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Neurophysiological signatures of pain in patients with cancer, in healthy humans and in patients with brain damage

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Abstract

Several lines of evidence suggest that chronic pain can affect the electroencephalogram (EEG) at rest. Most of the studies focus on the alterations of alpha rhythm, reporting an enhancement in patients with chronic pain. Today it is not clear the pain specificity of alpha alteration, instead it seems that alpha rhythms could be influenced by other medical conditions, such as depression.

Although the high prevalence of depression and psychological distress in chronic pain syndromes, the psychological comorbidities are not taken sufficiently in account.

To testing the pain specificity of alpha rhythm alteration, we have compared patients with cancer with and without pain. Importantly the two groups do not differ for depression level. Our results show an enhancement in alpha band for the patients without pain.

To better characterize the influence of psychological symptoms on EEG brain rhythms we have evaluated a group of depressed oncological patients before and after an effective treatment on mood. Our results show an increasing of alpha rhythm after the treatment.

Taking together the results of these two studies, they make a critical point on the use of alpha rhythms enhancement as a possible marker of pain.

In parallel with the patients' evaluation, I have tested an experimental model of central sensitization, a hallmark of chronic pain, in healthy subjects. We have evaluated Aδ-fiber and C-fiber response after a protocol of high frequency electrical stimulation (HFS) of the skin that lead a secondary hyperalgesia of the conditioned skin.

At last, I have investigated the relationship between the pain perception and the spatial cognition in a group of patients with brain damage.

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Introduction

Neuroscience is probably one of the most fascinating discipline today. Despite the continuous technical progress in neurophysiological and neuroimaging fields, our knowledge in brain functioning is today highly limited. Because of this limitation, the picture becomes more complicated when we focus on the pathology.

The study of brain changes in pathologies is one of the hardest challenge in the modern neurosciences. Their potential clinical relevance is crucial for those symptoms that are not objectively measurable, such as pain and the psychological symptoms. Even using the most sophisticate technique, it will be probably impossible to properly measure such symptoms, which for their nature are characterized by a subjectivity experience. However, in the last decades many researcher have tried to characterize the brain functioning in chronic pain condition and psychiatric disorders. The growing of this body of knowledge will lead in future to improve the diagnosis and treatment of such symptoms. However, I am sceptical on the substitution of the self-report instrument or the clinical interview by possible neuroimaging or neurophysiological biomarkers. Indeed, I think that reliable indicators could help the clinician to better characterize the patients symptoms, integrating informations from what the patients report, his/her clinical experience and possible neuroimaging or neurophysiological evidences.

In the following chapters, I will present the results from our group in this field, focusing on the exploration of the possible neural correlate of pain and depression. We have used the electroencephalography (EEG) technique to assess the brain activity in patients and in healthy subject after an experimental conditioning of the pain pathways. At last I will present a side project on the integration of spatial cognition and pain perception.

The studies presented in the next chapters are the results collected during my PhD at the Clinical Psychological and Oncological unit, under the supervision of the Professor Riccardo Torta. The data presented in the third chapter were collected during my stay at the Nocions Lab, Institute of Neuroscience, Université catholique de Louvain.

CHAPTER 1 : Alpha rhythm as possible marker of pain

The subjectivity of pain nature is considered an important challenge in determine disease severity and treatment effectiveness in clinical practice. The finding of biological markers of pain will improve the development of new treatment strategies and the evaluation of their effectiveness. In the last years, many studies have reported a relationship between the chronic pain and the electroencephalographic (EEG) rhythms. Most of these studies focus on the alpha band, that is the rhythm range from 8 to 13 Hz and that dominates the waking EEG at rest, especially when the subject closes his eyes. In this chapter, I will review the main studies that have tried to identify the relationship between the pain symptoms and the EEG brain rhythms. I will also present our data on alpha band and pain in a sample of patients with cancer.

Alpha band alteration in chronic pain: The Sarnthein Model

Sarnthein and collegues (2006) first proposed a specific relationship between alpha rhythm and chronic pain. They compared neuropathic pain patients versus healthy control subjects. They successfully discriminated the patients group using the characteristics of their alpha peak in a resting state EEG recording. They found that neuropathic pain patients showed a higher alpha peak, and that it was shifted towards the lower frequency. In addition, they found that the patients group shows a higher amplitude in the theta and in the beta band. Importantly, they evaluated the same patients after a neurosurgery intervention that results in pain relief. It consists in a therapeutic lesion of the central lateral nucleus of the thalamus. After the surgery, the authors have observed a normalization of the EEG in the patients group. Their interpretation of these results focuses on the thalamocortical interplay. It is on the field of the so called Thalamo-Cortical Dysrhythmia (TCD) (Llinás et al., 1999). They proposed that a

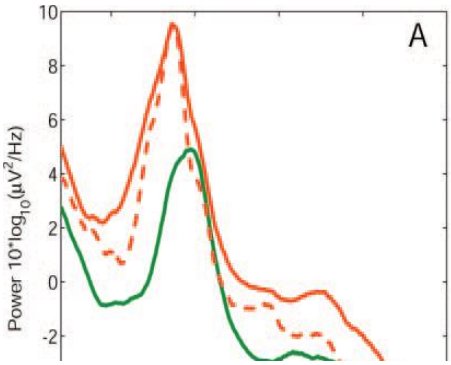

Figure 1. Grand average for the patients before the surgery (orange) and healthy subjects (green). Figure from Sarnthein el al 2006.

lesion to the peripheral or the central nervous system might lead to a loss of excitatory input at the thalamic level, thereby causing a lower frequency fire of the thalamic neurons, via a T-calcium mediated mechanism. The last effect of this lower frequency fire is proposed to be an overactivation for disinhibition of the cortical area involved in the pain processing.

Supporting this hypothesis, the same research group evaluated the possible cortical sources of the EEG alterations in patients. Stern and colleagues (2006) has substantially replicated the previous findings on neuropathic pain patients (Sarnthein et al., 2006). In order to explore the possible sources of the EEG patients overactivation they have used the Low Resolution Electromagnetic Tomography (LORETA) (Pascual-Marqui et al., 2002). LORETA can be used to locate the most probable source of different frequency band from the EEG continuous signal. The results show an overactivation in the theta and beta rhythms in the patients group. For the theta band, the peri-insular parietal cortex showed the highest overactivation. For the beta band, the midfrontal areas and the dorsolateral prefrontal cortex were highly overactivated. Insular cortex and anterior cingulate cortex were both overactivated for the beta and the theta band. Interestingly, after a therapeutic lesion of central lateral nucleus of the thalamus, the overactivations were no more detected. The possible sources emerging from the analysis are congruent with the central role proposed for the same areas in the pain processing (Apkarian et al., 2005; Peyron et al., 2000), even if nowadays the pain specificity of these area is still debated (Legrain et al., 2011).

Michels and collegues (2011) evaluated the relationship between the neurosurgery's clinical outcome (i.e. pain relief) and the normalization of the EEG in the patients. They evaluated a larger sample of neuropathic pain patients, before and after the neurosurgical lesion of the central lateral thalamus nucleus. They used quite the same procedure as Sarnthein (2006). They furthermore added an important information: the clinical outcome. In fact, they divided the patients using their reported pain relief 12 month after the surgery. They defined the patients as High Pain Relief (HPR) if they reported a pain relief higher than 50%, otherwise the patients were defined as Low Pain Relief (LPR).

The authors replicated the Sarnthein results, but they found that only the HPR group normalizes its EEG.

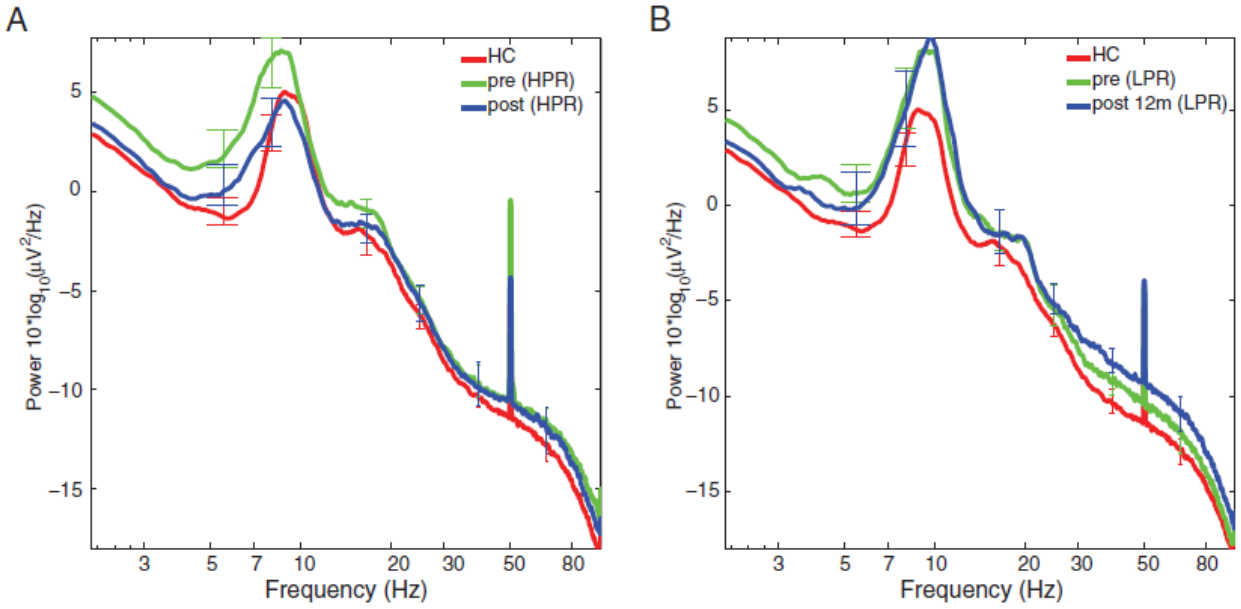

Figure 2. Panel A shows the grand average for the patients that report high pain relief before and after the surgery. Panel B shows the same for the patients that report low pain relief. Figure from Michels et al 2011.

Taking together, this three studies point towards the possible role of EEG rhythms as hallmark of pain in neuropathic pain patients. Sarnthein (2006) have shown the alpha enhancement in this patients and the normalization after a therapeutic neurosurgery; Stern (2006) have found that the possible sources of the EEG alteration in patients are proposed as crucial areas in the pain processing; and Michels (2011) have shown that the EEG normalization after the therapeutic neurosurgery is detectable only in those patients that have the best clinical outcome.

The attempts to generalize the Sarnthein model in other chronic pain syndromes

The results reported in the previous section are referred to a restricted group of patients. With restricted I mean that all the patients have a pain of neuropathic origin, and that the pain symptoms are so severe that lead to a neurosurgery intervention, that is surely not the first option in pain management.

For the possible use of these observations in the clinical practice, it is crucial to test the possibility that the proposed alpha alterations are detectable also in other chronic pain conditions. The attempts to replicate and generalize Sarnthein results' in other chronic pain syndromes led to contradictory results (Pinheiro et al., 2016). In this section, I will try to summarize the major findings.

Spinal cord injury (SCI)

The most important contribution of the study in patients with spinal cord injury is in my opinion the possibility to evaluate patients with and without pain, but with a comparable nerve lesion. Indeed, the possible role of neuropathy itself on alpha alteration could be a crucial factor. It has been shown that patients with SCI, even if they have no pain, show a lower alpha peak, compared with healthy subjects (Boord et al., 2008; Wydenkeller et al., 2009). However, the same studies showed that patients with SCI and pain have a lower alpha peak compared with both healthy subjects and patients with SCI but no pain. Taking together, this results point towards a potential role of the neuropathy in itself in alpha peak slowing, but they suggest a potential adjunctive role of the pain symptoms. Looking at the amplitude enhancement in alpha band, Jensen and colleagues (2013) found a reduction of alpha amplitude in patients with SCI and pain, compared with both patients with SCI and without pain and healthy subjects. However, the association between the alpha reduction and the pain symptoms is not clear. Indeed, the correlation between the reported pain intensity and the alpha amplitude points towards the opposite direction (i.e. more alpha correlates positively with the high pain level).

Low back pain

Patients with low back pain represent the largest group of patients with chronic pain; moreover, low back pain is considered as one of the condition causing the largest financial damage to the economy in terms of treatment and work days loss (Ricci et al., 2006). For these reasons it is interesting to study this population from a clinical point of view.

Schmidt and colleagues (2012) evaluated a group of patients with low back pain, considering the alpha band characteristics, the pain rating and the psychological comorbidities. They found no difference between the patients group and healthy subjects in the EEG rhythms. Interestingly they

found a negative correlation between the frequency at which the alpha peak occurs and the quality of life in the patients. They suggested that probably the shifting of the alpha peak observed previously in chronic pain patients could be related more with the quality of life (that is compromised by the pain symptoms) than with the pain in itself. Looking at the alpha amplitude, the authors have selected a subsample of patients with the most severe symptoms (with a pain rating of at least 7, in a scale from 0 to 10). Interestingly, this subsample shows a higher alpha amplitude compared to the control subjects, even if the differences do not reach the statistical significance. The authors proposed that the alpha enhancement is probably a characteristic of those patients with the most severe symptoms.

Chronic Pancreatitis

Chronic pancreatitis (CP) is a disease with progressive destruction of the pancreatic gland, characterized by an intense abdominal pain. Historically the focus of the pain origin in this syndrome has been on the pancreatic gland, assuming pain to originate in the pancreas or its surrounding organs. Recent findings indicate that both peripheral and central pain processing are abnormal in CP patients (Drewes et al., 2008; Pasricha, 2012). It has been proposed that electrophysiological methods, such as the spectral analysis of spontaneous EEG and evoked potentials, may be useful to unravel the pain origin in patients and to assist the clinician in determine individualized treatment (Lelic et al., 2014). Looking at the alpha band characteristics in patients with CP, Olesen and colleagues (2011) found an enhancement for the theta and the alpha band in the patients compared with healthy subjects. In contrast with other studies, the authors have computed the relative amplitude, i.e. the amplitude of each frequency band normalized with the amplitude of the entire spectrum and expressed as a percentage. The use of a different method in alpha amplitude calculation lead to some difficulties in comparing these results with other studies. More comparable are probably the results from de Vries and colleagues (2013). They compared patients with CP and healthy subjects. Their analysis not only focus on alpha amplitude (computed not as percentage) but also on the frequency of alpha peak. Their results showed no difference between the two groups for the alpha amplitude. Moreover, the alpha peak in these patients results shifted towards the lower frequency, compared to the healthy subjects.

The authors did not report an evaluation on pain intensity for the patients, but they reported a negative correlation between the pain duration and the frequency at which the alpha peak occurs (i.e. longer is the pain, higher is the alpha peak shift towards the lower frequency).

Cancer Pain

The only study that explores the alpha band alteration in patients with cancer was carried out by van den Broeke and colleagues (2013). They focused on the effect of breast cancer treatment, such as mastectomy, lumpectomy and axillary lymph node dissection. In fact, from the 25 to 60 percent of the patients suffer of persistent pain after surgery (Andersen and Kehlet, 2011; Jung et al., 2003). They compared patients with persistent pain and patients that have been treated with the same intervention, but that do not report pain. In this way, the authors have reduced the variability between the patients group and the control one. Their results showed an enhancement in the alpha band for the pain group. In contrast with previous findings, they found no difference for what concerns the frequency at which the alpha peak occurs.

Fibromyalgia

Fibromyalgia is a chronic syndrome characterized by musculoskeletal pain, in absence of apparent organic disease to justify it (Mease, 2005). Pain is characterized by hyperalgesia and allodynia. Patients with fibromyalgia has a high prevalence of psychological symptoms (Bradley, 2005; Castelli et al., 2012) and cognitive impairment (Tesio et al., 2015).

The EEG studies that have compared fibromyalgia patients and healthy subjects lead to not conclusive results. Rossellò and collegues (2015) found an enhancement for the alpha band in patients compared with healthy subjects. Moreover, such enhancement seems to be not specific for the alpha band, but it seems to involve in general all the frequency bands. A second study did not replicate the alpha enhancement for the patients (González-Roldán et al., 2016), whether the authors found a reduction in delta amplitude and an enhancement for the beta rhythm. However, in these patients, only the delta rhythm seems to correlate with the pain duration, whether the beta amplitude seems to be linked with the anxiety. It has been reported an enhancement in the theta rhythm, for the frontal electrodes,

without concomitant changes in other frequency band (Fallon et al., 2017). Moreover, the authors found a positive correlation between such enhancement, the severity of pain and also the reported fatigue.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic pain syndrome that affects the synovial membranes of joints, leading to pain, and joint deformities (McInnes and Schett, 2011). Meneses and colleagues (2016) evaluated the EEG in patients with RA compared with healthy control. They found an enhancement for the alpha and the theta rhythms in the patients group. Indeed they found no correlation between the pain intensity and the EEG amplitude in neither of the frequency band.

The pain specificity of alpha rhythm alteration

Taking together the main findings in EEG characteristics of chronic pain patients, it emerges a not concordant picture. Moreover, even when a difference between the chronic pain patients and the control group has been reported, only few cases show a significative relationship between the EEG indexes and the pain evaluation. Of course, the complexity of the chronic pain syndromes in itself plays a crucial role. In fact, chronic pain patients' clinical condition is characterized by a large use of drugs, a high prevalence of psychological symptoms and a poor quality of life. A way to control this variability is the choice of an appropriate control group. Indeed, only few study have not chosen healthy subjects as control group (Boord et al., 2008; Jensen et al., 2013; van den Broeke et al., 2013; Wydenkeller et al., 2009). Another important aspect is the presence of psychological comorbidities. In fact, especially depression and pain are strictly related and they have a reciprocal influence. Depressive mood reduces the pain threshold and increases, emotionally and cognitively, the pain perception, while chronic pain first induces demoralisation, then true depression (Torta and Munari, 2010).

Few of the previous reported studies have considered the psychological symptoms as a possible confounding factor. When evaluated, it has been found a difference between the patients and the

control group (Schmidt et al., 2012) or an influence of the psychological symptoms on the EEG rhythms (Fallon et al., 2017; González-Roldán et al., 2016; Schmidt et al., 2012).

Moreover, it has been proposed that depression itself can influence the EEG brain rhythms (Olbrich et al., 2015; Olbrich and Arns, 2013) and the alpha band in particular (Henriques and Davidson, 1990). Some studies report that patients with mood depression have an higher alpha amplitude than healthy subjects (Grin-Yatsenko et al., 2010, 2009; Jaworska et al., 2012); but others report an alpha reduction in depressed patients (Begić et al., 2011; Jiang et al., 2016). In the second chapter, I will deeply review the literature on the relation between the depression and the EEG brain rhythms.

Study 1: Cancer pain is not necessarily associated with alpha enhancement in spontaneous EEG

Assuming that the previous studies on alpha alterations in chronic pain patients do not take sufficiently in account the possible role of psychological symptoms, and assuming that the psychological symptoms can have an influence on the EEG rhythms; we have decided to study two groups of patients, one with pain and another one without pain, but with the same level of depression and anxiety. We evaluated patients with cancer that do or not report pain. Cancer patients report pain symptoms mostly due to the neurotoxic side effect of anticancer treatment, such as chemotherapy, or due to the compression of the cancer mass to other tissues (Esin and Yalcin, 2014).

Material and Method

Patients

Patients were enrolled from the Clinical and Oncological Psychology Unit, Città della Salute e della Scienza, Molinette Hospital, Turin, Italy. The inclusion criteria were diagnosis of cancer and having at least one psychological symptom in comorbidity (anxiety, depression or distress) measured during the psychological assessment. Patients were excluded if showing neurodegenerative diseases in comorbidity, brain cancer and previous traumatic, ischemic or haemorrhagic lesions. Using these criteria, we have enrolled 42 patients (39 women and 3 men, age average 56.6 ± 12.4, age range 22-

76). One patient was excluded from the subsequent analysis due to technical problem with the EEG recording, leaving a sample of 41 patients.

The patients were divided in 2 groups (a 'Pain' and a pain-free 'Control' group) using the Pain subscale of European Organization for Research and Treatment of Cancer Quality of Life Questionnaire 30 (EORTC qlc-30) (Aaronson et al., 1993). This questionnaire is a 30-item instrument that measures the quality of life in cancer patients. Each item is composed by a Likert scale, with four levels (not at all, a little, quite a bit, very much). It has a global score, five functional scales (Physical functioning, Role functioning, Emotional functioning, Cognitive functioning and Social functioning) and nine symptom scales (with a scale specific for pain). Every scale is represented by a score ranging from 0 to 100. For the functional scales and the global score, the higher is the score the better is the quality of life or the function evaluated. For the symptoms scale, the higher is the score, the higher is the symptom severity.

The pain scale is composed by two items: the first one concerns the pain intensity in the past week, and the second one concerns the interference of pain in the daily activities. In this way, the scale did not evaluate just the pain intensity but also the impact of the symptom on the daily living.

Patients were considered 'controls' if they scored ≤ 16.67. This score indicates that the patients did not experience pain in the previous week, or that their pain is so low that did not interfere with their daily activities. Using the EORTC Pain scale, 14 patients are included in the Pain group and 27 in the Control one. In the Pain group, four patients had a chronic pain syndrome in comorbidities (two patients with migraine and two patients with fibromyalgia).

Table 1 shows the clinical and demographic data of the sample.

Group	Sex	Age	Cancer Type	Other chronic pain syndrome	Active Cancer Treatment	Antidepressive Drugs	Anxiolitic Drugs	Analgesic Drugs
Control	F	40	Breast		YES	NO	NO	NO
Control	F	65	Melanoma		YES	NO	YES	NO
Control	F	49	Breast		YES	NO	NO	NO
Control	F	49	Breast		YES	YES	NO	NO
Control	F	62	Pancreas		YES	NO	NO	NO
Control	F	25	Bones		NO	NO	NO	NO
Control	F	54	Breast		YES	NO	NO	NO
Control	F	35	Melanoma		YES	YES	NO	NO
Control	F	68	Breast		YES	YES	NO	NO
Control	F	48	Breast		YES	NO	NO	NO
Control	F	57	Tonsil		NO	YES	YES	NO
Control	F	55	Breast		YES	YES	YES	NO
Control	F	76	Breast		NO	YES	YES	NO
Control	M	74	Bladder		NO	YES	YES	NO
Control	M	65	Liver		YES	YES	YES	NO
Pain	F	45	Breast		YES	NO	YES	NO
Pain	F	71	Myeloma		NO	YES	YES	NO
Pain	F	66	Breast		YES	NO	NO	NO
Pain	F	68	Bladder	Fibromyalgia	NO	NO	NO	NO
Pain	F	49	Breast	Migraine	YES	YES	YES	NO
Pain	F	60	Colon		NO	YES	YES	NO
Pain	F	61	Breast		NO	YES	YES	NO
Pain	M	56	Lung		NO	NO	YES	NO
Pain	F	61	Larynx		NO	NO	YES	NO
Pain	F	45	Breast		NO	NO	YES	NO
Pain	F	43	Breast		YES	NO	YES	NO
Pain	F	70	Breast		NO	NO	NO	YES
Pain	F	65	Breast		NO	YES	YES	NO
Pain	F	59	Colon		NO	YES	NO	NO
Pain	F	66	Breast	Fibromyalgia	NO	YES	YES	NO
Pain	F	53	Breast		NO	NO	NO	NO
Pain	F	57	Breast		YES	YES	YES	NO
Pain	F	69	Breast		NO	YES	YES	NO
Pain	F	56	Uterus		NO	YES	YES	NO
Pain	F	64	Uterus		NO	NO	YES	NO
Pain	F	69	Breast		NO	YES	YES	NO
Pain	F	42	Hodgkin Lymphoma		NO	YES	YES	NO
Pain	F	69	Breast		NO	YES	YES	NO
Pain	F	56	Liver		NO	NO	NO	NO
Pain	F	57	Uterus		NO	NO	YES	NO
Pain	F	48	Breast		NO	NO	NO	NO
Pain	F	22	Ovary	Migraine	NO	YES	YES	NO

Table 1 Sample characteristics divided for the Pain and the Control group

Psychological questionnaires

For the psychological assessment, in addition to the EORTC qlq-30 we have also used self-report questionnaires including:

1. Hospital Anxiety and Depression Scale (Hads, (Costantini et al., 1999; Zigmond and Snaith, 1983)), a 14-item instrument for anxiety and depression. It is composed by two scales, one for anxiety and one for depression (ranging from 0 to 21 each). The cut-off score for patient with cancer is ≥ 8 (Castelli et al., 2011, 2009) for each scale.
2. Distress Thermometer (DT, (National Comprehensive Cancer Network, 2003)), a visual analogue scale for distress. It has two anchors, 0 no distress and 10 the most distress that is possible to imagine. The cut-off score is ≥ 4 (Grassi et al., 2013). It contains also a list of stressor in different domains (practical, relational, emotional, spiritual, and physical problems). The patients have to mark all the stressors that have an influence on the reported distress value.

EEG recording

EEG was recorded in a quiet room, where the patients were sat on a comfortable chair. Due to technical reasons, the EEG of 23 patients was recorded with a Micromed system (Modigliano Veneto, Italy) and the EEG of 18 patients was recorded with Galileo (EBNeuro, Florence, Italy). Both the systems have 19 active electrodes. Signals were acquired with an electrode cap (ElectroCap, Eaton, OH) in accordance with the 10-20 international system and referenced to FCz. Impedance was less than 10 kΩ in each of the 19 active leads. Data were collected and digitalized at a sampling rate of 256 Hz with Micromed and 1024 Hz with Galileo. Eyes movements were detected using a diagonal electrooculogram (EOG) recording. It has been recorded 5 minute with eyes closed for each subject.

EEG Data Processing

All the EEG data processing was made offline using Letswave 6 (http://www.nocions.org/letswave). Data processing consists in the following steps:

i) data acquired with Galileo were downsampled to 256 Hz,

ii) band pass filter (Butterworth 0.5-40 Hz),

iii) Independent Component Analysis (ICA) to remove artefacts,

iv) rereference to the average of all electrodes,

v) segmentation in 4 second epoch,

vi) Fast Fourier Trasformation (FFT),

vii) average of the different epochs.

For each subject we considered 4 indexes for the alpha band (8-13 Hz). First, we considered the mean amplitude. Secondly, we computed three indexes concerning the alpha peak: i) the maximal amplitude (*peak amplitude*), ii) the frequency in which it occurs (*peak frequency*) and iii) the center of gravity (*CoG*). CoG represents the frequency at which the amplitude in the alpha band is divided in two equal parts It has been proposed as a more stable measure than the peak frequency, especially when the subject do not have a clear peak. For each index we computed the average of all the electrodes (scalp level), of the frontal ones (Fp1, Fp2, F7, F3, Fz, F4, F8) and of the posterior ones (P3, Pz, P4, O1, O2). In fact, the alpha rhythm is mostly expressed in the posterior electrodes, and it has been proposed an effect of depression in the frontal alpha.

Statistical Analysis

For statistical analysis, we used SPSS for windows 22 (SPSS Inc, Chicago, Il).

We evaluated the difference between the two group both for the psychological questionnaires and the EEG indexes. We tested all variables (both the EEG ones and the psychological ones) for normal distribution by the Kolmogorv-Smirnoff Test. If they proved to be normally distributed, we applied the t-test for independent samples; otherwise, we used the non-parametric Mann-Whitney U-Test.

To control possible differences in drugs assumption between the two groups, we used a Chi square test to evaluate the use of anxiolytics, antidepressant and anticancer treatment across the two groups.

To control the effect of medications per se on EEG brain rhythms we performed a multivariate analysis of variance (MANOVA), with the EEG indexes as independent variables and with three

factors: anticancer treatment, antidepressant treatment and anxiolytic treatment, with two level (Yes for that patients that are in treatment and No for that patients that are not in treatment).

Results

Patients

The Pain group and the Control group do not differ in age (t (1,39) = 0.503, p = 0.618).

15 out 41 patients are in active anticancer treatment (10 Control group, 5 Pain group), 21 out 41 patients are in antidepressant treatment (7 Control group, 14 Pain group), 26 out 41 patients are in anxiolytics treatment (6 Control group, 20 Pain group). Figure 3 shows the percentage of patients in treatment for each group. A Chi square analysis shows a significative association between Group and being in active cancer treatment ($\chi(1) = 11.125$, $p = 0.002$). No significant associations were found between Group and being in antidepressant treatment ($\chi(1) = 0.013$, $p = 0.585$), and between Group and being in anxiolytics treatment ($\chi(1) = 3.873$, $p = 0.086$), although the association for the use of anxiolytics is near to the statistical significance.

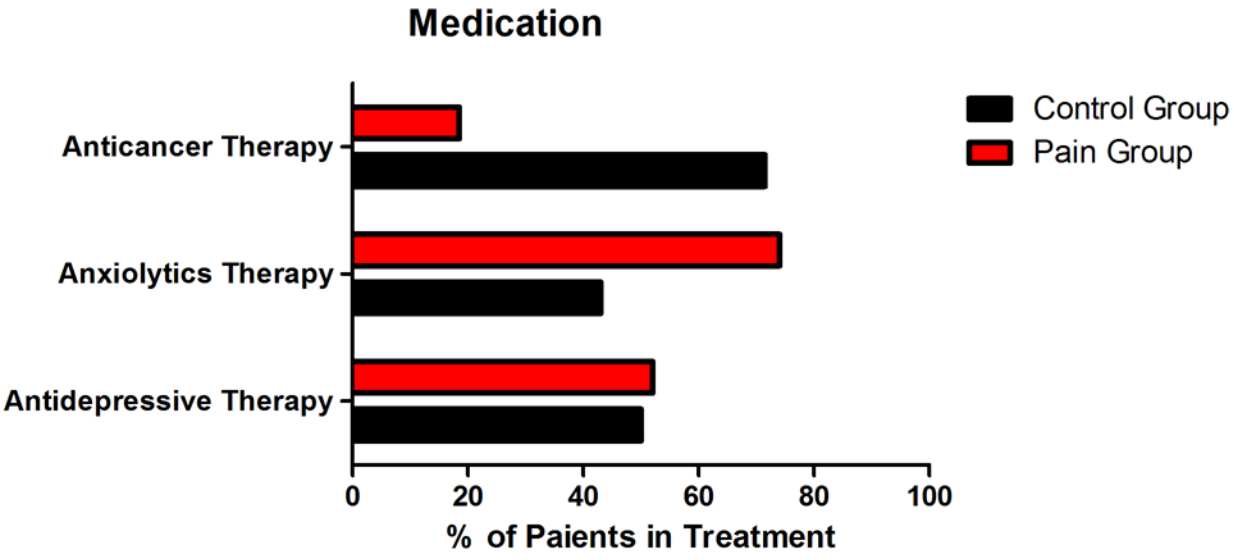

Figure 3 Percentage of patients in anticancer, antidepressant, anxiolytic treatment.

Psychological questionnaires

34 out 41 patients have a Hads Depression above the cut off (9 patients in the Control group, 25 Pain group); 30 out 41 patients have a Hads Anxiety score above the cut off (11 Control group, 19 Pain group); 38 out 41 patients have a DT score above the cut off (12 Control group, 26 Pain group). Figure 4 summarizes the score at each scale for the two groups.

The pain group and the control group do not differ in anxiety levels (Hads Anxiety: $U = 178.5$, $p = 0.271$), nor in depression levels (Hads Depression: $t(1,39) = 1.411$, $p = 0.166$). The pain group has higher levels of distress (DT: $U = 130.00$, $p = 0.019$) and a lower quality of life (EORTC: $t(1,39) = 3.094$, $p = 0.004$).

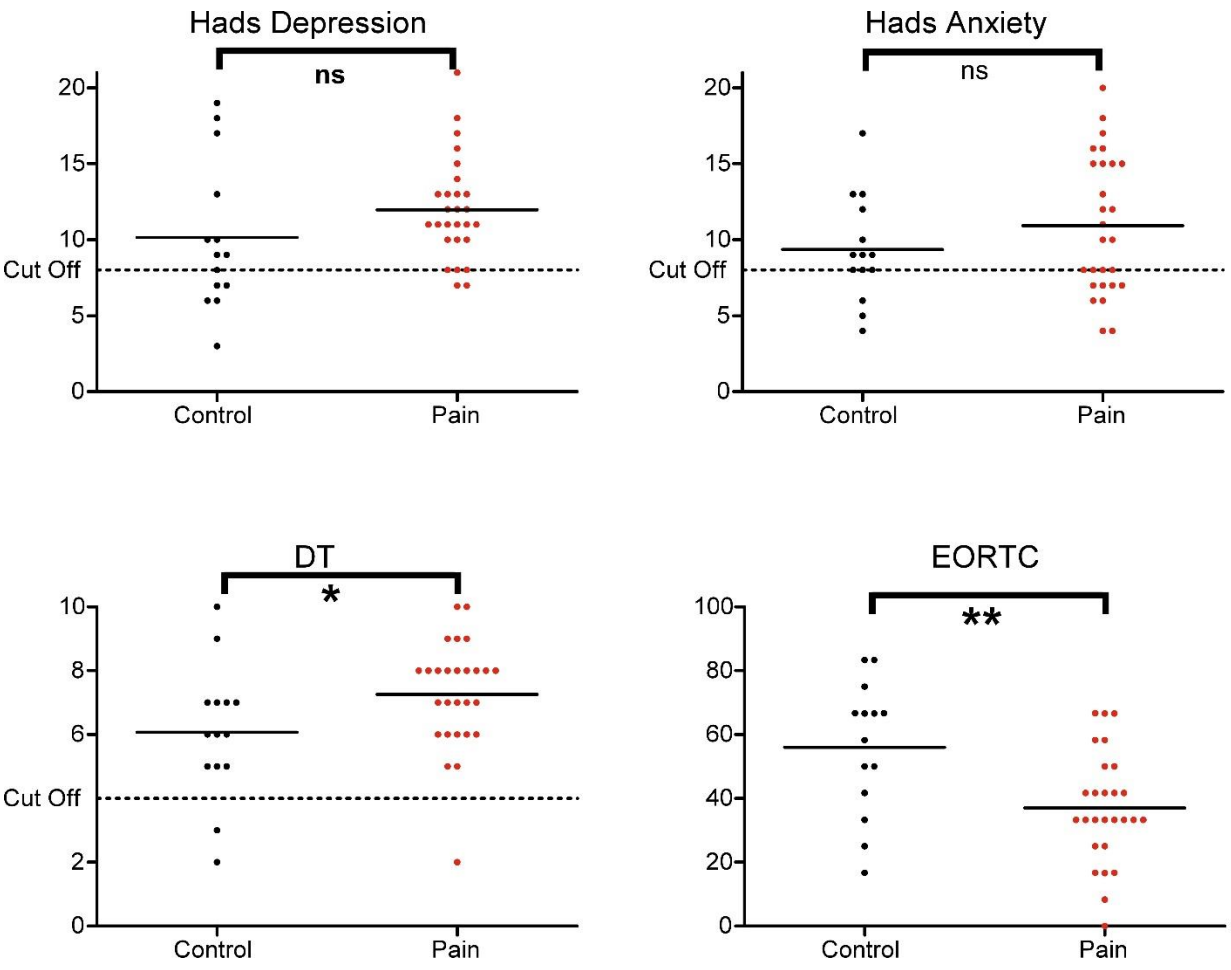

*Figure 4 Comparison between the Control group (in black) and the Pain one (in red) for the psychological questionnaire. Each point represents a patient. *p<0.05; **p<0.01.*

EEG indexes

Figure 5 and 6 show the results for the EEG indexes in the two groups.

The analysis show no difference between the two groups on the peak frequency and on the Cog.

Looking at the figure 5 it seems that the Control group has a higher alpha peak than the Pain one. The results show that this difference is significative for the frontal electrodes (peak amplitude: $U = 136$, $p = 0.032$; mean amplitude: $t(1, 39) = 2.226$, $p = 0.032$). For the posterior electrodes the difference between the two group is near but do not reach the statistical significance (peak amplitude: $U = 150$, $p = 0.073$; mean amplitude: $t(1, 39) = 1.762$, $p = 0.083$). The same trend is observed looking at the average of all the electrodes (peak amplitude: $U = 145$, $p = 0.055$; mean amplitude $t(1, 39) = 1.969$, $p = 0.056$).

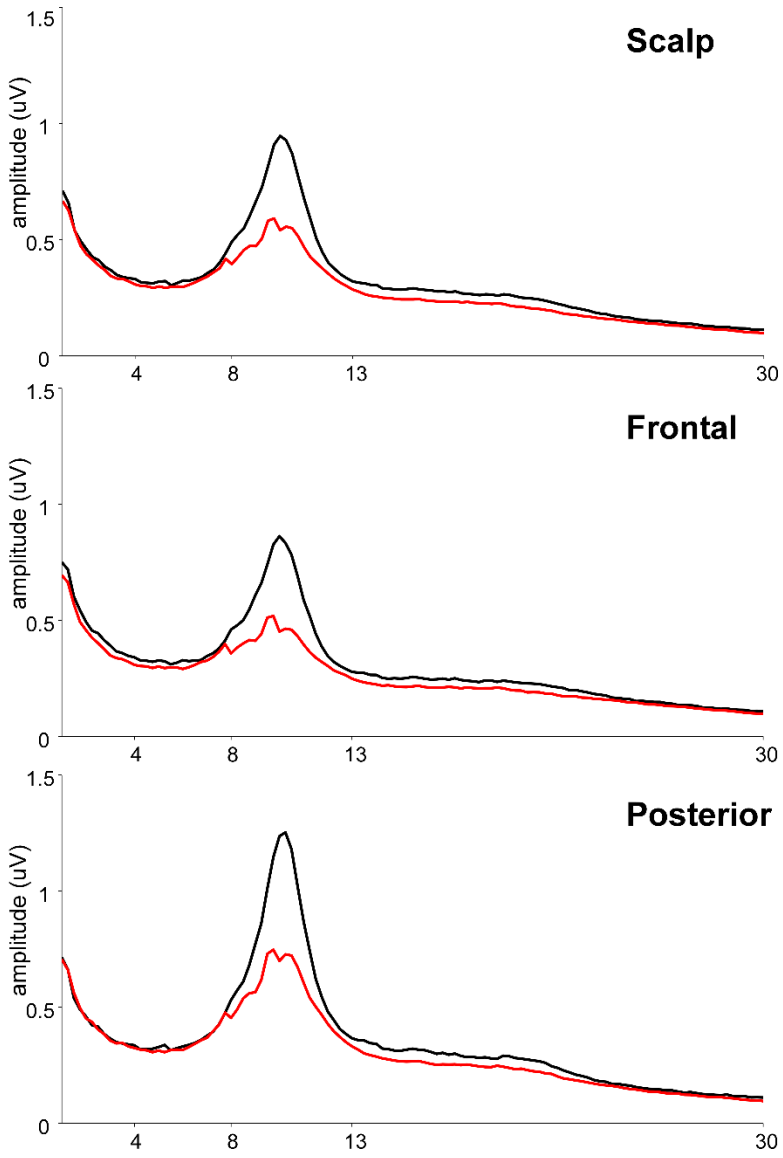

Figure 5 Grandaverage of the spectrogram for the Pain group (in red) and the Control one (in black)

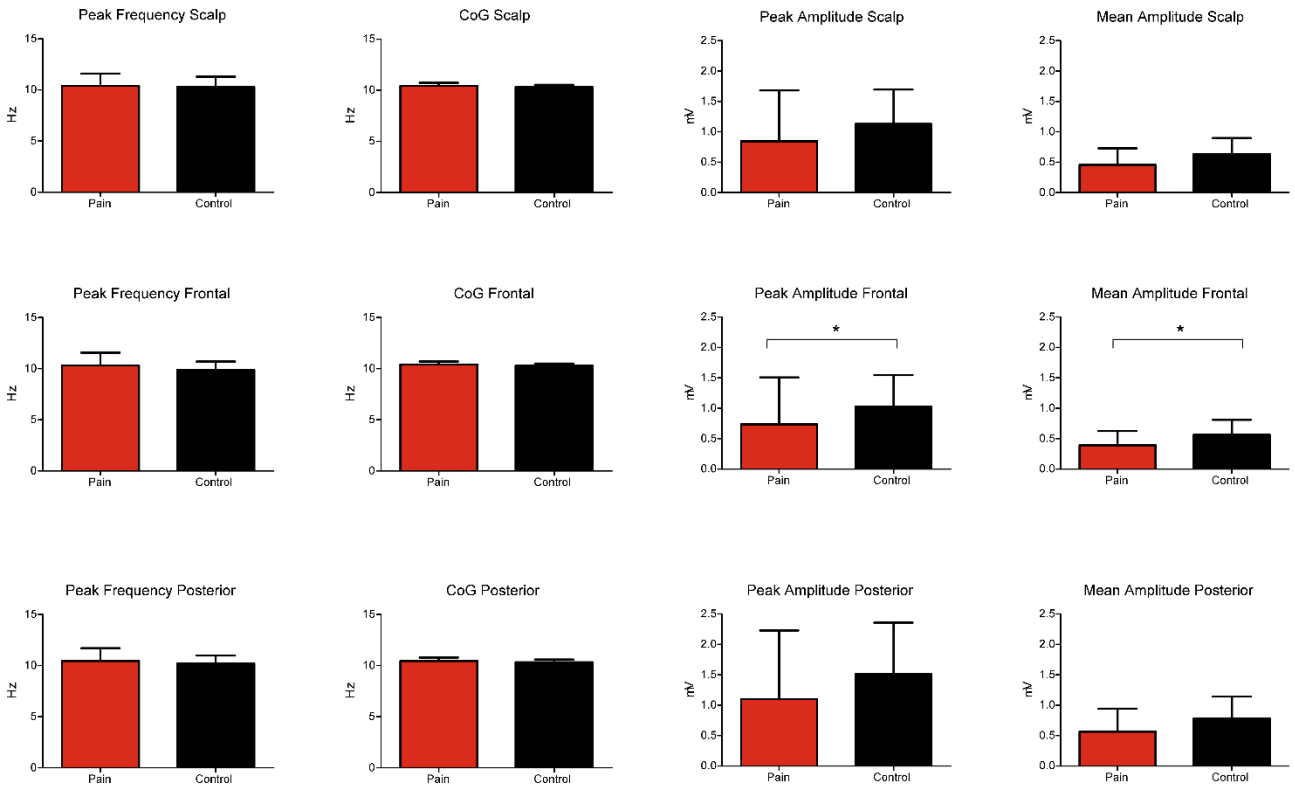

*Figure 6 Average for the EEG indexes in the Pain group (in red) and the Control one (in black). *p<0.05.*

Medication effects on EEG indexes

The MANOVA results show no significative main effects of the antidepressant, anxiolytic or anticancer treatments on none of the EEG alpha indexes (all p value > 0.3). Looking at the interaction effects between the different treatments, we found an interaction between the anticancer treatments and the antidepressant ones (posterior mean amplitude: $F(1, 36) = 4.749$, $p = 0.036$; scalp mean amplitude: $F(1,36) = 4.070$, $p = 0.051$) and between the antidepressant ones and the anxiolytic ones (posterior mean amplitude: $F(1,36) = 3, 861$, $p = 0.057$; posterior peak frequency: $F(1, 36) = 4.948$, $p = 0.032$; scalp peak frequency: $F(1, 36) = 5.380$, $p = 0.026$).

Discussion

Aim of our study was to investigate the pain specificity of the alpha alterations, which previous studies proposed as hallmark of chronic pain.

The two main characteristics, proposed as possible hallmark for the chronic pain syndrome, are the alpha enhancement and the shift of the alpha peak towards the lower frequency.

Our results show no difference between the Pain group and the Control one for what concerns the peak shift. Looking at the enhancement in the alpha band, our results seem to point towards the opposite direction (i.e. an alpha reduction in the Pain group).

Our results do not seem to support the previous finding in chronic pain patients. A possible explanation is that the proposed alpha alteration are maybe not generalizable to all the chronic pain patients. It is possible that only a subgroup of patients shows a clear alpha enhancement and a clear peak shift. Indeed, the first studies that reported this alteration (Sarnthein et al., 2006; Stern et al., 2006) focus mostly on neuropathic pain patients, with such severe symptoms that lead to a functional neurosurgery to treat it. It is possible that the only partial replication that have followed the first studies are partially due to the different kind of patients evaluated (in term of pain severity and neuropathic origin of pain).

Here we are interested on the pain specificity of alpha alterations. Indeed, we did not select the patients in base of the origin of their pain symptoms. Our aim was to evaluate if any pain can influence the alpha rhythm. Moreover, previous studies evaluated the specific role of the neuropathy itself on alpha rhythm. From their results, it seems that the neuropathy per se could affect mostly the peak frequency than the alpha amplitude (Boord et al., 2008; Wydenkeller et al., 2009). It is possible that the alpha peak shift is detectable only when the patients are selected form the neuropathic origin of their symptoms, and it is not the case of our study.

For what concerns the alpha amplitude, our results do not show any enhancement in the Pain group. Moreover, we have found a reduction in the peak amplitude and the mean amplitude for the frontal electrodes. An alpha amplitude reduction is already reported in patient with SCI (Jensen et al., 2013). Recently, Camffermann and colleagues (2017) evaluated the EEG characteristics of the largest sample of chroinc pain patients until today (more than 100 patients). The authors did not evaluate the difference between the patients and a control group, but they tried to better characterize the

relationships between the EEG indexes and the pain symptoms. They found that the more severe are the pain symptoms, the lower was the alpha amplitude in the frontal (F3 and F4) and the central electrodes (Cp3 and Cp4). The authors proposed that the frontal alpha amplitude reduction could characterize the chronic pain patients. Our results seem to partially support their conclusion.

An important factor to take in account is the patients' medication assumption. It can have a crucial role especially when healthy subject composes the control group. In our sample we controlled the anxiety and depressive symptoms across the two group, in these way we have compared two group with a similar assumption rate of antidepressant and anxiolitics drugs. However, the Pain group seems to have a higher percentage of patients that use anxiolitics drugs, even if the difference do not reach the statistical significance. In addition, the Control group have a higher percentage of patients that are in active cancer treatment. To evaluate the possible role on the alpha rhythm of the antidepressant, anxiolytic and anticancer treatment, we performed a MANOVA comparing the patient that are or not in treatment, despite the group (Pain or Control). The results show no main effect of neither of the treatments on the EEG indexes that we have considered. We found only interaction effects, but mostly for the peak frequency and for the CoG. We found no effect on the peak amplitude and the mean amplitude at the frontal electrodes, which are the indexes in which the difference between the two groups is more evident. These results allow us to suppose that the drug assumption in our sample does not seem to influence crucially the EEG indexes that we have considered for the analysis.

Taken together our results do not support the pain specificity of alpha band alterations. Looking deeply the previous studies, it is possible to note that most of them have not found a significative relationship between the severity of pain symptoms and the EEG indexes. It is possible that other factors can have a greater influence than pain on the alpha rhythm. Another possible explanation is that the alpha enhancement and the peak shift is a hallmark of a specific subgroup of patients, that share the same pathophysiology and with severe symptoms. The two hypothesis are not mutually exclusive. Here we are interested on the possibility that any pain can influence the alpha rhythm. The characterization of the pain symptoms in the patients go further the aim of our study. Future

researches need to explore the possible application of alpha alteration as possible marker of pain, even only on a subgroup of chronic pain patients.

Limitation and future perspective

For what concerns the EEG recording, using more active electrodes (32 or more) and an higher temporal resolution (with a sampling rate of 1024 Hz) could lead to a more accurate EEG investigation.

Moreover, almost all of the cited studies refers to patients that suffer from chronic pain. Here we assessed the pain symptoms using the EORTC pain subscale, which do not evaluate the pain duration. Cancer is a chronic disease, and its somatic symptoms are often long lasting. However, we did not evaluated formally the pain duration, and we can not assess its possible effects on our sample. Future studies will address this issue. An interesting model to evaluate the role of the chronification process could be the neuropathies due as side effect of chemotherapy. Indeed, peripheral neuropathy, usually located in the upper and lower limbs, is a quite common side effect of some chemotherapy treatments. It is possible to evaluate the patients before and after the onset of the therapy, estimating in this way the early effects of a predictable neuropathy against those modifications induced by the chronification of the symptoms.

Moreover, in our study we have not evaluated the neuropathic origin of pain. Indeed, looking at the literature, the neuropathy itself can play a crucial role in the proposed EEG alteration. Moreover, here we have investigated if the proposed EEG alterations were detectable despite the pain origin, and if they are specifically related with pain symptoms. The discrimination between the origin of the reported pain, neuropathic or not, go further the aim of the present study.

At last, we have evaluated patients suffering from different cancer types, which require different treatments. The most representative group of patients are breast cancer ones. Evaluating a larger group of patients could explore the possible role of the different treatments and the different pathologies on the EEG rhythms.

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CHAPTER 2 : The use of EEG brain rhythms as possible markers of depressive symptoms

In the previous chapter, I have discussed the limit in considering the alpha rhythm as possible marker of pain. In our study, we have controlled patients for depression and anxiety levels, because we are aware that these psychological symptoms may affect the EEG at rest.

In this chapter, I will discuss the evidence supporting the influence of depression on the EEG rhythms. I will also present our data, collected on a sample of patients with depression and cancer. Here we have explored the relationship between the EEG changes before and after an effective treatment and the response to the treatment itself.

The influence of depression on EEG brain rhythms

Many studies have tried to discriminate depressed patients from healthy subjects using the EEG brain rhythms and specially the amplitude in the different frequency band (Olbrich et al., 2015; Olbrich and Arns, 2013). Here, I will summarize the main findings reported on the relationship between the depressive symptoms and the EEG spectral indexes.

Frontal alpha asymmetry

Historically, one of the first line of research focused on the frontal alpha asymmetry. Davidson and colleagues (1992) proposed that the left and the right frontal cortices are specialized respectively for approach and withdrawal processes. They proposed that the activity of the two hemispheres is measurable using the alpha activity, assuming that the alpha activity correlates with the cortical inhibition (Neuper and Pfurtscheller, 2001); indeed it seems that the alpha amplitude is negatively correlated whit the cortical perfusion (Leuchter et al., 1999). Depressed subjects seem to have a greater left frontal alpha compared with non-depressed subjects (Schaffer et al., 1983). Anyway, it was assumed that the alpha asymmetry is not linked with depression itself, but it has been proposed as a risk factor. Indeed, previously depressed subjects showed a higher left frontal alpha compared

with healthy subjects, even if they had no symptoms at the time of the EEG evaluation (Henriques and Davidson, 1990). After these first studies, the alpha asymmetry hypothesis in depressed patients is under discussion.

The frontal alpha asymmetry (i.e. more left than right alpha) was found in infants of depressed mothers (Dawson et al., 1999), and it seems to be related with the mother depression score (Lusby et al., 2014). An higher left frontal alpha predicts first depressive episode in college students (Nusslock et al., 2011), and it has been proposed as a possible marker of psychomotor retardation in depressed patients (Cantisani et al., 2015).

However, it has been proposed that the anxiety comorbity could play a crucial role in the alpha frontal asymmetry. It seems that only the patients with anxiety symptoms in comorbidity with depression show a greater left frontal alpha (Bruder et al., 1997). Moreover, the anxiety score seems to explain more of the variance of frontal asymmetry than the depression score, in patients with major depression disorder (Adolph and Margraf, 2017).

Despite the controversial results, the link between alpha frontal asymmetries and depression is accepted by some authors (Allen and Reznik, 2015). Moreover, it seems that the difference between healthy subjects and depressed patients are easily detectable when the subject are tested during an emotional task, rather than in a resting state condition (Stewart et al., 2011).

Taking together, these results seem to suggest that, even if the relation between the frontal alpha asymmetry and depression is accepted, it could better consider the frontal alpha asymmetry more as a risk factor than a marker.

Alpha amplitude

If frontal alpha asymmetry is proposed as a possible risk factor of depression, the alpha amplitude is proposed as a possible marker of current depression. Moreover, today there is no agreement on the possible relation between alpha amplitude and depression; indeed some authors have found an enhancement, whereas others have reported a reduction for the depressed patients compared with

healthy subjects. Moreover, even if alpha amplitude seems to discriminate between depressed patients and healthy subject, the correlation between the depression severity and the alpha amplitude is not always reported.

Grin-Yatsenko and colleagues (2009) tested the possibility to discriminate depressed subjects from healthy ones on a quite large sample (101 depressed patients against 526 healthy subjects). They focused on patients at the early stages of depression, and most of them are not in treatment with antidepressant drugs. The authors discriminated successfully the patients from the healthy subjects using their EEG spectral indexes. Moreover, it seems that the alpha amplitude in an eyes closed condition is the best discriminant index, classifying correctly the 90% of the sample. Nevertheless, also the alpha amplitude in an eyes open condition and the theta and beta amplitude in both eyes open and close conditions, show a good discriminative performance, but with less accuracy (ranging from 78 to 82%). These results show that depressed patients have a higher alpha amplitude, especially in the parietal and posterior electrodes. In addition, the authors found an enhancement also in the beta and the theta band in the depressed patients. To better characterize the difference between the depressed patients and the control group, a second study on the same sample evaluated the possible cortical sources that determined the differences between the two groups (Grin-Yatsenko et al., 2010). The results show that the main differences are probably due to the posterior region and that are not lateralized (i.e. are present bilaterally). There is an exception for the beta band, which seems to be diffuse not only in the posterior but also in the frontal region.

An enhancement in alpha amplitude on the frontal and the parietal electrodes was found also in long lasting depressed patients (Jaworska et al., 2012). The sample evaluated is composed by patients with moderate depression and most of them are not at their first depressive episode. The results show a higher alpha amplitude in the depressed patients, compared with healthy subjects.

The alpha enhancement in depressed patients was not always found. Begic and colleagues (2011) found a decrease in alpha amplitude in depressed patients compared with healthy subjects. They found

no difference for the posterior electrodes. The alpha reduction in their sample was selective for the frontal ones, and it seemed to be bilateral.

Less alpha in depressed patients was found also by Jiang and colleagues (2016). Using MEG, they shown that depressed patients have a reduced alpha amplitude, compared with healthy subjects. In addition, their results show a reduction in frontal theta and an enhancement in frontal beta in the depressed patients.

Taking together, these results support the idea that depression influences the alpha rhythm, whereas it is not clear the direction of this influence. To clarify this aspect, it is possible to consider those studies that have evaluated the correlation between the depression severity and the alpha amplitude. For instance, Jiang and colleagues (2016) found that, despite depressed patients differ from healthy subjects for the theta, alpha and beta amplitude, only the alpha band has a significative correlation with the depression severity. Indeed, the results show that the most severe is the depression, the lower is the alpha amplitude. Moreover, using alpha amplitude, it is possible to correctly estimate the depression severity. The same negative correlation between alpha amplitude and depression severity was found by Zoon and colleagues (2013).

To summarize, despite it seems that depressed patients differ from healthy subjects not only for one spectral parameter, alpha amplitude seems to characterize better the depressed patients (Grin-Yatsenko et al., 2009) and the depressive symptoms (Jiang et al., 2016). The contrasting results suggest that probably the alpha rhythm in these patients is not only influenced by the depressive symptoms. It is possible that other factors can explain the difference with the healthy subjects.

However, it seems that, within the depressed patients, lower alpha amplitude is associated with an higher depression severity.

Other frequency band

Some studies reported EEG modifications in depressed patients for other frequency bands, even if these modifications are less frequently reported than alpha band one.

For the theta band, it has been reported an enhancement (Arns et al., 2015; Jaworska et al., 2012; Kwon et al., 1996) and in some case a reduction (Jiang et al., 2016) in depressed patients. A positive correlation between depression severity and theta amplitude is also reported (Arns et al., 2015).
For the beta band, it has been reported an enhancement in depressed patients (Begić et al., 2011; Jiang et al., 2016; Kwon et al., 1996), even if it has been proposed that the beta rhythm can be related most to the anxiety symptoms rather than depression (Grin-Yatsenko et al., 2009).

Study 2: S-Adenosyl Methionine effectiveness in patients with cancer and depression

Despite the previous studies are not conclusive on which are the possible EEG markers of depression, there is a general agreement on the possible use of the EEG technique to characterize the depressive symptoms in patients. In the present study, we evaluated a group of cancer patients with depression, before and after a 2 weeks intravenous daily treatment with S-Adenosyl Methionine (SAMe).

The use of SAMe as antidepressant

SAMe is a molecule that naturally occurs in the central nervous system. It was discovered in the early 50s (Cantoni, 1952). SAMe appears uniformly distributed in the brain where it serves as the major donor of methyl groups required in the synthesis of neuronal messengers and membranes (Baldessarini, 1987). Variations in SAMe levels in the central nervous system have been detected across neuropsychiatric disorders (Bottiglieri and Hyland, 1994), including depression (Bottiglieri et al., 2000). In particular, patients with depression appear to have lower serum (Bottiglieri et al., 1988) and cerebrospinal fluid SAMe levels than control subjects (Bottiglieri et al., 1990).
The SAMe effectiveness as antidepressant was investigated from many studies (Papakostas et al., 2003). Different meta-analyses (Bressa, 1994; Delle Chiaie and Boissard, 1997; Hardy et al., 2003) shown that SAMe is more effective than placebo, and that it is effective as standard antidepressant treatments but with less side effects. In patients that show a partial response or that do not respond to standard antidepressant treatments, it has been shown that an add-on of SAMe in their therapy can

improve the depressive symptoms (Alpert et al., 2004; De Berardis et al., 2013; Levkovitz et al., 2012).

SAMe effects on EEG brain rhythms

The effect of SAMe on EEG brain rhythm was investigated in only three studies (Arnold et al., 2005; Saletu et al., 2002; Torta et al., 1994).

Torta and colleagues (1994) evaluated a group of patients with major depression and one of healthy subjects. They observed the EEG modification after 5 day of SAMe daily infusion (400 mg/day). They also pointed out a higher latency in P300 for the depressed patients, which normalizes after the SAMe treatment. In both groups they observed an enhancement for the alpha band after the treatment. The other two contributions on EEG changes after SAMe treatment are not on a clinical population. They consist in a double blind, placebo-controlled crossover study, on the acute and subacute effects of SAMe in healthy subjects.

Saletu and colleagues (2002) evaluated a group of healthy subjects, during a treatment consisting in 7 days of SAMe daily infusion (800 mg/day) or placebo. They evaluated both the acute (1, 3, 6 hours after the infusion) and subacute (comparing the subjects at the enrolment and before the infusion at the last treatment day) SAMe effect on EEG. Their results show:

- For the total spectrum and the alpha band, it was observed a reduction in amplitude after the infusion, but an increase after 7 days of treatment. Interestingly, after a superimpose dose at the day seven the alpha band is reduced compared whit the placebo.
- A general increase of delta/theta amplitude.
- An increase for the beta band after the treatment.

Using the same design, the same research group (Arnold et al., 2005) reported congruent EEG modification also on a group of healthy elderly subjects, after 15 days of oral treatment of SAMe (400 mg/day and 1600 mg/day).

To summarize the results reported on EEG changes after SAMe treatment, it has been observed a general increase in amplitude after 5, 7 and 15 days of SAMe assumption at different dosages. For the alpha band and the total spectrum, it seems that the acute effect (1 hour after the infusion) has the opposite direction (i.e. a reduction). The only modification observed in a clinical population involved the increasing of the alpha amplitude after the treatment. Moreover, it is necessary to highlight some limitation of the reported studies. First, they have a small sample size, around 10 subjects per study (except Torta and colleagues, which have evaluated two arms with 10 subjects each). Second, only Torta and colleagues have evaluated a clinical sample. Third, in the only study on depressed patients, the authors do not report the clinical outcome; and in this way, it is not possible to evaluate the relationship between the EEG changes and the modification on the depressive symptoms.

Depression in oncology

The mean prevalence of depression in cancer patients ranged from 8% to 58% and differed by the type of instrument, type of cancer and treatment phase (Krebber et al., 2014; Massie, 2004). It is known that the prevalence of depression in cancer patients is higher than in general population (Härter et al., 2007). Depression has been shown to have negative consequences on both the patient and family, with data indicating a role of this clinical condition in reducing adherence to treatments, in increasing subjective perception of physical symptoms and, possibly, in worsening prognosis (Grassi et al., 2015; Sotelo et al., 2014).

The use of antidepressants in patients with cancer is necessary when the persistent presence of mood depression interferes with the patient's quality of life or with adherence to oncological treatment (Torta and Ieraci, 2013). However, the choice between the several classes of antidepressants should be followed by a careful evaluation of the balance between the effectiveness and safety, considering the possibility of interactions between antidepressants and oncological treatment (Torta et al., 2012).

Aim

Despite there is a large literature on the effectiveness of SAMe as antidepressant, there is only one study, conducted by our group, that evaluates the SAMe on cancer patients (Scalabrino et al., 2009). Nevertheless, SAMe could be a good option in depressed cancer patients' treatment, for its fast onset of action and for its safety profile.

In the present study, we evaluated the SAMe effectiveness in a population of cancer patients. The experimental protocol comprises both psychological questionnaire and EEG evaluation. Aim of our study is double. First, to confirm the previous data on the effectiveness of SAMe as antidepressant in an oncological population. Second, to evaluate the EEG modifications after the SAMe treatment on a larger clinical group, and explore their relationship with the clinical outcome.

Material and Methods

Patients

Patients were enrolled from the Clinical and Oncological Psychology Unit, Città della Salute e della Scienza, Molinette Hospital, Turin, Italy.

Inclusion criteria were diagnosis of cancer and presence of depressive symptoms in comorbidity, measured with the Hospital Anxiety and Depression Scale (Hads). Patients were enrolled if they have a Depression score ≥ 8. For the study were eligible both patients not adequately responders to ongoing antidepressant treatment, in which SAMe was used as add-on therapy; and patients that are not in antidepressant treatment, in which SAMe was used as starter treatment, especially when a rapid antidepressant response was required.

Exclusion criteria were presence of neurodegenerative disease in comorbidity and all kind of brain injury, such as brain cancer and previous traumatic, ischemic or haemorrhagic lesions.

27 cancer patients (3 man) with a diagnosis of depressive disorder, in according to DSM-V, were included in this study.

The clinical and demographic characteristics of the patients are reported in Table 1.

All procedures and the nature of the study were fully explained to the subjects and written informed consent was obtained for each patient.

Cancer Type	Sex	Age	Arm	Active cancer treatment	Antidepressive drugs	Anxiolitycs drugs
Hodgkin's lymphoma	F	42	1200 mg	NO	YES	YES
Breast	F	69	800 mg	NO	YES	YES
Bladder	M	74	800 mg	NO	YES	YES
Breast	F	33	-	NO	YES	NO
Breast	F	49	800 mg	NO	YES	YES
Uterus	F	64	-	NO	NO	YES
Bladder	F	68	800 mg	NO	NO	NO
Breast	F	68	1200 mg	NO	YES	NO
Breast	F	76	-	NO	YES	YES
Liver	F	56	-	NO	NO	NO
Tonsyl	F	57	800 mg	NO	YES	YES
Breast	F	65	1200mg	NO	YES	YES
Colon	F	59	800mg	NO	YES	NO
Melanoma	F	65	1200 mg	YES	NO	YES
Breast	F	55	1200 mg	YES	YES	YES
Breast	F	69	1200 mg	YES	YES	YES
Breast	F	66	1200 mg	NO	YES	YES
Breast	F	46	1200 mg	YES	YES	YES
Breast	F	37	-	YES	YES	YES
Myeloma	F	71	1200 mg	NO	NO	YES
Breast	F	61	1200 mg	NO	YES	YES
Liver	M	65	1200 mg	YES	YES	YES
Uterus	F	56	1200 mg	NO	YES	YES
Breast	F	44	-	NO	YES	NO
Bladder	M	59	1200 mg	YES	YES	YES
Breast	F	57	1200 mg	NO	YES	YES
Breast	F	59	800 mg	NO	YES	NO

Table 1 Clinical and demographic characteristics of the sample. The empty value in the "Arm" column represent those subjects that have not finished the treatment.

Study design

Patients were divided into 2 arms characterized by different dosage (see figure 1):

- arm A: 400 mg/day at day one, 800 mg/day from day two to twelve.

- arm B: 400 mg/day at day one, 800 mg/day from day two to seven, 1200 mg/day from day eight to twelve.

Patients were included in the arm B if they did not show any signs of treatment response at the day eight.

Patients were evaluated at four time points: T0 (baseline), T1/2 (day 8), T1 (day 12) and T2 (day 24). Patients of both groups complete a full evaluation at T0, T1 and T2 with the psychological scales. At T1/2 patients complete a short evaluation (only HADS and Distress Thermometer). At T0 and T1 patients complete an EEG recording at rest.

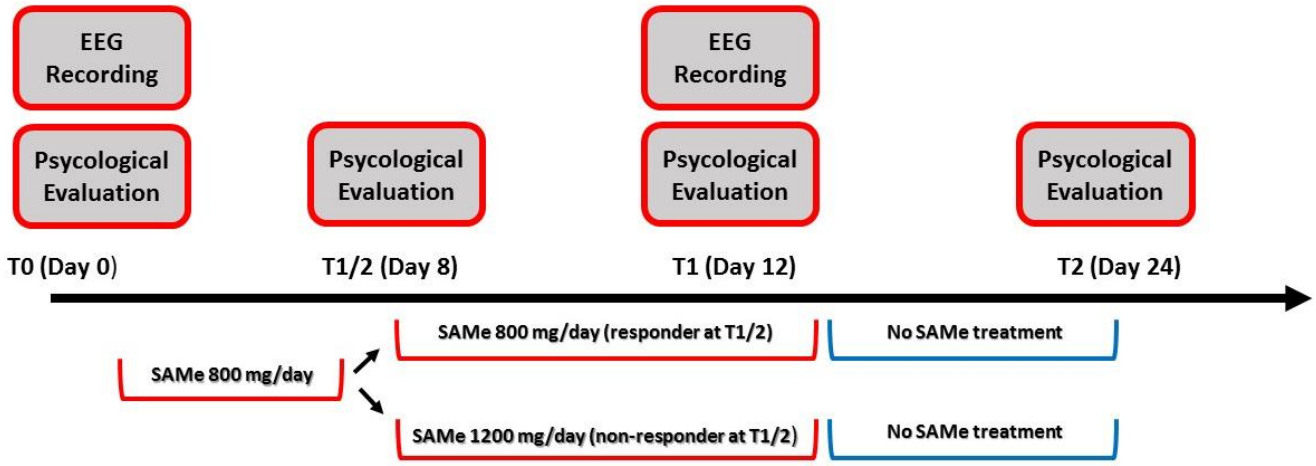

Figure 1 Schematic representation of the experimental design

Psychological Questionnaires

For the psychological assessment, we used the following scales:

1. Hospital Anxiety and Depression Scale (Hads) (Costantini et al., 1999; Zigmond and Snaith, 1983), a 14-item self-report questionnaire for anxiety and depression. It is composed by two scales, one for anxiety and one for depression (ranging from 0 to 21 each). The cut-off score for patient with cancer is ≥ 8 (Castelli et al., 2011, 2009) for each scale.
2. Distress Thermometer (DT) (National Comprehensive Cancer Network, 2003), a visual analogue scale for distress. It has two anchors, 0 no distress and 10 the most distress that is possible to imagine. The cut-off score is ≥ 4 (Grassi et al., 2013). It contains also a list of stressor in different domains (practical, relational, emotional, spiritual, and physical problems). The patients have to mark all the stressors that have an influence on the reported distress value.

3. Montgomery Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979) is a semi-structured clinical interview administered by an expert clinical psycho-oncologist. The cut off score for depression is ≥ 11 (Montgomery, 1994).
4. Dosage Record and Treatment Emergent Symptom Scale (DOTES), scale that evaluates side effects, their possible relation with pharmacological treatment and any measure adopted for their management.
5. European Organization for Research and Treatment of Cancer Quality of Life Questionnaire 30 (EORTC qlc-30) (Aaronson et al., 1993). This questionnaire is a 30-item instrument that measures the quality of life in cancer patients. Each item is composed by a Likert scale, with four level (not at all, a little, quite a bit, very much). It has a global score, five functional scales (Physical functioning, Role functioning, Emotional functioning, Cognitive functioning and Social functioning) and nine symptom scales (such as fatigue, pain, and nausea). Every scale is represented by a score ranging from 0 to 100. For the functional scales and the global score, the higher is the score the better is quality of life or the function evaluated. For the symptoms scale, the higher is the score, the higher is the symptoms severity.

EEG recording

EEG was recorded in a quiet room. The patients were seated on a comfortable chair. The EEG was recorded with Galileo system (EBNeuro, Florence, Italy). We have used 19 active electrodes. The electrodes were applied to the scalp with an electrode cap (ElectroCap, Eaton, OH) in accordance with the 10-20 international system and referenced to FCz. Impedance was less than 10 kΩ in each active lead. Data were collected and digitalized at a sampling rate of 1024 Hz. It has been recorded 5 minute with eyes closed at T0 (enrolment) and at T1 (day 12).

EEG Data Processing

All the EEG data processing was made offline using Letswave 6 (www.nocions.org/letswave).

Data processing consists in the following steps:

i) band pass filter (Butterworth 1-30 Hz),

ii) Independent Component Analysis (ICA) to remove artefacts,

iii) rereference to the average of all electrodes,

iv) segmentation in 4 seconds epochs,

v) Fast Fourier Trasformation (FFT),

vi) average of the different epochs.

For each subject we calculated the mean amplitude and the relative amplitude (i.e. the percentage of amplitude at each frequency band respect to the entire spectrum) on the four frequencies band delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz) and beta (13-30 Hz). All the indexes are computed for the average of all the 19 active electrodes.

Statistical analyses

For statistical analysis, we used SPSS for windows 24 (SPSS Inc, Chicago, Il).

To evaluate the difference on the psychological symptoms after the treatment, we used a repeated measure ANOVA, with a factor between subjects (the 'arm', 800 mg vs 1200 mg) and a factor within subject (the 'time', with 3 levels or 4 levels for the questionnaire administered at T ½). The Hyundt-Feldt correction was used in case of violation of sphericity.

For the EORTC questionnaire, we decided to focus the evaluation on the global score and on the Fatigue scale, because it is one of the most reported symptoms in cancer patients and very difficult to treat.

The same design is used to evaluate the modification in the EEG indexes.

To explore the relationship between the EEG modifications and the clinical outcome, we planned to compute the correlations between those EEG indexes that are significatively modified after the treatment and the score of depression measured with the psychological questionnaire (MADRS, Hads-D).

Results

Patients

Out of 27 patients, 21 have completed the therapy. The drop-out are due to factor external to the treatment, such as familiar concerns, difficulties to reach the hospital for the daily infusion, worsening of the general medical condition. No patient have reported serious side effect in relation with the treatment.

The following results are referred to the 21 patients that have completed the therapy.

7 patients have completed the treatment at the dosage of 800 mg/die, 14 ones at the dosage of 1200 mg/die.

Psychological Questionnaires

Figure 2 and Table 2 show the mean score and the standard deviation at each evaluation time point for each psychological questionnaire.

We observed a significative decrease in HADS total score after the beginning of the SAMe treatment (main effect 'time' $F(3, 54) = 4.866$, $p = 0.005$). The same trend is observed for the HADS-D ($F(3, 54) = 6.387$, $p = 0.001$), for the DT (main effect 'time' $F(3, 54) = 4.668$, $p = 0.006$), and for the MADRS (main effect 'time' $F(2, 32) = 14.775$, $p < 0.001$). An improvement of the quality of life is observed in the patients after the treatment (EORTC Qol, main effect 'time' $F(2, 36) = 3.202$, $p = 0.052$), such as for the EORTC Fatigue scale (main effect 'time' $F(2, 36) = 6.536$, $p = 0.004$).

No change is observed for the HADS-A ($F(3, 54) = 1.858$, $p = 0.148$).

It is not observed any significative interaction between the 'arm' (800mg vs 1200mg) and the 'time' for none of the psychological questionnaire.

Scale	T0	T1/2	T1	T2	Pairwise Comparison
HADS Total	22.24 (±6.42)	20.33 (±6.57)	16.66 (±5.66)	18.45 (±8.52)	T0>T1*; T1/2>T1*
HADS Depression	12.76 (±3.66)	11.52 (±3.89)	9.19 (±4.09)	10.85 (±4.88)	T0>T1**
HADS Anxiety	9.48 (±3.85)	8.86 (±3.84)	7.48 (±2.98)	8.1 (±4.59)	
DT	6.62 (±1.72)	5.52 (±2.29)	5.38 (±1.8)	5.25 (±2.67)	T0>T1**; T0>T2*
MADRS	21.74 (±4.98)	-	14.29 (±7.93)	13.45 (±7.74)	T0>T1**; T0>T2**
EORTC Global Score	45.64 (±19.99)	-	54.36 (±19.48)	51.24 (±19.54)	
EORTC Fatigue	51,95 (±5.38)	-	36,37 (± 4.5)	37,64 (±5.95)	T0<T1*; T0<T2*

*Table 2 Mean score and standard deviation of the psychological questionnaire. The last column shows the difference between the different time points. *p<0.05; **p<0.01; Bonferroni correction for multiple comparison was applied.*

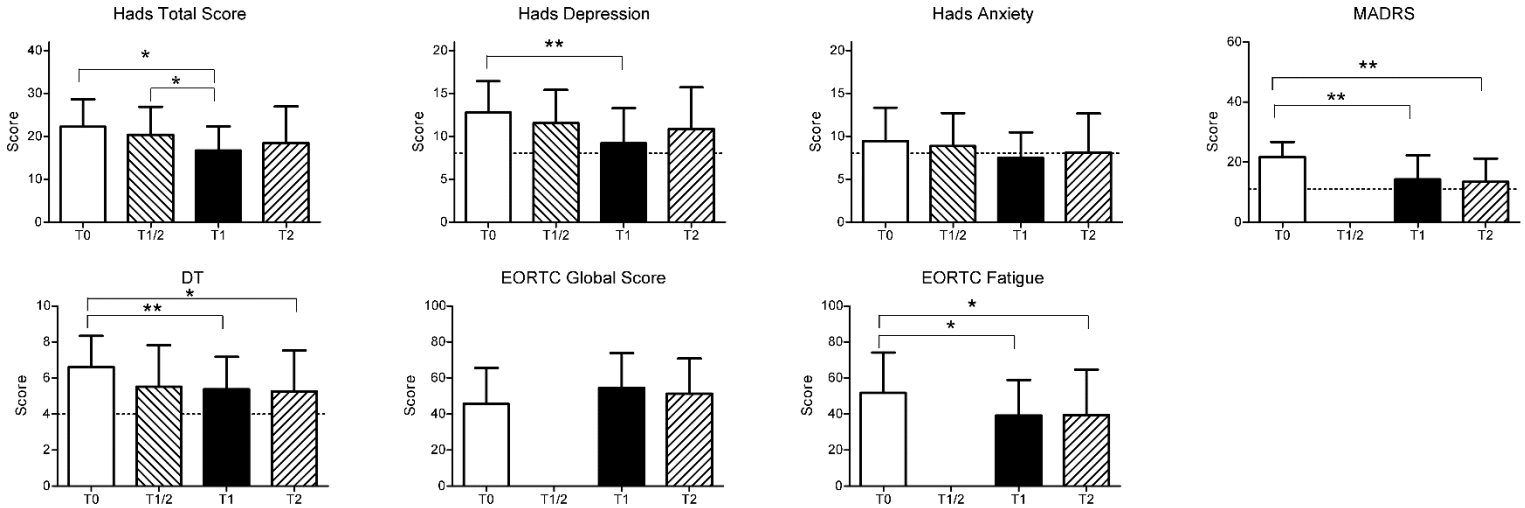

*Figure 2 Patients' score for the psychological questionnaire at the different evaluation time points. *p<0.05; **p<0.01; Bonferroni correction for multiple comparison was applied.*

EEG indexes

Figure 3 and Table 3 show the mean amplitude for each frequency band at the beginning and at the end of the treatment. Only relative alpha is modified after two weeks of treatment. No differences were found after the treatment for the mean amplitude in none of the frequency band. For the relative amplitude the patients have more alpha at T1 than T0 (main effect 'time' $F(1, 19) = 9.840$, $p = 0.005$). The relative beta shows a reduction after the treatment that is near to the statistical significance (main effect 'time' $F(1, 19) = 4.167$, $p = 0.055$). No significative difference were found in relative amplitude

for the other frequency band, neither any significative interaction between the 'arm' (800mg vs 1200mg) and the 'time'.

	T0	T1	p		T0	T1	p
1-30 Hz	0.27 (±0.1) μV	0.27 (±0.08) μV	0.8				
Delta	0.38 (±0.14) μV	0.36 (±0.08) μV	0.569	**Relative Delta**	15.15 (±3.48) %	14.68 (±4.01) %	0.369
Theta	0.32 (±0.2) μV	0.3 (±0.1) μV	0.837	**Relative Theta**	15.9 (±4.63) %	15.67 (±3.48) %	0.915
Alpha	0.49 (±0.24) μV	0.52 (±0.24) μV	0.173	**Relative Alpha**	29.77 (±8.21) %	31.63 (±8.43) %	0.005
Beta	0.18 (±0.06) μV	0.17 (±0.05) μV	0.731	**Relative Beta**	39.37 (±7.23) %	38.11 (±6.54) %	0.055

Table 2 Mean score and standard deviation of the EEG index. The last column shows the p value for the main effect of 'time' in the repeated measure ANOVA.

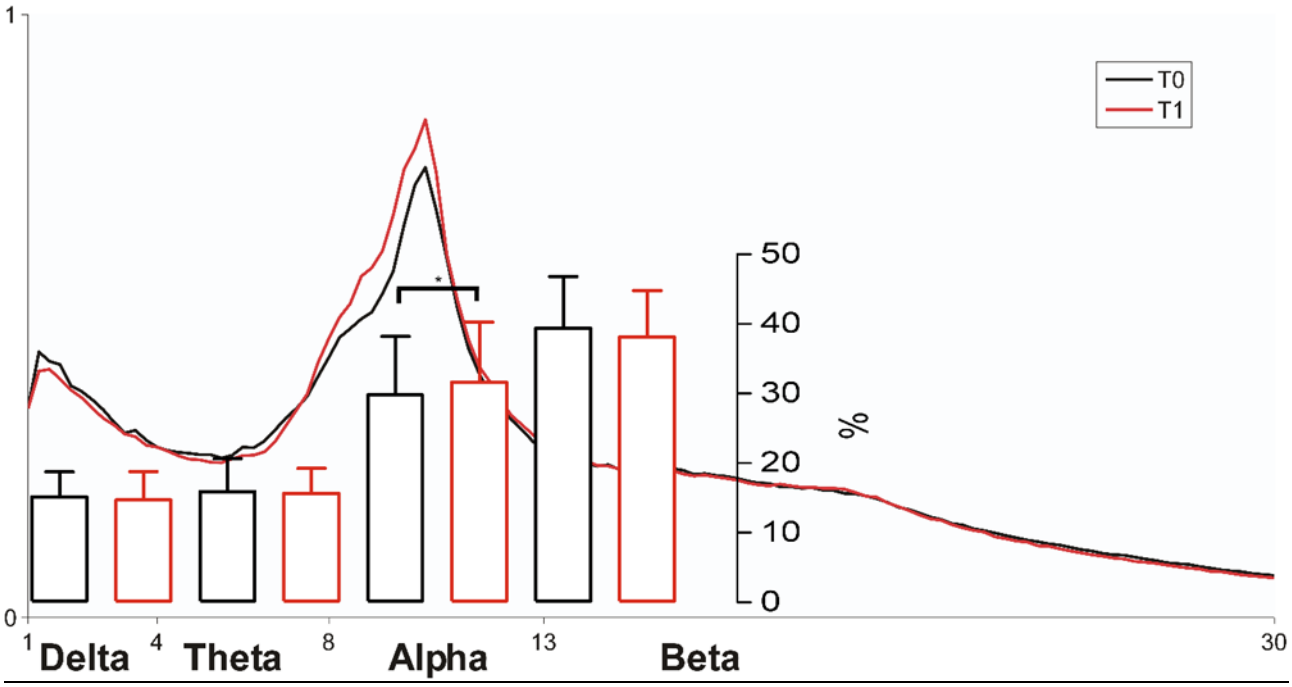

Figure 3 Granaverage of the spectrogram for all the patients at T0 (in black) and T1 (in red).The X axis represents the Hz, from 1 to 30; the Y axis represents the μV, from 0 to 1. The histogram represents the relative amplitude for each frequency band

Correlation between EEG indexes and the clinical outcome

From our results, only alpha band has been modified after 12 day of SAMe treatment. Indeed, we observed an increase of relative amplitude at T1 compared with T0. Here we are interested to explore

the relationship between the alpha increase and the decrease of depression score. We computed the difference in the alpha band between T1 and T0 (T1-T0, i.e. the alpha enhancement) for both the mean amplitude and the relative one. In the same way we computed the decrease of depressive score, computing the difference between T0 and T1 (T0-T1, i.e. the depression score reduction) for both the MADRS and the Hads-D.

Our results show a positive correlation between the alpha mean amplitude enhancement and the MADRS score reduction ($r = 0.473$, $p = 0.030$). No significative correlations were found for the Hads-D, neither for the relative alpha amplitude.

Discussion

Our data show three main results:

- a general improvement for depression, distress, quality of life and fatigue in patients with cancer after a two-week treatment of SAMe;
- in line with previous studies we have found an enhancement in the alpha rhythm after the treatment;
- for the first time, we have reported a correlation between the alpha enhancement after the SAMe treatment and the clinical outcome.

Improvement of psychological symptoms

In this study we evaluated patients with cancer and depression. Most of them are already in treatment with antidepressant, but with no or partial response. It was already shown that in depressed patients with the same characteristics (i.e. not o partial responder), the depressive symptoms are improved after SAMe treatment in add-on to the ongoing treatment (Alpert et al., 2004; De Berardis et al., 2013; Levkovitz et al., 2012). Our data confirm and generalize the finding in depressed patients on a sample of oncological ones.

After two weeks of SAMe treatment, we observed a decrease of depression symptoms, indeed, as the MADRS score shows, the improvement persists after the end of the treatment (T2, day 24).

The patients' improvement is not limited to the depressive symptoms; in fact, we observed a reduction in the distress and in the fatigue. Importantly, the reduction of the two symptoms persists after the end of the treatment. We observed also a slight improvement of the quality of life, which is near to the statistical significance.

Despite the general improvement, we did not observe any variation for the anxiety measured with the Hads. It is not surprising, in fact an increasing of anxiety and a behavioural activation is reported as SAMe side effects, and it can lead to a switch from a depressive to a maniacal/ipomaniacal phase in patients whit bipolar disorder (Carney et al., 1987, 1983).

Finally, we observed no interaction between the dose (800 vs 1200 mg) and the clinical improvement. It means that the same significative response was obtained in the group of non-responders at the dose of 800 mg/day, increasing the dose at 1200 mg/day in the second week.

In line with the previous literature, our data confirm the high tolerance profile of SAMe, the low incidence of side effects, and the relatively fast onset of action.

Taking together, our results suggest that SAMe could be a good option for the treatment of depressive symptoms in cancer patients. The use of psychotropic drugs in oncology need a careful balance between the effectiveness and the safety. Our data confirm the safety and the good tolerance profile of SAMe, even in a population of cancer patients. Indeed, antidepressants have potential adverse effects that might worsen cancer and treatment related symptoms. Our data show not only the effectiveness of SAMe on depressive symptoms, but we have also observed an improvement on other cancer related symptoms, such as distress and fatigue.

EEG modifications

Only few studies investigated the SAMe induced modifications in EEG brain rhythms: two of them evaluating healthy subjects (Arnold et al., 2005; Saletu et al., 2002) and only one evaluating a clinical sample (Torta et al., 1994). We found an enhancement of the relative alpha after the treatment. It is in agreement with the previous findings. Whereas in healthy subjects it was found a difference also in other frequency bands, in depressed patients, SAMe seems to influence only the alpha band (Torta

et al., 1994). Moreover, we found a significative difference only on the relative amplitude and not for the mean amplitude. Looking at the spectrogram (Figure 2), it seems that the grandaverage of all the patients after the treatment has a higher alpha peak, however, the mean amplitude is far from the statistical significance. Moreover, the relative amplitude represents the percentage of each frequency band respect to the entire spectrum. So the enhancement in the relative amplitude is not only influenced from the enhancement in the alpha band (that from our data it is not significative in itself), but also by the concomitant variations in the other frequency bands. We can speculate that with a larger sample size, it could be possible to observe modification also in other frequency bands, as reported in healthy subjects.

The previous studies did not explore the possible relationship between the alpha band modification and the clinical outcome. We found a positive correlation between the alpha mean amplitude enhancement and the reduction of depression score (i.e. the higher is the alpha enhancement, the higher is the depression reduction). As a post-hoc analysis, the positive correlation should be considered as explorative. However, these results can be read in the context of the literature on the EEG rhythms characterization in depressed patients (Olbrich et al., 2015; Olbrich and Arns, 2013). Indeed, it is not surprising that the alpha rhythm is modified after SAMe treatment. Most of the studies on depressed patients proposed the alpha rhythm as possible marker of depression (Begić et al., 2011; Grin-Yatsenko et al., 2010, 2009; Jaworska et al., 2012; Jiang et al., 2016). Moreover the alpha enhancement after the treatment and its correlation with the clinical outcome are in agreement with the proposed negative correlation between the alpha amplitude and the depression severity (Jiang et al., 2016; Zoon et al., 2013)

Limitations

The lack of a control group with a placebo treatment is surely one of the limits of the study. Indeed, there are ethical issues that have determined the choice of the study design. Most of the patients that we have evaluated needed a fast antidepressant response. Moreover, the peculiar characteristics of

SAMe (i.e. endovenous administration and the fast onset of action) do not allow to simply compare the results obtained with this treatment versus a standard antidepressant one. A way to solve this problem and corroborate our preliminarily results could be to carry out a placebo controlled study, using the SAMe as add-on treatment to a standard antidepressant. However, many studies proved the SAMe effectiveness in double blind placebo controlled studies (even if not on cancer patients). Moreover, our results show an improvement for the depressive symptoms, but not for the anxiety. This is in line with the expected effect of SAMe, that has activating properties.

Another limitation is the small sample size. Indeed our study should be considered explorative, as it is the first that have evaluated the difference after a SAMe treatment in a clinical sample, taking together the psychological evaluation and the neurophysiological modifications measured with the EEG. Future studies with a larger sample size should corroborate our findings.

Conclusions

Our data confirmed the safety profile and the antidepressant effectiveness of SAMe in a sample of cancer patients. Taking together the fast onset of action, the safety profile and the effectiveness, our results support the proposal that SAMe can be a good option in treatment of depression in cancer patients. For the first time we reported a correlation between the clinical outcome and the previously reported alpha enhancement after SAMe treatment.

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CHAPTER 3: Experimental model for hyperalgesia and central sensitization

In the previous two chapters, I have discussed the proposed EEG markers for pain and depression and the results of our data in this field. Here I will focus on the central sensitization, one of the core mechanisms at the base of the development and the maintenance of chronic pain. After an overview on the definition and the clinical relevance of central sensitization, I will discuss an experimental model of central sensitization in healthy subjects. In the last part I will present our data testing this model, and in particular the role of C fibers.

Central sensitization

Central sensitization can be defined as "an amplification of neural signalling within the central nervous system that elicits pain hypersensitivity" (Woolf, 2011). It is a top-down process, which involves the central nervous system at the spinal or at the cortical level. It is the result of a use-dependent plasticity of the synapses, which could increase their strength or efficacy. It can lead to two different phenomena, the hyperalgesia and the allodynia. Hyperalgesia is the enhancement of the response to a noxious stimulus; allodynia refers to a somatosensory innocuous stimulus that is perceived as painful. In other words, central sensitization represents a condition where input in one set of nociceptor sensory fibers (the conditioning input) amplified subsequent responses to other even non-stimulated fibers, which can be nociceptive fibers (hyperalgesia) or not (allodynia). For the hyperalgesia, it is possible to distinguish in primary or secondary one. When a tissue is damaged, such as injured skin, we can refer to primary hyperalgesia for the enhancement of the sensation on the lesioned tissue, and as secondary hyperalgesia the modification in sensations on the surrounding area.

An important implication of central sensitization is that the pain that we can perceive is not necessarily driven by the presence of a peripheral noxious stimulus, and even if it is present, its perceived

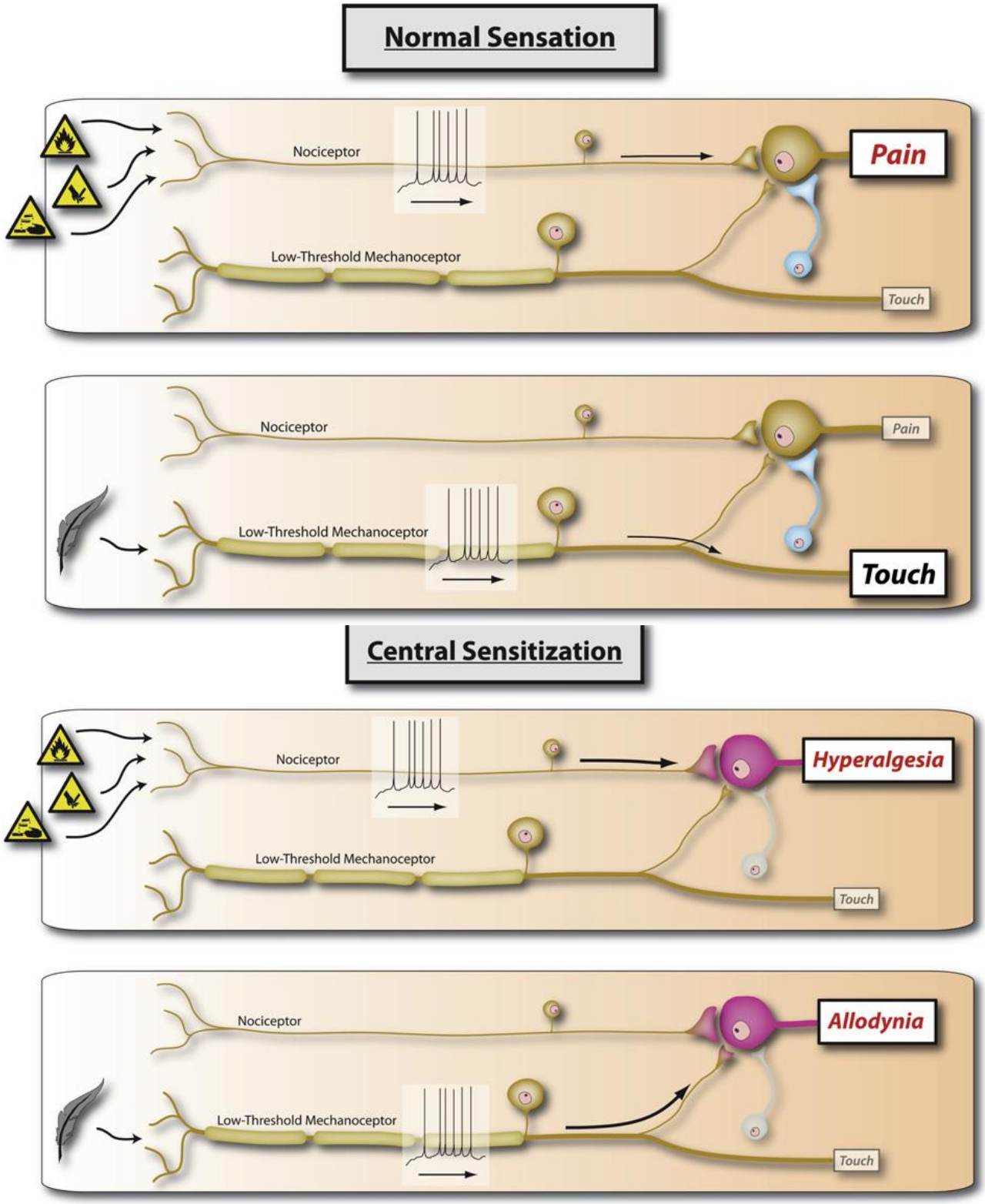

Figure 1 Schematic representation of Hyperalgesia and Allodynia induced by Central Sensitization. Figure from Wolf 2011.

intensity is not necessarily dependent from its physical characteristics. The amplification of the sensation on an injured area has a protective function; however, in chronic pain syndromes it can become dysfunctional. Experimental evidences shown many characteristics of central sensitization in different chronic pain syndromes, such as osteoarthritis (Arendt-Nielsen et al., 2010; Gwilym et al.,

2009), headache (Buchgreitz et al., 2006), fibromyalgia (Staud et al., 2007), neuropathic pain (Fernández-de-las-Peñas et al., 2009; Zanette et al., 2010), and others. Furthermore, the central sensitization processes are some of the core features in the transition from the acute to the chronic pain, as suggested from the modifications at the central nervous system level in the chronic pain syndromes (Vachon-Presseau et al., 2016).

Experimental model of central sensitization in healthy subjects

It is possible to study the central sensitization even in healthy subjects. Using experimental models, it is possible to induce a reversible sensitization of the nociceptive pathway at a central level. The most wide used models are the induction of sensitization by capsaicin injection and the stimulation of the peripheral nerve with electric high frequency stimuli. Experimental models give scientists the possibility to deeply explore the fiber involved in the sensitization process and the alteration in perception of stimuli of different nature. However, by definition, all the experimental models are a sort of acute effect of sensitization. They are useful to understand the underlying neurophysiological processes, but their interpretation and application in a clinical setting, should be done with caution. Indeed, in patients the sensitization of the nociceptive pathway is chronic, instead of being acute; nevertheless is often combined with a peripheral one (Staud, 2010).

Here I will deeply discuss the high frequency electrical stimulation (HFS) model, as it is the one we used in our study.

The cortical responses to noxious and not noxious stimuli after HFS

HFS stimulation is one of the conditioning technique used to sensitize the nociceptive system. It consists in the administration of trains of electrical stimuli at high frequency (usually 100 Hz).

It has been shown that HFS of peptidergic C-fibers induces long-term potentiation of excitatory synaptic transmission between peripheral C-fibers and spinal neurons, both in vitro (Ikeda et al., 2003) and in vivo (Liu and Sandkühler, 1997).

In humans, it has been shown that HFS enhances the perception of mechanical punctuate stimuli (van den Broeke et al., 2016a, 2016b, 2015, 2010; Vo and Drummond, 2013), and that such enhancement persists for at least 60 minutes after the conditioning (Klein et al., 2004) or even more (Pfau et al., 2011). The enhancement of perception was reported in both the conditioned skin and the surrounding area, even if the enhancement seems to be larger in the first site (Klein et al., 2008). In addition with the behavioural data on the enhanced perception after HFS, recently it has been shown that even the neurophysiological response is modified after the sensitization (van den Broeke et al., 2016a). Using pinprick evoked potentials (PEPs)[1] it has been shown and enhancement in the latest PEP components (over 350 ms), 20 and 45 minutes after HFS. In parallel, it was observed the opposite trend for the untreated skin, i.e. a reduction of the PEP response after the first stimulation when it was repeated after 20 and 45 minutes.

If the HFS induced hyperalgesia is clearly present using mechanical punctuate stimuli, it does not seem to be the same for other sensory modalities. Using electrical non-painful stimuli, it has been shown a lack of habituation after HFS compared with untreated skin. Repeating the electrical stimulation after 30 minutes it has been observed a reduction in intensity rating and evoked potentials (EP) on untreated skin, but not after HFS (van den Broeke et al., 2010). The same trend is reported with electrical painful stimuli for the EP, but not for the intensity ratings, that seem decrease in both sensitized and untreated skin (van den Broeke et al., 2012). Somatosensory vibrotactile stimuli are not sensitive to HFS for the reported intensity rating, but it seems that HFS induces a concomitant enhancement for the EP response (van den Broeke and Mouraux, 2014a).

HFS sensitization for mechanical punctuate stimuli seems to be a robust phenomenon, detected with both reported intensity rating and EP. Other kinds of stimuli seem to have a non-congruent effect on sensation and neurophysiological response. A possible explanation could be that mechanical painful stimuli can activate mechanosensitive fibers, even not pain specific, as suggested by the enhancement

[1] Pinprick Evoked Potentials (PEPs) are the brain responses to the Pinprick stimulator. It consist in a stainless inner tube with a retractile needle that exerts on the skin a constant force.

of vibrotactile somatosensory EP; and that those fibers can have a crucial role on the HFS induced hyperalgesia. Another possible explanation could be that the HFS long-term potentiation is selective for the nociceptive pathway, and it is not present with non-painful stimuli. To help to discriminate between the two hypotheses, it is interesting to look at those studies that have tested the HFS effects on painful heat stimuli. Indeed, using a laser stimulator it is possible to active the nociceptive pathway, without any mechanical stimulation.

The use of Laser Evoked Potentials (LEPs) to characterize the HFS induced hyperalgesia

Laser evoked potentials (LEPs) are the brain response to laser heat stimuli recorded with the EP technique. The cortical response is mediated by Aδ and C fiber (Plaghki and Mouraux, 2005). Aδ fibers are mielined, they have a fast conduction and generate an EP complex around 160-390 ms after the stimulus. They are involved in the perception of the "first pain" characterized by a pinprick sensation. C fiber are unmielined, they have a slower conduction and they generate an EP complex around 750-1150 ms after the stimulus. They mediated the perception of the "second pain" characterized by a burn sensation.

HFS seems to induce also heat hyperalgesia (van den Broeke and Mouraux, 2014a). After HFS healthy subjects report an enhancement in pain rating 20 and 45 minutes after a CO2 laser stimulation. Surprisingly, the concomitant recorded LEP is not modified after the sensitization. The authors proposed that the recorded LEP is probably due to an Aδ fibers activation rather than C one. Indeed, the reported latencies are in line with the Aδ conduction speed. They conclude that it is possible that C fibers rather than the Aδ ones could mediate the enhancement of the pain perception. Even though the reasons are still being debated, it is difficult to record C fiber LEPs simultaneously with the Aδ activation (Garcia-Larrea, 2004; Mouraux et al., 2004). One of the easily way to obtain a C fiber LEP response is using stimulus intensities above the C fiber activation threshold but below the Aδ fiber one (Jankovski et al., 2013), indeed C fibers have a lower activation threshold than Aδ. Using lower intensities it has been shown that after HFS sensitization, healthy subjects report an higher pain rating

20 and 45 minutes after the sensitization (van den Broeke and Mouraux, 2014b). However, the C fiber LEP seems to enhance only 20 minute after the sensitization and no difference are detected 45 minute after the sensitization, compared with the baseline. The authors conclude that if HFS did not enhance C fiber LEPs at both time points, probably HFS-induced enhanced heat sensitivity is mediated by afferents that do not significantly contribute to C fiber LEPs. However, one possible criticism on this method for activate C fibers selectively, could be that using low-intensity non-painful stimuli, the elicited intensity of perception and C fiber LEPs are mainly mediated by warm receptors instead of nociceptors.

Study 3: Transcutaneous high frequency electrical stimulation in humans enhances heat pain perception but exerts no effect on concomitantly recorded C fiber related brain potentials

To be sure that the LEP responses are elicited by the activation of nociceptors instead of warm receptors, it is necessary to use relatively high intensity stimuli, above the Aδ activation threshold. Recently, it has been shown the possibility to register concomitant Aδ and C fiber LEPs, using stimuli generated by an infrared Neodymium: Yttrium-Aluminum-Perovskite (Nd:YAP) laser stimulator (Hu et al., 2014). Aim of the present study is to evaluate the HFS effects using high intensity laser stimuli on the perceived intensity and the concomitant C fiber LEP response, using the method proposed by Hu and colleagues.

Material and methods

Participants

Sixteen healthy volunteers took part in the experiment (6 men and 10 women; mean age 24.5 ± 3.8 years). All participants signed an informed consent form and received financial compensation for their participation.

Experimental design

The design of the experiment is summarized in Figure 2. During the experiment participants were comfortably seated in an adjustable chair with their arms resting on a table in front of them. Painful heat stimuli were delivered to both arms before (T0), twenty minutes after (T1) and forty-five minutes

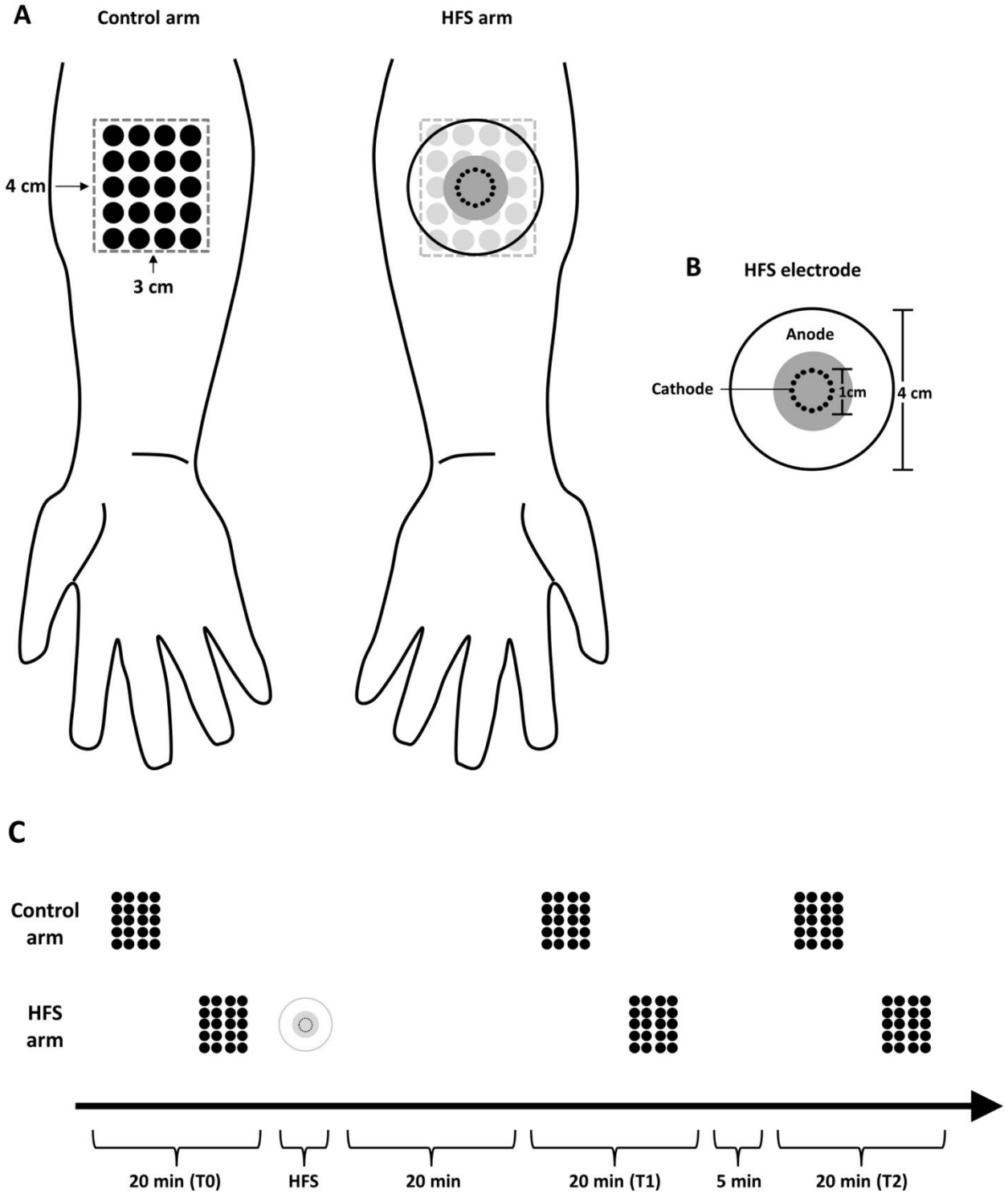

Figure 2 Brief overview of the experimental design. Panel A shows the stimulation site. Panel B represents the HFS electrodes used for the conditioning. Panel C shows the different evaluation time point for the control and the HFS arm.

after (T2) transcutaneous high frequency electrical stimulation (HFS) as showed in Figure 2. The heat stimuli were applied within and surrounding the area onto which HFS was delivered, and to the corresponding skin area of the contralateral arm which served as control. Concomitantly with the heat stimulation we also recorded brain responses (evoked potentials, EPs). Moreover, the intensity of perception elicited by the heat stimuli was evaluated after each stimulus, using a numerical rating scale (NRS) ranging from 0 (no perception) to 100 (maximal pain), with 50 representing the transition from non-painful to painful domains of sensation. Before the start of the experiment, subjects were familiarized with the heat stimuli.

High frequency electrical conditioning stimulation (HFS)

Transcutaneous HFS was applied to one of the arms. To avoid any confounding effect of handedness, the arm onto which HFS was applied (dominant vs. non dominant) was counterbalanced across participants. Handedness was assessed using the Flinders Handedness Survey (Nicholls et al., 2013). The HFS protocol consisted of five trains of 100 Hz (pulse width: 2 ms) lasting 1 second each. The time interval between each train was 10 seconds. The intensity of stimulation was individually adjusted to 20 times the detection threshold to a single pulse (0.29 ± 0.07 mA; mean ± sd). The electrical pulses were triggered by a programmable pulse generator (Master-8; AMPI Israel), generated by a constant current electrical stimulator (Digitimer DS7A, Digitimer UK), and delivered to the skin using a specifically designed electrode designed and built at the Centre for Sensory-Motor Interaction (Aalborg University, Denmark). The cathode consists of 16 blunt stainless steel pins with a diameter of 0.2 mm protruding 1 mm from the base. The 16 pins are placed in a circle with a diameter of 10 mm. The anode consists of a surrounding stainless steel ring having an inner diameter of 22 mm and an outer diameter of 40 mm (see figure 1B).

Heat stimulation

A block of twenty single heat stimuli were administered to each arm (HFS and control arm) at each time point (T0, T1 and T2). The heat stimuli (duration: 4 ms) were generated by an infrared Neodymium: Yttrium-Aluminum-Perovskite (Nd:YAP) laser stimulator which had a beam diameter

of ~7mm. The intensity of the stimulation was individually adjusted (4.0 ± 0.5, mean ± sd) to an energy that elicited a perception qualified as clearly painful, i.e. supra pain threshold (NRS > 50). The stimuli were delivered using a random inter-stimulus interval ranging from 3 to 5 seconds. The twenty single heat stimuli were applied, without any overlap, within an area of 3 x 4 cm (see Figure 1A). Before the start of the experiment, twenty circles were drawn on each arm indicating the spots to be stimulated. In order to prevent that for each block the heat stimulation started at the same spot, the order of stimulation was balanced across participants. During stimulation the laser pointer was hand-held perpendicular to the skin with a distance of approximately 5 cm between the laser and the skin surface. The laser stimuli were triggered manually via a pulse generator (Master-8; AMPI Israel) that at the time of stimulation also send a trigger to the EEG for marking stimulus onset. The sound that is normally produced at the onset of stimulation, for this type of laser stimulator, was switched off during the whole experiment. Before the start of each block the baseline skin temperature was measured with a thermometer (Tempett IR-Thermometer, Senselab, Sweden).

EEG recording

The EEG was recorded using three electrodes (Fz, Cz and Pz) which were applied with an elastic electrode-cap (Multitrodes, Brain products GmBH, Germany). Participants were instructed to keep their gaze fixed on a black cross displayed at a distance of approximately 2.0 m and to sit as still as possible without making any movement. The EEG signals were amplified and digitalized using a sampling rate of 1000 Hz and linked mastoids (M1M2) reference (HS64; Advanced Neuro Technologies, The Netherlands). The ground electrode was placed at electrode AFz. Eye movements were recorded using two surface electrodes placed at the upper-left and lower-right sides of the left eye. Electrode impedances were kept below 20 kΩ.

EEG data processing

All the EEG data processing was made offline using Letswave 6 (www.nocions.org/letswave). We have focused all the analysis on Cz, because it has been shown that C fiber LEPs are maximal at the vertex (Hu et al., 2014).

Data processing consists in the following steps:

1. band pass filter (Butterworth 0.3-30 Hz)
2. segmentation in epochs from 500 ms before the stimulus onset to 2000 ms after it
3. removing of ocular related artifacts using Gratton and Cole method (Gratton et al., 1983)
4. baseline correction (reference interval from -500 ms to 0)
5. rejection of epochs with amplitude exceeding 100 μV
6. grandaverage for each subject for each experimental condition (HFS arm T0, HFS arm T1, HFS arm T2, Control arm T0, Control arm T1, Control arm T2).

Statistical analysis

To assess the effects of HFS on pain perception we have performed a repeated measures ANOVA using two within-subject factors: time (T0, T1 and T2) and treatment (control vs. HFS arm). The Greenhouse-Geisser correction was used in case of violation of sphericity. The statistical analyses were conducted using SPSS 18 (SPSS Inc., Chicago, IL, USA). The effect of HFS was assessed using the interaction between the factors time and treatment.

The effect of HFS on the C fiber LEPs was assessed using a non-parametric cluster-based permutation approach (Maris and Oostenveld, 2007), which is implemented in Letswave 6 (www.nocions.org/letswave). As a first step, we computed, for each subject, difference waveforms assessing the change in LEP waveform at T1 vs. T0 and T2 vs. T0 at the control arm (control arm T1 – control arm T0; control arm T2 – control arm T0) and at the HFS-treated arm (HFS arm T1 – HFS arm T0; HFS arm T2 – HFS arm T0). Then, we performed the cluster-based permutation test on the difference waveforms of both arms, in the time window 500-1000 ms, thereby testing the interaction between the time and the treatment.

Results

Intensity of perception

Figure 3 shows the results of the reported stimuli ratings. The repeated measures ANOVA, based on the average ratings of all the 20 stimuli, revealed a significant interaction between time and treatment ($F(1.407, 21.110) = 5.843$, $p = .016$). This means that the average pain rating is different between the two arms at the different time points. The univariate within-subject contrasts revealed that the pain intensity was almost significantly different between the two arms at T1 ($F(1, 15) = 4.473$, $p = .052$) and significantly different at T2 ($F(1, 15) = 7.379$, $p = .016$). Post-hoc tests (Bonferroni corrected for multiple comparison) revealed a statistically significant decrease of pain perception at T2 (paired t-test; $t(15) = 3.584$, $p = .003$) on the control arm.

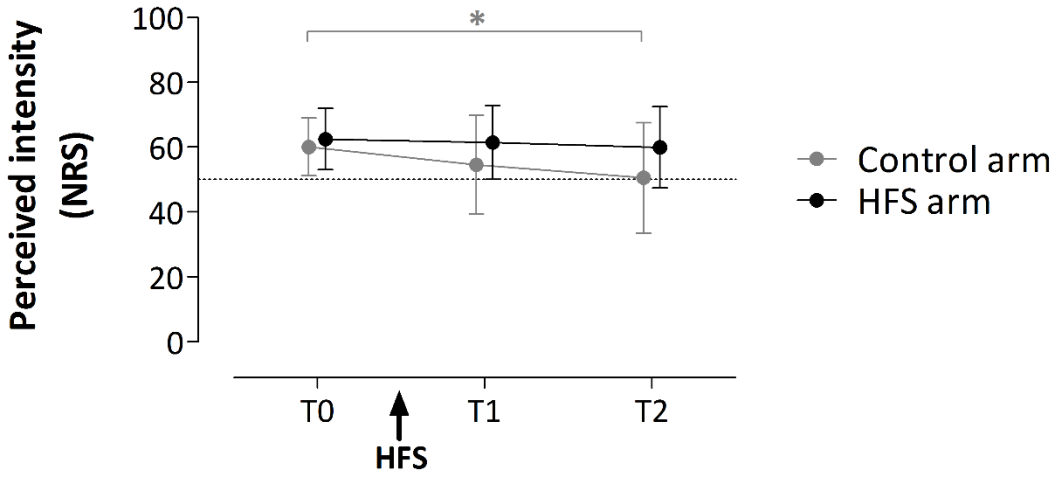

Figure 3 Results of stimuli rating at the different time points (T0, T1, T2) for each arm (HFS and Control)

C fibre related laser evoked potentials

The grandaverages for each time point for both the HFS and the control arm are shown in Figure 4. The non-parametric cluster-based permutation test revealed no statistically significant difference between the C fiber related LEPs of the HFS arm vs. control arm after HFS, at T1 or T2.

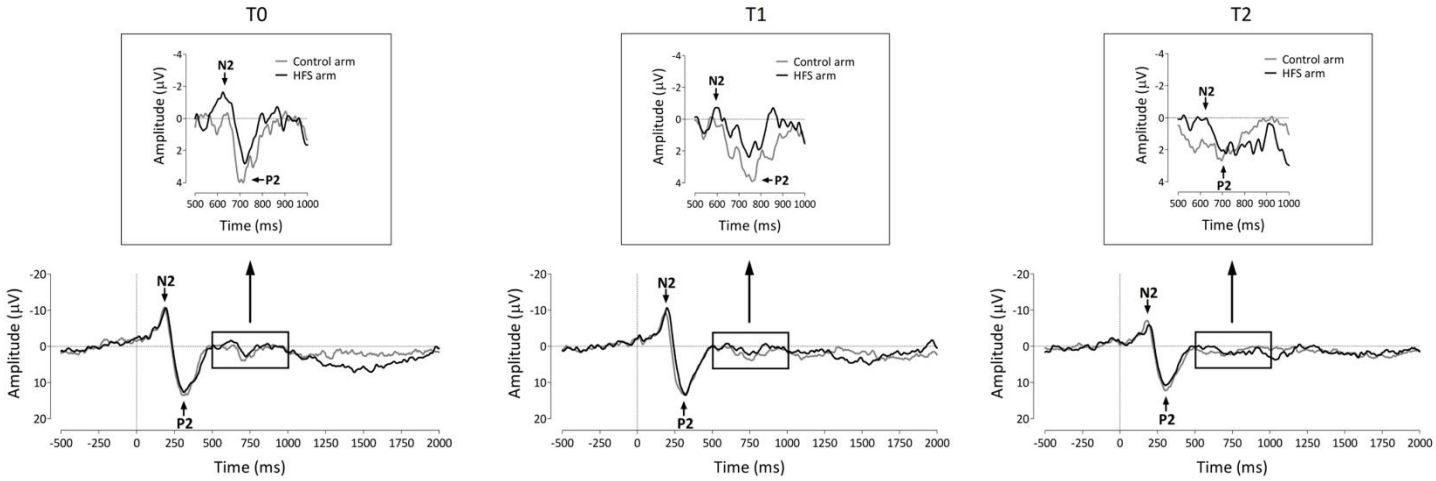

Figure 4 Grandaverages across all the subjects at each time point for both HFS and control arm. The first larger N2-P2 complex reflects the Aδ fiber response; the second one reflects the C fiber response.

Baseline skin temperature

The baseline skin temperature observed at both arms (control and HFS) at the different time points (T0, T1 and T2) is shown in Figure 5. The repeated measures ANOVA revealed no statistically significant changes in baseline skin temperature after HFS.

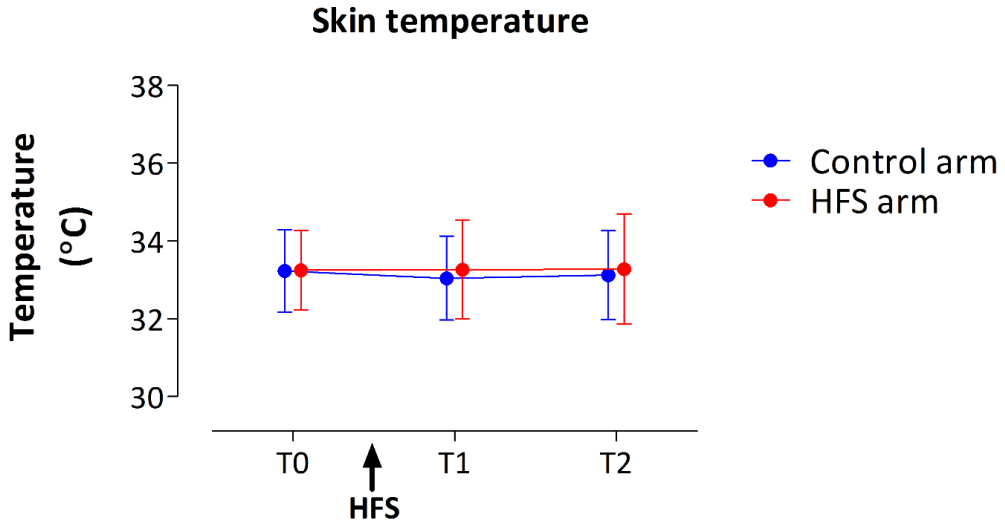

Figure 5 Mean skin temperature at each time point for both HFS and Control arm.

Discussion

Two main findings emerge from our results. First, as already reported (van den Broeke and Mouraux, 2014a, 2014b), our results show that, after HFS, the heat stimuli are perceived as more intense. Second, we have not observed any difference after the HFS sensitization on C fiber LEPs.

The lack of modification after HFS on LEP, seems to point against the possible role of C fiber on the increasing of pain ratings. The same conclusion was carried out after a selective activation of C fiber with low intensity stimuli (van den Broeke and Mouraux, 2014b), which lead only to a short lasting effects on LEP. However, the possibility that the recorded LEP was elicited by warm receptors instead of nociceptors, is not excluded by the previous study. We used relatively high intensity stimuli, and all the subjects perceived them as painful. Our results show that neither higher intensity stimuli can affect the C fiber LEP response. Taking together these results seem to exclude that the enhancement of reported pain intensity is mediated by the C fiber signalling.

If C fibers do not mediate the enhancement in perception, which mechanism underlies such enhancement? The lack of consistence between the increasing in pain rating and the unaltered neurophysiological response can be explained with almost two hypotheses.

The first concerns the specificity of the HFS induced heat hyperalgesia. Today, it has been observed a congruence between the behavioural and the EP responses after HFS only for mechanical punctuate stimuli (van den Broeke et al., 2016a). As proposed by the authors, it is possible that HFS lead to different changes, which can be unspecific or strictly related to the induced long-term potentiation. It is possible that the response to mechanical punctuate stimuli is directly mediated by HFS sensitization, as shown by the congruence between the behavioural and EP data. On the other hand, the enhancement in perception reported with heat stimuli, as in our results, can be mediated by unspecific mechanism, probably based on attentional processes. Recently, it has been shown that HFS modulates non only somatosensory stimuli, but also visual ones in the sensitized area (Torta et al., 2017). The authors shown an enhancement of visual evoked potentials on the sensitized skin 20 minutes after HFS, but not 45 minutes after HFS compared with the baseline. The timecourse of the

visual EP modification (enhancement after 25 minutes and not after 45 minutes) is similar to the one reported activating selectively the C fibers (van den Broeke and Mouraux, 2014b). Taking together, these results seem to support the idea that HFS induced short lasting unspecific effects (probably linked with attentional processes) and long lasting pain specific effects. Moreover, it is possible that these unspecific effects mediated the enhancement on heat stimuli perception reported here and elsewhere (van den Broeke and Mouraux, 2014a, 2014b).

A second hypothesis focuses on the high intensity of the stimuli used in this study. Our results show no effect on C fiber LEP, neither long nor short lasting one. Similar results with high intensity stimuli are already reported on Aδ LEP (van den Broeke and Mouraux, 2014a). If the high intensity stimuli guarantees the activation of nociceptors instead of warm receptors, it probably leads to a ceiling effect. It is possible that the LEP response has already reached a maximum, which do not allow a further increase after the sensitization. It has been shown that after capsaicin-induced sensitization, the relation between the pinprick EP response and the force applied by the stimulator, is not linear (van den Broeke et al., 2015). Indeed, the enhancement in pinprick EP, after the capsaicin sensitization, reach a maximum with the force of 64 mN, and it is less marked using higher forces. Moreover, even using HFS, after the sensitization an enhancement has been reported using the force of 64 mN but not with 90 mN (van den Broeke et al., 2016a). We used a fixed intensity for each subject and it is not possible from our data to exclude that LEP response after the sensitization is less sensitive to the enhancement, as it has been shown with the PEPs.

However, the two hypotheses are not mutually exclusive. With lower intensity stimuli, it has been reported only short lasting modifications in LEP after HFS. Moreover, it is proposed that the short lasting effects are probably related to unspecific attentional-based processes. We can speculate that the lack of even short last effects, is probably due to a ceiling effect and that the enhancement in stimuli perception is mediated by unspecific HFS induced modifications. Moreover, both the hypotheses seem to exclude the role of C fibers as mediator of the HFS induced heat hyperalgesia. Following the ceiling effect hypothesis, it is unlikely that C fibers mediate the perceived enhancement

if they have already reached their maximum response. On the other hand, if the perceived enhancement is due to unspecific attention related modifications, it is more likely that the effect is mediated at a higher cortical level.

Limitations

The possible ceiling effect is surely one of the limit of this study. Moreover, the use of a fixed intensity prevents the possibility to evaluate the changes in HFS LEP response with different intensities. Here we have preferred to apply a different intensity stimulation based on the subject perception, in order to be sure to elicit a clear painful perception.

Another limitation is the signal to noise ratio. Indeed, looking figure 4, Aδ response seems to be better defined than the C-fiber one. The LEP for each condition is obtained by the average of 20 stimuli. It is possible that the number of stimulation is sufficient to obtain a clear Aδ response, but less efficient to obtain a C fiber one. However, the use of the cluster based permutation test for the assessment, partially overcomes this aspect with its high sensitivity. Moreover, it is possible that with more stimuli the C fiber LEP could be better defined.

At last, today is still debated the reliability of the method proposed by Hu and collegues (2014) that we have tested. The latencies that we have reported for the N2 component is earlier compared with the attended one (600 ms vs 800-1000 ms), and it is possible that the N2-P2 complexes are the result of a mixed activation rather than a selective activation of the C fiber.

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Conclusions

For both depression and chronic pain, the analysis of the literature suggests that the EEG is not a reliable marker for these symptoms today. Our data on cancer patients show that those ones that report pain have a reduction in alpha amplitude instead of an enhancement. Our results, but also other studies that point in the same direction (Camfferman et al., 2017; Jensen et al., 2013), rise important question on the specificity of alpha band enhancement in chronic pain. Looking at our results on SAMe treatment, the enhancement in alpha rhythm at the end of the therapy highlights that the alpha band can be modulated by factors unrelated with pain, as it has been shown by the studies that report alpha alteration in depressed patients.

The amplitude in the different frequency band seems to be influenced by many factors, and it can partially explain the variability from the different studies. However, it is difficult to take in account all the possible sources of variability, especially when they are highly correlated, like pain and depression.

If many factors can influence the EEG rhythms at rest, a way to partially reduce this problem could be using experimental designs that can reduce the impact of such factors.

EP seems to be an useful tool to characterize central sensitization processes. From the data presented in the HFS study and the others discussed in the chapter 3, pinprick evoked potentials seem to measure effectively the hyperalgesia induced changes in pain pathway. They are a promising tool for the evaluating of treatment effects in chronic pain patients. Evoked potentials give by definition a more specific response than the analysis of spontaneous EEG, because they register only the time locked response to the stimulus.

Recording brain responses to stimuli should be a way to reduce the possible sources of variability. Moreover, it is possible to get better results even recording the spontaneous EEG. The typical instruction before a resting state recording is to relax and try to think about nothing specific. This approach do not take in account the possible role of the attentional processes in pain modulation, that

have an impact on pain perception and in pain related brain activation (Torta et al., 2017). Indeed, investigating formally the thoughts of healthy subjects during an EEG recording, it has been reported specific clusters of thoughts that seem to have an impact on the brain rhythms (Diaz et al., 2013). If specific thoughts can affect the EEG, we can speculate that asking the patients to focus on their ongoing pain, could be a good strategy to enhance the pain related EEG rhythm during the recording. A similar approach was already used in an fMRI study (Baliki et al., 2011), in which the patients were asked to rate the variation on their ongoing pain with a specific device. An online monitoring of pain sensation was also used for the evaluation of long last thermal stimuli in healthy subjects (van den Broeke et al., 2016).

In conclusion, the alpha amplitude does not seem to be a reliable marker for depression or pain. The large variability of the studies present in the literature and our data suggest that probably the resting state condition alone is not the best way to identify possible marker of psychological or pain symptoms.

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Collateral project: The influence of space perception on nociception, an exploration on the cross-hands analgesia phenomenon

Here I will present a collateral project, which focuses on the role of space perception on nociception. As I have already shown in the previous chapters, the idea that pain perception is influenced by many factors, such as mood disorders and cognitive function, is widely accepted. One of the function of the nociceptive system is to protect the body integrity. The strong link between pain perception and spatial perception is not surprising. Indeed, the ability to correctly identify a possible source of damage and its localization is one of the nociceptive system core feature. It is today debated what are the processes that underlie such integration between the pain and space perception. It is possible to investigate the contribution of different frames of reference in pain and somatosensory perception, inducing the so called cross-hand analgesia (CHA) (Gallace et al., 2011), using a paradigm in which are compared the response to stimuli given in an anatomical position or with the hands crossed over the body midline. In this chapter I will briefly report the most relevant studies that have explored the CHA phenomenon and I will also present our data on a group of patients suffering from unilateral spatial neglect (Vizzari et al., 2017).

Cross-hand analgesia (CHA): experimental evidences and possible explanations

When a stimulus is delivered to the hand, the ability to localize it depends from the integration of two set of coordinates, a somatotopic one (where the stimulus is on the skin) and a body-centred one (where the stimulus is in the space). Both somatosensory and nociceptive stimuli are coded in both the frames of reference (Heed and Azañón, 2014; Sambo et al., 2013). It seems that tactile stimuli are encoded first in a somatotopic frame of reference, and that the remapping in a body-centred frame consists in a second processing step (Azañón and Soto-Faraco, 2008). When the arms are crossed, there is a mismatch between the two coordinate systems. Indeed, usually the right arm occupies the right side of external space, and the opposite is for the left one. To solve this mismatch, it is required

an update of the current hand position, that can affect the perception of somatosensory and painful stimuli.

The effect of crossing the arms in stimuli localization has been evaluated through a temporal order judgments task (TOJ). It consists in a double asynchronous stimulation to both the hands. The subjects are required to indicate which hand is stimulated first. It has been observed a worsening in TOJ performance in a crossed position (Badde et al., 2014; Sambo et al., 2013; Soto-Faraco and Azañón, 2013).

Beside the data on stimuli localization, when the hands are crossed it has been observed a reduction on the stimulus perceived intensity. Crossing the arms over the body midline reduced both intensity of perception and evoked potentials (EPs) response for somatosensory (electric) and painful (heat) stimuli (Gallace et al., 2011). Interestingly the authors found a reduction only in the latest EP component, suggesting an involvement of cognitive processes rather than sensory ones, and according with the notion that at the early stages the stimuli are mapped using somatotopic coordinates. An fMRI study (Torta et al., 2013) shown that crossing the arms increases the activation of frontal multimodal area and reduces the activity of the posterior parietal cortex, during a mechanical punctuate stimulation. The authors also reported a reduction in stimuli perception in the crossed position. These results support the hypothesis that cognitive processes, and not sensory ones, mediate the crossed hand analgesia. It seems that crossing the arms can influence the brain activity itself. It has been shown an increased activity of the left posterior parietal cortex when subjects adopted a cross-hand posture at rest (Wada et al., 2012). Interestingly, after the scanning the subjects undergo to a TOJ task, in both uncrossed and crossed condition. It has been reported a significative association between the posterior parietal activation and the worsening in TOJ performance in the crossed position.

A possible explanation for the crossed hand analgesia focuses on the mismatch of the different frames of reference. In this view, the reduction in perception is a consequence of the cognitive cost, associated to the realignment of the neural representation based on the different frames of reference

(i.e. somatotopic and body-centred). On the other hand, the reduction in perception could be related to brain activity modifications, due to crossing arms itself.

Study 4: Mechanical pinprick pain in patients with unilateral spatial neglect: the influence of space representation on the perception of nociceptive stimuli

The previous mentioned hypotheses are not mutually exclusive. The first hypothesis postulated that crossing the arms can lead to a mismatch between the two frames of reference. The cost to realign the two frames of reference is possible to be the base for the CHA. However, a necessary condition for a mismatch is that both the representations should be present. Otherwise, we could expect that no representation will lead to no mismatch, and the lack of mismatch will lead to no CHA.

To test this hypothesis we evaluated two group of patients with brain damage. One group has no deficit in spatial cognition, whether the second group is affected by unilateral spatial neglect. Neglect represents a unique opportunity to test this hypothesis. In fact patients with neglect are impaired to detect, attend and respond to contra-lesional stimuli. Patients do not show any sign of primary sensory deficit, indeed the syndrome involves deficits in spatial representation, at higher level of stimulus processing (Bisiach and Luzzatti, 1978; Driver and Vuilleumier, 2001). It is the first time in which the CHA is tested on neglect patients. We expect to find a CHA in those patients without spatial deficit, but not in neglect patients that have an impairment on contra-lesional spatial representation.

In addition it has been reported that crossing the arms improves the ability to detect tactile stimuli in patients with extinction (Aglioti et al., 1999). Patients suffering from extinction do not show the same marked deficits that neglect patients do; for instance extinction patients have a preserved ability to detect, attend and respond to contra-lesional stimuli. However, when stimuli are presented concomitantly in the contra-lesional and ipsi-lesional sides, patients with extinction are impaired at detecting the contra-lesional stimulus, which competes with the ipsi-lateral one. Neglect and extinction have been considered by some authors as different levels of severity of the same disorder, but more often as two distinct syndromes associated with different neuronal substrates (de Haan et

al., 2012; Umarova et al., 2011; Vallar et al., 1994). For the close relation between neglect and extinction, a second aim of our study is to evaluate if crossing the arms has an influence either in stimuli detection, as reported in extinction patients.

Material and Methods

Patients

Table 1 shows the demographic and clinical characteristics of the sample. Patients were enrolled from the Division of Neurology and the Stroke Unit of the Città della Salute e della Scienza, Molinette Hospital and the Neuropsychology Unit of the San Camillo Hospital, both in Turin, Italy. Patients were tested for cognitive decline by expert neuropsychologists.

Inclusion criteria were: presence of an haemorragic or hischemic brain lesion, ability to clearly understand the task (i.e. lack of aphasia) and a score above the cut-off for the Mini Mental State Examination (MMSE > 23) (Carlesimo et al., 1996; Folstein et al., 1975) or Montreal Cognitive Assessment (MOCA) (Nasreddine et al., 2005).

Exclusion criteria were: the presence of primary somatosensory deficit, brain tumor, presence of aphasia, for both the difficulty in clearly understand the task and/or the inability to produce verbally the intensity rating of the stimuli (see below).

Patients recruited at the Division of Neurology and the Stroke Unit of the Città della Salute e della Scienza Hospital were tested with the MMSE. Patients recruited at the San Camillo Hospital were tested only with the MOCA and not the MMSE. Post-stroke patients were tested from one week to more than one year after the stroke, depending on the centre where they were recruited. All patients were right-handed according to self-report and administration of the Edinburgh Inventory Scale (Oldfield, 1971).

Group	Age	Sex	Education	Tactile Extinction	Visual Extinction	Lesion	Lesion side	Lesion site
N+E+	75	F	5	-	-	Ischemic	R	P
N+E+	85	M	18	-	+	Ischemic	R	F, P
N+E+	69	M	13	-	-	Haemorrhagic	R	P
N+E+	70	F	5	-	-	Ischemic	R	F,P,T
N+E+	49	F	8	-	-	Ischemic	R	F, P
N+E+	71	F	5	-	-	Ischemic	R	F, P, O
N+E+	83	M	5	-	-	Ischemic	R	F
N+E+	46	M	13	-	-	Ischemic	R	F, T, P
N+E+	64	F	18	-	-	Brain Tumor	R	T
N+E+	40	M	13	-	+	Ischemic	R	F, T, P
N+E+	55	M	13	+	-	Ischemic	R	BG
N+E+	86	F	8	-	-	Ischemic	R	F, P, T
N+E+	81	F	5	-	-	Ischemic	R	P
N+E+	72	F	8	-	-	Ischemic	R	F, T, P
N+E+	63	F	8	-	-	Ischemic	R	T
N-E-	79	M	16	-	-	Ischemic	R	F
N-E-	89	F	3	-	-	Haemorrhagic	L	F, T, P
N-E-	86	M	13	-	-	Ischemic	L	F, P, I
N-E-	64	M	12	-	-	Ischemic	L	BG
N-E-	75	M	6	-	-	Ischemic	R	T, O
N-E-	67	M	5	-	-	Ischemic	R	BG
N-E-	60	F	13	-	-	Haemorrhagic	L	P
N-E-	70	M	8	-	-	Ischemic	R	F
N-E-	61	M	13	-	-	Ischemic	L	BG
N-E-	54	M	8	-	-	Haemorrhagic	R	F, P
N-E-	72	F	7	-	-	Haemorrhagic	R	T, O
N-E-	52	M	11	-	-	Ischemic	R	BG
N-E-	56	M	11	-	-	Ischemic	R	F, T, P
N-E-	75	M	5	-	-	Haemorrhagic	L	T, P
N-E-	71	M	5	-	-	Ischemic	R	F
N-E-	72	M	5	-	-	n.a.	R	n.a.

Table 1 Demographic and clinical characteristics of the sample. Education is expressed in years. For the lesion site: F = Frontal lobe; P = Parietal lobe; T = Temporal lobe; O = Occipital lobe; I = Insula; BG = Basal ganglia. Data on the lesion site of the last subject are not available.

Neuropsychological evaluation

The spatial awareness was assessed with the following instruments:

- Bells test (Gauthier et al., 1989), a cancellation test in which the subject have to mark target stimuli in a context of visual distractors. The target stimuli are represented by bells, whereas

the visual distractor are other figures, such as cars, keys, trees and others. Omission target in the left but not in the right side are considered sign of neglect. The scoring was made computing the target stimuli omission for the left side. The cut off is 6 out of 15.

- Behavioral Inattention Test (BIT) (Halligan et al., 1991) consists in six conventional subtests (BIT-C) and nine behavioral ones (BIT-B), which asses aspects of daily life. BIT-C was composed by: Line Crossing, Letter Cancellation, Star Cancellation, Figure and Shape Copying, Line Bisection, and Representational Drawing. BIT-B was composed by: Picture Scanning, Telephone Dialing, Menu Reading, Article Reading, Telling and Setting Time, Coin Sorting, Address and Sentence Copying, Map Navigation, and Card Sorting. Cut-off scores were of 129/146 for the BIT-C, and 67/81 for BIT-B
- Diller Test (Diller and Weinberg, 1977) is a cancellation test. It consists in a sheet of A3 paper that has 104 uppercase Hs (targets) in a context of 386 different letters (distractors). Letters are arranged in 6 horizontal lines. The patient was asked to cross out all the Hs. The cut-off is a difference of ≥4 between omissions on the left and on the right side

Patients recruited at the Division of Neurology and the Stroke Unit of the Città della Salute e della Scienza Hospital were screened for the presence or absence of neglect with the Bells test. The BIT-C, BIT-B and Diller tests were performed only at the San Camillo Hospital.

In addition to awareness of space we evaluated the presence of primary sensory deficits and extinction using Bisiach and collaborators' tasks (Bisiach et al., 1986). Participants showing no sign of neglect at these tests were further screened for the presence of tactile and visual extinction. Visual extinction was assessed by a confrontation technique (Bisiach et al., 1986). The experimenter, in front of the patient, moved one or both index fingers in the left, right or both visual hemifields. To assess tactile extinction, the experimenter precluded the vision of the hands to the patient and delivered single or double light touches to the right, left or both hands. Series of 30 stimuli (10 unilateral left, 10 unilateral right and 10 bilateral) were used for both vision and touch. Visual and tactile extinctions were diagnosed when 30% of double stimuli were omitted, but at least 80% of single contralesional

stimuli were detected (Aglioti et al., 1999; Bartolomeo et al., 2004). If more than 80% of single stimuli were omitted the patient was considered as having a primary sensory deficit, i.e. hemianopia and/or hemianaesthesia.

Figure 1 summarizes the enrolment process. Using these criteria, we enrolled 31 patients of which 16 were free of any sign of unawareness of space (control group). Twelve patients were diagnosed with neglect (neglect group), but one neglect patient was discarded as the symptoms were due to a tumour. Two additional patients showed extinction to visual stimuli (but not tactile), and one showed extinction to tactile stimuli (but not visual).

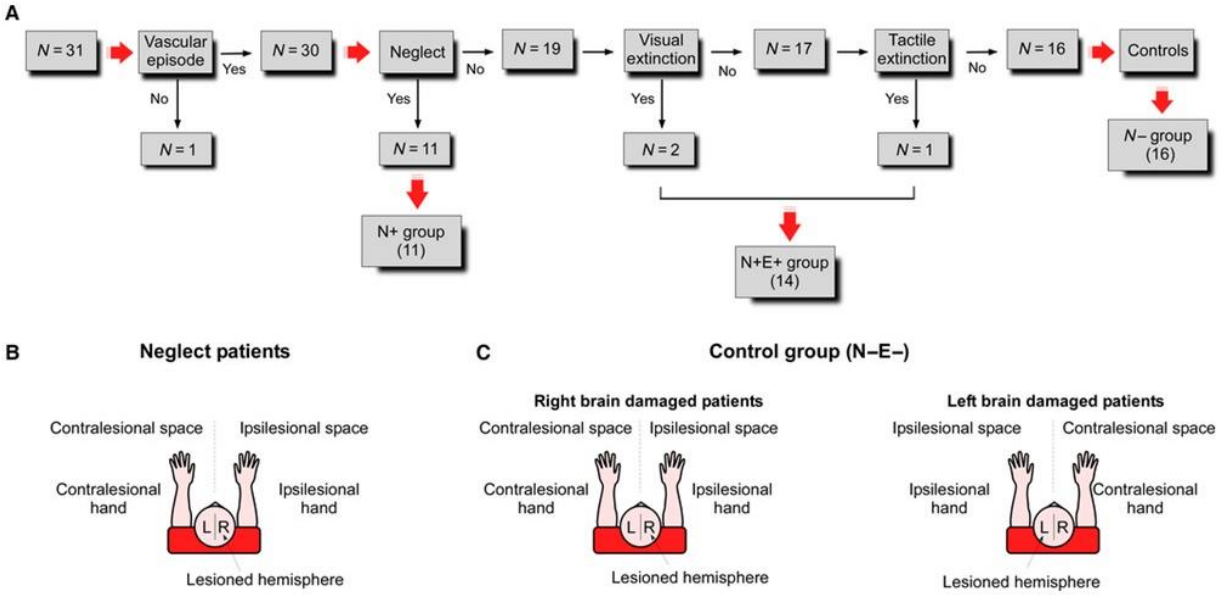

Figure 1 Panel A shows the patients selection process. Panel B and C represent graphically the notion of contra and ipsi-lateral and and space for each group.

Experimental procedure

Patients were tested, blindfolded. When in bed, a pillow or a rigid tray was placed below the arms to keep them at the same level. Patients able to move from the bed were tested on wheel-chair, while sitting in front of a table, with arms positioned on it. We applied mechanical punctuate stimuli on both hands, which were kept uncrossed or crossed over the body midline. Mechanical punctuate stimuli were applied with a pinprick stimulator. It consists in a stainless inner tube with a retractile

needle that exerts on the skin a constant force. We used, in all patients, two different intensity, 256 mN and 512 mN, that are the higher available in our set. The task comprised four blocks of 20 stimuli each (10 stimuli of the 256 mN and 10 of the 512 mN), for a total of 80 stimuli for the whole experiment. Patients were asked to rate verbally the intensity of the stimulus after each administration on a numerical rating scale from 0 (no pinprick sensation) to 100 (the most intense pinprick sensation). Before the beginning of the experimental procedure, a short familiarization block of 5 stimuli per hand was delivered to assess sensory deficits and to explain the task. The first block, uncrossed or crossed, was balanced across patients and no more than 3 stimuli were consecutively applied to the same hand. The experimental procedure is illustrated in figure 2.

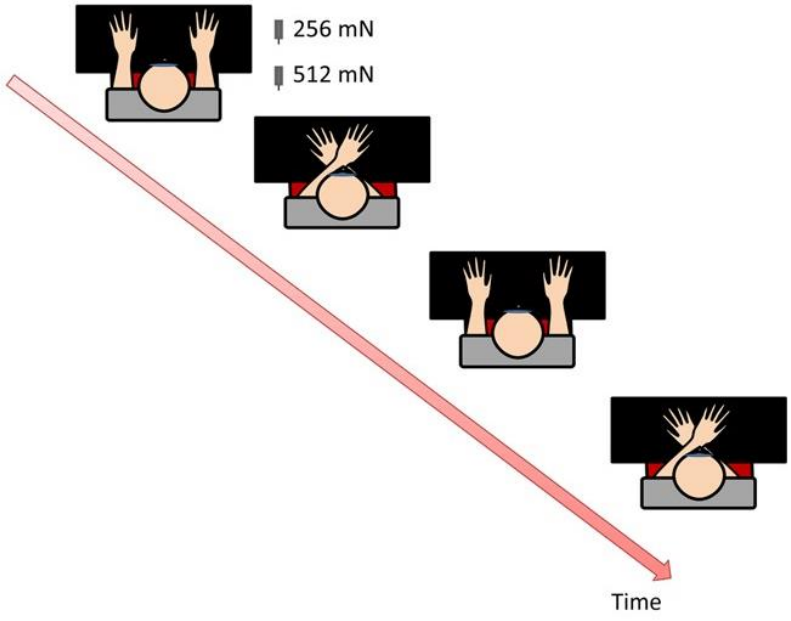

Figure 2 Representation of the experimental protocol. Each of the four block comprised 20 stimuli, 10 for each intensity.

Data analysis

To study the effect of neglect on the 'crossed hand analgesia' we first normalized the data within each patient. To normalize the data, we standardized the subject response using the average and the standard deviation of each subject independently from the experimental condition (as suggested with attitude thermometers by Marradi (1979)). It consists in a sort of row-wise standardization, and its

aim is to compare the subjects' response reducing the variability due to the different use of the numerical rating scale. For each patient we calculated the mean and standard deviation of ratings provided for the 80 stimulations, despite of the arm onto which the stimuli were applied. Importantly, only stimuli that were rated >0 (e.g. that were perceived) were included in the analysis. Then, each rating was subtracted to the average score of the patient, and divided by the standard deviation. In this way, for each patient, 0 represented his/her average rating, a normalized value >0 meant that the stimulus was perceived as more intense than average, a normalized value < 0 the opposite.

To investigate the effect of crossing the hands on the detection of the stimuli, we calculated, for each patient and each condition, the percentage of perceived stimuli.

Both the measurement were carried out from the same task.

Statistical analysis

To test the effect of the unawareness of pain on the perceived stimuli intensity, we carried out a repeated measure ANOVA with three factors within subjects, 'Intensity' (256 mN vs 512 mN), 'Position' (crossed vs uncrossed), 'Side' (contra vs ipsi-lesional), and a factor between subjects 'Group' (neglect vs control). The same design was used to evaluate the detection of the stimuli, with the percentage of detected stimuli instead of their perceived intensity.

Because neglect and extinction partially overlap but are often considered as separate syndromes, the analyses were performed both including and excluding the three patients with extinction as part of the neglect group. Indeed, analysis of neglect and extinction patients together aimed at investigating the extent to which any form of spatial deficit influences nociceptive processing. On the other hand, separate analyses were able to provide a clearer picture of the effect of these two different pathologies on nociceptive processing. Considering that only three patients were diagnosed with extinction, no formal testing was carried out on that group.

Results

Neuropsychological profile

The neglect and control groups did not differ in terms of age ($t(26) = 0.066$, $p = 0.948$), education ($t(26) = 0.38$ $p = 0.70$) and general cognitive impairment as measured by the MMSE ($t(20) = 1.95$, $p = 0.065$).

Crossed hands effect

Neglect group (N+) vs Control group (N-E-)

Figure 3 summarizes the main results. In the control group 81% of the patients reported reduced perceived intensity for stimuli applied onto the contra-lesional hand when this was placed in a crossed position. We observed an opposite pattern in the neglect group. Indeed, 72% of neglect patients increased their ratings for stimuli applied onto the contra-lesional hand when this was positioned in the healthy space, i.e. in the crossed position. A chi-square analysis showed that there was a statistically significant association between the 'Group' and the possibility of showing 'crossed-hands analgesia' for stimuli applied onto the contra-lesional hand ($\chi(1) = 4.030$, $p = 0.04$). Such an association was not observed for stimuli applied onto the ipsi-lesional hand ($\chi(1) = 0.580$, $p = 0.44$). The results of the 4-way ANOVA showed main effects of 'Intensity' ($F(1,25) = 38.050$ $p < 0.001$), 'Position' ($F(1,25) = 8.189$ $p = 0.008$) and 'Side' ($F(1,25) = 9.876$ $p = 0.004$). A significant interaction 'Group' x 'Intensity' x 'Position' was also observed ($F(1,25) = 6.593$ $p = 0.017$). Three-way ANOVAs carried out separately for each group revealed, in the control group, a main effect of 'Position' indicating that crossing the hands reduced the perceived intensity of the stimuli ($F(1,15) = 4.743$ $p = 0.053$). This effect was not observed in the neglect patients group ($F(1,10) = 3.975$ $p = 0.086$). The main effect of 'Intensity' was observed in both groups (Controls $F(1,15) = 21.400$ $p < 0.001$, Neglect $F(1,10) = 16.724$ $p = 0.002$). In addition, the neglect group showed a main effect of 'Side' $F(1,10) = 5.722$ $p = 0.038$).

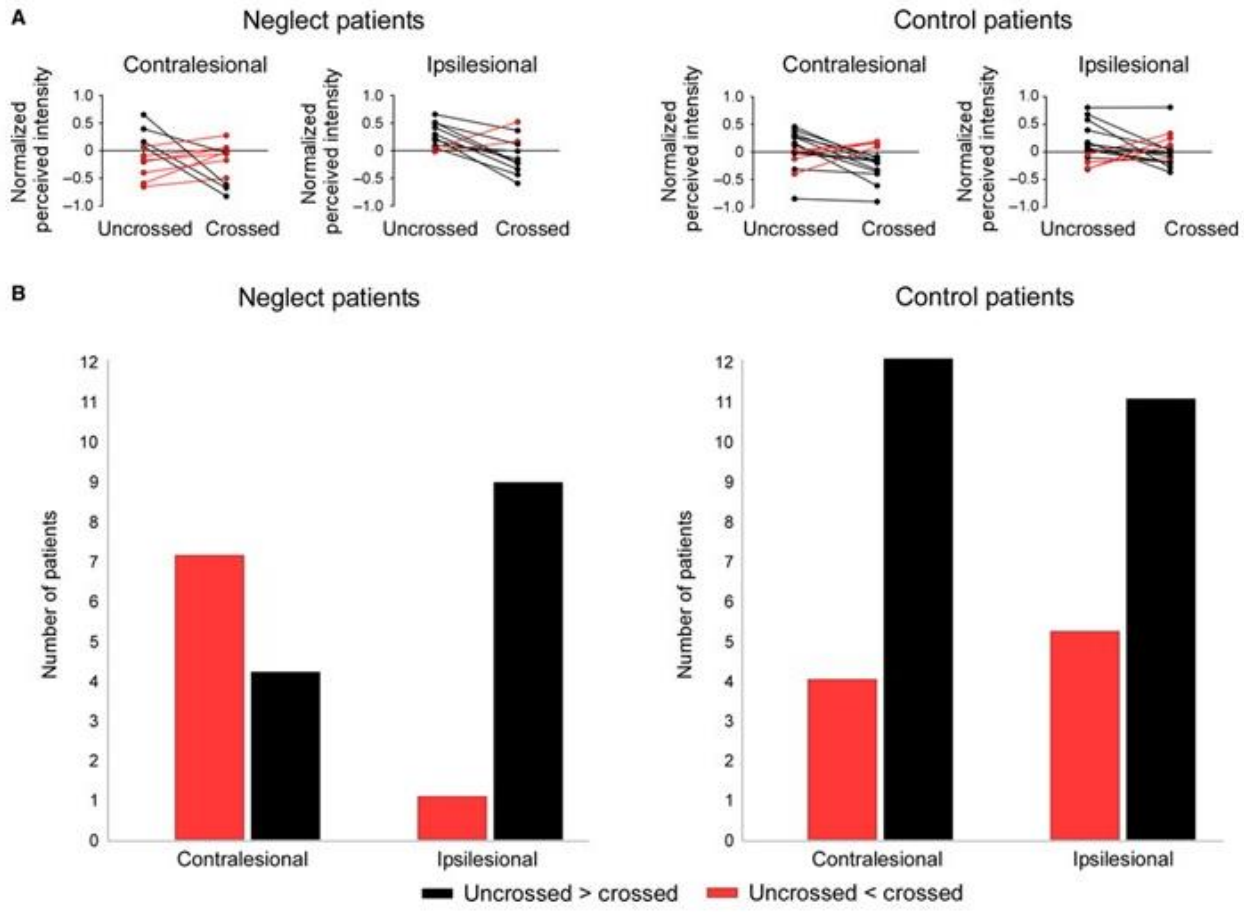

Figure 3 Representation of the perceived stimuli intensity. Panel A shows the data at a single subject level. Panel B represents the number of patients of each group in which is or not present CHA.

Extinction and Neglect patients (N+E+) vs Control Group (N-E-)

Adding the three patients with extinction to the Neglect group leads to similar results compared with those obtained considering neglect patients alone. Four-way ANOVA shows that stimuli exerting a higher force (512 mN) were rated as more intense (main effect of 'Intensity' ($F(1,28) = 30.939$ $p<0.001$). The 'Position' was a significant source of variation ($F(1,28) = 5.233$, $p = 0.030$), with stimuli applied onto the crossed hands perceived on average as less intense. The 'Side' was significant as well ($F(1,29) = 4.760$ $p = 0.038$), with contra-lesional stimuli perceived as less intense. A significant interaction 'Group x Intensity x Position' was also observed ($F(1,29) = 6.286$ $p = 0.018$). All other factors and interactions were not significant. Separate three-way ANOVAs confirmed that

crossing the hands reduced the perceived intensity of the stimuli in the control group ($F(1,15) = 4.743$ $p = 0.046$), but not in the neglect and extinction group ($F(1,14) = 1.443$, $p = 0.251$).

Stimuli detection

Comparing neglect group (N+) against the control one (N-E-), we observed significant main effects of 'Intensity' ($F(1,25) = 6.623$, $p = 0.016$) and 'Side' ($F(1,25) = 7.310$, $p = 0.012$) suggesting that the stimuli applied with the higher force and those applied to the ipsi-lesional hand are easily detected. The interaction 'Group' x 'Position' x 'Intensity' was also significant ($F(1,25) = 5.299$, $p = 0.030$ partial). Three-way ANOVAs carried out separately for each group did not reveal further differences. The three patients with extinction have detected all the 80 stimuli.

Discussion

Our data are the first that formally explore the possibility that unawareness of space influences the detection and the perception of mechanical punctate stimuli. The patients of both groups have correctly discriminate the two different intensity used. It is an indicator that the patients have correctly understood the task and that they have reliably used the numerical rating scale.

Our results show two main findings. First, the crossed-hand analgesia is reduced in patients with neglect. Second, crossing the arms have no effect on stimuli detection in patients with neglect.

Our results confirm the presence of CHA in the Control group, as it has been reported in healthy subjects (Gallace et al., 2011; Torta et al., 2013). Looking at the Neglect group, we have found a CHA only for the ipsilesional hand. Neglect patients report lower rating for the stimuli applied on the contra-lesional hand, but moving the hand in the healthy space (i.e. when the arms are crossed) we have observed an increasing of the reported rating for the 72% of the patients. Our results are in agreement with the notion that the spatial representations are crucial for the detection of the CHA phenomenon. Following this hypothesis, our results support the idea that the unawareness of contra-lesional representation will lead to no mismatch between the different frames of references, and the lack of mismatch will lead to no CHA. We can speculate that in neglect patients the conflict between

the two frames of references (somatotopic and body-centred) is probably solved in favour of the ipsi-lesional, unaffected side.

However, the absence or the presence of CHA in both groups does not seem to be a clear cut phenomenon. Also in healthy volunteers, it has been shown that the CHA is not always present, and that it can be influenced by small variations in the experimental condition (Valentini et al., 2015). Nevertheless, even neglect is not a monolithic syndrome, and it can affect mainly the personal or the peripersonal space (Rode et al., 2017), and it is possible that different form of neglect can have different effects on the CHA phenomenon. From our data, we can not classify the patients in different subtypes of neglect, that anyway go further the aim of the present study.

In contrast with previous data on patients with extinction (Aglioti et al., 1999), we did not found any effects of crossing the arms on stimuli detection. Patients with extinction seem to improve their performances when the arms are crossed in a double simultaneous stimulation protocol (Aglioti et al., 1999), even if it seems that the improvement is less evident in tasks with higher cognitive demand (Bartolomeo et al., 2004). From our data does not emerge any modification in stimuli detection in the crossed position. It is probably due to the different stimuli used. Whereas the previous studies have used a light touch, we tested mechanical punctuate stimuli, with relatively high intensity (256 and 512 mN). Moreover, our task includes only single stimulation, instead double, that can facilitate the detection. Indeed, most of the patients of both groups detected all the 80 stimuli, as all the three patients with extinction.

To conclude our results support the importance of the integrity of spatial representation for CHA phenomenon. Our data suggest that crossing the arms have no effect on the detection of mechanical punctuate stimuli, even if they do not exclude the possibility to modulate low force stimuli.

Limitations

One of the limitation of the study is the small sample size, especially for the neglect group. Moreover, only three patient show signs of extinction, and this prevent a formal evaluation on the characteristics

in perception and detection of pinprick stimuli in these patient. Future studies should corroborate our findings, also exploring the possible effect of different subtypes of neglect (such as personal or extrapersonal) on the CHA phenomenon.

Finally, we have asked the patients to rate the stimuli, without investigate the hand in which the stimulus was perceived. It has been shown that neglect patients tend to report thermal painful stimuli delivered to the contra-lesional hand to the ipsi-lesional one (Liu et al., 2011). At the best of my knowledge, the effect of this mislocalization on perceived intensity has not been explored yet. We can not exclude that in our sample this mislocalization has taken place, and if it can exert an effect on perceived intensity, or if it can be modulate by crossing the arms. However, we have tried to keep the task as simple as possible, in order to avoid a cognitive overload. Future studies should formally test this hypothesis.

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Personal Consideration

During the last four years I have followed the PhD program in Neuroscience at the Clinical and Oncological Unit, Città della Salute e della Scienza, Molinette Hospital, under the supervision of the Professor Riccardo Torta.

During my PhD, I have acquired technical skills in electroencephalography (EEG).

In particular, I have acquired the principles of the EEG recording for both resting state and Evoked Potentials (EPs).

For what concerns the EP technique, during my PhD I had direct experience in recording and analysing:

- Somatosensory Evoked Potentials (SSEP)
- Laser Evoked Potentials (LEP)
- Cognitive Evoked Potentials, i.e. the evaluation of the P300 using auditory and somatosensory oddball task.

I have acquired advanced skills in EEG analysis, using different software, such as Letswave and Eeglab.

Finally, I had the unique opportunity to work in a multidisciplinary team, composed by experts in psychology, psychiatry and electrophysiology.

My Publications

Articles published

Vizzari V, Barba S, Gindri P, Duca S, Giobbe D, Cerrato P, Geminiani G, Torta DM (2017) Mechanical pinprick pain in patients with unilateral spatial neglect: the influence of space representation on the perception of nociceptive stimuli. Eur J Pain 2017 Apr;21(4):738-749.

Poster presentations

Vizzari V, Huang G, Ieraci V, Zizzi F, Molinaro S, Torta R, Torta DM (2016) Cancer pain is not necessarily associated with enhanced alpha activity in spontaneous EEG. Poster presentato a Neuronus 2016 - IBRO & IRUN Neuroscience Forum - 22-24 April 2016, Krakow, Poland.