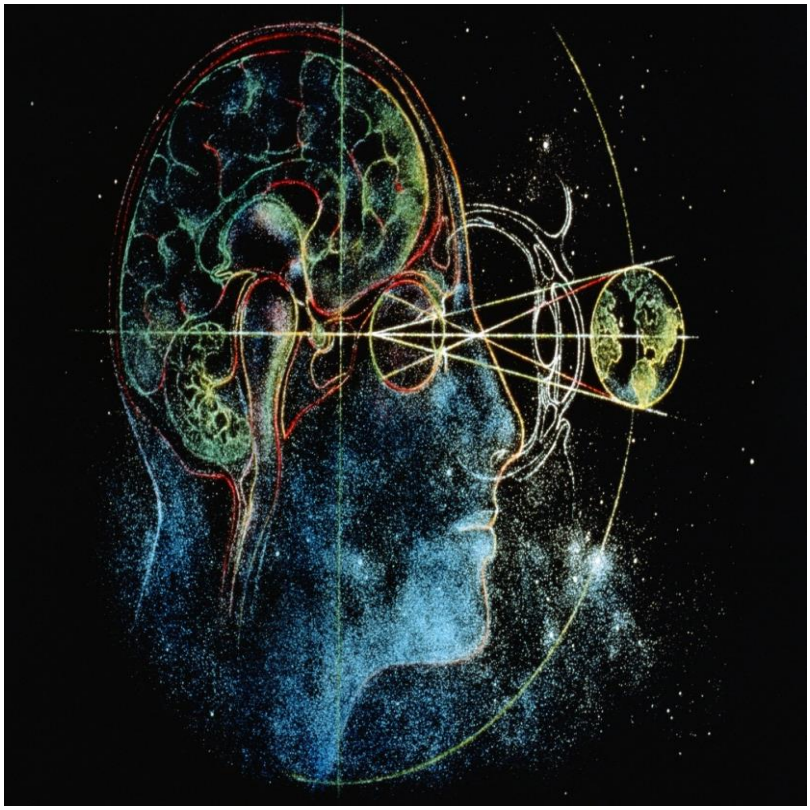
Directors:

Dr. Tomás López Alburquerque

Dra. Mª Dolores E. López García

Salamanca, 2015

INDEX



(Brain Universe, Anonymous)

INTRODUCTION

I. Introduction

Neurodegenerative diseases are defined as those that result in slowly progressive neuronal loss due to various genetic and environmental factors, and become clinically manifest by cognitive, sensory or motor changes. In recent decades, due to the increased life expectancy and longevity, especially in developed countries, there is higher prevalence of various neurodegenerative diseases. The most frequents among these diseases with high social impact are Parkinson's disease (PD) and Essential Tremor (ET), that in some cases combine motor and cognitive disorders (Samuels et al., 2007), as well as Mild Cognitive Impairment (MCI) and Alzheimer's disease (AD), in which predominate cognitive impairments (Valls-Pedret et al., 2010). The following paragraphs summarize the most relevant aspects of these neurodegenerative diseases and its relation with the techniques used in this thesis dissertation.

1. Parkinson's disease

PD belongs to a group of conditions called motor system disorders, which results from the damage of dopaminergic neurons in the substantia nigra pars compacta (Braak et al., 2003). PD is difficult to diagnose accurately as early symptoms occur gradually and differ among patients that experience motor problems after 50% of dopaminergic cell death (Stoessl, 2011). Motor abnormalities lead to the diagnostic criteria that include bradykinesia, rigidity, resting tremor or postural instability. Also, these motor dysfunctions reflect a loss of dopaminergic neurons within the nigrostriatal system that might be traceable to extranigral impairment of the motor system (Braak et al., 2000, 2003; Albin et al., 1989; Braak and Braak, 2000).

In addition to the hallmark symptoms of PD (Blumin et al., 2004; Sewall y et al., 2006), a significant and progressive deficit in the phonatory system is often experienced by PD patients (Sewall et al., 2006). Phonetics involves speech motor activities, transmission through air and speech perception (Saxena et al., 2014). The speech and voice deterioration can be explained by a sensory processing deficit related to speech (Ho et al., 1999; Ramig et al., 2007). Patients are often described as having a high-pitched, monotone, and monoloud voice with a restricted range pitch when compared with normal subjects (Canter, 1963; Griffiths et al., 1989). Vocal impairment may be amongst the earliest PD symptoms, detectable up to five years prior clinical diagnosis (Harel et al., 2004; Tsanas et al., 2012). The loss of dopaminergic input to the striatum and subsequent deregulation of the basal ganglia produce motor deficits that adversely affect all the subsystems related to speech motor control. Any alteration in one of these systems affects the voice and the proper coordination of speech (Martínez-Sánchez, 2010). Neuroimaging studies have demonstrated the involvement of the striatum in speech initiation and production (Price, 2009).

It has been suggested that speech motor deficits in speakers with PD could contribute to hypokinetic dysarthria (Ramig et al., 2011; Walsh and Smith, 2012) that is an integral part of the motor changes in PD patients. Disturbances of prosody, articulation and mobility, correlate with the disease's severity and these disturbances are secondary to the degree of the patient's rigidity and hypokinesia. The decrease in expiratory volume with a consequent alteration of the velopalatal resonance and a decrease in vocal intensity cause the disturbances in the prosody. Abnormalities described in patients with PD, as disruptions in prosody, differences in the rate of speech, voice inflection, hesitations, pauses, monoloud voice and a decrease in the intensity variations of the voice, have been observed during the reading task (Rusz et al., 2011).

2. Essential Tremor

ET is characterized by the presence of postural and kinetic tremor in the upper limbs and head, which usually does not exist at rest (Cohen et al., 2003). It is a slowly progressive process that eventually produces motor and functional disabilities. It seems possible to identify different subtypes of ET according to the progression rate of the disease's coexistence with PD, and the degree of cognitive impairment. It is the most common movement disorder in clinical practice, especially after the age 40. Almost half of patients have a family history with autosomal dominant inheritance. Linkage studies identify alteration in dopamine receptors to alterations in ET (García-Martín et al., 2009). Currently, about 5% of Spanish elderly suffer from this disease (Benito-León et al., 2003; Labiano-Fontcubertaa et al., 2012), and its incidence increases with age (Benito-León et al., 2005; Labiano-Fontcubertaa et al., 2012).

ET has been considered as a disease characterized by monosymptomatic kinetic tremor in the upper limbs, however, in recent years, we have seen that its clinical spectrum is wider (Labiano-Fontcubertaa et al., 2012). That its clinical spectrum is wider, including symptoms usually identified as motor symptoms like gait disturbance, intention tremor and rest tremor, and non-motor symptoms like olfactory, cognitive and personality disorders (Benito-Leon and Louis, 2006; Labiano-Fontcubertaa et al., 2012). Cognitive changes are initiated with impaired executive function and memory. When the ET starts in the elderly, there is an increased risk of developing dementia (Bermejo-Pareja et al., 2007). Pathological location in most cases of ET sits in the cerebellum, while a third of the brains of patients suffering from this pathology showed neurodegenerative changes, including neuronal death (Axelrad et al., 2008; Louis et al., 2007; Louis, 2010), and abundant Lewys bodies (LB), especially in the Locus Coeruleus (LC) (Louis et al., 2005, 2007; Shill et al., 2008).

It has been postulated that PD and ET are close in their evolution. The tremor may precede the onset of other extrapyramidal symptoms, having come to regard the ET as a risk factor in the development of PD. Considering these two entities as part of a neurodegenerative *continuum* is not new (Critchley and

Greenfield, 1948), and have a common anatomical basis the LC (Frisina et al., 2009). The LC is the principal site for production of noradrenaline (Swanson and Hartman, 1975), as well as monitoring and alarm functions, presenting statements for the most active (Aston-Jones and Bloom, 1981).

3. Mild Cognitive Impairment

MCI is considered a transitional state between the cognitive impairment and the physiological aging and dementia (Petersen et al., 2001; Morris et al., 2001). MCI is clinically manifested as impaired memory maintaining cognitive and functional abilities (Petersen et al., 2001; Morris et al., 2001). Recently, the diagnostic criteria for MCI, dementia and AD have been updated by the National Institute of Aging and the Alzheimer's Association (Albert et al., 2011; McKhann et al., 2011). According to them, the MCI is characterized by deficits in higher cognitive functions, maintaining independence and accompanied by concern of the family or the patient himself. These deficits must occur in the absence of other disorders that cause similar signs and symptoms. The main difference concerns between MCI and dementia is the presence of impairment in activities of daily living (Albert et al., 2011; Mora-Simon et al., 2012).

Because some patients with MCI progress to AD, it is a medical priority to have sensitive techniques that allow us to identify the disorders, for further pharmacological interventions that can slow or stop the progression of the disease (Sherwin, 2000; Morris et al., 2001).

4. Alzheimer's Disease

AD is characterized, in its typical form, by progressive memory loss and other mental abilities, accompanied by disturbances in behavior such as aggression, depression and errant behavior (Costa et al., 1996; Francis et al., 1999; Williams et al., 2003).

The AD shows a decrease in weight and volume of the brain, with enlarged sulci and gyri. Also, the cerebral cortex is thinned and slightly enlarged ventricles (cerebral atrophy). The pathological hallmarks of AD are: loss of neurons in the cerebral cortex, senile or neuritic plaques and neurofibrillary tangles. The most vulnerable anatomical regions to suffer these neuropathological changes are: hippocampus, parahippocampal gyrus, basal nucleus of Meynert, SN, LC, periaqueductal substance and raphe nuclei (Braak and Braak, 1991). Furthermore, an overall loss of synaptic connections, particularly those of the cholinergic system, occurs in AD. These alterations have been extensively studied in animal models that have shown degeneration of cholinergic, noradrenergic, serotonergic, GABAergic and glutamatergic neurons in different brain areas involved in memory such as the hippocampus, the association cortex and the limbic system (Manzano et al., 2009).

In AD patients, alterations in lexical semantic and pragmatic domains of language (Hodges et al., 1992) are frequently observed, while the articulatory, phonological and syntactic aspects of language production

remain well preserved until later stages of the disease (Caramelli et al., 1998). The most prominent characteristics of the voice in AD patients in early stages are related to prosody as well as acoustic and temporary measures, including alterations in rate (reduced or fluctuating language production rate, frequent pauses to find words), volume, phonological errors, and articulatory apraxia (Taler et al., 2008). In short, if speech impediments in people with AD differ qualitatively from the voice changes caused by normal aging or other conditions, such impairments may be isolated for early diagnosis of AD (Venneri et al., 2005).

The different mechanisms of action by which neuropathological features of AD arise may be the likely reason for the lack of effective treatment that could prevent the onset and progression of the disease. That's why we have to find biomarkers that reflect neurophysiological changes in pre-symptomatic stages, to find treatments that target these physiological mechanisms involved in disease.

5. DNA analysis

In humans, the most common form of genetic variation is the single base change in the sequence of a gene. This condition is known as single nucleotide polymorphism (SNP) (Cargill et al., 1999; Tindall et al., 2007; Schleinitz et al., 2011). One of these variations should affect at least 1% of the population to be considered a SNP. According to the International HapMap Consortium (catalog of common genetic variations that are present in the human species) (2003), SNPs occur with a frequency of 1% and can occur in either of the two copies of the genome. Although most of these SNPs have no effect on the genome, they can affect some function of genes/proteins and therefore lead to phenotypic differences between the carriers of different alleles. SNPs can be related with other diseases responsible for or associated with a higher risk for any health disorder, which can act as markers to locate the sequences responsible genes. For these reasons, SNPs are potentially valuable both to help biomedical research, and for the development of pharmaceutical products or medical diagnoses.

The etiology of many neurodegenerative diseases is not well defined, so many researchers advocate a multifactorial origin, a combination of genetic predisposition and environmental factors (Jenner et al., 1992). There is a wide range of described polymorphisms associated with PD (Ben-David and Tu, 2014), AD (Chouraki et al., 2014), and ET (García-Martín et al., 2009), but we will restrict ourselves to those who have been associated to changes in sensorimotor filtering.

Genetic mutations associated with deficits in sensorimotor gaiting in patients with schizophrenia and AD, among other pathologies has been reported (Zhang et al., 2011). Some of these mutations are related to the gene encoding neuregulin-1 (*NRG*-1) (Hong et al., 2007), dopamine receptors (Wong et al., 2000) and the gene encoding the catechol-O-methyltransferase (*COMT*), among others (Giakoumaki et al., 2008).

Neuregulin-1 is one of four neuregulin family proteins identified in vertebrates (Diez et al., 2014), which target the EGFR family (Epidermal growth factor receptor) receptors. It is essential for normal development of the nervous and cardiovascular system and is encoded by the *NRG-1* gene. Normofunctionality of this gene is one of the factors in schizophrenia diagnoses (Stefansson et al., 2002; Diez et al., 2014.). Studies by Hong et al., 2007 considered this polymorphism as an important marker in sensory processing.

Dopamine is a major central nervous system neurotransmitter, an endogenous catecholamine that influences various cell activities (Cavallotti et al., 2004). The dopaminergic system is involved in motor control, endocrine function, reward system and cognition (Tome et al., 2004). Dopamine receptors are widely expressed in neurons and in certain populations of non-neuronal cells (Tome et al., 2004). There are five subtypes of dopamine receptors, and among these, the Drd2 receptors (McGuire et al., 2011; Dai et al., 2014) and Drd3 (Lee et al., 2009; Kiyohara et al., 2011; Dai and al., 2014), are the most studied, because alterations in genes that encode them are associated with neurodegenerative diseases.

The *COMT* gene, located on chromosome 22, encodes catechol-O-methyltransferase (COMT) enzyme linked to dopamine degradation, particularly in the prefrontal cortex of mammals (Gennatas et al., 2012), and its activity is associated with levels of cognition, behavior (Swerdlow et al., 2013), and mechanisms of learning and memory (Gennatas et al., 2012).

Polymorphism rs4680 in which G is exchanged for A at position 158 of the coding region of the COMT polymorphism or Val158Met, causes an amino acid substitution in the protein, producing a decrease in the enzyme activity (Martínez et al., 2009), which can induce the formation of radicals that contribute to cytosolic neurodegeneration (Torkaman-Boutorabi et al., 2012). Also, it has been reported that this change in the enzyme causes an increase in the concentration of dopamine in the prefrontal cortex (Vallelunga et al., 2012), linking this with the decline in cognitive function in patients with MCI and AD (Harrison et al., 2008; Talledo et al., 2009; Quednow et al., 2009).

6. Startle Reflex

Sensorimotor filtering is also affected by anatomical and physiological changes observed in many neurodegenerative diseases. Studies that evidence the involvement of the sensorimotor system in the pathophysiology of certain movement disorders, this makes it essential to consider the potential contribution of changes in sensorimotor integration in motor execution (Abbruzzese and Berardelli, 2003). Disturbances in motor control can be measured through the analysis of muscle electromiographic activity (Valls-Sole and Valldeoriola, 2002). The acoustic startle reflex (ASR) is a rapid muscle contraction evoked by a sudden and intense acoustic stimulus. It is a simple test that serves as a tool for assessment of behavioral plasticity mechanisms of sensorimotor response (Koch, 1999). ASR reflects a protective

behavior against the injury and the magnitude of this reflex can be modulated by external and internal conditions (Walker and Davis, 1997). Prepulse inhibition (PPI) is the reduction of the magnitude of ASR when the startle-eliciting stimulus (pulse) is preceded 30-500 ms by a weak non-startling stimulus (prepulse) (Hoffman and Ison, 1980). PPI is an operational measure of sensorimotor gating and reflects a protector mechanism where the information carried by the prepulse is analyzed early avoiding the interference of other stimuli, which limits the ingress of trivial stimuli to cognitive centers or motor output pathways (Swerdlow et al., 2001). When the prepulse-to-pulse intervals are longer than 500 ms, the phenomenon is known as prepulse facilitation (PPF) (Graham and Murray, 1977), and reflects sensory enhancement and selective attention (Anthony and Graham, 1985).

(Igor Morski)

HYPOTHESIS AND OBJECTIVES

II. Hypothesis and Objectives

II.a. Hypothesis

The neurophysiologic tests (ASR, PPI and PPF) provide quantifiable data, are affordable and safe for patients without any adverse effects. These tests are commonly used for the evaluation of the alterations in brainstem circuits and their neurotransmitters. Based on the anatomical connections between structures belonging to sensory filtering circuit, substantia nigra (SN) and basal ganglia, we hypothesized that the dysfunction between these circuits affect the sensorimotor gating in neurodegenerative disease. Thus, it is possible to assess the alteration in this sensorimotor filtering using the ASR and its modulation through the PPI and PPF.

The use of potential biomarkers such as ASR and PPI, or polymorphisms associated could contribute to early detection and improved classification of diseases with similar clinical manifestations.

The modifications of the acoustic and perceptual parameters of voice and speech also could be used as a complement to assess initial symptoms in different neurodegenerative diseases.

Thus, this dissertation is motivated by the following research question: Could the neurophysiologic tests (ASR, PPI and PPF), single nucleotide polymorphism and speech analysis be used as diagnostic tools in neurodegenerative diseases?

II.b. Objectives

The main objective is to demonstrate the existence of an alteration of the ASR/PPI in the early stages of certain neurodegenerative diseases and validate this test as an auxiliary tool for early diagnosis.

1- Determine if the ASR/PPI is altered in patients with MCI, AD, PD and ET compared to a control group.

2- Determine the degree of cognitive impairment in patients with neurodegenerative diseases.

3- Analyze the changes of speech in PD patients with AD.

4- Determine the prevalence of different genetic polymorphisms in neurodegenerative diseases.

5- Establish correlations between markers studied.

MATERIALS AND METHODS

III. Materials and Methods

1. Subjects:

Patients diagnosed with Parkinson's Disease, Essential Tremor, Alzheimer's Disease, Mild Cognitive Impairment and healthy Controls.

1a. Parkinson's Disease patients

PD patients were clinically diagnosed according to the criteria of the Brain Bank of London (Hughes et al., 1992). They were outpatients from the Neurology Service of the University Hospital of Salamanca, who were seen by at least two senior neurologists experienced in movement disorders. PD patients include 52 patients (mean age, 68.44 ± 9.89), who were clinically evaluated using Unified Parkinson's Disease Rating Scale (Goetz et al., 2008) and Hoehn and Yahr scale (Hoehn and Yahr, 1967). The study was conducted at the time of their medication's minimal effect.

1b.Essential Tremor patients

ET patients were clinically diagnosed according to the criteria of the Consensus statement of the Movement Disorder Society on Tremor (Movement Disorders, Deuschl et al., 1998). ET patients include 34 patients (mean age, 63.46 ± 12.56), who were clinically evaluated using TETRAS. Patients included in this group showed a visible and persistent postural tremor in the upper extremities, for more than 5 years. The study was conducted at the time of their medication's minimal effect.

1c.Mild Cognitive Impairment patients

Thirty-three Patients diagnosed with MCI were part of the study (mean age 72,21± 8.39). Patients were diagnosed according to the criteria established by Petersen et al., 2001 and Grundman et al., 2004 and the Spanish Society of Neurology (GENCD-SEN, 2003).

1d.Alzheimer's Disease patients

Twenty-one patients with a mean age of 72.64 ± 7.51, diagnosed with AD according to the criteria developed by the NINCDS-ADRDA (McKhann et al., 1984, 2011) and the criteria for SEN took part in the study. Only patients in stage 1 with a Minimental State Examination (MMSE)

(Folstein et al., 1975) between 20 and 24 points and maintained their autonomy in activities of daily living. The control group was formed by 37 healthy controls, recruited from the general community in recreational centers for elderly people (mean age, 73.74 ± 13.84).

1e.Control subjects

Exclusion criteria included: neurological disorders other than the selected neurological disease, head injury, hearing disorders with significant reduction of the auditory threshold, diagnosis of psychiatric disorder other than depression, frequent use of illicit substances or alcohol consumption. All patients received written inform consent according to the ethical standards laid down in the 1964 Declaration of Helsinki before the test.

2. Cognitive assessment

We acquired cognitive assessment using the direct scores from the following subscales of **MMSE** (Folstein et al., 1975), verbal fluency **Isaac Test categories** (Isaacs and Akhtar, 1972), **Geriatric depression Scale (GDS)** (Yesavage et al., 1983) and **Daily living Activities** (Pfeffer, 1982). For the assessment of attention, working and spatial memory we used the Spanish version of the **Wechsler III scale**.

3. Voice Analysis

The speech task consisted of asking subjects to read on a screen (48-point font size and multiple lines to facilitate reading), the first paragraph of the novel “Don Quixote” by Miguel de Cervantes (405 syllables): “In a village of La Mancha, the name of which I have no desire to call to mind, there lived not long since one of those gentlemen that keep a lance in the lance-rack…” (English translation of Don Quixote by John Ormsby, 1885). This paragraph is well known by all Spanish speakers, like the famous passage "to be or not to be" of Shakespeare used in English equivalent tests. The recordings were made in a room quietly placing the microphone to 8 cm and an angle of 45 ° from the mouth to prevent aerodynamic noise. The recordings were obtained with a portable professional voice recorder (Sony PCM-M10), and the analyses were done according to the methodology described in previous studies (Meilán et al., 2014). Analysis focused on common acoustic measures of speech, including temporal aspects of the speech sample, pitch or fundamental frequency (F0), volume (intensity), and voice quality. To characterize the fluctuations in the amplitude of sound, we computed the intensity in dB of voice and unvoiced signals, and measured phonatory stability shimmer period perturbation stability (shot term, cycle to cycle,

perturbation in the amplitude of the voice): local shimmer (shimmer loc) and shimmer amplitude perturbation quotient 3 (shimmer apq3). Prosodic patterns were quantified by automatic prosodic transcription of a recording, using the algorithms implemented by Mertens (Mertens, 2004) on the Praat program (Boersma and Weenink, 2013). To characterize the temporal aspects of the speech sample, we computed the duration of the voice sample used (total duration of the paragraph from Don Quixote, the phonation time, and the reading and articulation speed), the interruption of sound (proportion and number of pauses of voice, percentage of the recording without voice, and number and percentage of voice breaks), and the periods of voice (number of pulses analyzed as voice, and mean number of periods of voice). To characterize the F0, we analyzed the mean F0, maximum and minimum values of F0, high and low global pitch and autocorrelation measures. Detection range of 65-650 Hz for F0, on windows of 0.005 s duration; for automatic segmentation threshold intensity was used in the styling of the algorithm that determines the presence of a vowel (Glissando = 0.32/T2 semitones / s., DG = 30, dmin = 0.05). While the standard psychoacoustic threshold for isolated voice is G = 0.16/T2, during natural speech voice flow is rarely linear, so that the value assigned is the better-modeled prosodic voice variables. Finally, we computed measures of the speaker's voice quality, and one spectral noise measure, the noise-to harmonics ratio (NHR).

4. DNA Analysis

Genomic DNA was extracted from saliva of patients and control subjects by Saliva Gene kit Intervect. Genotyping of the Neuroreguline-1, catechol-O methyltransferase and dopamine receptors D2 and D3 polymorphisms (Table 1), was performed using TaqMan 5'-exonuclease allelic discrimination assays (Applied Biosystems) that contain sequence-specific forward and reverse primers to amplify the polymorphic sequences and two probes labeled with VIC and FAM dyes to detect both alleles of each polymorphism (Schleinitz et al., 2011) (Figure 1). PCR reactions were carried out using TaqMan universal PCR Master Mix (Applied Biosystems) following manufacturer's instructions in a Step-One Plus Real-time PCR system (Applied Biosystems). To assess reproducibility, a randomly selected 5% of the samples were re-genotyped, and all of these genotypes matched with genotypes initially designated.

Gene	Abreviation	Polymorphism	Probe used	Brand
Catechol-O-methyltransferase	*COMT*	rs4680	C_25746809_50	Applied Biosystems
Dopamine D2 receptor	*Drd2*	rs6277	C_11339240_10	Applied Biosystems
Dopamine D2 receptor	*Drd2*	rs1800497	C_7486676_10	Applied Biosystems
Dopamine D3 receptor	*Drd3*	rs6280	C_949770_10	Applied Biosystems
Neuregulin-1	*NRG-1*	rs3924999	C_359159_10	Applied Biosystems
Neuregulin-1	*NRG-1*	rs6994992	C_22019_10	Applied Biosystems

Table 1. Summary of polymorphisms of the genes studied.

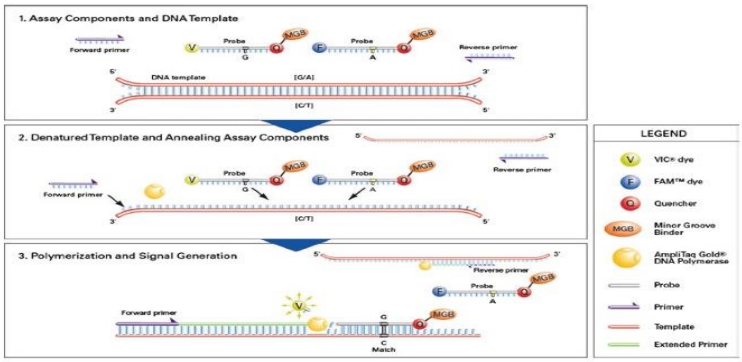


Figure 1. Principle of allelic discrimination using TaqMan ® SNP Genotyping Assays. (1) Test components and template DNA: forward and reverse primers to amplify the polymorphic sequence of interest. (2) Step of denatured and the assay components. (3) Generation of polymerization and signal: Taq polymerase begins to synthesize a new chain and meets the probe annealing. 5 '- fluorescent dye attached to the appropriate probe is separated (5' → 3 'exonuclease activity of Taq polymerase). No fluorescence signal is turned off and now can be detected by laser excitation.

5. Startle reflex and Prepulse Inhibition Measurement

Subjects remained comfortably seated in a chair with armrests, during the 15 minutes that lasted the test. The binaural auditory stimulation is provided through headphones (Sony MDR-V6) connected to the reflection measurement device (SRH-LAB trending system), and electromyographic registration of the right orbicularis oculi (Figure 2) was done using two small

silver electrodes filled with conductive paste placed beneath the right eye and the ground electrode placed in the right mastoid. The test starts after acclimation period of 4 minutes with background noise (70 dB white noise), which is maintained during the entire test. ASR trigger pulses are bursts of 40 ms of white noise with an intensity of 115 dB. The prepulse, a non-startling stimulus, have duration of 20 ms and intensity of 85 dB white noise. Intertrial intervals (ITIs) were assigned with durations of 9 to 23 ms to avoid habituation. The session had four blocks of pulse and prepulse with interstimulus intervals (ISIs) of 60, 120, and 1000 ms. The initial and final blocks were composed of single pulses (5 in each block). The second and third block, each one contained 6 pulses alone and 9 prepulse-pulse with ISI of 60 ms, 9 prepulse-pulse with ISI of 120 ms and 9 prepulse-pulse with ISI of 1000 ms.

Figure 2. ASR measurement equipment SR-HLAB.

The onset measurement was in ms, using the beginning of the maximum amplitude response (in microvolts) that occurs within 18-120 ms after acoustic stimulus (Figure 3). PPI is calculated using the mathematic formula PPI= (amplitude ASR without prepulse- Amplitude ASR after prepulse/amplitude ASR without prepulse x 100).

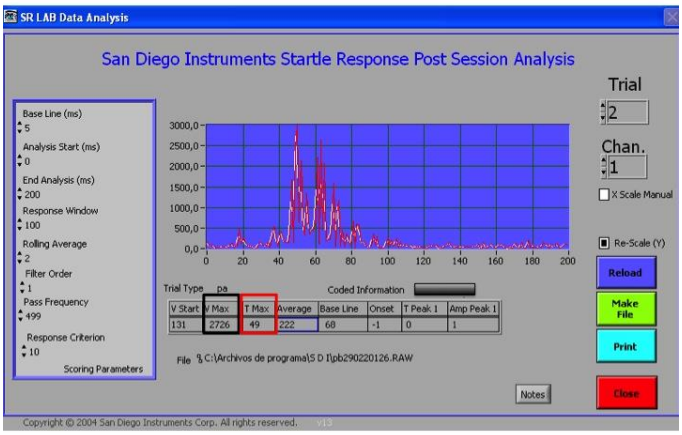


Figure 3. Electromyographic recording of muscle response obtained with the SR-HLab Program

6. Statistical Analysis

All statistical analyses were performed using SPSS (version 20.0 for Windows; SPSS Inc., Chicago, Illinois, USA). The description of the data was performed using the mean ± standard error of the mean (SEM) for both continuous variables as absolute and relative frequencies for categorical variables.

The results were compared between groups using ANOVA and MANOVAs module (contrasts between and within subjects) and in appropriate cases, post-hoc multiple comparisons (Pearson correlations and corresponding regression model and Spearman) were performed and Student t-test or Mann Whitney test to compare differences in quantitative and qualitative variables between groups.

In longitudinal studies, the results were compared between groups using ANOVA test mixed split-plot, with Shceffe comparisons (inter-subject analysis) and Bonferroni (intra-subject analysis) pairs. The level of statistical significance was $p < 0.05$ accepted.

The voice analysis were performed initially, compliance with the assumption of normality of the distribution of scores on the variable speed of elocution was evaluated; the results show that this variable is normally distributed through the goodness of fit test Kolmogorov-Smirnov ($z = .046$; $p = .20$). Test for comparison of means of independent samples Student t-test was used to compare both groups, and thereby define the differential profile in the prosodic aspects between the two experimental groups.

The analyses of polymorphisms were performed using the chi-square or Fisher's exact when appropriate. Multiple comparison tests were performed using logistic regression tests and Bonferroni test. To determine whether the presence of a variable is a risk factor for a particular, the percentage of appearance was calculated using odds ratios (OR) of each genotype. To estimate the accuracy of the OR a 95% confidence interval (IC) is used.

(Igor Morski)

RESULTS

V. Results:

1. Cognitive Assessment

We conducted a cognitive evaluation of patients using various test measuring patient independence (*Functional Scale Pfeffer*), cognitive impairment (*MMSE*), depression (*GDS*), semantic verbal fluency (*tests of Isaac*), and assessment of attention and auditory and spatial memory (*Wechsler test III*) work.

1.a. Functional Assessment

In *Pfeffer functional scale*, measuring the functional activities of the subjects in reverse, with 0 being autonomous and 33 the most dependent people we found that there was a significant difference between controls and AD patients (p=0.001). We also found significant differences between AD patients and MCI patients (p=0.006) among patients with AD and PD (p=0.00) and between patients ET and AD patients (p=0.000). Table 2 shows the scores obtained by patients in detail.

1.b. Cognitive Impairment

As shown in Table 2, AD patients showed a further deterioration in cognitive function, obtaining a low score on the *MMSE*, with a significant difference from controls (p=0.000). In the between-subjects analysis, patients with AD were significantly different from other groups (p=0.015), patients with MCI (p=0.000) PD (p=0.000) and the ET.

Also, patients with MCI had higher significantly cognitive decline than patients with ET (p=0.019).

1.c. Geriatric Depression Scale

We analyzed the degree of depression in patients using the *GDS*. A score of 0 to 10 should be considered as normal; 11 or more, as a possible indicator of depression; 11-14 points (moderately depressed) and 15-39 points (severely depressed). The results do not indicate differences between the groups (Table 2).

1.d. Verbal Fluency

Verbal fluency, as measured by the Test of Isaac, only displayed significant alterations in AD patients compared to controls (p=0.000), and with other groups of patients with

neurodegenerative diseases (p=0,00) (Table 2). Also, patients with MCI showed significant differences with patients with ET (p=0.013) in this parameter.

In the case of the *Letter P item*, MCI patients differed from controls (p=0.010). Upon intra-group analysis, we found that AD patients have a lower number of responses compared to patients with ET, with a significant difference (p=0.031). MCI patients also differed significantly compared to patients with ET (p=0.00) and patients with PD (p=0.005).

1.e. Wechsler test

This test is a general tool for assessing memory in different coding processes, retention and retrieval. We found in the analysis of variance, logically, AD patients showed lower scores on all tests used to respective controls and other patients (Table 2). This occurred both in measurements of memory as *text recognition I and II* (p = 0.01), *Numbers and Letters* (p=0.001); *Verbal pairs* (p=0.05) and *recognition of Verbal pairs* (p=0.001). The analysis of pairwise comparisons showed a significant worse performance in the subscale texts I recall, in AD patients compared to patients with PD and ET (p=0.01 respectively). Also, we found significant differences between the group of patients with MCI compared to patients with ET (p=0.001) and between the ET and PD group (p=0.01). Finally, the *slope of auditory learning* variable, the difference between recovery in the first and second time that the story is heard, we found differences between the AD group versus PD group (p=0.05) and ET (p=0.001). MCI patients also differed in this variable from the AD patients (p=0.05). In memory of *texts II*, ET patients scored higher than patients with AD (p=0.001), MCI (p=0.001) and PD (p=0.001). AD patients had significantly lower than patients with PD (p=0.01) scores. A similar result was obtained with the test for recognition of ideas in the texts. ET patients scored significantly higher than AD patients (p=0.001) MCI (p=0.001) and PD (p=0.05). The score for AD patients was significantly lower than that obtained by PD patients (p=0.05). We conclude that the encoding and retention of auditory information is severely impaired in AD patients compared to patients with PD and ET, and that this process also shows a severe impairment in PD patients versus patients with ET.

As for the subscale *Letters and Numbers*, AD patients obtained lower scores than patients with PD (p=0.01) and patients with ET (p=0.01). MCI patients also had lower scores than patients with ET (p=0.05). Thus, we can conclude that patients with AD and MCI showed significant deficits in executive processes of short-term memory compared to PD patients and ET.

As for *Verbal pairs* subscale, patients with ET obtained the highest score, significant differences compared to AD patients (p=0.01), with MCI (p=0.01) and patients with PD (p=0.05). MCI patients had lower scores than patients with PD (p=0.006). As for the *learning slope* difference between the first and the fourth test, patients with ET scored higher compared to patients with AD (p=0.001), MCI (p=0.01), and PD (p=0.05). The *learning slope* results appear to show clear deterioration in patients with AD, MCI and PD regarding the ability to encode and retrieve associations, in addition to retaining through repetition. In the recognition test of Verbal pairs, again ET patients scored higher compared to patients with AD (p=0.001), MCI (p=0.001) and PD (p=0.05). Therefore, it is not so much a problem of recovery, but deterioration in encoding processes of new associations. The same results were obtained in the analysis of the percentage of retention of *Verbal pairs*. ET patients scored significantly higher compared to patients with AD (p=0.001), MCI (p=0.001) and PD (p=0.05). AD patients were lower than the other groups (p=0.001) scores. Thus, the deficit is confirmed in the processes of encoding and retention of patients with AD, MCI and PD versus patients with ET.

		Control (n=35)	AD (n=22)	MCI (N=34)	PD (N=52)	ET (N=34)	[a] F	[b] p
Daily Living Activities		1,4±1,4	7,92±0,8	9,3±1,2	10,7±1,0*	7,5±0,9	7,4	0,000**
Geriatric Depression Scale		6,6±1,5	3,5±0,7*	9,0±1,9*	3,1± 0,9	0,9± 0,4	2,4	0,05*
MMSE		27,2±0,9	24,5±0,9	20,7±1,0*	26,5± 0,4	27,6±0,5	14,9	0,00**
Isaac Test		36,7±1,2	34,8± 0,9	27,3±1,7*	37,1± 0,5	38,9±0,4*	18,6	0,00**
Isaac Test (P)		8,5±0,6	6,6± 0,5*	7,1± 0,6	8,1±0,3	9,1±0,3	4,4	0,002**
Wechsler	Text	22,0±2,8	13,6±2,2*	8,3± 2,1*	20,1±1,7	29,0±2,1	12,7	0,00**
	Learning Slope	3,1±0,6	2,1± 0,5	0,8± 0,5*	2,9±0,4	4,2±0,5	5,7	0,00**
	Text recall	12,2±2,2	5,9± 1,6*	1,6± 1,5*	10,5±1,4	17,8±1,8	12,1	0,00**
	Text Recognition	19,4±1,0	18,1± 0,8	16,1± 0,8*	19,8± 0,7	22,6±0,7*	8,8	0,00**
	Text Recognition Percentage	65,7±8,2	36,2±7,6*	10,7±7,5*	60,6±5,5	73,4±5,5	14,7	0,00**
	Letters Number sequencing	4,6± 0,7	3,0± 0,5	1,57±0,6*	4,1±0,4	5,1± 0,5	6,8	0,00**
	Verbal Pairs	9,2±1,5	3,1±1,2*	4,0±1,3*	8,3±1,0	12,5±1,2	12,0	0,00**
	Learning Slope	1,2±0,4	1,0±0,3	1,5±0,4	2,2±0,3	3,5±0,3*	9,2	0,00**
	Verbal Pairs Recall	2,5±0,5	1,1± 0,4*	1,1±0,5	2,6±0,3	4,1±0,4*	9,2	0,00**
	Verbal Pairs Retention Percentage	66,3±10,9	31,2±8,6*	33,9±11,5	55,2±6,7	84,6±6,3	7,7	0,00**
	Verbal Pairs Recognition	22,9±0,7	21,5±0,7	17,8± 0,9*	22,8 ±0,4	23,8±0,2*	15,4	0,00**

Table 2. Average scores on cognitive tests (± SEM) in patients and controls (n = number of subjects). a value of F lower limit to the level of significance. b p value of statistical significance, significant differences between groups (*) for p <0.05 was observed and (**) p <0.001).

2. Voice Analysis

2.a. Parkinson's Disease

For the analysis of the different variables of the parameters of the voice in PD patients and controls, different statistical tests were performed to verify that there were no significant differences between groups in clinical and sociodemographic variables. No differences between the two groups in the variable "age" were observed (t 82 = 1.07), nor in the distribution of gender group ($\chi 2=3.11$). We also found no difference in cognitive abilities measured by the MMSE (t 82= 1.33). Finally, we found there were no difference in verbal fluency in both groups, measured both by a semantic verbal fluency task (test Isaacs, t 82= 0.71) and phonological ("p", t82 = 0.47).

The existence of differences between groups in four variables related to verbal fluency was found. The results show that the PD group, shows lower rate of utterance (t 82 = 2.41; p = 0.05) and articulation (t 82= 2.68; p=0.01) than the control group; similarly, the average duration of the syllable is higher in the group with PD (t82 = -2.81; p = 0.01). In relation to the number of breaks, group patients with PD performed breaks under 100 ms, with a significantly higher average duration (t 82 = -2.05; p = 0.05) than the control group, but no differences were found in those pauses longer than 300 ms duration. These results define a profile of PD patient, with a language slowed in the syllabic and prosodic articulation. By contrast, no differences were found in other variables such as the number of pauses (t 82 = -0.08), the phonation time (t 82 = 1.08), or the percentage of phonation (t 82 = -0, 85). In Table 3, we can observe the conversion results in scores for each of the prosodic variables used. Thus, the prosodic profile is highlighted in both experimental groups.

	Control (SD)	PD (SD)	t	p
Task Duration	46,05(22,94)	43,77(15,96)	0,52	0,604
Number of Breaks ≥300ms	16,91(13,42)	17,13(13,93)	-0,08	0,936
Average Length of the Pause (≥300ms)	0,61(0,18)	0,66(0,16)	-1,28	0,201
Number of Micropauses ≥10ms	26,87(17,90)	23,82(13,36)	0,87	0,386
Average Length of Micro pause (≥10ms)	0,46(0,16)	0,53(0,15)	-2,15	0,033
Phonation Time	34,02(12,40)	31,38(7,29)	1,19	0,237
Proportion of Phonation	78,70(12,63)	75,22(10,81)	1,34	0,181
Speed of Elocution	3,49(0,80)	3,10(0,68)	2,41	0,017
Speed of Articulation	4,40(0,47)	4,09(0,53)	2,80	0,009
Average Duration of the syllable	0,22(0,02)	0,24(0,03)	-2,91	0,005

Table 3. Descriptive statistics and mean of the variables studied in patients with PD and Control. SD = standard deviation.

The results of this study were published in the journal of Neurology.

Martínez-Sánchez F, Meilán JJ, Carro J, Gómez Íñiguez C, ***Millian-Morell L***, Pujante Valverde IM, López-Alburquerque T, López DE. (2015) Speech rate in Parkinson's disease: A controlled study.*Neurología*. pii: S0213-4853(14)00264-3. doi: 10.1016/j.nrl.2014.12.002.

2b.Alzheimer's Disease Speech analysis

Group	Parameters	Control Mean (SD)	AD Mean (SD)
Temporal aspects of the speech sample	Total duration (seconds) Phonation Time (seconds) Reading Speed Articulation Speed	44.39 (18.32) 34.50 (13.16) 3.66 (.71) 4.39 (.45)	82.19 (53.05) 46.72 (19.86) 2.55 (.63) 4.05 (.43)
Analysis of fundamental frequency (F0)	Mean F0 (Hz) Minimum value F0 (Hz) Maximum value F0 (Hz) Autocorrelation High Pitch Global Low Pitch Global	179.08 (29.06) 68.16 (3.98) 539.80 (127.75) .89 (.04) 478.12 76.82	159.42 (25.12) 67.66 (3.78) 596.63 (68.76) .90 (.05) 571.25 82.50
Space full of sound	N Pulses N Periods N Mean Periods	4207 (1835) 4113 (1805) 6.47 (1.10)	6520 (2872) 6379 (2830) 5.76 (.88)
Interruption of sound	Percentage without voice N voice breaks Percentage of voice breaks Proportion of pauses of voice N pauses of voice	35.40 87.46) 84.08 (30.15) 39.29 (6.87) 28.67 (12.887) 14.33 (9.3)	47.83 (12.28) 118.93 (68.27) 51.27 (12.03) 51.76 (13.55) 34.5 (27.04)
Parameters of the fluctuation in frequency	Jitter (loc) (Hz) Jitter (loc, abs) (ms.) Jitter (rap) (Hz) Jitter (ppq5) (Hz)	2.83 (.65) 184.92 (57.24) 1.2 (.41) 1.51 (.48)	2.80 (.95) 165.22 (75.41) 1.34 (.54) 1.54 (.61)
Fluctuations in the amplitude of sound (Intensity of Sound)	Shimmer (loc) Shimmer (loc, db) Shimmer (apq3) Shimmer (apq5) Shimmer (apq11) Intensity of unvoiced dB Intensity of voiced dB	10.40 (1.84) 1.59 (.44) 5.35 (2.17) 7.84 (3.04) 14.16 (4.52) 56.78 (4.42) 71.16 (4.10)	11.67 (2.95) 1.60 (.51) 6.01 (2.74) 7.92 (3.43) 12.66 (4.58) 53.27 (5.15) 70.35 (2.74)
Harmonic/ Noise Ratio	Noise to Harmonic Ratio Harmonic to Noise ratio	.17 (.65) 12.38 (2.85)	.15 (.07) 12.72 (2.64)

Table 4. The Wilks' lambda and Fisher coefficients structure matrix for each introduced variable.

Linear discriminant analysis results made it possible to obtain a highly significant discriminant function (percentage of variance explained = 100%; eigenvalue = 1.095, canonical correlation = 0.723; Wilks' lambda = 0.477, Chi-squared = 45.488, df = 5, p<0.001), containing five factors (see table 5). The discriminant function, in standardized coefficients, selected the following measures: Analysis of space full of sound as periods numbers of voice (SC= 0.874); analysis of breaks of

voice as percentage of voice breaks (SC=0.787) and voice breaks numbers (SC= -0.683); parameters of the fluctuation of amplitude of sound as Shimmer apq3 (SC= 1.381); and finally, noise to harmonic ratio (SC = -1.127). Table 2 shows the Wilks' lambda and Fisher coefficients of structure matrix for each variable. The non-standardized centroids are to Alzheimer's disease =1.129 and to Control group = -.941.

Parameters	Wilks' lambda	Structure Matrix: Function
Percentage of voice breaks	25.717**	.654
N Periods	17.940**	.471
N voice breaks	14.886**	.330
Shimmer (apq3)	13.162**	.131
Noise to Harmonic Ratio	13.142**	-.138

Table 5. Speech Parameters were measured and descriptive data.

Once the discriminant function was obtained, the participants in the sample were classified according to the scores obtained by each of them in the function. The results, show how the cases correctly classified by this procedure, total 56 of the 66 participants thus are providing 84.8% sensitivity in correct classification. Only 6 participants in AD group and four in control group were classified incorrectly. In the cross validation, the cases correctly classified by this procedure total 55 of the 66 participants, thus are providing 83.3% sensitivity in correct classification. Seven participants in AD group and four in control group were classified incorrectly. The results speak for themselves regarding the accuracy in the diagnosis of AD with the measures used.

Table 4 shows the speech parameters that were measured and descriptive data. The results of this study are published in the journal Dementia and Geriatric Cognitive Disorders

Meilán JJ, Martínez-Sánchez F, Carro J, López DE, ***Millian-Morell L***, Arana JM. Speech in Alzheimer's disease: can temporal and acoustic parameters discriminate dementia? Dement Geriatr Cogn Disord. 2014;37(5-6):327-34. doi: 10.1159/000356726. Epub 2014 Jan 30.

3. DNA Analysis

We analyzed SNPs in different genes, from DNA extracted from saliva samples from study subjects. An analysis of the frequency distribution, in which the different alleles of each gene polymorphisms studied, using the chi-square test to analyze their frequency of distribution in the (Table 6).

	NRG-1 rs3924999	NRG-1 rs6994992	COMT rs4680	Drd2 rs6277	Drd3 rs6280	Drd2 rs1800497
N	112	132	113	124	132	122
X^2, gl 8	4,176,62	4,74	13,30	5,72	11,63	3,17
p	P=0,847578	p=0,784	p=0,043*	p=0,706	p=0,168	p=0,923

Table 6. Values of chi-square analysis on the distribution of the different allelic genotypes in the study groups. N- Number of cases studied. The differences are significant at p <0.05.

3.a. Polymorphism rs3924999

The genotype frequencies for rs3924999 polymorphism of *NRG-1* are reflected in Figure 4. The three genotypes did not differ in relation to the groups studied. This shows that the most frequent genotype is homozygous G/G, normal genotype in this gene. The homozygous A/A, which would be having more partnerships with different pathologies, has a lower frequency in all study groups.

3.b. Polymorphism rs6994992

The polymorphism rs6994992 of the *NRG-1* gene, the genotype distribution analyzed shows no difference between the different study groups.

3.c. *COMT gene polymorphisms*

In analyzing the distribution of the *COMT* gene polymorphism, we found that there are significant differences (p=0.043) in the frequency of the different genotypes in the study groups. The genotype A/A, which is associated with risk for different pathologies, has lower frequency of occurrence (Figure 4). The peer distribution analysis indicated that there are significant differences between patients with MCI and controls (p = 0.017), with an OR: 1.26.

3.e. *Dopamine receptor polymorphisms*

3.e.1a.Polymorphisms Drd2 rs6277

Distribution analysis of the polymorphism of the gene encoding the dopamine Drd2 rs6277, homozygous T/T (normal) had a higher genotype distribution in the study groups representing the erroneous coding (C/C). No differences between the frequencies of the different allelic forms between the study groups were found.

3.e.1b.*Drd2 polymorphisms rs1800497*

The distribution of the different genotypes Drd2 polymorphism, rs1800497, to appreciate that the T/T genotype, which represent the coding error appears in very low percentages or zero (control group and ET patients) in some cases in our group of subjects. The genotype C/C is the most frequent, so we did not obtain significant differences in the frequency distribution analysis.

3.e.2. Drd3 polymorphism rs6280

Analysis rs6280 polymorphism distribution (Figure 4) of the *Drd3* gene, showed that the presence of the altered genotype T/T, appears in a greater percentage with PD in our group of subjects, with a statistically significant frequency in relation to controls (p=0.010), with the risk factor in this disease OR: 11.08 with 95% CI 1.96 to 62.5. No significant differences were found between the frequencies of the different allelic forms among the other study groups.

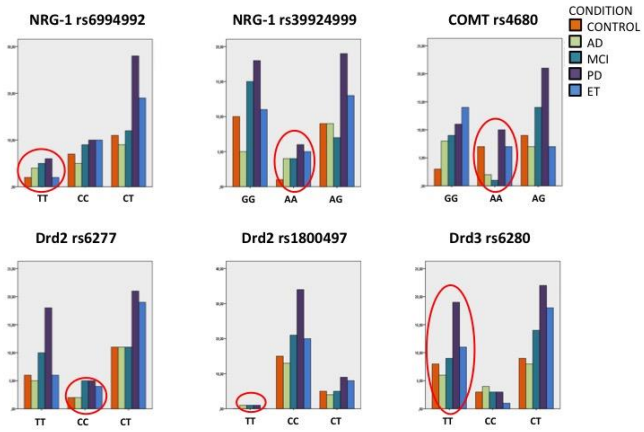


Figure 4. Distribution of genotype frequencies of the polymorphism *NRG*-1 gene, *COMT*, *Drd2*, and *Drd3* in neurodegenerative diseases.

4. Startle reflex and Prepulse Inhibition

We studied the auditory startle reflex in controls (35), patients with AD (21), patients with MCI (33), patients with PD (52) and patients with TE (34).

The amplitude of the ASR was greater in patients with MCI than in all other neurodegenerative diseases studied, although the difference is not statistically significant (Figure 5). Regarding ASR latency, no significant differences between groups were found. In the analyses by group and gender, we found significant differences in the latency of the ASR in AD patients (p=0.04). Also,

in this condition, the differences were greater in men than in women, but the differences did not reach statistical significance.

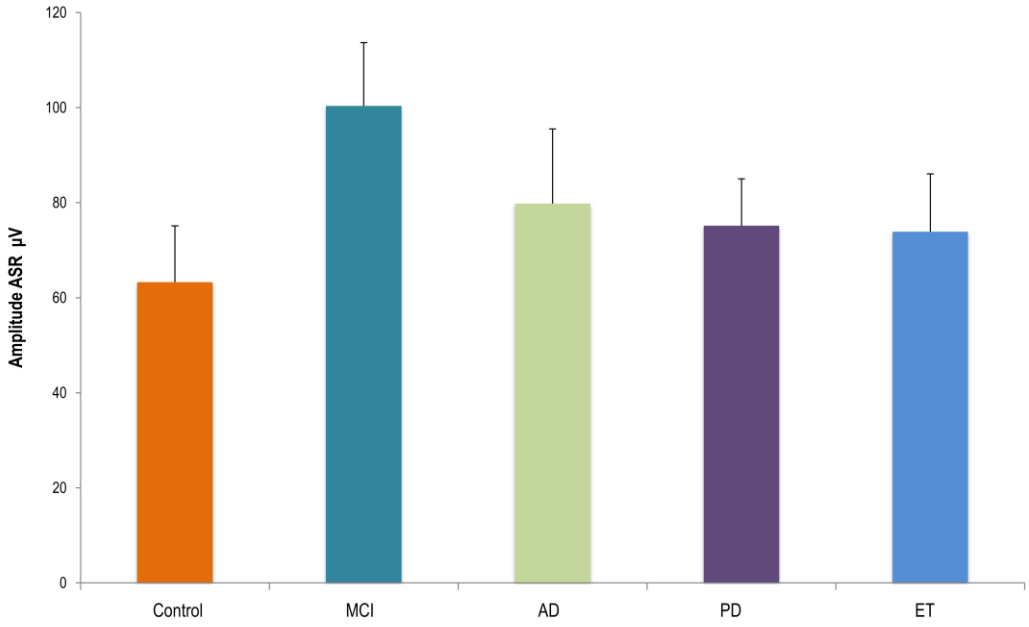


Figure 5. Startle reflex amplitude in neurodegenerative diseases. Significant differences (*) between groups ($p < 0.05$) were observed. Error bars show ± SEM.

4.1. Prepulse Inhibition

We examined the PPI (within-subjects factors) using ANOVA with repeated measures. A significant main effect on PPI at 60 ms was found between MCI and PD patients ($p = 0.017$). There was a significant effect on the PPI in PD group relative to controls ($p=0.05$ F 1, 82 = 8.52) (Figure 5), when the prepulse-to-pulse was 120ms. Also, a significant effect was found at the interval of 120 ms between PD patients ($p= 0.000$), EA ($p = 0.000$) and ET ($p = 0.000$), and MCI. (Figure 5).

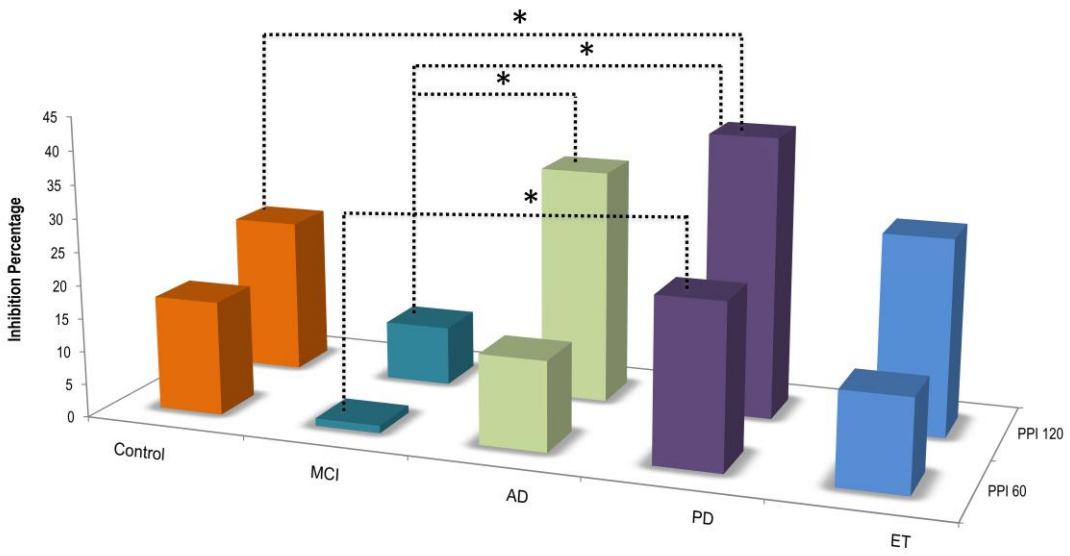


Figure 6. Startle reflex modifications in neurodegenerative diseases prepulses before the trigger pulse reflection. Abbreviations: PPI 60, percent inhibition of ASR when the interval between prepulse-pulse is 60 ms 120 PPI, percent inhibition of ASR when the interval between prepulse-pulse is 120 ms. (*) Significant differences were observed between groups ($p < 0.05$).

4.2 Prepulse Facilitation

Analyzing the facilitation of the auditory startle reflex (PPF), (Figure 7), we found differences between controls and PD patients ($p=0.045$) and between patients with PD and MCI ($p=0.001$).

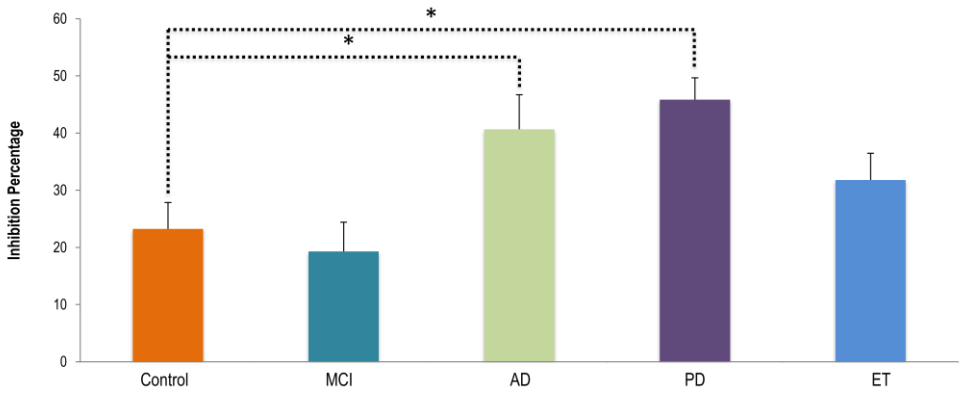


Figure 7. Startle reflex modifications in neurodegenerative diseases prepulses before the trigger pulse reflection. Abbreviations: PPF percent facilitation of ASR when the interval between prepulse-pulse is 1000 ms. (*) Significant differences was observed between groups ($p < 0.05$).

5. Correlations between PPI / PPF and DNA polymorphisms

We analyzed the different parameters of the PPI depending on the SNPs genotype of patients and controls. The different genotypes of polymorphisms of *NRG-1* gene had different values in different parameters of the PPI. In Figure 8, it can be seen significant differences between PD patients, between the distribution of carriers and non-carriers of the mutated allele, and different intervals of PPI and PPF in the rs6994992 polymorphism.

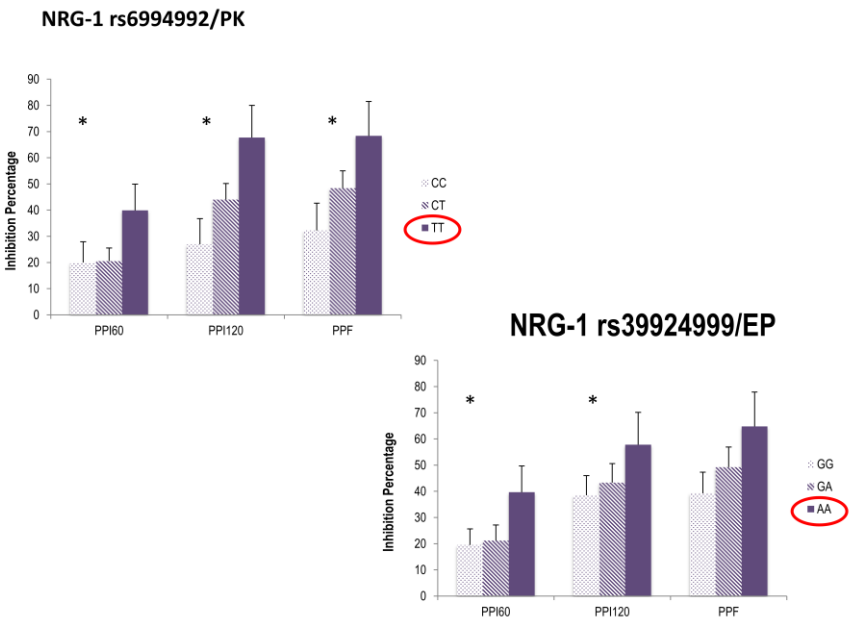


Figure 8. Values of the PPI and PPF grouped by genotype of rs6994992 and rs39924999 polymorphism of *NRG-1* gene. Error bars represent mean values ± SEM.

In the polymorphism rs39924999 significant differences between genotypic distribution and PPI_{120ms} and PPF were among PD patients (Figure 8). In cluster analysis differences were found among carriers of the mutant allele and the PPI when we analyzed the *NRG-1* rs6994992 polymorphism in patients with MCI and AD, finding a higher percentage of inhibition in AD patients carrying the mutant allele (Figure 9).

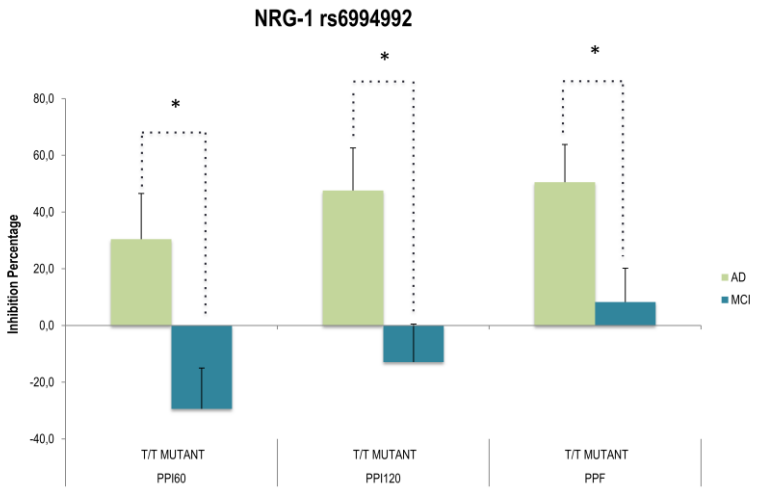


Figure 9. Values of the PPI and PPF grouped by genotype of rs6994992 polymorphism of *NRG*-1 gene. Error bars represent mean values ± SEM.

Regarding the Drd3 receptor polymorphism and different parameters of PPI, we found significant differences between patients with PD who possess the allele carrier and controls when we analyzed the PPI_{120} and PPF (Figure 10).

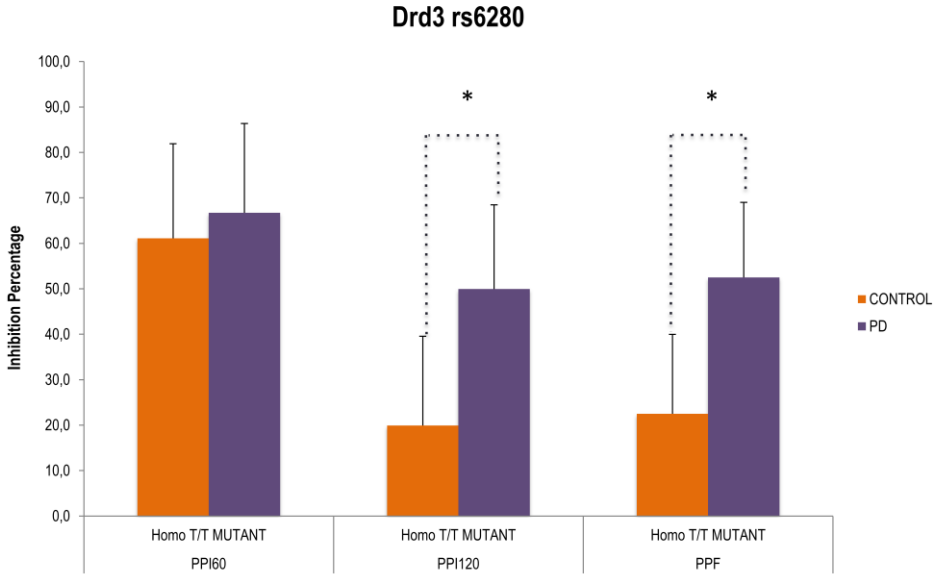


Figure 10. Values of the PPI and PPF grouped by genotype of rs6280 polymorphism of Drd3. Error bars represent mean values ± SEM.

(Igor Morski)

DISCUSSION

VI. Discussion

1. Acoustic startle reflex and its modulations

We analyzed the variations in the ASR, PPI and PPF behavioural paradigms in patients with neurodegenerative diseases. Furthermore, we studied the differences between groups with different neurodegenerative diseases and compared with a control group and each other. Our results show no significant differences in the amplitude of ASR between patients and controls. Latency of the ASR appears to increase with age, showing this trend in all groups analyzed. We have not found differences between controls and patients in latency; we only found differences in latencies between AD patients between men and women.

In the present study, it was found that patients with neurodegenerative diseases and control subjects had similar amplitudes in the ASR. PD patients show longer latencies in the ASR in comparison with other subjects. Our results are consistent with the literature (Kumari et al., 2004; Ellwanger et al., 2003; Ludewig et al., 2003; Kumari et al., 2008), which suggests that with age there is less startle reaction, a phenomenon affecting healthy subjects. The differences observed in the startle response between these diseases can be interpreted in the context of the degenerative neuronal loss, including structural changes that occur at different levels within the nervous system.

In the present study, we found that PD patients and control subjects had ASR with similar amplitudes and longer onset latencies. Several authors (Vidailhet et al.,1992; Kofler et al., 2001; Bowers et al., 2006) described longer latencies in *orbicularis* oculi muscle in PD, which was prolonged in ON states and in sitting positions. Our results are consistent with the literature (Kumari et al., 2004; Ellwanger et al., 2003; Ludewig et al., 2003; Kumari et al., 2008) that suggests that, with age there is less startle reaction and PPI, which would affect healthy subjects. The PPI demonstrates the control that the basal ganglia exert over the brainstem reflexes. In PD, there is an enhancement of the blink reflex, indicating an increased filtering of sensory information. PPI circuits are controlled through the pedunculo-pontine tegmental nucleus, which regulates the excitability of the startle related structures of the reticular formation (Kumari et al., 2008). When compared to controls, PD patients showed higher prepulse inhibition at 60 ms and 120 ms, confirming our hypothesis that PD have disturbances in sensorimotor gating. Periol and coworkers (Perriol et al., 2005) reported a significant difference in PPI_{120ms} when compared to

healthy controls. In their study, they also compare patients with AD and Dementia with Lewys bodies, and their results suggest an involvement of the dopaminergic subcortico-thalamo-cortical networks in PPI regulation, and more severe disruption of these networks in Dementia with Lewys bodies than in PD (Perriol et al., 2005). Also, Valls-Solé and coworkers (Valls-Solé et al., 2004) indicated an increased prepulse inhibition in PD patients. Excessive sensory gating in PD may reflect impaired integration of sensory inputs, resulting in impaired goal-related movements (Bischoff-Grethe et al., 2003). Bradykinesia in PD affects the ability to initiate and coordinate a series of motor tasks (Schindler and Kelly, 2002). This impaired ability to inhibit motor movements justified both slowing the onset of motor responses, as well as disruption of movements once they have been initiated (Obeso et al., 2011). The impairment of the sensorimotor regions (basal ganglia, cerebral cortex, and other associated regions) to receive accurate afferents input may account for errors in the initiation, timing and range of movement (Bischoff-Grethe et al., 2003). The fact that individuals with PD often show an abnormal increase in muscle tone and rigidity can reflect a tendency of the central nervous system to remain in a state of alert and movement preparation, while awaiting the arrival of the afferent signal, resulting in a delayed or aberrant transition to movement execution (Bischoff-Grethe et al., 2003; Farrell et al., 2005).

We also found that PD patients showed less PPF than the control group. PPF is a less understood process associated with a later stage of generalized alerting or orienting (Dawson et al., 1982) as well as specific selective attention process (Graham, 1975). According to Graham (1975), the PPF reflects a generalized orienting or attention automatically elicited where there is no selective attention to specific stimuli. The study published by Wynn et al., 2004 about PPF in schizophrenic patients showed that these patients and their siblings had lower PPF than controls, imputing this finding to an orienting deficit. Bowen and Ison (2006), reported that PPF has a more peripheral motor consequence of a non-specific alerting reaction, suggesting that the pathways of the PPF may not depend on the sensory-motor processing. In order to explain our findings of less PPF in PD group, a cognitive evaluation of patients and their corresponding control subjects should be necessary to evaluate any type of cognitive impairment or attention disorders.

In patients with ET, we found they had larger amplitudes and less prolonged latencies in the ASR when compared to the control group. These findings could be due to a loss of the early LC compensatory mechanism over the SN, which in turn might cause dysfunction of NPP-NCPR circuit with subsequent manifestations in the ASR (Lavoie and Parent, 1994). In the current

literature, we did not find studies of the ASR and PPI in patients with ET, so the results are not compared in this dissertation. Since PD and ET share a common pathological substrate and that we found different results, we hypothesized that there is a difference in the subcortical structures and reflex neuronal pathways that are affected in each of the pathologies.

The results obtained in the case of MCI and AD levels reflect a dysfunction circuit structures that are involved in attention processes. MCI patients had greater amplitude of the ASR that controls subjects and the other patient groups. We atribute this difference to the neuronal loss in the hippocampal formation, that occurs in these patients because as the amygdala, hippocampus and anterior cingulate cortex play an important role in the modulation of reflex (Lee and Davis, 1997; Medford and Critchley, 2010).

The results of our study are consistent with a previously published study by Hejl and colaborators (2004), as there are no differences in PPI at different ISIs among MCI patients and AD patients when compared to control subjects. As the study published by Hejl et al., (2004), our results contradict the study published by Ueki et al. (2006), in which they found PPI facilitation in patients with AD. This rather contradictory results can be explained by differences in the methodology applied. Thus, an accelerometer was used to measure muscle contraction in the study of Ueki et al., 2006, whereas our study and Hejl et al. (2005) used an electromyographic recording. We hypothesized that these results might be due to the anticholinergic drugs that are use to treat these patients. Unpublished studies of our research group using experimental animals models, indicate that a modification of the PPI exists after treatment with anticholinergic. This effect found in experimental animals might be extrapolated to our patients.

2. Speech analysis

2.1 Parkinson's disease

Control of specific frequencies is learned in childhood and requires coordination of the vocal structures adjusting acoustic resonances of the vocal tract (Saxena et al., 2014; Bunton, 2008; Wolfe et al., 2009), which is a process of motor coordination affected in PD.

An estimated 70%–90% of patients with PD also develop speech or voice disorders (Darley et al., 1968) specifically hypokinetic dysarthria, characterized by monopitch, monoloudness, under articulation, and hoarseness in voice (Forrest et al., 1989). This is consistent with our results as we have observed significant differences between controls and patients with Parkinson's. As

reflected in the usual language studies in PD (Canter, 1963; Griffiths and Bough, *1989;* Cannizzaro et al., 2004), we found that there were disruptions in temporal aspects of the speech sample like a breakage in the prosody, poorly controlled phonation time and fewer pauses and voice breaks. Our results reflected a sharper voice, less control of low frequencies, which prevents on the other hand the noises characteristic of elderly people in the voice. Finally, the lack of control increases the intensity of sound, with a substantial increase in decibels, but at the same time, the lack of emission control causes high monotone, barely accentual variations. An inhibitory deficit means more voice breaks, percentage of periods without voice emission and emission ratio of syllables per time lowest phonation. The pathways that follow the vocalization process consist primarily of three components: laryngeal activity, respiratory movements and supralaryngeal. The most important is the extrapiramydal pathway that connects the motor cortex – putamen - substancia nigra - parvocellular reticular formation - phonatory motoneurons (Jürgens, 2002). The loss of motor control that holds the function of the vocal folds could explain the fluctuations of voice frequency. The position of the reticular formation and ventral parabrachial regions suggests that this area plays a crucial role in vocal motor coordination. Among the hierarchically control of vocal behavior pathways, the phonatory motoneurons input are of two types. One for motor coordination, which comes from the motor cortex and basal ganglia via the pyramidal and extrapyramidal pathway, and the other for the learned vocal patterns, that has a gaiting function, becomes from the periaqueductal grey and cingulated cortex, structures that represent different levels of sensorimotor gaiting control.

The damage of neuronal pathways due to neuronal loss in PD might explain the deficits in startle modulation and speech.

2.2. Alzheimer's disease

The findings suggest that speech measures may indeed be valuable in detection of AD. The results showed that the two groups performed qualitatively differently in the language production task. The participants were classified into the disease and the control groups depending on only five factors: Percentage of voice breaks, N Periods of voice, N voice breaks, Shimmer (apq3) and Noise to Harmonic Ratio. Thus, the prosodic values analyzed are of enormous value given that they allow us to define the profile of individuals with AD (Meilán et al., 2012)

The variables were interpreted using he Fisher coefficients of matrix structure. In the analysis of space full of sound, it was found that in the speech of the participants with AD there was a higher number of periods sound. This implied that the frequencies used are lower, with fewer cycles per

second, presenting the AD group a deeper voice, slower, with a slower rate of speed or rhythm of the glottal pulses and giving rise to a monotonous voice. In the interruptions of voice, it was found that in the speech of the participants with AD there was a higher proportion and numbers of voice breaks. Recently, Meilán et al. (2012) found that the increase in the percentage of voiceless segments in patients' speech is a sign that explains more than 34% of the variance in the scores obtained in a specific language and memory test. Roark et al. (2011) found that the frequency of pauses were useful in discriminating between healthy elderly subjects and subjects with MCI. The interruptions of voice show a series of phonetic features of speech such as narrowing of the phonacional range, voice breaks and excessive reduction of the fundamental frequency of speech, as the result of difficulties of laryngeal muscle accommodation to rapid changes in frequency. The voice breaks is a voice disorder where the pitch of the voice changes suddenly and the number of distances between consecutive pulses are longer than 1.25 divided by the pitch floor. With that, the AD speech turn to be contaminated, and characteristic noises like bubbles or tremor in the voice start showing. Moreover, in the Noise/ Harmonic Ratio (NHR), which provides an indication of the overall periodicity of the voice signal by quantifying the ratio between the aperiodic (noise) and periodic (harmonic part) components, the group with AD had a lower ratio than the control group, with the execution of greater noise frequencies in the AD patients than in the control group. In conclusion, the noise and voice break related parameters give information regarding the amount of noise component in the voice signal. In the analysis of fluctuations, the amplitude of sound, the speech of the participants with AD there was a higher amplitude perturbation quotient between the amplitude of a period and the average of the amplitudes of its neighbors, divided by the average amplitude. The group with AD showed greater variation in the intensity of the successive waves produced continuously, especially in the variation of the amplitudes of their two closest neighbors. Fluctuations in the amplitude of sound refer to period-to-period amplitude variation in the voice signal. The patients with AD showed a tremulous voice, with less intensity and less control of airflow than the control group. In conclusion, we have designed a direct test for measuring language production through an objective and ecological task that can be applied in a very short period of time. It is a method for automatically measuring the speech characteristics of spoken language samples and we have examined the usefulness of these measures for discriminating between patients with AD and control groups, helping in a more accurate diagnosis of AD. The speech measures may indeed be valuable in detection of AD. Spoken language examination is a relatively inexpensive and an easy technique, which involves minimum discomfort for the patient. The use of spectral analysis

tools yields an objective description of voice output that will allow specialists to unify concepts.

3. SNPs analysis

We analyzed the SPSs of the *NRG-1* gene, the *COMT* gene and dopamine receptors *Drd2* and Drd3.

3.1. NRG-1 SNPs

The analysis of genotype rs3924999 and rs6994992 for *NRG-1* gene polymorphisms showed no significant differences between the neurodegenerative disease patients studied, and the control group. Alterations of different polymorphisms of *NRG-1* have been identified as predictors of schizophrenia (Hong et al., 2008) and the mutant genotype of this gene has been linked to abnormalities in sensory gating in schizophrenia (Roussos et al., 2011). Although our results do not show that the mutant genotype is a marker of pathologies, we found an association between the mutant genotype and abnormal patterns of PPI in degenerative diseases. This can be further evidence of the association of the PPI as quantifiable neurophysiological measure to be associated with genetic alterations (Braff et al., 1990; Cadenhead et al., 2000; Geyer et al 2001; Anokhin et al., 2003; Kumari et al, 2005; Hong et al., 2008).

3.2. COMT SNPs

In our analyses for rs4680 polymorphism of the *COMT* gene, we found no significant differences in the frequency of occurrence of different genotypes. Our results are consistent with studies previously published by Quednow et al. (2009), that *COMT* rs4680 influences the clinical and anatomical characteristics of patients with dementia. This is particularly observed in patients with MCI and AD when compared to controls. It is known that the A allele (Met) is related to cognitive functions (Harrison et al., 2008; Talledo et al., 2009; Quednow et al., 2009). Studies in healthy controls suggest that *COMT* genotype influences cognitive processing, the system of reward and emotion (Gennatas et al., 2012). Numerous studies have associated the rs4680 polymorphism with the presence of AD with psychosis (Sweet et al., 2005; Borroni et al., 2006, 2007; DeMichele-Sweet et al., 2010). In our study, we have not addressed the presence of psychosis in patients because in early stages there is no presence of psychosis. Our results show a lower prevalence of genotype A/A for patients with MCI, which could indicate that carriers of this

genotype have a risk factor for developing the disease.

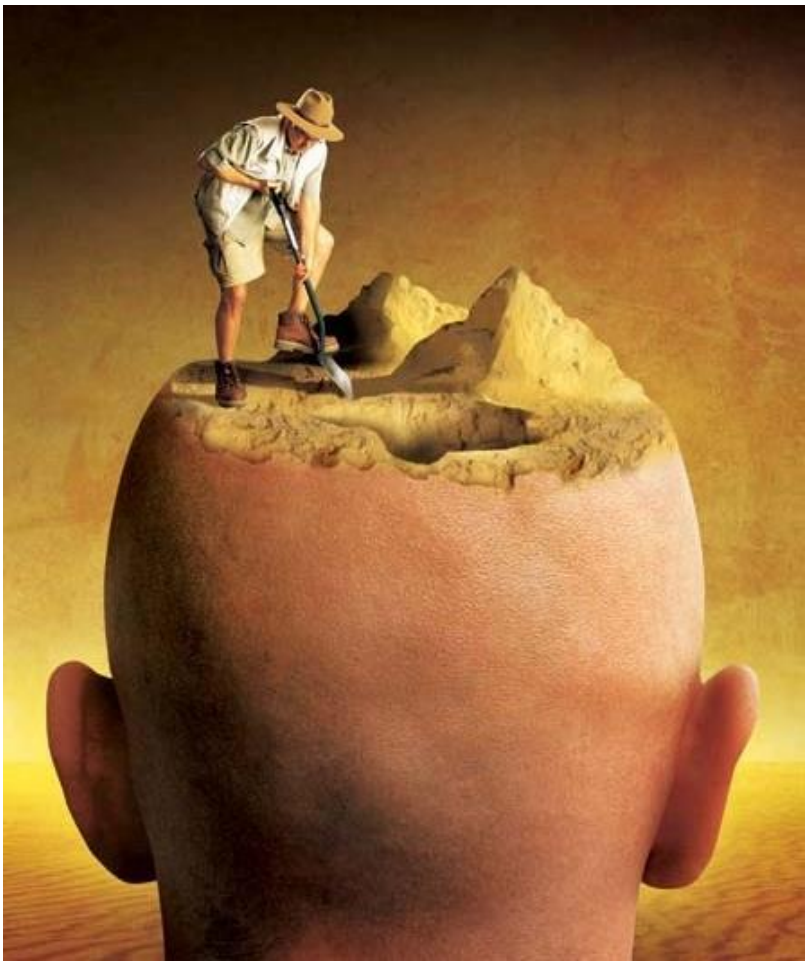
3.3. Dopamine receptor SNPs

Polymorphisms of genes encoding dopamine receptors *Drd2* (Drd2rs6277 and Drd2 rs1800497) did not show significant results in the study sample. The SNP rs6277 the normal genotype (T/T) was the predominant frequency. For the SNP rs1800497, we did not find the mutant genotype in control subjects and in patients with ET and, for this genotype the frequency was low in the other pathologies. The results of our study are in agreement with the literature, in which no association between neurodegenerative diseases and polymorphisms of dopamine receptors *Drd2* (Singh et al., 2008; De Palma et al., 2010; Tan et al., 2003; Costa-Mallen et al., 2000; Comings et al., 1991; Kiyohara et al., 2011). This was an unexpected finding since Drd2 receptors play an important role in dopaminergic control of the movement and behavior (Van Ham et al., 2007).

For the *Drd3* receptor, the rs6280 polymorphism was found as a risk factor in PD, showing higher levels of the mutant allele.

Correlating the mutated genotype distributions of Drd3 with the PPI, we found significant differences between PD patients and control subjects with PPI_{120ms} and with PPF, showing higher percentage in booth parameters in the PD patients with the mutant allele. Our results show relationship between SNPs and the percentage of PPI as published by other studies (Völter et al., 2011; Roussos et al., 2008). Signaling D2 and D3 receptors in the striatal system may be responsible for the deficits of PPI (Völter et al., 2011). Although we did not find significant association with genotype of the D2 SNPs and pathology, the fact that we found association with the values of PPI genotype and pathology can be used as in early diagnosis. The association with dopamine and PPI has been studied mostly in patients with schizophrenia and animal models for schizophrenia, and studies in healthy subjects confirm the association between PPI and D2 and D3 receptors, (Roussos et al., 2008; Völter et al., 2011).

Studies show the involvement of the sensory system in the pathophysiology of certain movement disorders, makes it essential to consider the potential contribution of changes in sensorimotor integration in motor execution (Abbruzzese and Berardelli, 2003).



(Igor Morski)

CONCLUSIONS

VII. CONCLUSIONS:

1. Sensorimotor integration capacity measured by the PPI is impaired in most neurodegenerative diseases.

2. The prepulse-pulse interval of 120 ms is the best setup for discriminating patient groups with the PPI behavioural paradigm.

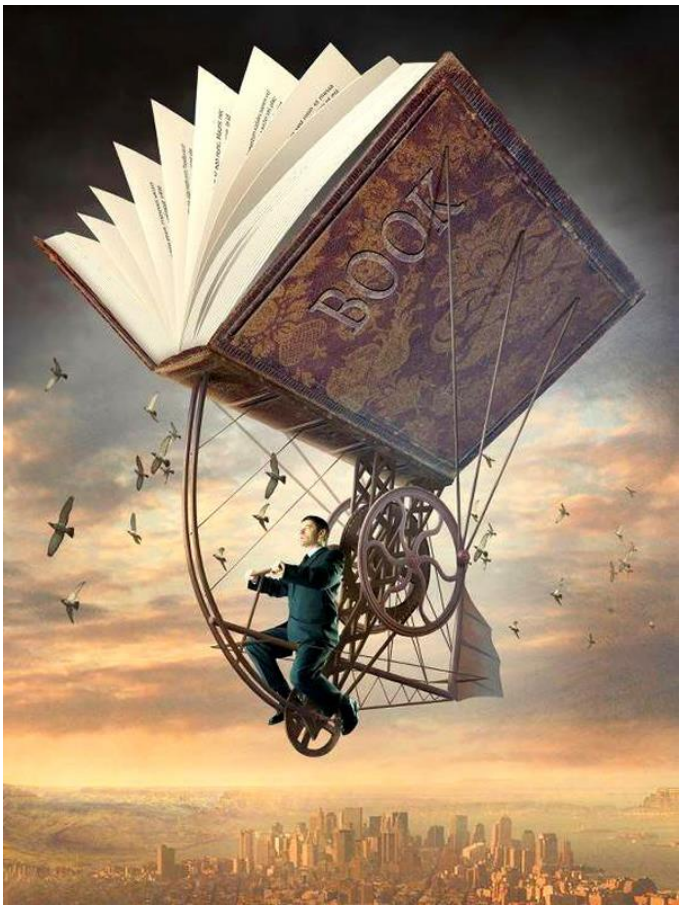
3. Patients with AD and MCI, show greater cognitive impairment than the rest of the groups.

4. Patients with PD have slowed language syllabic and prosodic joint.

5. AD patients show differences in language production tasks in the study of temporal and acoustic parameters of the voice, having these patients a discriminating voice profile.

6. The presence of the mutated rs4680 polymorphism of the *COMT* gene differentiates between patients with MCI and healthy individuals.

7. The rs6280 SNP of the dopamine D3 receptor, genotype and allelic variants could be related with the risk of PD.



(Igor Morski)

REFERENCES

VII. REFERENCES

- Abbruzzese G, Berardelli A, (2003). Sensorimotor integration in movement disorders. *Mov Disord.* 18(3): 231-240.
- Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH. (2011). The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7(3):270-9. doi: 10.1016/j.jalz.2011.03.008.
- Albin RL, Young AB, Penney JB. (1989). The functional anatomy of basal ganglia disorders. *Trends Neurosci.* 12(10): 366-375.
- Anokhin AP, Heath AC, Myers E, Ralano A, Wood S. (2003). Genetic influences on prepulse inhibition of startle reflex in humans. *Neurosci Lett.* 15;353(1):45-48.
- Anthony BJ, Graham FK. (1985). Blink reflex modification by selective attention: Evidence for the modulation of automatic processing. *Biological Psychology*. 21(1): 43-59.
- Aston-Jones G, Bloom FE. (1981). Norepinephrine containing locus coeruleus neurons in behaving rats exhibit pronounced responses to non-noxious environmental stimuli. *J. Neurosci.*1, 887–900.
- Axelrad JE, Louis ED, Honig LS, Flores I, Ross GW, Pahwa R. (2008). Reduced purkinje cell number in essential tremor: a postmortem study. *Arch Neurol*. 65: 101-107.
- Ben-David E, Tu R. (2014). Genetics of Parkinson Disease. *Am J Neuroradiol*. 2014.
- Benito-León J, Bermejo-Pareja F, Louis ED. (2005). Neurological Disorders in Central Spain (NEDICES) Study Group. Incidence of essential tremor in three elderly populations of central Spain. *Neurology*. 64:1721–1725.
- Benito-León J, Bermejo-Pareja F, Morales JM, Vega S, Molina JA. (2003). Prevalence of essential tremor in three elderly populations of central Spain. *Mov Disord*.18:389–94.
- Benito-León J, Louis ED. (2006). Essential tremor: emerging views of a common dis- order. *Nat Clin Pract Neurol*. 2:666–678.
- Bermejo-Pareja F, Louis ED, Benito-Leon J. (2007). Risk of incident dementia in essential tremor: a population-based study. *Mov Disord*. 22: 1573-1580.
- Bischoff-Grethe A, Crowley MG, Arbib MA. (2003). Movement Inhibition and Next Sensory State Prediction in the Basal Ganglia, In Graybiel A, Delong M, Kitai ST Eds. *The Basal Ganglia VI Advances in Behavioral Biology* Springer US. 54:267-277.
- Blumin JH, Pcolinsky De Fau - Atkins JP, Atkins JP, (2004). Laryngeal findings in advanced Parkinson's disease. *Ann Otol Rhinol Laryngol*.113(4): 253-258.
- Boersma P, Weenink D. (2013). Praat: doing phonetics by computer (versión 5.1.42). [Software] Available: http://www.praat.org/.
- Borroni B, Grassi M, Agosti C, Costanzi C, Archetti S, Franzoni S, Caltagirone C, Di Luca M, Caimi L, Padovani A. (2006). Genetic correlates of behavioral endophenotypes in Alzheimer disease: Role of COMT, 5-HTTLPR and APOE polymorphisms. *Neurobiol Aging*. 11:1595–1603.
- Bowen GP, Ison JR. (2006). Excision of visual cortex does not affect the facilitatory and inhibitory effects of a light flash prepulse on the acoustic startle reflex in the rat. *Behav Brain Res*.168(1):114-119.
- Bowers D, Miller K, Mikos A, et al., (2006). Startling facts about emotion in Parkinson's disease: blunted reactivity to aversive stimuli. *Brain*. 129(12): 3356-3365.
- Braak H, Braak E. (2000). Pathoanatomy of Parkinson's disease. *J Neurol*. 247 (2): 3-10.
- Braak H, Rüb U, Sandmann-Keil D, Gai WP, de Vos RA, Jansen Steur EN, Arai K, Braak E. (2000). Parkinson's disease: affection of brain stem nuclei controlling premotor and motor neurons of the somatomotor system. *Acta Neuropathol*. 99(5): 489-495.
- Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur E N, Braak E. (2003) Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging. 24(2): 197-211.
- Braak H, Braak E. (1991). Demonstration of amyloid deposits and neurofibrillary changes in whole brain sections. *Brain Pathol*. 1(3):213-216.
- Braak H, Braak E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol*.82(4):239-59.
- Braff DL, Geyer MA. (1990). Sensorimotor gating and schizophrenia. Human and animal model studies. *Arch Gen Psychiatry*. 47(2): 181-188.

- Bunton K. (2008). Speech versus nonspeech: different tasks, different neural organization. *Semin Speech Lang*. 29(4):267-275.
- Cadenhead KS, Light GA, Geyer MA, Braff DL. (2000). Sensory gating deficits assessed by the P50 event-related potential in subjects with schizotypal personality disorder. *Am J Psychiatry*.157(1):55-59.
- Canter GJ. (1963). Speech Characteristics of Patients with Parkinson's disease: I. Intensity, Pitch, and Duration. *J Speech Hear Disord.* 28: 221-229.
- Cannizzaro M, Harel B, Reilly N, Chappell P, Snyder PJ. (2004). Voice acoustical measurement of the severity of major depression. *Brain Cogn*. 56(1): 30-35.
- Caramelli P, Mansur LL, Nitrini R. (1998). Language and communication disorders in dementia of the Alzheimer type. In B. Stemmer, & H. A. Whitaker (Eds.), *Handbook of neurolinguistics* (p.463-473). Academic Press, San Diego, CA.

- Cargill M, Altshuler D, Ireland J, Sklar P, Ardlie K, Patil N, Shaw N, Lane CR, Lim EP, Kalyanaraman N, Nemesh J, Ziaugra L, Friedland L, Rolfe A, Warrington J, Lipshutz R, Daley GQ, Lander ES. (1999). Characterisation of single-nucleotide polymorphisms in coding regions of human genes. *Nat Genet*. 22:231–238.
- Cavallotti C, Cavallotti D, Tonnarini G, Leali FM. (2004). Dopamine receptors in the human dura mater. *Neurochem Res*. 29(8):1499-1504.
- Cohen O, Pullman S, Jurewicz E, Watner D, Louis ED. (2003). Rest tremor in patients with essential tremor: prevalence, clinical correlates, and electrophysiologic characteristics. *Arch Neurol.* 60: 405–410.
- Comings DE, Comings BG, Muhleman D, Dietz G, Shahbahrami B, Tast D, Knell E, Kocsis P, Baumgarten R, Kovacs BW, Levy DL, Smith M, Borison RL, Evans DD, Klein DN, MacMurray J, Tosk JM, Sverd J, Gysin R, Flanagan SD. (1991). The dopamine D2 receptor locus as a modifying gene in neuropsychiatric disorders. *JAMA*. 266:1793–1800. doi: 10.1001/jama.266.13.1793.
- Costa PT Jr, Williams TF, Somerfield M. (1996). Clinical Practice Guideline No. 19: Recognition and Initial Assessment of Alzheimer's Disease and Related Dementias. AHCP publication 97-0702 (4th ed.)US Dept of Health and Human Services, Agency for Health Care Policy and Research, Rockville, Md (1996).
- Costa-Mallen P, Costa LG, Smith-Weller T, Franklin GM, Swanson PD, Checkoway H. (2000). Genetic polymorphism of dopamine D2 receptors in Parkinson's disease and interactions with cigarette smoking and MAO-B intron 13 polymorphism. *J Neurol Neurosurg Psychiatry*. 69(4):535-537.
- Critchley M, Greenfield JG. (1948). Olivo-pontocerebellar atrophy. *Brain* 71: 343-364.
- Chouraki V, Seshadri S. (2014). Genetics of Alzheimer's disease. Adv Genet. 87:245-94. doi: 10.1016/B978-0-12-800149-3.00005-6.
- Dai D, Wang Y, Wang L, Li J, Ma Q, Tao J, Zhou X, Zhou H, Jiang Y, Pan G, Xu L, Ru P, Lin D, Pan J, Xu L, Ye M, Duan S. (2014). Polymorphisms of DRD2 and DRD3 genes and Parkinson's disease: A meta-analysis. *Biomed Rep.* 2(2):275-281.
- Darley FL, Aronson AE, Brown JR. (1968). Motor speech signs in neurologic disease. *Med Clin North Am.* 52(4): 835-844.
- Dawson ME, Schell AM, Beers JR, Kelly A. (1982). Allocation of cognitive processing capacity during human autonomic classical conditioning. *J Exp Psychol Gen*. 111(3): 273-295.
- DeMichele-Sweet MA, Sweet RA. (2010). Genetics of psychosis in Alzheimer's disease: a review. *J Alzheimers Dis*. 2010;19(3):761-80. doi: 10.3233/JAD-2010-1274.
- De Palma G, Dick FD, Calzetti S, Scott NW, Prescott GJ, Osborne A, Haites N, Mozzoni P, Negrotti A, Scaglioni A, Mutti A. (2010). A case-control study of Parkinson's disease and tobacco use: gene-tobacco interactions. *Mov Disord*. 25:912–919. doi: 10.1002/mds.22980.
- Deuschl G, Bain P, Brin M. (1998). Consensus statement of the Movement Disorder Society on Tremor. Ad Hoc Scientific Committee. *Mov Disord*. 13 Suppl 3:2-23.
- Díez A, Cieza-Borrella C, Suazo V, González-Sarmiento R, Papiol S, Molina V.(2014). Cognitive outcome and gamma noise power unrelated to neuregulin 1 and 3 variation in schizophrenia. *Ann Gen Psychiatry*.13:18. doi: 10.1186/1744-859X-13-18.
- Ellwanger J, Geyer MA, Braff DL. (2003). The relationship of age to prepulse inhibition and habituation of the acoustic startle response. *Biol Psychol*. 62(3): 175-195.
- Farrell A, Theodoros D, Ward E, Hall B, Silburn P. (2005). Effects of neurosurgical management of Parkinson's disease on speech characteristics and oromotor function. *J Speech Lang Hear Res.* 48(1): 5-20.
- Folstein MF, Folstein SE, McHugh PR. (1975). Minimental-State: A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res*. 12: 189-198.
- Forrest K, Weismer G, Turner GS. (1989). Kinematic, acoustic, and perceptual analyses of connected speech produced by parkinsonian and normal geriatric adults. *J Acoust Soc Am.* 85(6): 2608-2622.

- Francis PT, Palmer AM, Snape M, Wilcock GK. (1999). The cholinergic hypothesis of Alzheimer's disease: A review of progress. *J Neurol Neurosurg Psychiatr*. 66:137–147.
- Frisina PG, Thomas WT, Hälbig D, Libow LS. (2009). The Pattern of Cognitive-Functional Decline in Elderly Essential Tremor Patients: An Exploratory-Comparative Study With Parkinson's and Alzheimer's Disease Patients Journal of the American Medical Directors Association, Volume 10, Issue 4, 238-242.
- García -Martín E, Martínez C, Alonso-Navarro H, Benito-León J, Puertas I, Rubio L, López-Alburquerque T, Agúndez JA, Jiménez-Jiménez FJ. (2009). Dopamine receptor D3 (DRD3) genotype and allelic variants and risk for essential tremor. *Mov Disord*. 24(13):1910-5. doi: 10.1002/mds.22518.
- Gennatas ED, Cholfin JA, Zhou J, Crawford RK, Sasaki DA, Karydas A, Boxer AL, Bonasera SJ, Rankin KP, Gorno-Tempini ML, Rosen HJ, Kramer JH, Weiner M, Miller BL, Seeley WW. (2012). COMT Val158Met genotype influences neurodegeneration within dopamine-innervated brain structures. *Neurology*. 78(21):1663-1669. doi: 10.1212/WNL.0b013e3182574fa1.
- Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. (2001). Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology* (Berl). 156(2-3):117-154.
- Gioumaki SG, Roussos P, Frangou S, Bitsios P. (2008). Disruption of prepulse inhibition of the startle reflex by the preferential D(3) agonist ropinirole in healthy males. *Psychopharmacology* (Berl). 194(3):289-295.
- Graham FK. (1975). Presidential Address, 1974. The more or less startling effects of weak prestimulation. *Psychophysiology*.12(3): 238-48.
- Graham F, Murray GM.(1977). Discordant effects of weak prestimulation on magnitude and latency of the reflex blink. *Physiological Psychology*. 5(1): 108-114.
- Griffiths C, Bough ID Jr. (1989). Neurologic Diseases and Their Effect on Voice. *Journal of Voice.* 3(2): 148-156.
- Goetz CG, Tilley BC, Shaftman SR, et al., (2008). Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Scale presentation and clinimetric testing results. *Movement Disorders*. 23(15): 2129-2170.
- Grupo de Estudio de Neurología de la Conducta y Demencias. GENCD-SEN (2003). Guías en Demencias. Conceptos, criterios, y recomendaciones para el estudio del paciente con demencia: revisión 2002. Sociedad Española de Neurología. Barcelona: Masson.
- Harel B, Cannizzaro M, Snyder PJ. (2004). Variability in fundamental frequency during speech in prodromal and incipient Parkinson's disease: a longitudinal case study. *Brain Cogn*. 56(1): 24-29.
- Harrison PJ, Tunbridge EM. (2008). Catechol-O methyltransferase (COMT): a gene contributing to sex differences in brain function, and to sexual dimorphism in the predisposition to psychiatric disorders. *Neuropsychopharmacology*. 33(13):3037-3045.
- Hejl AM, Glenthøj B, Mackeprang T, Hemmingsen R, Waldemar G. (2004). Prepulse inhibition in patients with Alzheimer's disease. *Neurobiol Aging*. 25(8):1045-1050.
- Ho AK, Bradshaw JL, Iansek R, Alfredson R. (1999). Speech volume regulation in Parkinson's disease: effects of implicit cues and explicit instructions. *Neuropsychologia*. 37(13): 1453-1460.
- Hoehn MM, Yahr MD. (1967). Parkinsonism: onset, progression and mortality. *Neurology*.17(5): 427-442.
- Hodges JR, Salmon DP, Butters N. (1992). Semantic memory impairment in Alzheimer's disease. Failure of access or degraded knowledge? *Neuropsychologia.* 30, 301-314.
- Hoffman HS, Ison JR. (1980). Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. *Psychol Rev*. 87(2): 175-189.
- Hong LE, Wonodi I, Stine OC, Mitchell BD, Thaker GK. (2007). Evidence of missense mutations on the neuregulin 1 gene affecting function of prepulse inhibition. *Biol Psychiatry*. 1;63(1):17-23.
- Hughes AJ, Daniel SE, Kilford L, Lees AJ. (1992). Accuracy of clinical diagnosis of idiopathic Parkinson's disease. A clinico-pathological study of 100 cases. *JNNP*. 55:181-184.
- Isaacs B, Akhtar AJ. (1972). The set test: a rapid test of mental function in old people. *Age Ageing*. 1(4):222-6.
- Jenner P, Schapira AH, Marsden CD. (1992). New insights into the cause of Parkinson's disease. *Neurology*. 42(12):2241-2250.
- Kiyohara C, Miyake Y, Koyanagi M, Fujimoto T, Shirasawa S, Tanaka K, Fukushima W, Sasaki S, Tsuboi Y, Yamada T, Oeda T, Shimada H, Kawamura N, Sakae N, Fukuyama H, Hirota Y, Nagai M; Fukuoka Kinki Parkinson's Disease Study Group. (2011). Genetic polymorphisms involved in dopaminergic neurotransmission and risk for Parkinson's disease in a Japanese population. *BMC Neurol*. 11:8.
- Koch M. (1999). The neurobiology of startle. *Prog Neurobiol*. 59(2): 107-128.
- Kofler M, Müller J, Wenning GK, et al., (2001). The auditory startle reaction in parkinsonian disorders. *Mov Disord*. 16(1): 62-71.
- Kumari V, Aasen I, Papadopoulos A, et al., (2008). A comparison of prepulse inhibition in pre- and postmenopausal women and age-matched men. *Neuropsychopharmacology*. 33(11): 2610-2618.

- Kumari V, Das M, Zachariah E, Ettinger U, Sharma T. (2005). Reduced prepulse inhibition in unaffected siblings of schizophrenia patients. Psychophysiology. 42(5):588-594.
- Labiano-Fontcubertaa A, Benito-León J, Bermejo-Parejaa F. (2012). Alteraciones neuropsiquiátricas en el temblor esencial. *Med Clin (Barc)*. 138(4):171–176.
- Lavoie B, Parent A. (1994). Pedunculopontine nucleus in the squirrel monkey: cholinergic and glutamatergic projections to the substantia nigra. *J Comp Neurol*. 344(2):232-241.
- Lee Y, Davis M. (1997). Role of the hippocampus, the bed nucleus of the stria terminalis, and the amygdala in the excitatory effect of corticotropin-releasing hormone on the acoustic startle reflex. *J Neurosci*. 17(16):6434-6446.
- Lee JY, Lee EK, Park SS, Lim JY, Kim HJ, Kim JS, Jeon BS. (2009). Association of DRD3 and GRIN2B with impulse control and related behaviors in Parkinson's disease. *Mov Disord*. 24:1803–1810.
- Louis ED, Honig LS, Vonsattel JP, Maraganore DM, Borden S, Moskowitz CB. (2005). Essential tremor associated with focal nonnigral Lewy bodies: a clinicopathologic study. *Arch Neurol.* 62: 1004-1007.
- Ludewig K, Ludewig S, Seitz A, Obrist M, Geyer MA, Vollenweider FX. (2003). The acoustic startle reflex and its modulation: effects of age and gender in humans. *Biol Psychol*. 63(3): 311-323.
- Martínez MF, Martín XE, Alcelay LG, Flores JC, Valiente JM, Juanbeltz BI, Beldarraín MA, López JM, Gonzalez-Fernández MC, Salazar AM, Gandarias RB, Borda SI, Marqués NO, Amillano MB, Zabaleta MC, de Pancorbo MM. (2009). The COMT Val158 Met polymorphism as an associated risk factor for Alzheimer disease and mild cognitive impairment in APOE 4 carriers. *BMC Neurosci*. 10:125. doi: 10.1186/1471-2202-10-125.
- Martínez-Sánchez F. (2010). Trastornos del habla y la voz en la enfermedad de Parkinson. *RevNeurol*. 51(09): 542-550.
- Manzano S, González JL, Marcos A, Payno M, Villanueva C, Matias-Guih J. (2009). Modelos experimentales de la enfermedad de Alzheimer.*Neurología*, 24(4):255-262.
- McGuire V, Van Den Eeden SK, Tanner CM, Kamel F, Umbach DM, Marder K, Mayeux R, Ritz B, Ross GW, Petrovitch H, Topol B, Popat RA, Costello S, Manthripragada AD, Southwick A, Myers RM, Nelson LM. (2011). Association of DRD2 and DRD3 polymorphisms with Parkinson's disease in a multiethnic consortium. *J Neurol Sci*.307(1-2):22-29. doi: 10.1016/j.jns.2011.05.031.
- McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH. (2011). The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 7(3):263-9. doi: 10.1016/j.jalz.2011.03.005.
- McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology.* 34: 939-944.
- Medford N, Critchley HD. (2010). Conjoint activity of anterior insular and anterior cingulate cortex: awareness and response. *Brain Struct Funct*. 214(5-6):535-549. doi: 10.1007/s00429-010-0265-x.
- Meilán JJ, Martínez-Sánchez F, Carro J, Sánchez JA, Pérez E.(2012). Acoustic markers associated with impairment in language processing in Alzheimer's Disease. *Span J Psychol.* 15(2):487-94.
- Meilán JJ, Martínez-Sánchez F, Carro J, López DE, Millian-Morell L, Arana JM. (2014). Speech in Alzheimer's disease: can temporal and acoustic parameters discriminate dementia? *Dement Geriatr Cogn Disord*. 37(5-6):327-334.
- Mertens P. (2004). Le prosogramme: une transcription semi-automatique de la prosodie. *Cahiers de l'Institut de Linguistique de Louvain* . 30:7-25.
- Mora-Simon S, Garcia-Garcia R, Perea-Bartolome MV, Ladera-Fernandez V, Unzueta-Arce J, Patino-Alonso MC, Rodriguez-Sanchez E. (2012). Mild cognitive impairment: early detection and new perspectives. *Rev Neurol*. 54(5):303-310.
- Morris JC, Storandt M, Miller P, Mckeel DW, Price JL, Rubin EH, Berg L. (2001). Mild Cognitive Impairment Represent Early-Stage Alzheimer Disease. *Arch Neurol*. 58:397-405.
- Obeso I, Wilkinson L, Casabona E, et al., (2011). Deficits in inhibitory control and conflict resolution on cognitive and motor tasks in Parkinson's disease. *Exp Brain Res*. 212(3): 371-384.
- Perriol M, Dujardin K, Derambure P, Marcq A, Bourriez JL, Laureau E, Pasquier F, Defebvre L, Destée A.(2005). Disturbance of sensory filtering in dementia with Lewy bodies: comparison with Parkinson's disease dementia and Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 76(1):106-8.
- Petersen RC, Stevens JC, Ganguli M, Tangalos EG, Cummings JL, DeKosky ST. (2001). Practice parameter: early detection of dementia: mild cognitive impairment (an evidence-based review): report of the quality standards subcommittee of the American Academy of Neurology. *Neurology*. 56: 1133–1142.

- Pfeffer R I, Kurosaki TT, Harrah CHJr., Chance JM, Filos S. (1982). Measurement of functional activities in older adults in the community. *J. Gerontol*.37, 323–329. doi: 10.1093/geronj/37.3.323.
- Price C J. (2010). The anatomy of language: A review of 100 fMRI studies published in 2009. *Annals of the New York Academy of Sciences*.1191:62–88.
- Quednow BB, Schmechtig A, Ettinger U, Petrovsky N, Collier DA, Vollenweider FX, Wagner M, Kumari V. (2009). Sensorimotor gating depends on polymorphisms of the serotonin-2A receptor and catechol-O-methyltransferase, but not on neuregulin-1 Arg38Gln genotype: a replication study. *Biol Psychiatry*. 66(6):614-620. doi: 10.1016/j.biopsych.2009.05.007.
- Ramig LO, Fox C, Sapir S. (2007). Speech disorders in Parkinson's disease and the effects of pharmacological, surgical and speech treatment with emphasis on Lee Silverman voice treatment (LSVT(R)). *Handb Clin Neurol*. 83:385-399.
- Ramig L, Fox C, Sapir S. (2011). Speech and Voice Disorders in Parkinson's disease. In: Olanow CW, Stocchi F, and Lang AE, Eds, *Parkinson's disease: Non-Motor and Non-Dopaminergic Features First Edition*. Blackwell Publishing Ltd, Oxford, UK. 31:346-360.
- Roark B, Mitchell M, Hosom JP, Hollingshead K, Kaye J. (2011). Spoken language derived measures for detecting mild cognitive impairment. *IEEE trans audio speech.* 19, 2081-2090.
- Roussos P, Giakoumaki SG, Adamaki E, Bitsios P. (2011). The influence of schizophrenia-related neuregulin-1 polymorphisms on sensorimotor gating in healthy males. *Biol Psychiatry*. 69(5):479-486. doi: 10.1016/j.biopsych.2010.09.009.
- Roussos P, Giakoumaki SG, Rogdaki M, Pavlakis S, Frangou S, Bitsios P (2008). Prepulse inhibition of the startle reflex depends on the catechol O-methyltransferase Val158Met gene polymorphism. *Psychol Med*. 38(11):1651-1658. doi: 10.1017/S0033291708002912.
- Rusz J, Cmejla R, Ruzickova H, Ruzicka E. (2011). Quantitative acoustic measurements for characterization of speech and voice disorders in early untreated Parkinson's disease. *J. Acoust. Soc. Am.* 129, 350. http://dx.doi.org/10.1121/1.3514381
- Samuels MA. (2007). Brains and mysteries. *Newsweek*. 150(24):90.
- Saxena M, Behari M, Kumaran SS, Goyal V, Narang V.(2014). Assessing speech dysfunction using BOLD and acoustic analysis in parkinsonism. *Parkinsonism Relat Disord.* (14) 177-181.
- Schindler JS, Kelly JH. (2002). Swallowing disorders in the elderly. *The Laryngoscope*. 112(4): 589-602.
- Schleinitz D, Distefano JK, Kovacs P.(2011). Targeted SNP genotyping using the TaqMan® assay. *Methods Mol Biol*. 700:77-87. doi: 10.1007/978-1-61737-954-3_6.
- Sewall GK, Jiang J, Ford CN. (2006). Clinical evaluation of Parkinson's-related dysphonia. *Laryngoscope*.116(10): 1740-1744.
- Sherwin BB. (2000). Mild cognitive impairment: potential pharmacological treatment options. *J Am Geriatr Soc*.48:431-441.
- Shill HA, Adler CH, Sabbagh MN. (2008) Pathologic findings in prospectively ascertained essential tremor subjects. Neurology. 70(16):1452–1455.
- Singh M, Khan AJ, Shah PP, Shukla R, Khanna VK, Parmar D. (2008). Polymorphism in environment responsive genes and association with Parkinson disease. *Mol Cell Biochem.* 312:131–138. doi: 10.1007/s11010-008-9728-2.
- Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T., et al., (2002). Neuregulin 1 and susceptibility to schizophrenia. *Am J Hum Genet*. 71:877–892. doi: 10.1086/342734.
- Sweet RA, Devlin B, Pollock BG, Sukonick DL, Kastango KB, Bacanu S-A, Chowdari KV, DeKosky ST, Ferrell RE. (2005). Catechol-O-methyltransferase haplotypes are associated with psychosis in Alzheimer disease. *Mol Psychiatry*. 10:1026–1036.
- Swerdlow NR, Geyer MA, Braff DL. (2001). Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. *Psychopharmacology*.156(2-3): 194-215.
- Swanson LW, Hartman BK. (1975) The central adrenergic system.An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine-beta-hydroxylase as a marker. J Comp Neurol.15;163(4):467-505.
- Swerdlow NR, Hartman PF, Auerbach PP. (1997). Changes in sensorimotor inhibition across the menstrual cycle: implications for neuropsychiatric disorders. *Biol Psychiatry*. 41(4): 452-460.
- Swerdlow NR, Hines SR, Herrera SD, Weber M, Breier MR. (2013). Opposite effects of tolcapone on amphetamine-disrupted startle gating in low vs. high COMT-expressing rat strains. *Pharmacol Biochem Behav.* 106:128-131. doi: 10.1016/j.pbb.2013.03.015.
- Stoessl AJ. (2011). Neuroimaging in Parkinson's disease. *Neurotherapeutics*. 8(1): 72-81.
- Taler V, Phillips NA. (2007). Language performance in Alzheimer's disease and mild cognitive impairment: A comparative review. *J clin exp neuropsyc*. 30, 501-56.

- Talledo JA, Sutherland Owens AN, Schortinghuis T, Swerdlow NR. (2009). Amphetamine effects on startle gating in normal women and female rats. *Psychopharmacology* (Berl). 204(1):165-175. doi: 10.1007/s00213-008-1446-7.
- Tan EK, Chai A, Lum SY, Shen H, Tan C, Teoh ML, Yih Y, Wong MC, Zhao Y. (2003). Monoamine oxidase B polymorphism, cigarette smoking and risk of Parkinson's disease: a study in an Asian population. *Am J Med Genet B Neuropsychiatr Genet.* 120B:58–62. doi: 10.1002/ajmg.b.20035.
- Tindall EA, Speight G, Petersen DC, Padilla EJD, Hayes VM. (2007). Novel PlexorTM SNP genotyping technology: comparisons with TaqMan® and homogenous MassEXTENDTM MALDI-TOF mass spectrometry. *Hum Mutat*. 28(9):922–927.
- Tomé M, Jiménez AJ, Richter H, Vio K, Bermúdez-Silva FJ, Rodríguez EM, Pérez-Fígares JM. (2004) The subcommissural organ expresses D2, D3, D4, and D5 dopamine receptors. Cell Tissue Res. 317(1):65-77. Epub 2004 Jun 9.
- Torkaman-Boutorabi A1, Ali Shahidi G, Choopani S, Reza Zarrindast M. (2012). Association of monoamine oxidase B and catechol-O-methyltransferase polymorphisms with sporadic Parkinson's disease in an Iranian population. *Folia Neuropathol*.50(4):382-389.
- Tsanas A, Little MA, McSharry PE, Spielman J, Ramig LO. (2012). Novel speech signal processing algorithms for high-accuracy classification of Parkinson's disease. *IEEE Trans Biomed Eng*. 59(5): 1264-1271
- Ueki A, Goto K, Sato N, Iso H, Morita Y. (2006). Prepulse inhibition of Acoustic Startle Response in mild cognitive impairment and mild dementia of Alzheimer type. *Psychiatry and Clinical Neuroscience*. 60:55-62.
- Van-Ham II, Banihashemi B, Wilson AM, Jacobsen KX, Czesak M, Albert PR. (2007). Differential signaling of dopamine-D2S and -D2L receptors to inhibit ERK1/2 phosphorylation. *J Neurochem.* 102(6):1796-804.
- Vallelunga A, Flaibani R, Formento-Dojot P, Biundo R, Facchini S, Antonini A. (2012). Role of genetic polymorphisms of the dopaminergic system in Parkinson's disease patients with impulse control disorders. *Parkinsonism Relat Disord*.18(4):397-399. doi: 10.1016/j.parkreldis.2011.10.019.
- Valls-Pedret C, Molinuevo JL, Rami L. (2010). Diagnóstico precoz de la enfermedad de Alzheimer: fase prodrómica y preclínica. *Rev Neurol*. 51 (8): 471-480.
- Valls-Solé J, Muñoz JE, Valldeoriola F. (2004). Abnormalities of prepulse inhibition do not depend on blink reflex excitability: a study in Parkinson´s disease and Huntinton´s disease. *Clinical Neurophysiology*. 115:1527-1536.
- Valls-Solé J, Valldeoriola F. (2002). Neurophysiological correlate of clinical signs in Parkinson's disease. Clin Neurophysiol. 113(6): 792-805.
- Venneri A, Forbes-McKay KE, Shanks MF. (2005). Impoverishment of spontaneous language and the prediction of Alzheimer's disease. *Brain.* 128, E27.
- Vidailhet M, Rothwell J C, Thompson PD, Lees A J, Marsden CD. (1992). The auditory startle response in the Steele-Richardson-Olszewski syndrome and Parkinson's disease. *Brain*. 115 (4): 1181-1192.
- Völter C, Riedel M, Wöstmann N, Aichert DS, Lobo S, Costa A, Schmechtig A, Collier DA, Hartmann AM, Giegling I, Möller HJ, Quednow BB, Rujescu D, Kumari V, Ettinger U. (2012). Sensorimotor gating and D2 receptor signalling: evidence from a molecular genetic approach. *Int J Neuropsychopharmacol*.15(10):1427-1440. doi: 10.1017/S1461145711001787.
- Walker DL, Davis M. (1997). Anxiogenic effects of high illumination levels assessed with the acoustic startle response in rats. *Biol Psychiatry*. 42(6): 461-471.
- Walsh B, Smith A. (2012). Basic parameters of articulatory movements and acoustics in individuals with Parkinson's disease. *Mov Disord*. 27(7): 843-850.
- Williams BR, Nazarians D, Gill MA. (2003). A Review of Rivastigmine: A Reversible Cholinesterase Inhibitor. *Clinical Terapheutics*. 25(6):1634-1653.
- Wolfe J, Maëva G, John S. (2009).Vocal tract resonances in speech, singing, and playing musical instruments. *HFSP J*. 3(1):6-23.
- Wong AH, Buckle CE, Van Tol HH. (2000). Polymorphisms in dopamine receptors: what do they tell us? *Eur J Pharmacol.* 410(2-3):183-203.
- Wynn JK, Dawson ME, Schell AM, McGee M, Salveson D, Green MF. (2004). Prepulse facilitation and prepulse inhibition in schizophrenia patients and their unaffected siblings. *Biol Psychiatry*. 55(5): 518-523.
- Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey MB, Leirer VO.(1983). Development and validation of a geriatric depression screening scale: A preliminary report. *Journal of Psychiatric Research.* 17: 37-49.
- Zhang L, Hu XZ, Li H, Li X, Smerin S, Benedek DM, Ursano R. (2011). Startle response related genes. *Med Hypotheses.*77(4):685-691. doi: 10.1016/j.mehy.2011.07.018.