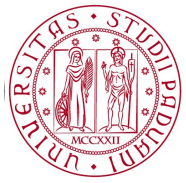


UNIVERSITÀ
DEGLI STUDI
DI PADOVA

Università degli Studi di Padova

Dipartimento di Scienze Medico-Diagnostiche e Terapie Speciali

Scuola di Dottorato di Ricerca in Scienze Mediche Cliniche e Sperimentali

Indirizzo: Neuroscienze

Ciclo: XXIII°

STUDY ON HIPPOCAMPAL BRAIN VOLUMES IN TREATMENT RESISTANT DEPRESSION DURING VAGAL NERVE STIMULATION (VNS)

Direttore della Scuola : Ch.mo Prof. Gaetano Thiene

Coordinatore d'Indirizzo: Ch.mo Prof. Corrado Angelini

Supervisore :Ch.mo Prof.ssa Giulia Ida Perini

Dottorando : Giovanni Ferri

Anno Accademico 2010-2011

INDEX

Abstract _____ 3

Riassunto_____ 5

PART I: INTRODUCTION

1.TREATMENT RESISTANT DEPRESSION_____ 7

a)Definition and Epidemiology 7

2.VAGUS NERVE STIMULATION 12

a)VNS: Historical Development 12

b)VNS: Clinical Data 14

c)VNS: Experimental Data 20

PART II: EXPERIMENTAL DATA

3.HYPOTHESIS 26

4.METHODOLOGY 26

a)Patients Selection 26

b)VNS intervention and follow-up 28

c)MRI study 30

d)Statistics 32

5.RESULTS 33

a)Clinical Data 33

b)MRI Results 38

6.DISCUSSION 41

REFERENCES 48

ABSTRACT

BACKGROUND: Volumetric changes in mood-relevant distributed limbic/paralimbic neurocircuitry have been reported in the recent literature on the course of Major Depression (MD). A decrease in hippocampal gray matter volumes is one of the possible mechanisms involved in pathogenesis of depression [Nifosì et al. 2010]. Converging evidences suggest that a reduced neurogenesis may occur in this area in recurrent depression and its positive modulation is fundamental for antidepressive action. Neurogenesis is stimulated both by plasticity-inducing stimuli (such as environmental enrichment, exercise, and electrical stimulation) and by antidepressant treatments. In functional imaging studies, Vagal Nerve Stimulation

(VNS), a novel approach for treatment resistant depression (TRD), demonstrated that its antidepressive function may be correlated to changes in cerebral blood flow in limbic and prefontal cortex [Zobel et al 2005]. We are exploring if volumetric changes in specific brain areas involved in depression may be obtained with VNS. ***METHODS***: Comparison of data on changes in brain volumes of specific regions of interest in 6 patients affected by TRD treated with VNS (VNS Therapy System™, Cyberonics, Houston, Texas –USA) and 5 TRD cases "treated as usual" (TAU) was performed. Brain MRI scans were detected before the implantation (t0) and after 12 months (t1). Psychopathology was assessed using patient self reports (BDI) and clinical evaluation (HDRS) every three months during the follow-up. ***RESULTS*** : Three out of six VNS treated patient showed a good clinical response (Reduction of depressive scores >50% from baseline) in three months of treatment. A significant increase of 22% and 14% of Left and Right hippocampal volumes was found after one year of Vagus Nerve Stimulation. No correlation were found between clinical outcomes measures and increase in volumes. Non responders showed no increase in hippocampal volumes. Preliminary findings from this case series seem to confirm the fundamental role of hippocampal remodelling as a marker for response in TRD.

RIASSUNTO

PREMESSE: Modifiche nella volumetria delle strutture limbiche-paralimbiche coinvolte nella regolazione del tono dell'umore sono state descritte nella letteratura più recente sui Disturbi Depressivi Maggiori (DDM). Una diminuzione nei volumi della sostanza grigia ippocampale è uno dei possibili meccanismi coinvolti nella patogenesi della depressione [Nifosì et al. 2010]. Evidenze convergenti sembrano confermare che in corso di depressione una ridotta neurogenesi possa verificarsi in questa area e che una modulazione positiva di tale processo sia fondamentale per l'azione antidepressiva. La Neurogenesi è stimolata sia da stimoli che rafforzano la plasticità neuronale (come stimolazioni ambientali esterne, esercizio fisico, stimolazione elettrica) sia dai trattamenti antidepressivi. In studi di neuroimaging funzionale, la Stimolazione del Nervo Vago, (VNS) una metodica di recente introduzione per il trattamento della Depressione farmaco Resistente (TRD), è stata dimostrato che la sua azione antidepressiva può essere correlata con modifiche nel flusso ematico cerebrale nella corteccia limbica e prefrontale [Zobel et al 2005]. Obiettivo di questo studio è valutare se modifiche delle volumetrie in specifiche aree coinvolte nella depressione, possano essere ottenuta mediante la VNS. **METODOLOGIA:** E' stato effettuato un confronto dei dati sulle modifiche di volume di specifiche regioni d'interesse in 6 pazienti con depressione farmaco resistente trattati mediante VNS (VNS Therapy System™, Cyberonics, Houston, Texas –USA) con i volumi delle stesse aree in 5 casi di TRD trattati in maniera convenzionale. Le Risonanze Magnetiche Nucleari (MRI) cerebrali sono state realizzate prima dell'impianto (t0) e dopo 12 mesi(t1). L'entità della depressione è

stata valutata mediante scale autosomministrate (BDI) o etero somministrate (HDRS) ogni tre mesi durante il del monitoraggio clinico. **RISULTATI:** Tre dei sei pazienti che hanno ricevuto la VNS hanno manifestato una buona risposta clinica (riduzione dei punteggi alle scale per la depressione > 50% dai punteggi iniziali) a tre mesi dall'inizio del trattamento. Un aumento significativo di circa il 22% e del 14% rispettivamente per ippocampo sinistro e destro è stato messo in luce dopo un anno di trattamento mediante VNS. Nessuna correlazione è stata trovata tra le principali variabili indicative dell'andamento clinico e l'aumento dei volumi cerbrali. I pazienti non responsivi alla VNS non hanno manifestato aumenti nei volumi ippocampali. I dati preliminari riscontrati in questo piccolo gruppo sembrano confermare il ruolo fondamentale del rimodellamento ippocampale come marker per la possibile risposta clinica nella TRD.

PART I: INTRODUCTION

1.TREATMENT RESISTANT DEPRESSION

Definition and Epidemiology

Worldwide, depression is the 4th leading cause of disability, with a prevalence of 2.3–3.2% in men and 4.5–9.3% in women [Kessler et al., 2003]. It is estimated that by 2020 major depression disorder (MDD) will be the 2nd most disabling condition in the world with largest number of Disability Adjusted Life Years (DALYs)[Murray and Lopez, 1997].

Main symptoms are presence of depressive feelings and thoughts, loss of interest and pleasure, neurovegetative alterations as sleep disturbances, changes in food intake, sexual dysfunction, somatic symptoms, psychomotor retardation or agitation, and cognitive impairment [DSM IV- TR, 2004]. The disability is mainly due to reduced daily activities and social retirement, to chronic or recurrent course, and nonetheless depression is associated with high rates of mortality secondary to suicide or indirectly due to increased mortality when depression co-occurs with general medical conditions for example myocardial infarction. As a complex disorder, most properly described by a multifactor ethiological model, but with clearly proven neurobiological correlates, actual milestone in treatment of depression is pharmacological treatment, with psychological, social and educational intervention playing a relevant role in management of disorder. Non pharmacological, especially brain stimulation treatment, are growing in use.

Regarding pharmacological therapies, despite the considerable efforts made over the past decades, there is a large proportion of patients with depression who still do not respond to the currently available treatments. Between 20 and 40% of patients with MDD do not show substantial clinical improvement on their first treatment with antidepressant medication [Fava and Davidson, 1996; Sackeim, 2001]. Treatment-resistant depression or TRD refers to major depressive episodes (unipolar or bipolar) from which patients do not recover fully after at least two and typically three or more adequately delivered but unsuccessful treatment attempts [Sackeim, 2001]. TRD are patients who do not respond in the acute phase of treatment or are those who, after achieving remission, have an early relapse, with difficulties in sustaining remission in long-term treatment, and consequently at high risk for development of chronicity and disability. For this reason actual therapeutic strategies used for TRD are multiple and sometimes “aggressive” including varying combinations of different agents,

psychotherapy and electro-convulsive therapy (ECT). In this contest the term TRD then refers to neither a homogeneous group nor a diagnostic entity, but it's possible to hypothesize that patients with TRD likely suffer from biologically heterogeneous disorders, so that different next-step treatments may be effective for some but not other patients with TRD. Rather, this category designates patients for whom treatments entailing greater risks or intolerance become more reasonable considerations, and providing such treatments offers a reasonable chance of greater efficacy [George MS et al 2008]. Even in treatment responders in fact the use of medication combinations is recommended in later treatment steps if less complex, better tolerated, simpler to use, monotherapies have previously failed [Crismon et al., 1999]. Analogously, despite its greater efficacy, electroconvulsive therapy is typically reserved for depressed patients without adequate benefit from one or often several medication monotherapies and combinations [AHCPR Guidelines, Vol. 2, 1993].

As a great challenge in "real world" psychiatric clinical practice, in recent years researchers focused attention on TRD to better describe, not only criteria to define it, but also data on its course and peculiarity in management of treatment. Different models to define TRD were proposed in the first 2000's and reviewed by Souery in 2006; what emerged from this work is first of all the need for a correct identification of specific factors that led to treatment resistance (for instance *pseudoresistance* is defined as wrong completion of correct treatment trial, both for inadequate dosing, discontinuation or non compliance) and nonetheless the fundamental identification of specific features of depressive episode possibly leading to treatment failure, because misdiagnosis may be the first cause of erroneous attribution of treatment resistance. In a two years follow-up study of 164 outpatients with a severe and/or resistant mood disorder Parker et.al [2005] identified 6 paradigm-errors leading to improper attribution of resistance, such as failure to diagnose and manage bipolar disorder, or psychotic depression, or melancholic depression, diagnosing and/or managing a non melancholic condition as if it were melancholic depression, misdiagnosing depression secondary to other relevant axis I disorders, failing to identify organic determinants. Starting from primarily exclusion of all these conditions, main TRD definition comprehend those from Committee for Proprietary Medial Products (CPMP) [VVAA 2005] guidance, the Thase and Rush staging method [Thase et al 1997], the Massachusetts General Hospital (MGH) staging method [Fava 2003] and

the Sourey et al operational criteria for TRD [Sourey D et al. 1999]. These models slightly differ in relevance given to response to different classes of antidepressant agents or to their sequence in use. They all convey in setting the landmark of TRD after the second treatment failure, and they all refer to general construct of response or remission based on decrease in scoring of main rating scales for depression commonly used in clinical practice, as summarized by Nierenberg and colleagues [2001]. (Tab 1)

DEPRESSIVE SCORING REDUCTION FROM BASELINE	
Recovery	**≥75 %**
Response	**50-74 %**
Partial response	**25-49 %**
Absence of Responce	**<25 %**
REMISSION SCORES IN MOST COMMON RATING SCALES	
HDRS (Hamilton Rating Scale for Depression)	**<7**
MADRS (Montgomery-Asberg Depression Rating Scale)	**<8**

Few studies examined natural course of TRD in long term and, despite a wide range of treatment options, 90% of TRD patients continue to experience substantial levels of depression symptoms after 2 years of active treatment [Dunner et al., 2006]

The STAR*D (Sequenced Treatment Alternatives to Relieve Depression) study, a multicenter trial sponsored by U.S. National Institute of Mental Health's (NIMH) is currently regarded as the trial with the best real-world setting, and therefore best generalizability of its results. It was designed to describe natural course of depression if treated with a fix scheme of sequenced treatments: Citalopram monotherapy (Level I); switching to another monotherapy with SSRi (Sertraline) or agents from another class (Venlafaxine or Bupropion), or in alternative, augmentation of Citalopram with Bupropion or Buspiron (Level II); switching to most effective compounds (Mirtazapine or Nortriptyline) or augmenting with Lithium or Triiodothyronine (Level III); combining two antidepressant with serotonin/norepinphrine dual action (Venlafaxine Extended Release + Mirtazapine) or MAOI (Tranylcypromine).

The results from the STAR*D programme, in which finally 4041 adult non psychotic major depressed outpatients were enrolled, indicate that remission is achieved within six weeks in only about one-third of depressed patients in response to treatment with Citalopram [Trivedi et al. 2006]. Moreover, although a second monotherapy is

usually recommended at the next level of treatment, the STAR*D study results indicate that less than 30% of patients will attain remission with a single medication, while higher rates of response of more than 30% were possible with augmentation strategy.[Rush et al 2006b]. The rate of remission drops precipitously among patients who did not respond to two treatment strategies, in fact the investigators found remission rates of 36.8% and 30.6% with the first two treatment steps, but only 13.7% and 13.0% with each of the next two steps. Not only remission was less likely in these later steps, but relapse during follow-up was much greater when more acute steps had been tried. Relapse rates (and time to relapse) were 40.1% (4.1 months) after the first step, and 55.3% (3.9 months), 64.6% (3.1 months), and 71.1% (3.3 months) after steps 2, 3, and 4 respectively [Warden et al., 2007]. Maintenance (after initial remission) data indicate that the broad majority of patients will relapse in the next year of treatment; therefore, treatment resistance is the rule rather than the exception. For the duration of the study, the cumulative sustained recovery rate was 43%[Rush et al 2006]. For all these reason it's possible to affirm that the main information coming from STAR*D results is not the confirm of the validity of the sequenced treatment guideline proposed, whereas the prove of the validity of construct of TRD as lack of response to at least two trials of antidepressants, that shall guide clinicians in fast recognition of this "special population" to avoid the risk of chronic depression or residual symptoms that lead to disability[Rush et al. 2009a].

2.VAGUS NERVE STIMULATION

a)VNS: Historical Development

Vagal Nerve Stimulation (VNS) was approved by CEM Trademark in 1994 and by FDA in 1997 for Treatment Resistant Epilepsy (TRE) [Groves 2005]. Starting from clinical evidence of improvement in mood and cognition of epileptic patients, an application in Treatment Resistant Depressed patient was studied. CEM approval for TRD was released in 2001 and FDA approval came in 2005, as "…adjunctive long-term treatment of chronic or recurrent depression for patients over the age of 18 who

are experiencing a major depressive episode that has not had an adequate response to two or more adequate antidepressant treatments" [PMA n° P970003, FDA 2005]. Actually Cyberonics Inc. (Houston, TX) company developing and marketing the device, estimates almost 60'000 people worldwide carrying a Vagus Nerve Stimulator for epilepsy or depression treatment.

First reports of vagal stimulation can be traced back in late 1883 in animal model by J.L. Corning but first human implant for clinical aims was described by Penry et al. in 1990. In the first 90s' a few studies with randomized controlled design for treatment of refractory epilepsy led to approval for clinical use of the device. First studies in TRE with partial onset seizures had an acute "dose finding" goal: in the US multicenter RCT study 54 patients intermittent high frequency stimulation (Current 0,25-3 mA; Freqency 20-50 Hz; Pulse Width 500 μsec; On time 30-90 sec; Off-time: 5-10 min) applied for 14 weeks led to a 24,5% reduction of mean seizure frequency, compared to previous 12 weeks of naturalistic observational phase of same sample, versus 6,1% mean seizures frequency reduction in 60 low frequency(Current 0,25-2,75 mA; Freqency 1-2 Hz; Pulse Width 130 μsec; On time 30 sec; Off-time: 60-180 min) stimulated patients observed with same design [VV.AA. 1995]. With an identical design, comparable results were published by the first international VNS study group in a european multicenter RCT, reporting statistically significant reduction in mean seizures frequency in VNS high frequency stimulation group compared both to baseline (same sample without VNS) and to VNS low frequency stimulation active arm, but no statistical significant reduction in seizures frequency in this last group compared to baseline [Ben-Menachem E, et al. 1994]. The same authors also produced a first report on tolerability and statistically significant side effects of VNS, distinguishing those limited to the periods in which the stimulator was actually delivering pulses (hoarseness, throat pain, and coughing) and other events that seemed to be VNS related (abdominal pain, nausea, shortness of breath, and chest pain)[Ramsay et al. 1994] and finally a follow-up study of 16-18 months after RCT phase, in an observational design, reporting increase in efficacy, achieving a 52.0% mean seizure frequency reduction as compared with baseline, with an unmodified tolerability profile over time [George R et al.1994].

Post marketing studies explored VNS application in patients with other forms of epilepsy and in longer term follow-up. The longest term data on a 12 years period in 48 TRE patients confirmed effectiveness of VNS, with a mean seizures frequency

reduction > 50% in 52% of cases at twelve years, especially remarking absence of tolerance phenomena over time [Uthman et al. 2004]. Morris and Mueller [1999] described 440 patients enrolled in previous studies with an open-label, long-term efficacy/tolerability observational design of 2-3 years: in this sample the largest part reached 50% seizures frequency reduction after 24 months showing an increase in efficacy over time, whereas adverse events became less common during the follow-up period. Low withdrawal rate was noted, even after battery depletion. This means that patients were willing to undergo renewed surgery to continue therapy. This is a clear indicator for the patient satisfaction concerning the therapy. For what concerns other forms of TRE, Labar et al [1999] studied 24 patients affected by generalized epilepsy demonstrating a mean reduction of 46% in seizure rate in the 3 months after VNS implantation compared with 1 month baseline before onset. Studies in paediatric population evaluated feasibility of VNS in Lennox-Gastaut syndrome or Lennox like epilepsy showing lower rates of response (after 24 months, a mean seizure reduction of 20.6% was found and four of the 19 patients had a seizure frequency reduction of > 50%) [Majoie et al. 2005] while others reported use of VNS in small case series of catastrophic epilepsy and status epilepticus [Zamponi et al. 2008] and refractory multifocal epilepsy [Blount et al. 2006].

In 2000 two studies started exploring effects on mood in TRE patients treated with VNS. Elger et al [2000] study considered a sample driven from EO3 study [Ben-Menachem et al. 1994] and compared psychiatric rating scales MADRS and SANS scoring in five patients of low intensity stimulation group versus six patients of high intensity stimulation group, at baseline, 3rd and 6th month of stimulation, finding a significant reduction in depressive symptoms after 3 and 6 months, which seemed independent both of seizure attenuation and of stimulation pattern. On the other hand Harden and colleagues [2000] compared 20 VNS patient with 20 epileptic patients in stable AED treatment, showing a significant decrease in depression rating scales in VNS group after three months stimulation, independent from seizures response, but not statistically significant changes in variance of depressive scores in the two groups over time. Beyond limits in sample-size and design of these studies, they may be considered the basis for next researches in proper mood effects of VNS

b)VNS: Clinical Data

After four years from FDA approval of VNS for TRD, John Rush, main author on this subject, reviewed in 2009 the principal studies that provided scientific basis for this application. An initial open label study **(D01)** enrolled 30 patients with non psychotic Major Depressive or Bipolar Disorder in a chronic index episode (> 2 years) or at least in the fourth MDE lifetime, which had not received satisfactory benefit from at least two adequate prior treatments in the current MDE. Twelve of them (≈ 40%) reached a Response defined as ≥50% reduction from baseline depressive scoring of HDRS[28] and MADRS and 17% Responded completely (HDRS score ≤ 10) after completion of 10 weeks of acute phase stimulation[Rush et al. 2000]. This sample was expanded to 59 patients in a later enlargement of multi-center enrolment, and beside a slight loss of significance in acute (10 weeks) VNS effect (Response rate 30,5% and Remission rate 15,3% according to HDRS scores reduction), a measure of improvement in functioning and quality of life, and a better definition of possible factors influencing VNS response was performed for the first time. Patients with history of "high" resistance, defined as more than 7 failed trials of antidepressant treatments in the index episode and/or failed response to ECT, were significantly less responsive to VNS instead of those who were resistant to more than three antidepressant but less than seven [Sackheim et al. 2001]. Data from one year follow-up of first 30 cases in Rush's study [Marangell et al. 2002] and from two year follow up of 59 cases in Sackheim's study [Nahas et al. 2005], confirmed the tendency to increase in VNS efficacy over time reporting 46% responders and 29% remitters at one year and 42% responders 22% remission at two years, respectively, and accordingly with loss of significance in sample enlargement. Moreover correlation with severity of resistance and response to VNS was confirmed in 1 year but not in two years follow-up. If considered that almost 39% of initial non responders showed substantial benefits at 24 months in Nahas et al. study, data from sustained follow-up confirm the possibility of gaining beneficial effects even in absence of early response, accordingly with data on treatment resistance epilepsy [Morris et al 1999].

Limits of this first series of study, as small sample size and naturalistic prospectic non randomized controlled design, led to **D02** study that was planned with a double blind randomized acute phase "sham" controlled design. Two hundred and twenty two Patients were enrolled, VNS implanted and studied in the acute phase, ten weeks after device activation. Patients were randomly and double blindly assigned to an

arm of VNS active stimulation or to Sham arm (VNS not turned on). Results in response rates (HDRS < 50% from baseline) were 15.2% for the 112 subjects of active VNS group and 10.0% for the 110 subjects of sham group; this difference didn't reach statistical significance but response rates of 17.0% and 7.3% for active and sham arm respectively, with a secondary outcome, the Inventory of Depressive Symptomatology –Self-Report (IDS-SR30), did. For ethical motivation it was not considerable to continue this design longer than the acute phase and at the end of ten weeks all sham participants were proposed active VNS. These results led to reconsideration of real acute effects of VNS, and authors attributed this resizing of response to sample differences (for example longer duration of illness, even if patients were enrolled if resistant to ≤6 antidepressants in index episode, and lower number of bipolar subjects than Sackeim [2001] study) and lower stimulation intensities applied in present study[Rush et al. 2005a]. Concomitantly the same authors published results from one year naturalistic follow up of almost all the same sample of D02 study (205 patients, 110 of those who continued active VNS stimulation and 95 of those that were "turned-on" from sham group) evidencing once more a significant but less striking percentage of response growing over time (55/202 = 27,2% of evaluable participants achieved a response at exit visit and 32/202 = 15.8% achieved a remission)[Rush et al 2005b]. Relevant aspects from this naturalistic observational study of the largest sample described nowadays of TRD patients VNS treated, are however reply of cases of manic/hypomanic switch that reinforces hypothesis of a direct antidepressant effect, and demonstration of progressive increase in efficacy and decrease in side effects along one year stimulation. Reinforcing "real world" limits of this study design, authors described 3 deaths, one due to suicide, 7 suicide attempts (4 of them were repeaters) and hospitalization in 30 patients, common events in patients with complicated mood disorders, but they excluded that this "hard to treat" population could have had a similar rate of improvement based on a placebo response or for other concomitant factors. For instance in VNS responders they described fewer medication changes or doses increases compared to VNS non responders.

To better define natural course of Treatment Resistant Depressed patient **D04** study was conceived comparing a cohort of 124 TRD patients treated as usual (TAU) and D02 cohort. Comparison of these group was made by George at al. [2005] and revealed that after 12 months the treatment as usual group's response rate was

significantly smaller with just 13% of the patients having a depression score reduction of 50% or more instead of 27% depressive scores reduction in VNS + TAU group. Even if two population were similar in principal demographic features, ECT exposure lifetime and for index episode was significantly higher in VNS+ TAU group while among TAU patients an higher number had a history of more than ten major depressive episodes lifetime. On the other hand responders to VNS+TAU had fewer dose increases or medication additions (56%, 31 of 55) than did VNS+TAU nonresponders (77%, 115 of 150) and more TAU responders had medication increases or additions (92%, 12 of 13) than did TAU nonresponders (80%, 87 of 109) highlighting the limited responsivity to pharmacological interventions of this patient population. Authors concluded that, even if quality of information driven from this study design is not comparable to those coming from a common RCT, and nonetheless ethical concerns in hypothesizing a long term RCT in VNS implanted patients were previously mentioned [Rush et al .2005 a], the relevance of difference in response of these two population should be understood in the context of the chronic, recurrent, treatment resistant nature of the sample TRD population. Finally follow up at two years was published in 2008 by Nierenberg and collegues, , with the primary outcome of evaluating differences in VNS response between Unipolar or Bipolar patients. Even if the last (12 Bip I e 13 Bip II) had less chronic index episodes, generally shorter but more frequently relapsing episodes lifetime, with a higher number of failed treatment/year and obviously a higher number of antidepressant-induced mania, authors confirmed results of one year data with almost 25% of responders.

For completeness **D03** Study were performed in European centres and comprehended Schlaepfer et al [2008] and Bajbuoj et al [2010] study, respectively acute (three months) and long (24 months) follow-up in a naturalistic observational design as original Rush 2000 and Sackeim 2001 studies of 74 initial subjects, finding percentage of responders of almost 50%. Authors explained this higher percentage of responders and remitters as linked to differences in baseline depression scores, higher stimulation parametrers, and differences in statistical methods, once again underlining limits of non randomized controlled trials.

What we actually know on effects in clinical population treated with Vagus Nerve Stimulation for Depression resistant to pharmacological therapy, may be summarized as follows:

- In acute (three months) data from the only RCT performed, don't confirm VNS efficacy in determining a higher percentage of response in TRD patients.
- In Open label Naturalistic Observational acute and long term (two years) trials, VNS, associated to usual treatment for depression, may lead to a 30% mean increase in number of responders and 15% mean increase in number of remitters.
- With VNS, percentage of responders is higher than in Treatment Resistant Depressed patients Treated as Usual and tends to increase over time, in contrast with natural course of disease, in which resistance to treatment if not contrasted by treatment increase or modification, is a predictor of relapse and worsening of functioning [Dunner et al 2006]
- In all studies a very low percentage of complication due to surgery and a low rate of discontinuation due to specific side effects of VNS reported in general a favourable tolerability profile.

Side effects commonly reported, a part from those correlated to surgical implant which generally resolved in two weeks, are hoarseness, cough, voice alteration, sore throat, neck pain and headache. These adverse events were generally mild and well tolerated, and most typically occurred when the generator was on. Application of VNS to left vagus nerve, made of 80% afferent fibers, relieved from main cardiac or gastrointestinal effects which very rarely reported. Changes in stimulation parameters permitted improvement in tolerability profile, and in main studies these side effects decrease over time. Table 1, adapted from O'Reardon and collegues [2006] summarizes frequency of side effects reported in main studies.

Tab 1: SUMMARY OF PRINCIPAL SIDE EFFECTS REPORTED IN MAIN ACUTE AND LONG TERM STUDIES OF VNS IN TRD POPULATION

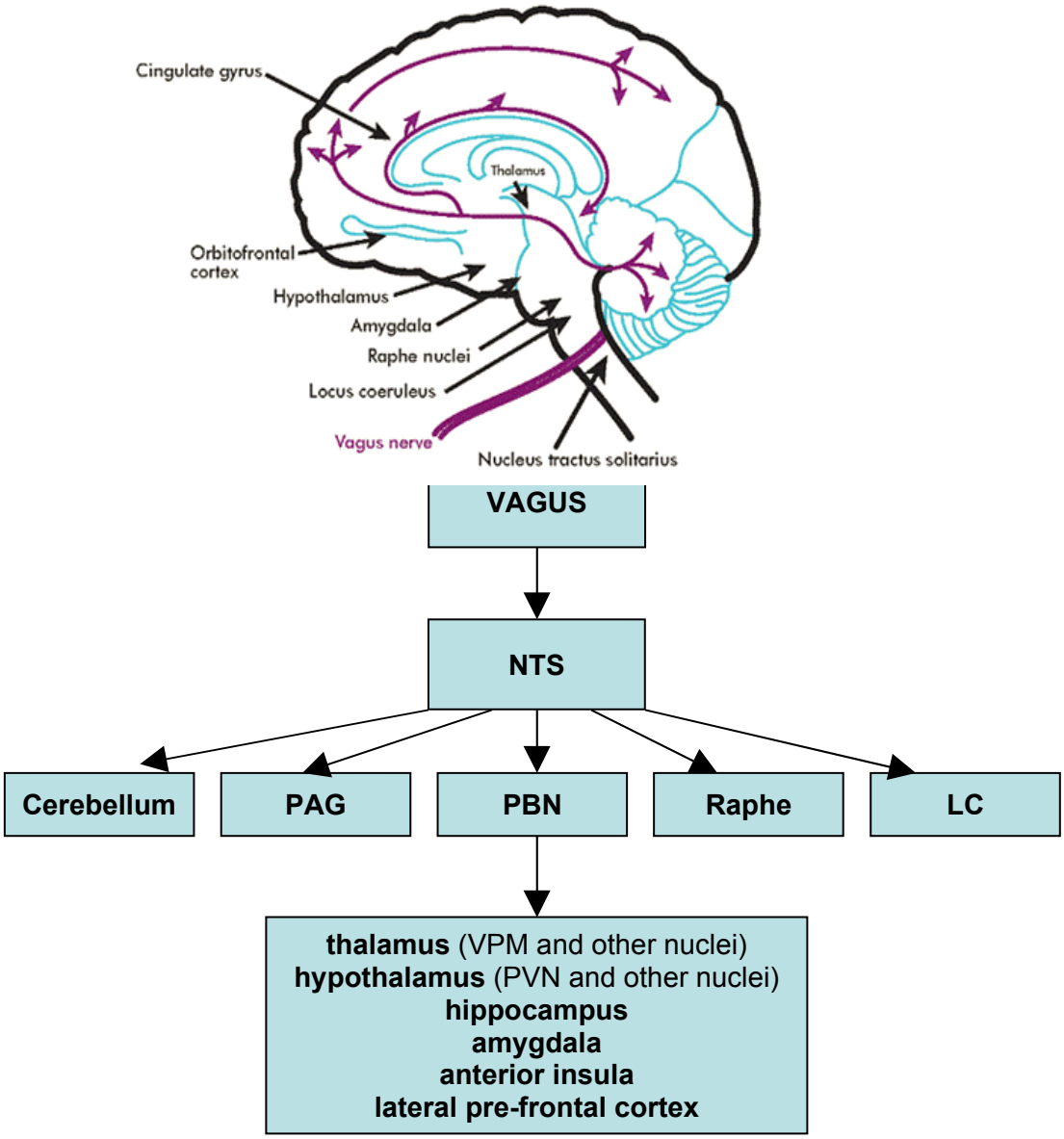
SIDE EFFECTS	SACKEIM ET AL,2001; N= 60; 10 WEEKS	RUSH ET AL, 2005; N= 119 10 WEEKS	RUSH ET AL, 2005 ; N=209 12 MONTHS	RUSH ET AL, 2005; N=59 24 MONTHS
Hoarsness	55%	68%	54%	27%
Cough	17%	29%	6%	--
Dyspnea	15%	23%	16%	8%
Neck Pain	17%	21%	13%	13%
Pain	13%	--	6%	--
Headache	22%	--	4%	--
Dysphagia	13%	21%	4%	--
Vomiting	--	21%	--	--
Nausea	7%	--	2%	--
Dyspepsia	10%	10%	16%	--
Palpitations	5%	5%	--	--
Paresthesiae	7%	16%	4%	--
Laryngismus	--	11%	5%	--
Pharyngitis	13%	--	5%	--

[O'Reardon et al, 2006]

c)VNS: Experimental Data

Neurobiological studies suggest that VNS might work through several putative antidepressant mechanisms, including its role as an anticonvulsant, or its ability to

directly modulate limbic structures known to be important in mood regulation [Henry 2002]. The vagus nerve is a large and predominantly afferent nerve (80%), with connections to the vagal nucleus in the brainstem, the nucleus tractus solitarius (NTS). Neuronal pathways tracking from the NTS in turn stimulate selective areas of the brain, many of which are involved in mood regulation, such as the amygdala, the hippocampus, and the locus coeruleus.



In addition to the neuroanatomic considerations, several lines of evidence provide the background for studies of VNS as therapy for treatment-resistant depression:

- ~ Known neuroanatomic projections of vagus nerve
- ~ Evidence of mood improvement in epilepsy studies
- ~ Neurochemical studies in animals and humans showing VNS effects on brain monoamines

- ~ The role of anticonvulsants (carbamazepine, valproic acid, and lamotrigine) and /or ECT (also an anticonvulsant) in treating mood disorders and EEG changes
- ~ Changes in the HPA axis
- ~ Effects on pro- and anti-inflammatory cytokines
- ~ Evidence by brain imaging studies that VNS affects the metabolism of limbic sructures relevant to mood regulation

Both clinical and animal studies indicate that VNS likely results in changes in serotonin [Ben-Menachem et al 1995], norepinephrine [Krahl et al 1998], GABA and glutamate [Walker et al 1999], which are all neurotransmitters implicated in the pathogenesis of major depression. VNS in animals activates the locus coeruleus, the main source of CNS norepinephrine-containing neuronal cell bodies [Naritoku et al 1995] In patients with epilepsy, VNS appears to increase 5-hydroxyindoleacetic acid, a metabolite of serotonin [Ben-Menachem et al 1995]. Carpenter et al [2004] evaluated CSF changes over time in 21 adults with treatment-resistant, recurrent or chronic major depression. Patients participated in the D-02 trial, and remained on stable regimens of mood medications. CSF collections were made at baseline (2 weeks after surgical implantation but before device activation), week 12 (end of the acute-phase study), and week 24. The authors failed to confirm serotonin metabolite changes, while, significant (mean 21%) VNS-associated increases in homovanillic acid (HVA) - a dopamine metabolite) were found. Mean CSF concentrations of norepinephrine (NE), 5-hydroxyindoleacetic acid (5-HIAA), 3-methoxy-4-hydroxyphenylglycol (MHPG) and GABA did not change significantly. Higher baseline HVA/5-HIAA ratio predicted worse clinical outcome. The two studies [Ben-Menachem et al 1995; Carpenter et al 2004] differed in their study subjects (epilepsy and depression), VNS parameters and concomitant medications, which might explain the discrepancy. Further studies are needed, but enough data have accumulated to date to conclude that VNS acts on norepinephrine and serotonin systems, and that this may be an important mechanism in explaining VNS antidepressant actions.

One study on the hypothalamic-pituitary-adrenal (HPA) axis stress system in chronic depression VNS treated patients, examined the corticotropin-releasing hormone (CRH) challenge test in a group of subjects with chronic depression (N=11), before and after 3 months of treatment with VNS, and a matched group of healthy control subjects (N=11)[O'Keane et al 2005]. Inclusion criteria were DSM-IV defined major depressive disorder, a history of current episode lasting for at least 2 years, and

unresponsiveness to at least two classes of antidepressant medications. There were significant reductions in depression scores over the study period. The CRH / ACTH (corticotropin-releasing hormone / adreno-corticotropic hormone) responses in the depressed group before VNS implantation were significantly higher than in the healthy group and were reduced to normal values after VNS treatment. Some measures of cortisol response were elevated before treatment and were reduced to normal over the study period

The only clinical measure correlated with HPA axis alterations was reduction in atypical depressive symptom scores. These preliminary results suggest that chronic depression, in contrast to acute melancholic depression, might be characterized by increased ACTH response to CRH challenge. Short-term treatment with VNS therapy was associated with normalization of this response. In this study, improvement was associated with probable altered secretion of CRH. This could have resulted from a direct stimulatory effect, transmitted from the vagus nerve through the NTS, to the paraventricular nucleus of the hypothalamus, resulting in altered CRH production and secretion. Alternatively, CRH secretion might have been modified through alterations in the central and peripheral immune system. Blood-borne proinflammatory cytokines seem to stimulate HPA activation through the vagus nerve, but the cytokine-induced HPA activation might also be centrally mediated.

Finally for what concerns functional imaging studies, in a preliminary SPECT (Single Photon Emission Computed Tomografy)study, Devous et al. [2002] compared 11 patients with TRD receiving VNS therapy (10 weeks) to healthy controls. Blood flow abnormalities were seen in limbic and cortical structures (insula, DLPFC, temporal cortex), structures known to be associated with depression. Moreover a correlation was found between HDRS scores and increased blood flow in the medial temporal cortex. Zobel et al. [2005] explored 12 patients with TRD at baseline and after 4 weeks of VNS. At this endpoint there was an increased rCBF in the left middle frontal gyrus, and reduced rCBF in the hippocampus/amygdala.

Hagen et al. [2003] presented preliminary results of Positron Emission Tomografy (PET) study of long-term VNS in a small group of 8 patients. After a year of VNS, a decreased metabolism was noted, compared to baseline, in the hypothalamus, middle and inferior frontal gyrus, insula/claustrum, superior temporal gyrus. Sheikh et al. [2003] found increased glucose metabolism, compared to baseline, in the

cerebellum. The regions of change were consistent with brain structures associated with depression. Finally, Conway et al. [2006] reported preliminary results for 4 patients with TRD who were scanned prior to VNS activation and after 12 weeks of VNS. An increased metabolic activity was reported in the orbitofrontal cortex, bilateral anterior cingulate cortex and right superior and medial frontal cortex, while decreases in rCBF were found in the bilateral temporal cortex and right parietal area. Functional Magnetic Resonance Imaging (fMRI) studies have demonstrated that changes in the parameters of the device such as frequency [Lomarevet al., 2002] or pulse width [Mu et al., 2004] produced dose-dependent modulatory effects on brain activity. Nahas et al. [2005] have shown that the impact of VNS on the brain varies with time. The changes induced by the VNS depend in the duration of exposure to VNS, especially in the medial/prefrontal limbic areas and therefore depend on the clinical improvements. Other factors such as depression level on scan day and intensity of the stimulation may influence the regional brain responses. The unmet medical needs in major depression, particularly in treatment-resistant forms, and the mechanism of action of VNS, provide a solid rationale for its use in clinical practice in those cases in which less invasive procedures have failed.

PART II

SPERIMENTAL STUDY

HYPOTHESIS

To authors' knowledge in recent literature on VNS, no clinical data were published on effects of VNS in brain structure of TRD patients. Our study aimed to find if Structural MRI analysis may highlight changes in brain regions involved in mood disorders, and especially if VNS may influence longitudinal modification in regions supposed to be target of its action. Moreover if clinical course of TRD may be related to changes in specific regions of interest for VNS action on mood regulation. The following results are driven from our pilot experience in a case series of TRD subjects VNS implanted, in an open label observational longitudinal design.

METHODOLOGY

Patients Selection

Since January 2008 all outpatients attending to Service for Affective Disorders in Psychiatry Clinics of Padua University in a TRD episode, were proposed for VNS intervention. The following Inclusion Criteria were used for enrollment:

- Outpatients ≥18 and ≤ 65 years of age of both sexes
- Diagnosis of Major Depressive Episode (MDE) Unipolar or Bipolar, according to DSM-IV TR diagnostic criteria derived from the Mini International Neuropsychiatric Interview(MINI-plus)
- Patients in a Chronic (≥ 2 years) current MDE and/or has had a history of Recurrent MDEs (at least 4 lifetime MDEs including the current MDE).
- Lack of acceptable Clinical Response due to failure (Resistance) with at least two treatments from different drug categories during the current MDE(SSRI, SNRI, TCAs, Mirtazapine, Trazodone, Reboxetine, Bupropion, Lithium, Carbamazepine, Lamotragine, ECT). Treatment Resistance will be measured by an Antidepressant Resistance Rating (ARR) score ≥ 3 using the modified version of the Antidepressant Treatment History Form (ATHF). [Sackeim, 2001].
- Patients with bipolar disorder with a demonstrated resistance to Lithium treatment or with medical contraindication to treatment with lithium, or known to be intolerant to lithium

- Score ≥18 on the 21 item Hamilton Rating Scale of Depression (HRSD).

Outpatients were not eligible if anyone of following Exclusion Criteria were met:

- Patients with a history of Schizophrenia, Schizoaffective or Delusional Disorders
- Patients currently having a secondary diagnosis of, or signs of, Delirium, Dementia, Amnestic or other Cognitive Disorders per DSM-IV TR.
- Patient having Alcohol or Substance Dependence or Abuse within the previous 12 months
- Patient with history of Suicide Attempts o Acute Suicidal Behaviour or Ideation
- Patients meeting the following "non Psychiatric" Exclusion Criteria to VNS device application:
  - History of neurosurgical intervention or CNS damage or disease; Neurological Disorders, apart from epilepsy; Cardiac Arithmias or other relevant Heart Diseases (Tested by Holter EKG); History of Vegetative Nervous System Disorders; History of Pulmonary Disorder; History of gastric ulcer; History of vasovagal syncope; Presence of monolateral unique vagal nerve; Presence of other concomitant brain stimulation devices
- Presence of pre-existing chronic Hoarseness

If eligible for VNS intervention and before informed consent was given, patients were monitored for clinical course of disease and optimization of pharmacological therapy for at least eight weeks. This time was necessary to educate patients on VNS procedure, efficacy and side effects ; on the other hand defining the most effective drug therapy was important to face the first three months of VNS (rump-up phase) with unmodified pharmacological therapy. Medication and dosage continued unmodified as long as possible after implantation, according to global clinical conditions, with the goal of full remission.

The following rating scales have been administered before VNS implantation, and at 3,6,9,12 months after device activation: HAM-D, MADRS,HAM-A, CGI, BDI, STAI, DAI-30. Following D-04 study model from George and collegues [2005] a sample of TRD patients Treated As Usual coupled for main demographic variables, were chosen to compare results from structural MRI study.

VNS intervention and follow-up

The Vagus Nerve Stimulation is delivered by a NeuroCybernetic Prosthesis (NCP) System (Cyberonics™, Houston). The device includes an implantable and multiprogrammable pulse generator that delivers electrical signals to the left vagus nerve (10th cranial nerve) via the bipolar lead. Left vagus nerve is composed of 80% afferent fibers, so that electrical impulse delivered at cervical level follows in orthodromal direction different regions of interest through vagus nerve nuclei. Moreover left application reduces the risk of interferences on vagal parasympathetic function on heart or gastroenteral function, that are mainly exploited by right vagus nerve. The pulse generator is programmed via a programming wand attached to a computer, which sets or adjusts stimulation parameters.

Surgical intervention for device application was performed in Neurosurgery Clinics of Padua University, by neurosurgeons experienced in performing surgery in the carotid sheath and trained in the surgical technique relating to implantation of the VNS Therapy System in pectoral region, and most of all in . Patients were hospitalized for 48 hours to perform pre-surgical routine examination, anaesthesiologic visit an MRI acquisition in baseline. Intervention took 1-2 hours in general anaesthesia; no surgical complications was observed and mean time to recover from surgical wounds was two weeks. After completion of the 2-week, postimplantation, single-blind"recovery period," the device was turned on with initial stimulation parameters of 0.25 mA, 20 or 30 Hz, and 500 msec, with stimulation on for 30 sec every 5 min. At this visit, the output current was increased gradually (in 0.25-mA increments) to allow accommodation to the stimulation until a comfortable tolerance level was reached. After a comfortably tolerated output current was attained, the patient left the clinic at these settings. Additional increases (in 0.25-mA steps) in output current were made anytime during the "stimulation adjustment period" over the next 2 weeks. Stimulation parameter settings were determined based on patient tolerance. Investigators were allowed by protocol a range of frequency (e.g., 20–30 Hz), pulse width (e.g., 250–500 msec), and on/off cycle parameters (e.g., off 3 or 5 min).

Fig 1. VNS implant

MRI study

MRI acquisition have been performed in baseline and after 12 months from VNS activation using a 1.5 T MRI system (Philips Medical Systems). Whole-brain T1-weighted three-dimensional images were acquired in the sagittal plane for volumetric measurements (time to repetition = 25 ms, time to echo = 4 ms, flip angle = 25°, field of view = 100 mm, slice thickness = 1.4 mm, matrix size = 256 × 192). The sagittal images were aligned approximately parallel to the anterior-posterior commissure line. Axial proton density and T2-weighted images were obtained to enable the exclusion of structural abnormalities on the MRI scan. The procedure was well tolerated by all subjects, and no sedation was used. During MRI study the device was deactivated and all stimulation parameters were lowered at minimum, while OFF time was set at 180 minutes. Brain MRI protocol for safe procedure in VNS implanted patient from Cyberonics was applied. The imaging data were transferred from the MRI unit to a PC workstation and analysed using the semi-automated software Analyze® (Mayo Clinic) using a stereologic method for detection of gray matter.

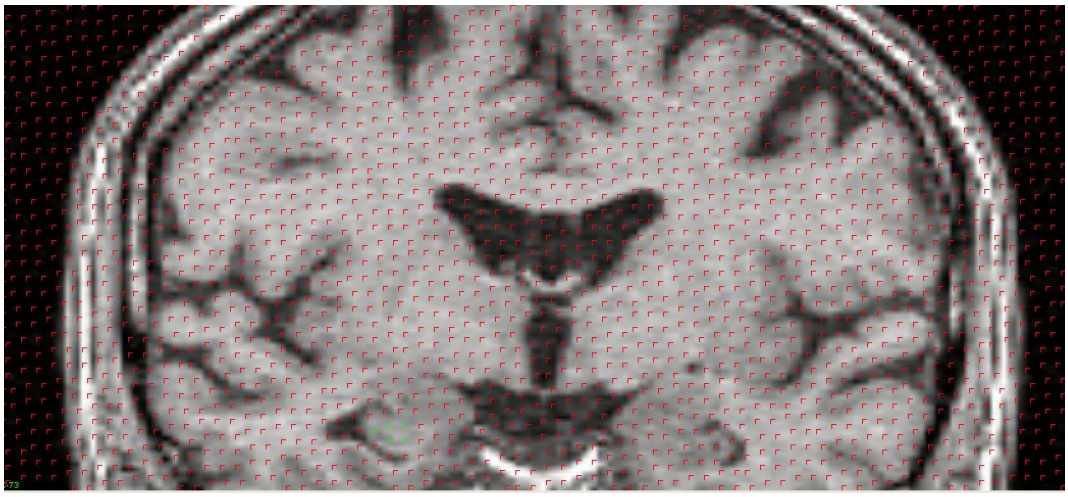


Figure 2: Anterior limit of hippocampus

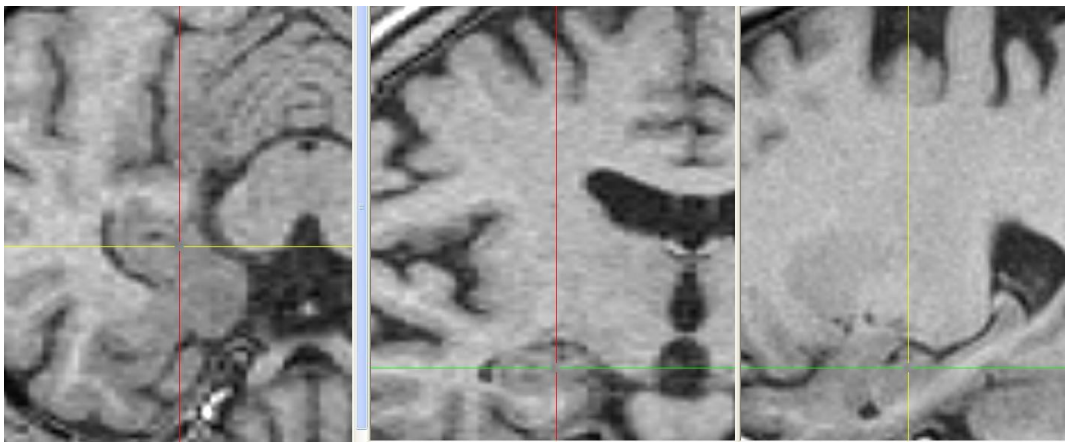


Figure 3: Hippocampus in the three axis

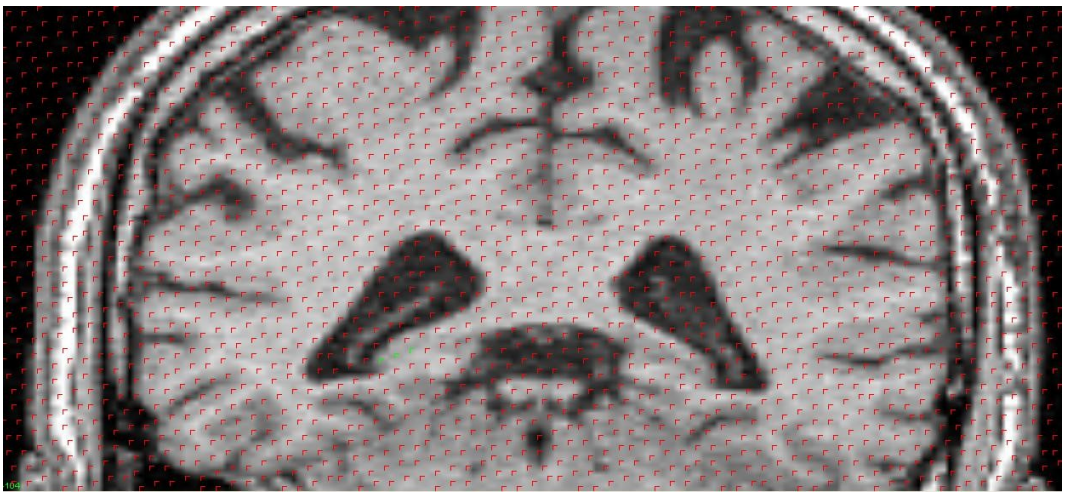


Figure 4: Posterior limit of hippocampus

Statistics

Given the small sample size all variables, a part from the discrete ones, were analyzed with non parametric models. Wilcoxon test was performed for comparison in test-re test analysis both for variables of clinical outcome and brain volumes in

VNS group. Comparison between groups was performed with Mann Whitney test, while correlation between clinical variables and brain volumes by mean of Spearman R coefficient.

RESULTS

Clinical Data

A total of 12 outpatients were evaluated for inclusion/exclusion criteria: 6 outpatients, 4 males and 2 females, were enrolled and, after giving informed consent, were implanted with VNS device. Two subjects were excluded for "non psychiatric" contraindication: atrial fibrillation was detected by means of Holter EKG during screening phase in a 64 years old female, whereas the second had a history of latero-cervical surgery for a previous lymphoma. One patient responded to changed medication, and two patients withdrew consent to intervention and follow-up. The

last patient is actually in the optimization of pharmacological treatment phase. Table n° 1 summarizes main demographic feature of the sample that gained VNS.

TAB 1: Demographic features of VNS sample

VNS Implanted Patients	*%*	*Mean ± SD*	*Median*	*Range*
Age		51,3 ± 9,5	52	40-64
Female	33% (2/6)			
MDD Recurrent	67% (4/6)			
Bipolar Symptoms	33% (2/6)			
Chronic episode	50% (3/6)			
Length of current MDE (months)		26 ± 23,1	24	4-60
Age at onset of Current MDE		46,2 ± 8,9	47	40-57
Length of illness (years)		22,3 ± 7,2	22	15-33
Age at onset of illness		29 ± 5,5	28	25-37

Table 2 summarizes main clinical features on course of disease and outcome with detail of the six VNS implanted cases.

TAB 2 : Detail of clinical course and outcome of VNS sample

	Sex	*Age*	*Length of illness (from 1st episode)*	*N° of Episodes*	*Length of current MDE (documented)*	*N° of AD lifetime (ATHF)*	*Duration of VNS*	*Baseline HAM-D*	*LOCF HAM-D*	*Time to Response or Remission*
Case 1	M	41	15 years	>7	5 years in a chronic episode	9	35 Months	38	9	Response at 6 months,no remission
Case 2	F	58	25 years	3	3 years in a chronic episode	8	29 Months	27	2	Remission at 3 months, sustained
Case 3	M	43	19 years	5	2 years in a chronic episode	6	19 Months	21	5	Remission at 3 months, Relapsed at 9 months
Case 4	M	49	15 years	5	6 Months	6	16 Months	20	7	Response at 3 months
Case 5	M	58	33 years	> 7	4 Months	7	13 Months	30	15	Partial Response at 3 months, Relapsed at 9 months
Case 6	F	52	27 years	4	6 Months	6	6 Months	18	12	Partial Response at 3 months

All patients met or exceeded eligibility criteria by failing at least two robust treatment trials in the current MDE according to the ATHF (Antidepressant Treatment History Form)[Sackeim et al 2000] . To qualify, the agent had to be used at doses with established efficacy for a sufficient period (e.g., at least 4 weeks) to establish that the agent was ineffective. Table 4 presents detail of pharmacological therapies assumed by 6 VNS implanted patients in the index episode. Before VNS each patient completed a mean of four trials with antidepressant monotherapies or in combination with other drugs mainly comprehending mood stabilizers or atypical antipsychotics.

Tab 4 Detail of therapies assumed by VNS patient during the study

Case 1	**Paroxetine**
Case 2	**Mianserine, Quetiapine, Venlafaxine**
Case 3	**Duloxetine Aripiprazole Valproic Acid**
Case 4	**Bupropion Valproic Acid Mirtazapin**
Case 5	**Bupropion Valproic Acid Mirtazapine**
Case 6	**Nortryptiline Mirtazapine Pramipexole Valproic Acid, Gabapentin**

Data of clinical outcome are presented in Figure 1 and 2 referring respectively to Hamilton rating scale for depression as main report from clinicians and Beck Depression Inventory as main self report scale.

Figure 1: Hamilton Depression Rating Scale

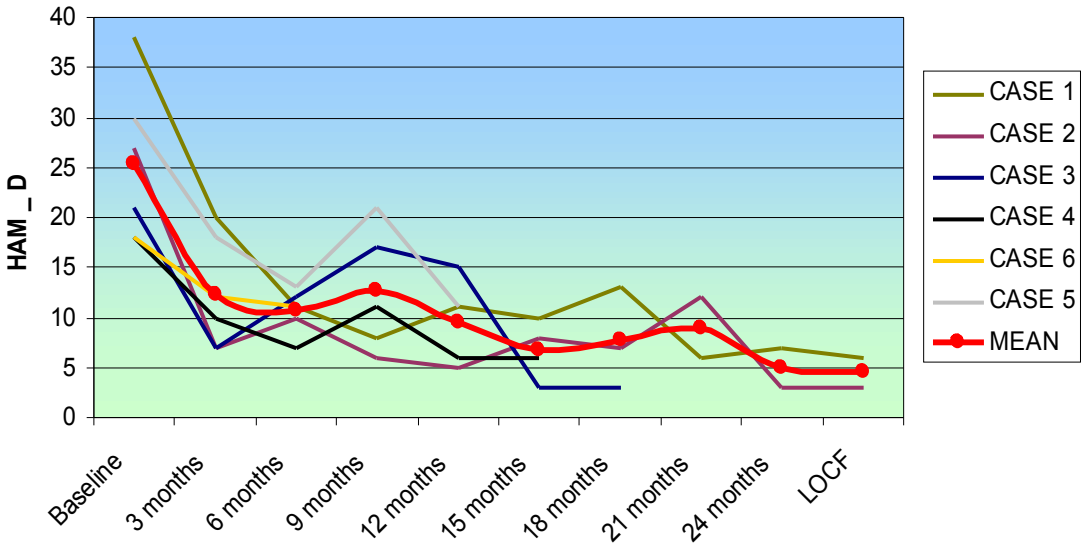
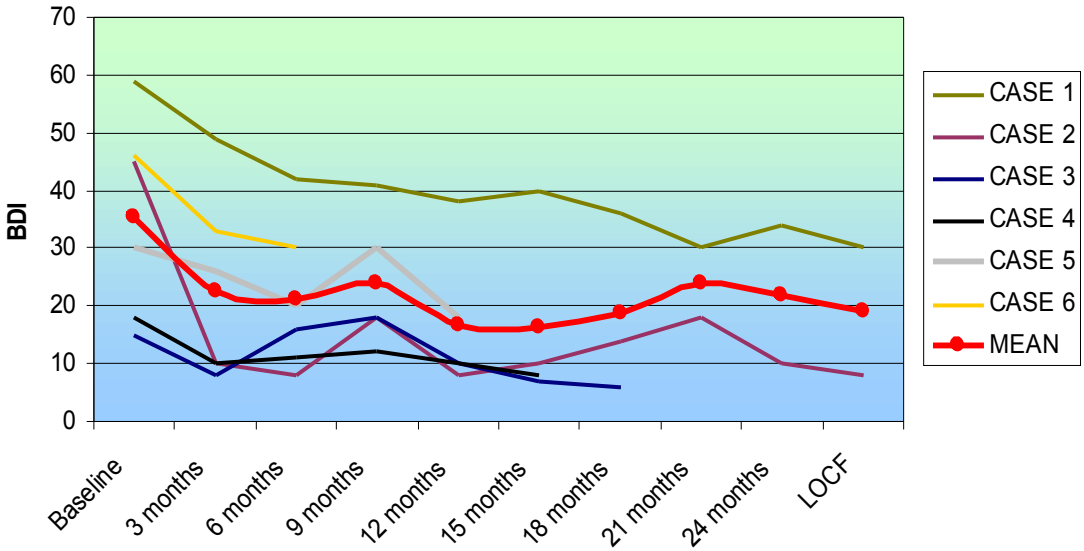


Figure 2: Beck Depression Inventory



All patients experienced substantial reduction in depressive symptoms after at least three months of VNS, in four out of the six cases reaching reduction from baseline depressive scores at HAM-D higher than 50%, qualifying them as responders, while last two patient implanted reached only a partial response. Patient 3 and patient 5 relapsed at 9 months from VNS activation and needed adjustment in pharmacological treatment, while increase of ratings for depression of patient 1 and patient 2 at month 15-18 and 21 respectively may be related to the attempt of reducing pharmacological burden motivated by sustained levels of response. Especially patient 2, in fact experienced a complete and sustained remission since the third month, that is even more remarkable if it is considered that this patient came from a three years chronic episode. Summarizing the outcome of treatment, three out of six (≈50%) patients showed a substantial response (case 1, 2 and 4) , one patient (case 3, Bipolar) responded but experienced a relapse of clinical relevance that needed adjustment in pharmacological treatment, and two out of six (≈30%) may be considered as non responders (case 5 and 6, even if the last is just in 6 months follow-up). Given the small size of the sample, but watching at the time course of disease in the follow up performed to date, it is possible to affirm that in the long term VNS reduced the whole burden of disease, not excluding the possibility of relapses. Patients reported the impression that when these happened their intensity tended to be less relevant and shorter in time, and this is confirmed by the fact that clinical management of relapses was faced with small changes in pharmacological treatments.

Most of the subjects observed were sent to consultation for the screening of inclusion/exclusion criteria by other clinicians external to our Clinic, that documented course of disease and if not eligible for VNS continued management of care. Among the four patients that were not eligible none gave consent to continue both clinical or neuroimaging follow-up, rather preferring to continue their treatment with mandatory clinician.

Data were compared with a sample of five TRD patients Treated As Usual (TAU) controls, following D-04 study model from George and collegues [2005]. These control group were chosen to compare MRI data of a sample with analogous clinical feature of disease not treated with VNS, and extracted from a data set of depressed studied for a previous work [Nifosì F et al 2010]. Table 5 summarizes main demographic and clinical data of these samples. No statistical differences, at t-test or

at Fisher exact test for continuous and discrete variables respectively, were found in the comparison of VNS group both with TRD treated as usual and healthy controls.

Tab.5: Demografic and Clinical features of Control Groups

	VNS				*TRD Treated as usual*			
	%	*Mean ± SD*	*Median*	*Range*	*%*	*Mean ± SD*	*Median*	*Range*
Age		51,3 ± 9,5	52	40-64		53,6 ± 15,5	59	26-64
Female	33% (2/6)				40%(2/5)			
MDD Recurrent	67% (4/6)				60%(3/5)			
Bipolar Symptoms	33% (2/6)				40%(2/5)			
Chronic episode	50% (3/6)				20%(1/5)			
Lenght of current MDE(months)		26 ± 23,1	24	4-60		29,4 ± 17	36	3-48
Age at onset of Current MDE		46,2 ± 8,9	47	40-57		51,2 ± 15,8	57	23-60
Lenght of illness(years)		22,3 ± 7,2	22	15-33		22,4 ± 12,5	21	9-40
Age at onset of illness		29 ± 5,5	28	25-37		31,5 ± 11,7	30	17-47

MRI results

Measurement of Whole Brain Volumes (WBV) and left and right Hippocampus gray matter for VNS group at baseline and after one year of follow-up, whereas a single image acquisition for TRD Treated As Usual were used for statistical analysis.

A statistically significant increase in volume of ≈14% and ≈22% in right and left Hippocampus respectively after one year of VNS treatment. If measures of hippocampal volumes were normalized on WBV increase of volume resulted significant only for left hippocampus. Data including *p*-levels form Wilcoxon test for non parametric repeated measures are reported in Table 6

Tab 6. Brain Volumes of VNS treated patients

Volumes are expressed In mm³	*Baseline (T0)*	*1 year (T1)*	*% Δ Volumes from baseline*	*p-level (Wilcoxon test)*
Whole Brain Volume (WBV)	1087003,000	1090218,500	0,29%	0,5
Right Hippocampus	2149,167	2453,673	14,17%	**0,043**
Left Hippocampus	2061,000	2531,308	22,08%	**0,043**
Right Hippocampus/ WBV%	0,198	0,225	13,63%	0,079
Left Hippocampus/ WBV%	0,191	0,234	22,51%	**0,043**

Detail of increase of hippcampi normalized on WBV in VNS implanted patient is presented in Table 7.

Tab.7: Detail of excursion in hippocampal volume in VNS implanted patient

	Right Hippocampus/WBV%	Left Hippocampus/WBV%	Δ Right hippo %	Δ Left hippo %
Case 1 T0	0,186	0,219	17,2 %	17,35 %
Case 1 T1	0,218	0,257	0,032	0,038
Case 2 T0	0,185	0,178	7,57 %	27,53 %
Case 2 T1	0,199	0,227	0,014	0,049
Case 3 T0	0,192	0,154	28,13%	61,03%
Case 3 T1	0,246	0,248	0,054	0,094
Case 4 T0	0,180	0,159	42,22%	40,25%
Case 4 T1	0,256	0,223	0,076	0,064
Case 5 T0	0,199	0,188	-6,03%	7,98%
Case 5 T1	0,187	0,203	-0,012	0,015
Case 6 T0	0,246	0,247	0 %	-1,62%
Case 6 T1	0,246	0,243	0	-0,004

Increase in hippocampi was found with different entities in patients 1, 2, 3 and 4, while patients 5 and 6 didn't increase or at least showed some decrease; interestingly, as previously reported these last two patients are those who didn't reach remission.
Correlations with clinical features and outcome were searched with non parametric Spearman R test and resulted in non significant correlation with rating scores for depression, but the only significant correlation was between Length of index episode and Normalized Volume of left Hippocampus after 1 year of VNS, which means paradoxically that patients with history of chronic or longer episode were those with bigger left hippocampus after one year (Spearman R= 0,9; p-value: 0,037)
Comparison with TRD treated as usual was methodologically limited by lack of MRI follow-up to 1 year in natural course of TRD treated as usual. However there was no significant difference in values of WBV and of Left and Right Hippocampus between baseline values of VNS and TRD, as presented in Table 7. This confirms that even if not randomly paired, the TAU group may be considered as a valid comparison. Actually follow up of TRD Treated as usual are ongoing.

TAB 7

Volumes expressed In mm^3	*Baseline (T0)*	*1 year (T1)*	*TRD treated as usual*	*p-level (U test Mann Whitney) T0vs TRD*	*p-level (U test Mann Whitney) T1 vs TRD*
Whole Brain Volume (WBV)	1087003,000	1090218,500	1026170,600	0,530870	0,210076
Right Hippocampus	2149,167	2453,673	1830,600	0,210076	0,060104
Left Hippocampus	2061,000	2531,308	1773,800	0,296271	**0,036715**
Right Hippo/ WBV%	0,198	0,225	0,180	0,916563	0,094694
Left Hippo/ WBV%	0,191	0,234	0,171	0,401966	**0,021177**

Based on the hypothesis of no evolution or at least decrease of hippocampus in normal course of depression, supported by data from Frodl and collegues [2004] that observed this in patients non remitting from depressive episode followed up to one year in natural setting, a primary and exploratory analysis was performed comparing T1 volumes of VNS with baseline volumes of TAU. This analysis demonstrated significantly higher volumes of left hippocampi both in absolute value (VNS: 2531,308 mm^3 vs TRD 1773,8 mm^3 ; Test U Mann-Whitney: *p*-value= 0,0367) both

in normalized value (VNS: 0,43 vs TRD: 0,171; Test U Mann-Whitney: *p*-value= 0,0211) in the VNS group. Data should be confirmed by real comparison with follow-up of patients treated as usual.

DISCUSSION

In this sample clinical efficacy and tolerability of VNS was in line with reports from its widespread use in TRD, with almost 50% of patients able to reach a clinically relevant response for depressive episode previously unresponsive to pharmacological therapies. Data from MRI study seem to confirm the primary hypothesis of a role of VNS in modulating structural changes in brain areas target of its action. A significant increase of 22% from baseline volume in left hippocampus was found, while in right hippocampus increase was significant but smaller (14% from baseline). First explanation of this asymmetry its obviously the specific laterality of VNS application on left Vagus nerve. This increase was not correlated to clinical outcome of depression response, nor to specific variables on course of disease (age at onset, length of illness, number of episodes, ATHF lifetime or in index episode). Baseline Hippocampal volumes resulted similar between VNS implanted patient and a sample of TRD patients with the same course of disease, and if hippocampal volume after one year of VNS was compared with TRD values, increase in left side resulted significant. This data was limited by lack of direct comparison to one year follow-up of controls. However if absolute values are considered and compared with normative values of hippocampal volumes driven from literature this data keeps its relevance. Normal volumes of right an left hippocampus in healthy subjects were studied in a sample of 619 healthy volouteers and distinguished in four classes of years from 40 to 90 years of age[Mu et al 1999]. Two main metanalysis of 2004 [Videbech and Ravnkilde, 2004; Campbell et al., 2004] and a recent one in 2009 [Mac Kinnon] reported volumes of left and right hippocampus in clinical population of depressed patients compared with healthy controls. Table 8 summarizes mean data derived from these studies.

Tab. 8: Summary of mean volumes of Hippocampus from principal studies in healthy and Depressed patients

	Depressed		***Healthy***	
	Left Hippocampus	***Right Hippocampus***	***Left Hippocampus***	***Right Hippocampus***

Mu et al . 1999	/	/	*Class of age* 40–60 61–70 71–80 81–90	*Vol. in cm3* 2.76 ± 0.08 2.40 ± 0.08 2.24 ± 0.08 2.08 ± 0.08	*Class of age* 40–60 61–70 71–80 81–90	*Vol. in cm3* 2.76 ± 0.08 2.41 ± 0.07 2.25 ± 0.08 2.10 ± 0.08
Campbell et al. 2004	2533,86 mm^3 (normalized on WBV=0,218)	2612,43 mm^3 (normalized on WBV=0,224)	2659,64 mm^3 (normalized on WBV=0,230)		2742,07 mm^3 (normalized on WBV=0,237)	
McKinnon et al. 2009	2764,92 mm^3 (normalized on WBV=0,245)	2838,59 mm^3 (normalized on WBV=0,252)	3055,7 mm^3 (normalized on WBV=0,227)		3017,96 mm^3 (normalized on WBV=0,224)	

Given methodological differences and different period to which they refer, especially for Mac Kinnon work in which more studies with 3 Tesla MRI, with consequent higher resolution of measures, were present, these data appear similar to those found in our VNS sample after treatment. Data from the study sample seem to indicate in fact that TRD patient tend to have lower hippocampal volumes, and the increase gained by the effect of VNS seems to take hippocampi to values comparable with normal population (see Tab 7 and 8). This finding seems to be reinforced by the work of Kempton [et al.2011] that in a recent metanalysis confirmed that patients with MDD in remission had a significantly larger hippocampal volume compared with patients who were currently depressed and there was no significant difference in volume between patients with remitted MDD and controls.

To authors' knowledge on literature on VNS in TRD patients, this is the first report of MRI structural changes in limbic structures, suggesting the possible role of VNS in increasing hippocampal volume. The real meaning of this finding should be carefully interpreted.

Reduction in hippocampal volumes of MDD patients compared to healthy controls has been widely replicated [McKinnon 2009, Campbell et al., 2004; Videbech and Ravnkilde, 2004, Steffens et al., 2000; Mervaala et al., 2000; Bremner et al., 2000; Frodl et al., 2002] especially in long duration and relapsing disease. Pathophisiology is linked to glucocorticoids mediating HPA stress response. They induce damage in HC by mechanisms that are only partially known. However, four processes have been suggested : 1) decreased glucose uptake with selective vulnerability of the

dentate gyrus to hypoglycaemia; 2) increased action of excitatory amino acids (glutamate), particularly on CA3 cells; 3) reduced neurotrophic factors (nerve growth factor-β and brain-derived neurotrophic factor); and 4) reduced neurogenesis [McKinnon, 2009, Szeszko,2006] Last one was largely established in both the rodent and primate hippocampus [Pittenger and Duman, Dranovsky and Hen, 2006], although no clear-cut data on a differential anterior-posterior gradient effect on neurogenesis are currently available. The literature supports a neuroendocrinological model of hypercortisolemic stress-related disorders in which the hippocampus may be the most vulnerable and neuroplastic structure of the brain [Duman and Monteggia 2006; Sapolsky, 2000a]. Moreover, the well-documented GC-mediated inhibition of neurogenesis may also explain the presence of hippocampal atrophy [McEwen, 2001]. Moreover it is widely demonstrated in preclinical models that antidepressant therapies increase proliferation of new neurons especially in the Dentate Gyrus of hippocampus. But the question of what role may be played by treatment in vivo samples is still open. Most of the studies of structural MRI for volumetric analysis compared MDD patients with healthy controls in a cross-sectional scan in order to detect a marker of disease. Most of the review and metanalysis in fact underline that the real role of treatment is considered more frequently as a confounding factor in interpretation of results [Campbell et al., 2004; Videbech and Ravnkilde, 2004], for one of the main objection is thatlong term treatment may have induced a recovery in hippocampal volume, starting from preclinical evidence of neurotrophic effect of antidepressants. On the other hands metanalysis converged not uniformly on affirming that hippocampal volumes were significantly reduced in population with long history of disease [Mac Kinnon et al 2009] assuming that at least repeated episode or chronic episode are necessary to produce a detectable loss in hippocampal gray matter. Studies investigating hippocampal volumes in first depressive episodes confirmed that first-episode patients had hippocampal volume comparable to controls, while patient with multiple-episode had smaller than firs episode and controls hippocampi [Mc Queen et al 2003].

Extending these notion to TRD, one could argue that hippocampal volumes should always be reduced in TRD. Actually specific data on MRI study of hippocampus in clinical population of TRD patient are scant. Smaller right hippacampal volumes before treatment in female non responders to fluoxetine assumed at 20 mg/day for

eight weeks was found in comparison to responderes by Vakili and collegues [2000]. In TRD patients ,MRI studies in cross sectional design could result even more complex than in common MDD patients. The construct itself of TRD however implies follow up along with time to confirm resistance, and may hardly be described by a cross sectional study, moreover if treated with VNS, which effects are known to have a latency. To our knowledge only three longitudinal studies with repeated MRI scans of the same sample [Frodl et al 2004; Frodl et al 2008 ; Ahdidan J et al 2011] Frodl and collegues [2004] reported that left and right hippocampal volumes were reduced at baseline and after one year follow-up in patients who were unresponsive to antidepressant treatment compared both to remitters and to healthy controls. This is consistent with observation from our sample in which patient that showed no response (case 5 and 6) were those whose hippocampi didn't change at all. In another study of three years follow-up of thirty MDD patients compared to thirty controls, same authors found that neither hippocampal nor amygdala volumes changed significantly among patients or participants in the control group. However, in the subgroup of patients who took antidepressants over the full 3 years, the left hippocampal volumes increased significantly. Patients with small hippocampal volumes and previous depressive episodes had a worse clinical outcome compared with patients with large hippocampal volumes and previous depressive episodes. These observation seem to confirm that small hippocampal volume could be considered a biological marker of non response. Finally the longest longitudinal study has been recently published and presents data from an 11 years follow up . Interestingly this report described smaller volumes of hippocampi and other regions of interest in depressed compared to controls at baseline, but these changes were not found at follow-up 11 years later. Moreover, these changes did not significantly correlate with the illness outcome. Authors concluded that brain structure changes seem to be state dependent in major depression, only occurring in acute episode of major depression and normalizing after remission. However a limit of this study was that only remitted patients were analyzed at follow-up [Ahdidan J et al 2011].

Even if limited by small sample size an lack of follow up in control group of TRD Treated as usual, data from this first study on effects of Vagus Nerve Stimulation on brain structure are inline with result from main studies of MRI volumetry that indicate reduced hippocampal volumes as a possible marker of non response. In the longitudinal observation the increase of volume surely is intriguing and is in line

with the neurogenesis hypothesis of depression [Aimone et al 2010; Steffens et al 2011]. Preclinical data from Reveszs [et al 2008] demonstrated neurogenesis in rat treated with VNS and effect of electrical stimulation has been demonstrated to reinforce neurogenesis [Duman et al 1996; Santarelli 2003]. A recent study confirmed increase in hippocampal volume studied with MRI one week before and one week after ECT [Nordanskog et al 2010]. However the lack of correlation with clinical outcome seems to indicate that as proposed by Aimone [2010] one possibility is that antidepressant action is delivered through neurogenesis-dependent and neurogenesis-independent mechanisms [David et al 2009]. VNS may represent a factor that determines associated with conventional pharmacological therapies an adjuvant factor activating both of this factor.

To authors opinion structural MRI, compared to other brain imaging methods (fMRI, PET) describing changes in brain function that take place in a short time , applied in this peculiar population, in which long term changes are expected may highlight more findings relevant to better understand Vagus Nerve Stimulation and contribute to better define pathophisiology of Depression.

REFERENCES

AHCPR Publication No. 93-0550 *Depression Guideline Panel: Depression in Primary Care: Volume 1. Detection and Diagnosis. (Clinical Guideline No. 5)*Rockville, MD: U.S. Department of Health and Human Services, Public Health Service, Agency for Health Care Policy and Research, 1993a

AHCPR Publication No. 93-0551 *Depression Guideline Panel: Depression in Primary Care: Volume 2. Treatment of Major Depression (Clinical Guideline No. 5)*. Rockville, MD: U.S. Department of Health and Human Services, Public Health Service, Agency for Health Care Policy and Research, 1993b

Ahdidan J, Hviid L. B.,Chakravarty M. M., Ravnkilde B., Rosenberg R., Rodell A., Stødkilde-Jørgensen H., Videbech P. *Longitudinal MR study of brain structure and hippocampus volume in major depressive disorder.* Acta Psychiatr Scand 2011: 123: 211–219

Aimone JB, Wei Deng W and Gage FH. *Adult neurogenesis: integrating theories and separating functions*. Trends in Cognitive Sciences 14 (2010) 325–337

Bajbouj M , Merkl A, et al. *Two-Year Outcome of Vagus Nerve Stimulation in Treatment-Resistant Depression.* J Clin Psychopharmacol 2010;30: 273-281

Ben-Menachem E, Mañon-Espaillat R, Ristanovic R, Wilder BJ, Stefan H, Mirza W, Tarver WB, Wernicke JF. *Vagus nerve stimulation for treatment of partial seizures: 1. A controlled study of effect on seizures. First International Vagus Nerve Stimulation Study Group.* Epilepsia. 1994 May-Jun;35(3):616-26

Ben-Menachem E, Hamberger A, Hedner T, et al. *Effects of vagus nerve stimulation on amino acids and other metabolites in the CSF of patients with partial seizures.* Epilepsy Res. 1995;20:221-7.

Blount JP, Tubbs RS, Kankirawatana P, et al. *Vagus nerve stimulation in children less than 5 years old.* Childs Nerv Syst. 2006;22:1167–1169.

Bremner J.D., Narayan M., Anderson E.R., Staib L.H., Miller H.L., Charney D.S., 2000. *Hippocampal volume reduction in major depression*. The american journal psychiatry 157, 115–118.

Carpenter LL, Moreno FA, Kling MA, et al. *Effect of vagus nerve stimulation on cerebrospinal fluid monoamine metabolites, norepinephrine, and gamma-aminobutyric acid concentrations in depressed patients*. Biol Psychiatry. 2004;56:418-26.

Conway, C.R., Sheline, Y.I., Chibnall, J.T., George,M.S., Fletcher, J.W., Mintun, M.A., 2006. *Cerebral blood flow changes during vagus nerve stimulation for depression*. Psychiatr. Res. 146, 179–184.

Crismon, M.L., Trivedi, M., Pigott, T.A., Rush, A.J., Hirschfeld, R.M., Kahn, D.A., DeBattista, C., Nelson, J.C., Nierenberg, A.A., Sackeim, H.A., Thase, M.E., *Texas Consensus Conference Panel on Medication Treatment of Major Depressive Disorder, The Texas medication algorithm project: report of the Texas consensus conference panel on medication treatment of major depressive disorder*. J. Clin. Psychiatry 1999.60, 142–156.

David, D.J. et al. (2009) *Neurogenesis-dependent and –independent effects of fluoxetine in an animal model of anxiety/depression*. Neuron 62, 479–493

Devous, M.D., Husain, M., Harris, T.S., Rush, A.J., 2002. *Effects of VNS on regional cerebral blood flow in depressed subjects*. Poster Presented at the 42nd Annual New Clinical Drug Evaluation Unit Meeting Boca Raton, FL, June 10–13.

Dranovsky A., Hen R., 2006. *Hippocampal neurogenesis: regulation by stress and antidepressants.* Biological psychiatry. 59, 1136–43.

Duman R.S., Monteggia L.M., 2006. *A neurotrophic model for stress-related mood disorders.* Biological psychiatry 59, 1116–1127.

Dunner, D.L., Rush, A.J., Russell, J.M., Burke, M.,Woodard, S., Wingard, P., Allen, J. P*rospective, long-term, multicenter study of the naturalistic outcomes of patients with treatment-resistant depression.* J. Clin. Psychiatry 2006. 67, 688–695.

Elger G, Hoppe C, Falkai P, et al. *Vagus nerve stimulation is associated with mood improvements in epilepsy patients*. *Epilepsy Res.* 2000;42:203–210.

Fava M, Davidson KG. *Definition and epidemiology of treatment resistant depression.* Psych Clin North Am 1996. 19(2) 179-200

Fava M. *Diagnosis and definition of treatment-resistant depression*. Biol Psvchiatry 2003;53:649-659

Frodl T., Meisenzahl E.M., Zetzsche T., Born C., Groll C., Jager M., Leinsinger G., Bottlender R., Hahn K., Möller H.J.,. *Hippocampal changes in patients with a first episode of major depression*. The american journal psychiatry 2002;159, 1112–1118.

Frodl T, Meisenzahl EM, Zetzsche T et al. *Hippocampal and amygdala changes in patients with major depressive disorder and healthy controls during a 1-year follow-up.* J Clin Psychiatry 2004;65:492–499.

Frodl T, Jager M, Smajstrlova I et al. *Effect of hippocampal and amygdala volumes on clinical outcomes in major depression: a 3-year prospective magnetic resonance imaging study.* J Psychiatry Neurosci 2008;33:423–430.

George, M.S., Rush, A.J., Marangell, L.B., Sackeim, H.A., Brannan, S.K., Davis, S.M., Howland, R., Kling, M.A., Moreno, F., Rittberg, B., Dunner, D., Schwartz, T., Carpenter, L., Burke, M., Ninan, P., Goodnick, P., *A one-year comparison of vagus nerve stimulation with treatment as usual for treatment-resistant depression.* Biol. Psychiatry. 2005.58, 364–373.

George MS, Sackeim HA. *Brain Stimulation, Revolutions, and the Shifting Time Domain of Depression* Biol Psychiatry 2008;64:447–448

George R, Salinsky M, Kuzniecky R, Rosenfeld W, Bergen D, Tarver WB, Wernicke JF. *Vagus nerve stimulation for treatment of partial seizures: 3. Long-term follow-up on first 67 patients exiting a controlled study. First International Vagus Nerve Stimulation Study Group*. Epilepsia. 1994 May-Jun;35(3):637-43

Groves A.D. and Brown V.J., *Vagus nerve stimulation: a review of its applications and potential mechanisms that mediate its clinical effects* Neurosci biobehav rev 2005

Hagen, M.C., Sheikh, J.S., Adson, D., Rittberg, B., Abuzzahab, F.S., Lee, J.T., Pardo, J.V., *Metabolic changes in treatmentresistant depression responsive to VNS therapy*. 2003.Poster Presented at the 58th Annual Scientific Convention of the Society of Biological Psychiatry, San Francisco, CA, May 15–17.

Henry TR. *Therapeutic mechanisms of vagus nerve stimulation.* Neurology 2002;59(Suppl 4):S3–S14

Harden CL, Pulver MC, Ravdin LD, et al. *A pilot study of mood in epilepsy patients treated with vagus nerve stimulation.* Epilepsy Behav. 2000;1:93–99.

Kessler, R.C., Berglund, P., Demler, O., Jin, R., Koretz, D., Merikangas, K.R., Rush, A.J., Walters, E.E., Wang, P.S.,. *The epidemiology of major depressive disorder:*

results from the National Comorbidity Survey Replication (NCS-R). JAMA. 2003; 289, 3095–3105.

Krahl SE, Clark KB, Smith DC, Browning RA. *Locus coeruleus lesions suppress the seizure-attenuating effects of vagus nerve stimulation*. Epilepsia. 1998;39:709-14.

Labar D, Murphy J, Tecoma E. *Vagus nerve stimulation for medication-resistant generalized epilepsy.* E04 VNS Study Group. Neurology. 1999;52:1510–1512.

Lomarev, M., Denslow, S., Nahas, Z., Chae, J.H., George, M.S., Bohning, D.E., 2002. *Vagus nerve stimulation (VNS) synchronized BOLD fMRI suggests that VNS in depressed adults has frequency/dose dependent effects*. J. Psychiatr. Res. 36, 219–227.

Mckinnon MC, Yucel K, Nazarov A, Macqueen GM. *A meta-analysis examining clinical predictors of hippocampal volume in patients with major depressive disorder.* J Psychiatry Neurosci 2009;34:41–54.

MacQueen GM, Campbell S, McEwen BS, et al. *Course of illness, hippocampal function, and hippocampal volume in major depression.* Proc Natl Acad Sci U S A 2003;100:1387-92.

Majoie HJ, Berfelo MW, Aldenkamp AP, et al. *Vagus nerve stimulation in patients with catastrophic childhood epilepsy, a 2-year follow-up study*. Seizure. 2005;14:10 –18.

Marangell, L.B., Rush, A.J., George, M.S., Sackeim, H.A., Johnson, C.R., Husain, M.M., Nahas, Z, Lisanby, S.H.,. *Vagus nerve stimulation (VNS) for major depressive episodes one year outcomes.* Biol. Psychiatry (2002) 51, 280–287.

Mervaala E., Fohr J., Kononen M., Valkonen-Korhonen M., Vainio P., Partanen K., Partanen J., Tiihonen H., Viinamaki H., Karjalainen A.K., Lehtonen J.,. *Quantitative MRI of the hippocampus and amygdala in severe depression.* Psychological medicine 2000; 30, 117–125.

Morris GL III, Mueller WM. *Long-term treatment with vagus nerve stimulation in patients with refractory epilepsy. The Vagus Nerve Stimulation Study Group* E01–E05. *Neurology.* 1999;53:1731–1735.

Mu, Q., Bohning, D.E., Nahas, Z., Walker, J., Anderson, B., Johnson, K.A., Denslow, S., Lomarev, M., Moghadam, P., Chae, J.H., George, M.S., 2004. *Acute vagus nerve stimulation using different pulse widths produces varying brain effects*. Biol. Psychiatry. 55, 816–825.

Murray, C.J., Lopez, A.D.,. *Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study.* Lancet 1997; 349, 1498–1504.

Nahas, Z., Marangell, L.B., Husain, M.M., Rush, A.J., Sackeim, H.A., Lisanby, S.H.., Martinez, J.M., George, M.S.,. *Two-year outcome for vagus nerve stimulation*

(VNS) of treatment of major depressive episodes. J. Clin. Psychiatry 2005 (66) 1097–1104.

Naritoku DK, Terry WJ, Helfert RH. *Regional induction of fos immunoreactivity in the brain by anticonvulsant stimulation of the vagus nerve.* Epilepsy Res. 1995;22:53-62.

Niernberg AA, De Cecco LM. *Definition of Antidepressant Treatment Response, Remission, Nonresponse, Partial Response and other Relevant Outcome: a Focus on Treatment Resistant Depression.* J Clin Psychiatry 2001; 62 (Suppl 16)5-9

Nierenberg AA, Alpert JE, Gardner-Schuster EE, et al. *Vagus nerve stimulation: 2-year outcomes for bipolar versus unipolar treatment-resistant depression*. Biol Psychiatry. 2008;64:455– 460.

Nifosì F, Toffanin T, Follador H, Zonta F, Padovan G, Pigato G, Carollo C, Ermani M, Amistà P, Perini GI. *Reduced right posterior hippocampal volume in women with recurrent familial pure depressive disorder* Psychiatry Research: Neuroimaging (2010): 184: 23–28

Nordanskog P, Dahlstrand U, Larsson MR, Larsson EM, Knutsson L, Johanson A. *Increase in hippocampal volume after electroconvulsive therapy in patients with depression: a volumetric magnetic resonance imaging study*. J ECT. 2010 Mar;26(1):62-7.

O'Reardon J.P. et al., *Vagus nerve stimulation (VNS) and treatment of depression: to the brainstem and beyond,* Psychiatry 2006

G.B. Parker, G.S. Malhia, J.G. Crawford, M.E. Thase. *Identifying paradigm failures contributing to treatment-resistant depression*. Journal of Affective Disorders 87 (2005) 185– 191

Penry JK, Dean JC. *Prevention of intractable partial seizures by intermittent vagal stimulation in humans: preliminary results*. Epilepsia 1990;31(suppl2):S40-S43.

Pittenger C., Duman R.S., 2008. *Stress, depression, and neuroplasticity: a convergence of mechanisms.* Neuropsychopharmacology 33,

Ramsay RE, Uthman BM, Augustinsson LE, Upton AR, Naritoku D, Willis J, Treig T, Barolat G, Wernicke JF *Vagus nerve stimulation for treatment of partial seizures: 2. Safety, side effects, and tolerability. First International Vagus Nerve Stimulation Study Group.* Epilepsia. 1994 May-Jun;35(3):627-36.

Rush AJ, George MS, Sackeim HA, et al. *Vagus nerve stimulation (VNS) for treatment-resistant depressions: a multicenter study.* Biol Psychiatry. 2000; 47:276 – 286.

Rush, A.J., Marangell, L.B., Sackeim, H.A., George, M.S., Brannan, S.K., Davis, S.M., Howland, R., Kling, M.A., Rittberg, B.R., Burke, W.J., Rapaport, M.H., Zajecka, J., Nierenberg, A.A., Husain, M.M., Ginsberg, D., Cooke, R.G. *Vagus*

nerve stimulation for treatment-resistant depression: a randomized, controlled acute phase trial. Biol. Psychiatry. (2005a)58, 347–354.

Rush AJ, Sackeim HA, Marangell LB, et al. *Effects of 12 months of vagus nerve stimulation in treatment-resistant depression: a naturalistic study*. Biol Psychiatry.2005b;58:355–363.

Rush AJ et al. *Acute and Longer-Term Outcomes in Depressed Outpatients Requiring One or Several Treatment Steps: A STAR*D Report*. Am J Psychiatry 2006; 163:1905–1917

Rush, A.J., Trivedi, M.H., Wisniewski, S.R., Stewart, J.W., Nierenberg, A.A., Thase, M.E., Ritz, L., Biggs, M.M., Warden, D., Luther, J.F., Shores-Wilson, K., Niederehe, G., Fava M., STAR*D Study Team,. *Bupropion-SR, sertraline, or venlafaxine-XR after failure of SSRIs for depression.* N. Engl. J. Med 2006b. 354, 1231–1242.

Rush A. J., Siefert S.E. *Clinical issues in considering vagus nerve stimulation for treatment-resistant depression*. Experimental Neurology 219 (2009) 36–43

Rush AJ, Warden D, Wisniewski SR, Fava M, Trivedi MH, Gaynes BN, Nierenberg AA. *STAR*D: revising conventional wisdom*. CNS Drugs. 2009a Aug 1;23(8):627-47.

Sackeim, H.A. *The definition and meaning of treatment-resistant depression*. J.Clin. Psychiatry 2001.62 (suppl 16), 10–17.

Sackeim HA, Rush AJ, George MS, et al. *Vagus nerve stimulation (VNS) for treatment- resistant depression: efficacy, side effects, and predictors of outcome.* Neuropsychopharmacology. 2001 ;25:713–728.

Samuels BA, and Hen R. *Neurogenesis and affective disorders* European Journal of Neuroscience, Vol. 33, pp. 1152–1159, 2011

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R. *Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants*. Science. 2003 Aug 8;301(5634):805-9.

Sapolsky R.M., 2000 b. *The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death*. Biological psychiatry 48, 755–765.

Sapolsky R.M., 2000a. *Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders.* Archives of general psychiatry 57, 925–935.
Sheikh, S., Hagen, M.C., Adson, D., Rittberg, B., Abuzzahab, F.S., Lee, J.T., Pardo, J.V., 2003. *Effects of VNS therapy on brain metabolism in severe, chronic treatment-resistant depression: oneyear outcome*. Poster Presented at the 58th Annual Scientific Convention of the Society of Biological Psychiatry, San Francisco, CA, May 15–17.

Schlaepfer, T.E., Frick, C., Zobel, A., Maier, W., Heuser, I., Bajbouj, M., O'Keane, V., Corcoran, C., Adolfsson, R., Trimble, M., Rau, H., Hoff, H.J., Padberg, F., Mülloer- Siecheneder, F., Audenaert, K., Van den Abbeele, D., Stanga, Z.,

Hasdemir, M.,. *Vagus nerve stimulation for depression: efficacy and safety in a European study.* Psychol. Med. 2008. 38, 651–661.

Souery D, Amsterdam J, de Montigny C, Lecrubier Y, Montgomery S, Lipp O, Racagni G, Zohar J, Mendlewicz J. *Treatment resistant depression: methodological overview and operational criteria.* Eur Neuropsychopharmacol. 1999 ;9(1-2):83-91.

Sourey D, Papakostas GI, Trivedi MH. *Treatment Resistant Depression*. J Clin Psychiatry 2006: 67 (suppl 6); 16-22

Steffens D.C., Byrum C.E., McQuoid D.R., Greenberg D.L., Payne M.E., Blitchington T.F.,. MacFall J.R., Krishnan K.R., *Hippocampal volume in geriatric depression.* Biological psychiatry 2000.48, 301–309.

Surget, A. et al. (2008) *Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal*.Biol. Psychiatry 64, 293–301

Uthman BM, Reichl AM, Dean JC, et al. *Effectiveness of vagus nerve stimulation in epilepsy patients: a 12-year observation.* Neurology. 2004;63:1124 –1126.

Szeszko PR, Betensky JD, Mentschel C, Gunduz-Bruce H, Lencz T, et al. *Increased stress and smaller anterior hippocampal volume.* Neuroreport 2006;17(17):1825-8

Thase M.E., Rush A.L., *When at first you don't succeed: sequential strategies for antidepressant nonresponders.* J Clin Psychiatry, 1997; 58 (Suppl 13): 23-29

Trivedi MH, Rush AJ, Wisniewski SR, et al. *Evaluation of outcomes with citalopram for depression using measurement- based care in STAR*D: implications for clinical practice*. Am J Psychiatry 2006 Jan; 163 (1): 28-40

VV.AA. The European Agency for the Evaluation of Medicinal Products Committee for Proprietary Medicinal Products. *Note for guidance on clinical investigation of medicinal products in the treatment of depression.* Available at: http://www.emea.eu.int/pdfs/human/ewp/051897en.pdf. Accessed Dec 21, 2005

VV.AA. "The Vagus Nerve Stimulation Study Group". *A randomized controlled trial of chronic vagus nerve stimulationfor treatment of medically intractable seizures.* Neurology 1995;45:224-230

Walker BR, Easton A, Gale K. *Regulation of limbic motor seizures by GABA and glutamate transmission in nucleus tractus solitarius.* Epilepsia. 1999;40:1051-1057.

Warden D, Rush AJ, Trivedi MH, Fava M, Wisniewski SR. *The STAR*D Project results: a comprehensive review of findings.* Curr Psychiatry Rep. 2007 Dec;9(6):449-59.

Zamponi N, Rychlicki F, Corpaci L, et al. *Vagus nerve stimulation (VNS) is effective in treating catastrophic 1 epilepsy in very young children.* Neurosurg Rev. 2008;31:291–297.

Zobel,A., Joe,A., Freymann,N., Clusmann,H., Schramm, J., Reinhardt, M., Biersack, H.J.,Maier,W., Broich, K., 2005. *Changes in regional cerebral blood flow by therapeutic vagus nerve stimulation in depression: an exploratory approach.* Psychiatr. Res. 139, 165–179.