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PERIPHERAL AND CENTRAL MECHANISMS INVOLVED IN POST-TRAUMATIC STRESS DISORDER AND ITS TREATMENT BY EYE-MOVEMENT DESENSITIZATION & REPROCESSING

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[PTSD & TREATMENT]

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ACKNOWLEDGEMENT

To everyone who has made this achievement scientifically and humanly possible….

ABSTRACT

Although most people encounter at least one traumatic event over their lifetime, not all of them will develop post-traumatic stress disorder (PTSD). Lifetime prevalence of full-blown PTSD, known as an anxiety disorder, is in fact around 10%. In addition to accidents, aggression, grief, rape, fires, traumatic events can be caused by natural (flooding, earthquake…) or man-made stressors (war, terrorism…). With the contemporary rise in traumatic sources the World Health Organization recent reports describe PTSD as an increasing global health issue, due to its high frequency, severity, comorbidity and cost. A body of research has thus started investigating various aspects of PTSD concerned with intrusive thoughts, hypervigilance, emotional deficits, cognitive disturbances and memory issues.

Similarly to other mental health problems, much remains unknown about PTSD, and similarly to other anxiety disorders it is marked by excessive fear. It comes as no surprise that the most prevalent hypothesis in PTSD is that of a fear-processing deficit. Conceptualizing PTSD as a fear disorder can be phenomenologically quite narrow. It has been nonetheless pragmatic in allowing thorough translational research from animal to bench-side and clinical studies. Most studies have suggested that central and peripheral impairments in PTSD revolve around altered neural fear processing network. These alterations involve mechanisms implicated in fear conditioning, as well as emotional and attentional processing, all of which are altered in PTSD.

We address PTSD as a pathological model of altered fronto-limbic processing after traumatic exposure, bearing in mind its correlation to anxious symptomatology. The aim of our study was to investigate central and peripheral mechanisms involved in fear conditioning, emotional attending and cognitive processing of threat in PTSD and explore their correlation to symptomatology and subsequently infer on their inherited v/s. acquired characteristics of PTSD. In the absence of twin studies differing for PTSD diagnosis, we chose to do so by monitoring deficits before and after symptom removal by a validated and quick psychotherapy. Patients' results would be compared to healthy controls and to a wait-list of PTSD patients.

Stemming from the surprising lack of research investigating how different emotional and attentional components of PTSD interact, we studied altogether threat-related amygdala hyperactivity and prefrontal cortex (PFC) hypoactivity in PTSD with other aspects of anxious responding such as emotional deficits, attentional bias and self-measures of distress.

We hypothesized that patients would initially have an overactive amygdala and a hypoactive PFC compared to controls, with symptoms worsened by an inadequate fronto-limbic connectivity. With known roles of amygdala and PFC in fear conditioning, emotion and attention orientation, we further hypothesized that their alterations in PTSD would manifest peripherally in increased fear sensitization and delayed fear extinction, in exaggerated emotional attending and inefficient emotional suppressing and in attentional bias toward emotional cues due to a disengagement difficulty.

To do so, we used an arsenal of techniques at the central and peripheral level. Tasks included classical conditioning paradigm, emotional attending and suppressing of film excerpts (inducing fear, sadness, joy and peacefulness) as well as attentional bias tasks and fMRI based emotional face matching.

Our results have confirmed our hypotheses of increased amygdala and decreased prefrontal activity in PTSD alongside altered connectivity between limbic and cortical areas. PTSD also seemed characterized by an exaggerated physiological responding to fear and difficulty controlling their emotions and detaching their attention from threat cues. We have further shown that these impairments are correlated with symptom severity and are restored after symptom removal.

We hereby reproduce central and peripheral alterations in PTSD and for the first time monitor them after therapy and show their intercorelation and causal dependence. We have shown these deficits remain in wait-list group and are restored after EMDR. As such this provides preliminary evidence that these could constitute acquired markers of the pathology.

We also provide support to improve PTSD diagnosis using subjective scales and attentional task correlated with biological variables. Those markers should be further examined in relationship to risk of relapse.

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LIST of ABBREVIATIONS

ACC	Anterior Cingulate Cortex
AC/PC	Anterior Comissure/Posterior Comissure
Ag	Silver
ANS	Autonomic Nervous System
APA	American Psychology Association
BA	Brodman Area
BDI	Beck Depression Inventory
CBT	Cognitive and Behavioral Therapy
CNS	Central Nervous System
CR	Conditioned Response
CS	Conditioned Stimulus
CS+	CS coupled with aversive US only at acquisition during fear conditioning
CS-	CS uncoupled with aversive US at any phase
DOT	Detection of Target
DSM	Diagnostic and Statistical Manual
EMDR	Eye Movement Desensitization and Reprocessing
EMG	ElectroMyoGram
e-STROOP	Emotional Stroop
fMRI	Functional Magnetic Resonance Imaging
GAD	Generalized Anxiety Disorder
HR	Heart Rate
IES	Impact of Events Scale
ITI	Inter-Trial Interval

MIN	Minute(s)
MINI	M ini International Neuropsychiatric Interview
MNI	Montreal Neurologic Institute
MPSS	Modified PTSD Symptoms Scale
OCD	Obsessive-Compulsive disorder
OFC	Orbito-frontal cortex
PCL-s	PTSD Check List, self filled
PFC	Pre Frontal Cortex
dPFC	Dorsal Pre Frontal Cortex
lPFC	Lateral Pre Frontal Cortex
vmPFC	ventromedial Pre Frontal Cortex
PTSD	Post Traumatic Stress Disorder
REM	Rapid Eye Movement
ROI	Region Of Interest
RT	Reaction Time
S	Second(s)
SC	Skin Response
SD	Standard Deviation
SPM	Statistic Parametric Map
STAI	State-Trait Anxiety Inventory
SVC	Small Volume Correction
T1	spin-lattice longitudinal relaxation time in fMRI acquisition
TR/TE	Repetition Time/Echo Time
US	Unconditioned Stimulus
V	Volts
µ	Micro

Salute to the past,

Pride in the present,

& Cheers to a brighter future…

CHAPTER I

INTRODUCTION

A. PTSD: Facts and Figures

Post traumatic Stress Disorder (PTSD) is an anxiety disorder that arises in the aftermath of a traumatic event; involving the death or threat of death to oneself or someone else, or a threat to one's or others' physical or psychological integrity.

Traumatic events that may trigger PTSD include violent personal assaults, accidents, natural catastrophes such as tsunami, earthquake, etc, or man-made disasters like wars, military combat, etc. In the 30 years since its diagnostic criteria was first outlined in the Diagnostic and Statistical Manual of Mental Disorders Third Edition (DSM-III), a body of research has emerged, to better characterize PTSD, understand its impairments, isolate its risk factors and tackle its treatment options. Inclusion of PTSD as a diagnostic criterion in mental health textbooks was mainly motivated by the need to capture the emotional distress and clinical phenomenology reported by many Vietnam War veterans exposed to severe combat conditions. It also paralleled the feminist movement protesting against intimate partner violence. The move from traumatic neuroses classification to DSM diagnosis in the early 1980s has boosted PTSD research with some 17,000 peer-reviewed articles on PTSD available on PUBMED in 2010.

According to the DSM-IV classification (American Psychiatric Association (APA), 1994), PTSD is characterized by an identifiable stressor producing intense fear, helplessness or horror (Criteria A); the original trauma to be persistently re-experienced with intrusive memories, flashbacks or nightmare associated with intense physiological and psychological responses (Criteria B); stimuli, people and situations associated with the trauma leading to distressing memories to be avoided (Criteria C); and there be hyperactivity with signs of increased arousal such as sleeplessness, irritability, racing heart, hypervigilance, exaggerated startle response and cognitive difficulties (memory, concentration) (Criteria D). A formal diagnostic criterion requires the 3 classes of symptoms (B, C and D) to last at least one month after trauma (Criteria E) and cause significant impairments and distress (Criteria F).

PTSD also involves emotional deficits and social dysfunctions (Bremner et al, 1999; Pole, 2007). Patients may feel numb, detached from their close environment; they decrease involvement in significant life activities, and lose interest in previously pleasurable ones. Their future seems somewhat more constrained. They mostly feel stressed even when they're no longer in danger.

With the contemporary rise in natural and man-made traumatic stressors, the World Health Organization recent reports describe PTSD as an increasing global health issue, due to its high frequency, severity, comorbidity and cost. The lifetime prevalence of any anxiety disorder worldwide is 28.8% (Kessler et al., 2005), and that of PTSD is around 8-11% in the USA and Europe (Jehel, 2006). It is associated with personal and professional impairments, diminished workplace performance and heavy economic weight. It is among the 10 medical conditions most likely to cause sufferers to miss work (WHO, 2001), and being an anxiety disorder, it is a risk factor for cardiovascular problems. PTSD has a comorbid rate of 85% with other mental health disorders (major depression, other anxiety disorders, substance abuse, suicidal risk…).

Although most people encounter trauma over their lifetime (50-90%) not all of them will develop PTSD. Risk and resilience factors, both biological and psychological, seem to mediate the development and maintenance of symptoms (Jovanovic &Ressler, 2010) (Fig.1).

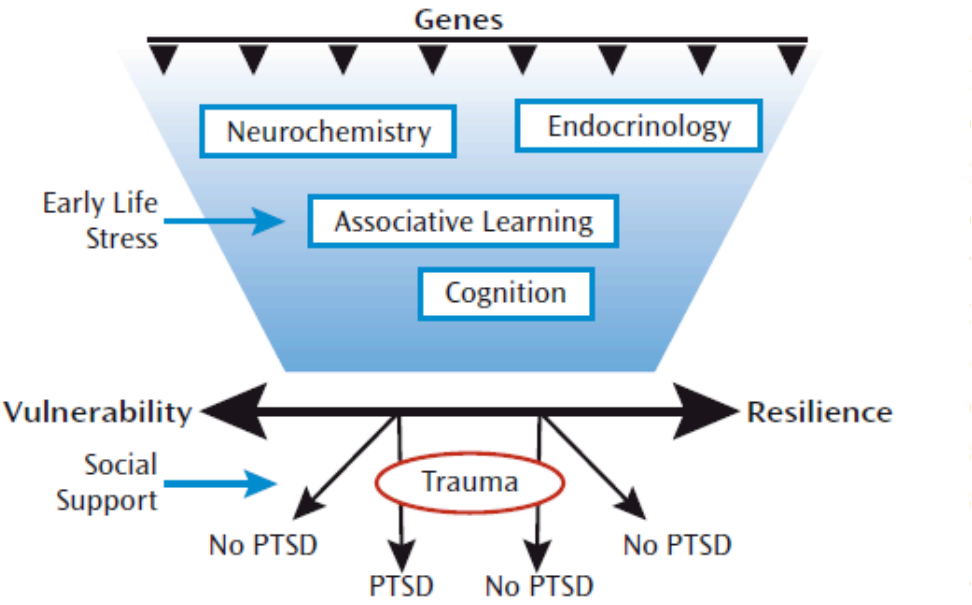

Fig. 1. Diagram of genetic, neurobiological and environment interactions that contribute to vulnerability or relation in relation to PTSD (Jovanovic &Ressler, 2010)

Research focused on assessing risk factors in PTSD, indicated that adverse outcomes after a trauma are associated with female gender, prior psychiatric problems, early and chronic life stressors, inadequate social resources, mass violence, severity of exposure, genetic risk, and IQ (Noris et al., 2002; Buckley et al, 2000). Conversely, resilience factors (although less studied) included strong social support, professional occupation, emotional acceptance, self-awareness and strong beliefs (Brewin et al., 2003). Personal beliefs seem particularly important. Although life threatening events are considered as powerful predictors of PTSD in combat veterans, political prisoners and assault victims, it is the subjective perception of threat that mostly modulates exposure outcome (Brewin et al., 2003).

PTSD is a significant problem in the community and stands out as the psychiatric condition with the most understood underlying critical neural circuitry (Jovanovic et al., 2010, Fig.2). Indeed, the most prevalent hypothesis in PTSD is indeed that of a fear-processing deficit. Conceptualizing it as a fear inhibition disorder has allowed researchers to make progress on animal models, and thus better define central and behavioral alterations in fear processing. Moving to the preclinical studies has subsequently focused on understanding central and peripheral dysfunctions in human and subsequently optimized diagnosis and treatment options.

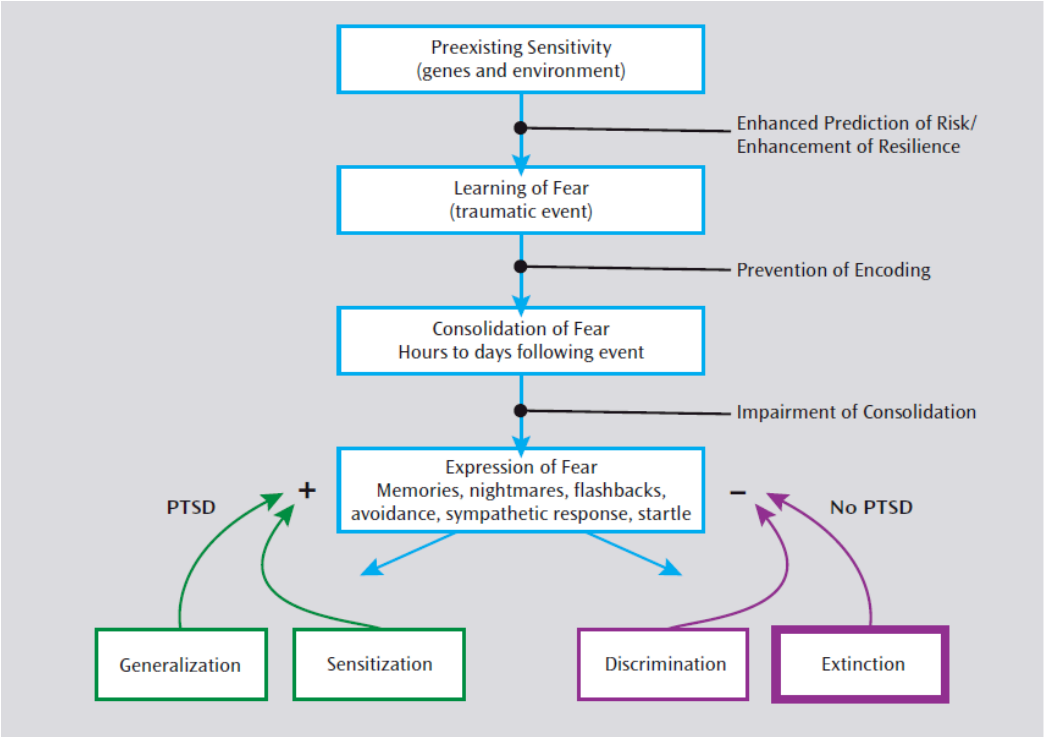

Fig. 2. The developmental progression of PTSD conceptualized as a fear inhibition disorder (Jovanovic &Ressler, 2010)

B. PTSD: State-of-the-Art Research

In all cases, with rising incidence of PTSD, growing research is addressing this anxious pathology. Studies on PTSD have mainly revolved around understanding cognitive and biological theories of PTSD, as well as associated information processing deficits, drawing on patients' behavioral impairments. It has long been known that exposure to life-threatening events can produce adverse psychological and physiological reactions in some individuals (Orr et al., 2002). These reactions are mostly concerned with the processing of the traumatic event and its reminders in PTSD, leading to an exaggeration of fearful responses in patients, even in safe settings.

In such perspective, and in line with the fear-processing deficit hypothesis, the recent blooming of neuroimaging has focused on the neural centers implicated in fear circuitry in PTSD. Alterations in PTSD seem to involve functional activity and connectivity of numerous areas. Alterations are further complexified by structural modifications of those areas (Francati et al., 2007). These mostly include the thalamus (gateway for sensory inputs), the hippocampus (short-term memory and contextual encoding), the amygdala (conditioning to fear responses), the parietal and motor cortex (visuo-spatial processing and evaluation of threat), the medial prefrontal cortex (mPFC) including the anterior cingulate cortex (ACC), orbitofrontal cortex (OFC) and subcallosal gyrus (extinguishing fear response) (Nemroff et al., 2006). The overall model of PTSD includes modified limbic (increased amygdala activity) and frontal structures (decreased PFC activity) as well as decreased frontal regulation of amygdala. Together with hippocampal dysfunctions, these central alterations might account for more complex attentional and emotional deficits in PTSD (Shin et al., 2010). Neural alterations underlying attentional bias are starting to be identified and encompass the amygdala and PFC (Shin et al., 2005; Monk et al., 2008). Again, these same cerebral structures seem to account for physiological impairments in PTSD in emotional settings.

In fact, ever since the early kick off of research on PTSD, investigations have defined cognitive and physiological features involved in emotional and attentional tasks in PTSD. Information-processing models, drawing its origins from cognitive psychology, postulated that PTSD patients show an attentional bias to trauma-relevant information in their environment (Litz et al., 1989). Under normal circumstances, this comes as a normative function to facilitate detection of danger in the environment and help the organism respond effectively to threatening situations. When exaggerated, biases in processing threat-related

information are assigned a prominent role in etiology and maintenance of anxiety disorders (Beck, 1976; Mogg et al., 1991), notably PTSD, generalized anxiety disorder (GAD), phobias, obsessive-compulsive disorders (OCD), and non-clinical populations with high level of anxiety.

Hypervigilance to reminders of a traumatic event, one of the main characteristics of PTSD, was the most robust finding in the PTSD literature (Orr et al., 2002). The presence of elevated physiologic responses to internal and external reminders of traumatic events and to conditioned and unconditioned (startle responses) aversive stimuli has been interpreted as a result of altered fear conditioning (Pitman et al., 1989; Peri, 2000). Patients would have an increased propensity to learn associative fearful stimuli and, have a deficient capability to extinguish them afterwards (Orr et al., 2002). It would manifest by hyperaroused autonomic nervous system (ANS) with elevated heart rate (HR), blood pressure, skin conductance (SC) and frowning electromyogram activity (EMG) in trauma survivors with PTSD (Blanchard et al 1982, 1991; Malloy et al 1983; Pitman et al 1987). Phelps et al., (2004) have also shown that amygdala activation predicted physiological hyperactivity in PTSD such as the increased skin conductance during fear conditioning.

Finally, clinical investigations have evaluated cognitive and behavioral approaches, alone or in combination with pharmacotherapy to better address treatment effectiveness of PTSD. Therapies fall into 3 general subtypes empirically validated for PTSD (Foa et al., 2002). These are exposure therapies (such as systematic desensitization and flooding) where patients confront their fear, anxiety management (including relaxation, stress inoculation and self-distraction) to improve patients' skills and finally cognitive therapies identifying erroneous cognitions and replacing them with realistic ones. In terms of medications, various drugs are commonly used to target subsets of PTSD symptoms or comorbidities.

In spite of extensive clinical and experimental investigations on PTSD, much remains to be done, to better integrate the various facets of the pathology. The need arises to understand common crosstalk for instance between attentional and emotional impairments at the central level, underlying behavioral and physiological alterations in PTSD, and potentially causing pathological symptomatology.

Therefore, we chose to study central alterations in PTSD, specifically functional activity in the frontal and limbic areas, in addition to their functional connectivity. We also studied the emotional and cognitive disorders induced by these central alterations and their peripheral manifestations.

To better understand central alterations in PTSD, with their emotional and cognitive correlates, it is important to define if these mechanisms are altered by the trauma itself, or if some of those mechanisms were previously altered, favoring subsequent emergence of PTSD following traumatic exposure.

To explore the innate/acquired aspect of underlying central and peripheral mechanisms involved in PTSD, authors have adequately used twin studies. An elegant monozygotic twin paradigm included twins discordant for trauma exposure. Its findings suggest that smaller hippocampal volume represents a risk factor for developing PTSD after trauma-exposure (Gilbertson et al 2002), rather than a marker of pathophysiology per se. An initial cortical parcellation study was recently performed and found that Vietnam combat-exposed nurses with PTSD versus without PTSD exhibited selectively reduced volumes in rACC and SC (Rauch et al 2003). In a voxel-based morphometry (VBM) study, Yamasue et al (2003) found smaller gray matter volumes in the dorsal ACC in PTSD. Furthermore, these gray matter volumes were inversely related to PTSD symptom severity.

Since it is extremely difficult to recruit PTSD twins with matched trauma exposed twins in our clinical setting, we chose to use the pre/post treatment model i.e when patients are symptomatic for PTSD and when they are symptom-free after therapy. This model stands out, as an alternative one, to approach the innate/acquired features of PTSD, monitoring patients before and after symptom amelioration.

Our study thus revolves around two main parts. The first one addresses emotional processes in PTSD before and after treatment through the exploration of physiological and cognitive (attentional) mechanisms. The second one tackles central mechanisms involved in PTSD, before and after symptom removal and their links with the cognitive alterations, and symptoms of PTSD.

To adress our aim within the focus of this manuscript, we will start defining central processes implicated in fear processing and that are known to be altered in PTSD. We will then review how these central alterations could manifest at the physiological and cognitive level, accounting for emotional and attentional deficits in PTSD. We will finally detail the validated treatment option we used for PTSD. We will ultimately aim to correlate aforementioned mechanisms with symptomatology and shed lights on its innate v/s. acquired features.

C. PTSD and fear processing deficit

As for other mental health disorder, much remains unknown about PTSD. Yet, similarly to other anxiety disorders, it is mostly marked by excessive fear. Given that exaggerated fear is a marked component of PTSD, it is not surprising that the search for its underlying brain circuits has mainly revolved around the fear circuitry, in animal and human models (Francati, 2007).

a) Central alterations of fear conditioning

The most prevailing hypothesis in PTSD is that of a hyperactive amygdala and hypoactive prefrontal cortex (Milad et al., 2006). Researchers have put forward the implication of functionally connected regions in PTSD; namely the amygdala, prefrontal cortex (PFC) (Quirk et al., 2006), as well as hippocampus and insular cortex. In a recent review, Shin (2010) have shown these structures have functional and structural alterations in PTSD, accounting for one or more facets of the pathology. It thus comes as no surprise that the most prevailing hypothesis accounting for the above-mentioned impairments in PTSD is that of a deficient peripheral and central processing of fear. Fear processing deficits are associated in PTSD with alterations in learning conditioned fear and its subsequent extinction. Enhanced fear sensitization and failure of fear extinction have been hypothesized as part of the dysfunction causing aetiology and maintenance of PTSD (Charney, 2004).

A much used way to mimic learned fear responses, both in animal and human model, is the fear conditioning and extinction paradigm. Classically, a habituation phase accommodates subjects to stimuli presentation. Next, an acquisition phase consists of pairing the neutral conditioning stimulus (CS) (e.g. an image) (Fig.3) to an aversive unconditioned stimulus (US) (e.g. an electric shock). After several trials, the CS, even presented alone, induces conditioned fear responses (CR) such as freezing in rats (Ledoux, 2000) and changes in autonomic nervous system such as HR and SC in humans (Orr et al., 2000). Ultimately during the extinction phase, repeatedly presenting the CS without the US extinguishes the learned fear CR (Berman & Dudai, 2001; Myers & Davis, 2002).

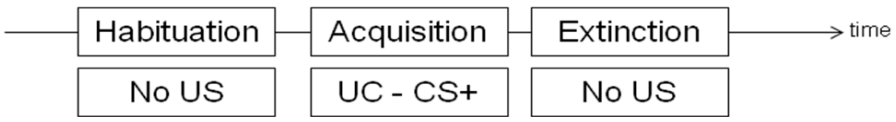

Fig. 3. Illustration of a classical Pavlovian conditioning

Extensive animal and human research point to the orchestrating role of amygdala in acquisition of associative fear learning in classical conditioning tasks (LeDoux et al., 1996; Orr et al, 2000) and the role of vmPFC and amygdala in fear extinction (Milad et al., 2007). A rat model clearly showed the implication of basolateral amygdala in stimulating efferent neurons in the central nucleus, provoking fear responses. After extinction, potentiated PFC would activate intercalated cells in the amygdala, inhibiting the central nucleus and subsequently annulling fear responses. This annulment could alternatively involve interactions of the PFC with the thalamus and hippocampus (Fig. 4) (Quirk et al., 2006).

Translational research has proven this animal model is fairly applicable to human fear conditioning and most importantly to the PTSD model. Using classical fear conditioning paradigms, human have shown the same pattern of amygdala activation and PFC implication throughout all conditioning phases. Moreover, PTSD patients were shown to have exaggerated amygdala activation at fear acquisition and decreased frontal activity at extinction compared to controls (Bremner et al., 2005). Patients also exhibited altered recall of fear extinction when tested the next day (Milad et al., 2006).

In a quick overview of human neurofunctional anatomy (Fig. 5), amygdala is seen as an assembly of sub-cortical nuclei, situated in the anterior temporal lobe of each hemisphere. It is largely implicated in emotional processing and expression, in human models (Ledoux, 2007). It has shown increased activity for negative stimuli compared to neutral ones, whether using paradigms with faces (Hariri et al., 2000), sounds (Zald, 1998) or words (Davis, 2001). It has also shown activation to positive stimuli (Liberzon et al., 2003). More broadly, amygdala is responsive to emotionally arousing and salient stimuli (Phan, 2002).

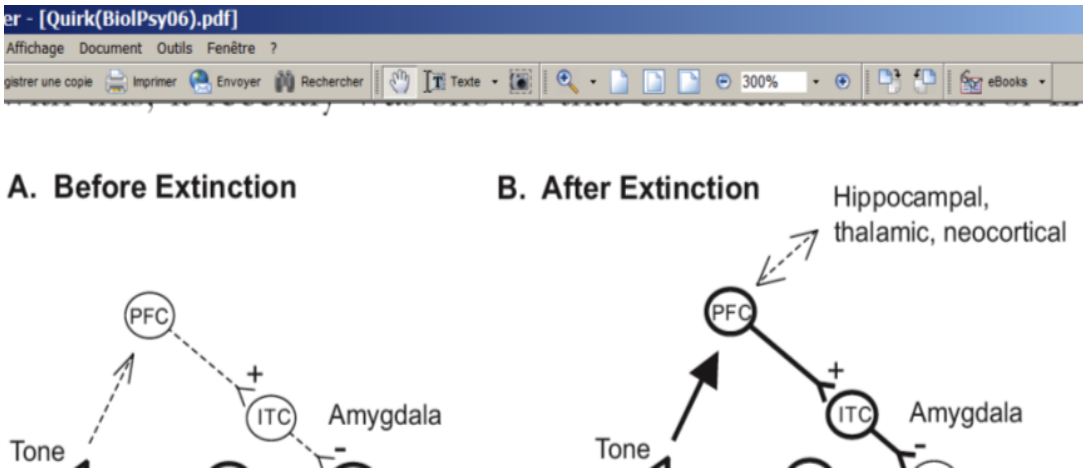

Fig. 4. Illustration of neuronal fear conditioning at A. Acquisition and B. Extinction in rats (Quirk et al., 2006), representing the differential implication of amygdala and PFC at various phases of the conditioning paradigm.

Anatomically, it is highly interconnected with the ventral portion of the prefrontal cortex (PFC) including the anterior cingulate cortex (ACC) and the orbitofrontal cortex (OFC) in primates (Carmichael &Price, 1995) and humans (Bracht et al., 2009; Johansen-Berg et al., 2008). PFC has a role in emotion integration and subsequent guidance of adapted behaviors and decisions (Bechara et al., 1999) and its ventral part is involved in attention and cognitive-emotional association (Bush et al., 2000). Its implication in fear extinction in animal and human studies has led to the elaboration of its role in top-down regulation of the amygdala (Garcia et al., 1998; Milad et al., 2007). Deficits in its ability to modulate the activity of the amygdala have been hypothesized to be instrumental in PTSD development (Hariri et al., 2000).

OFC's role in emotional processing is still under investigation (Frodl et al., 2010). To date, it is largely involved in controlling behavioral and emotional responses and seems altered in anxiety disorders (de Marco et al., 2006). According to these authors, there seem to be a directional flow from amygdala to ACC and OFC in incidental perception of fear, whereas for intentional perception the route followed is in reverse direction from OFC to ACC.

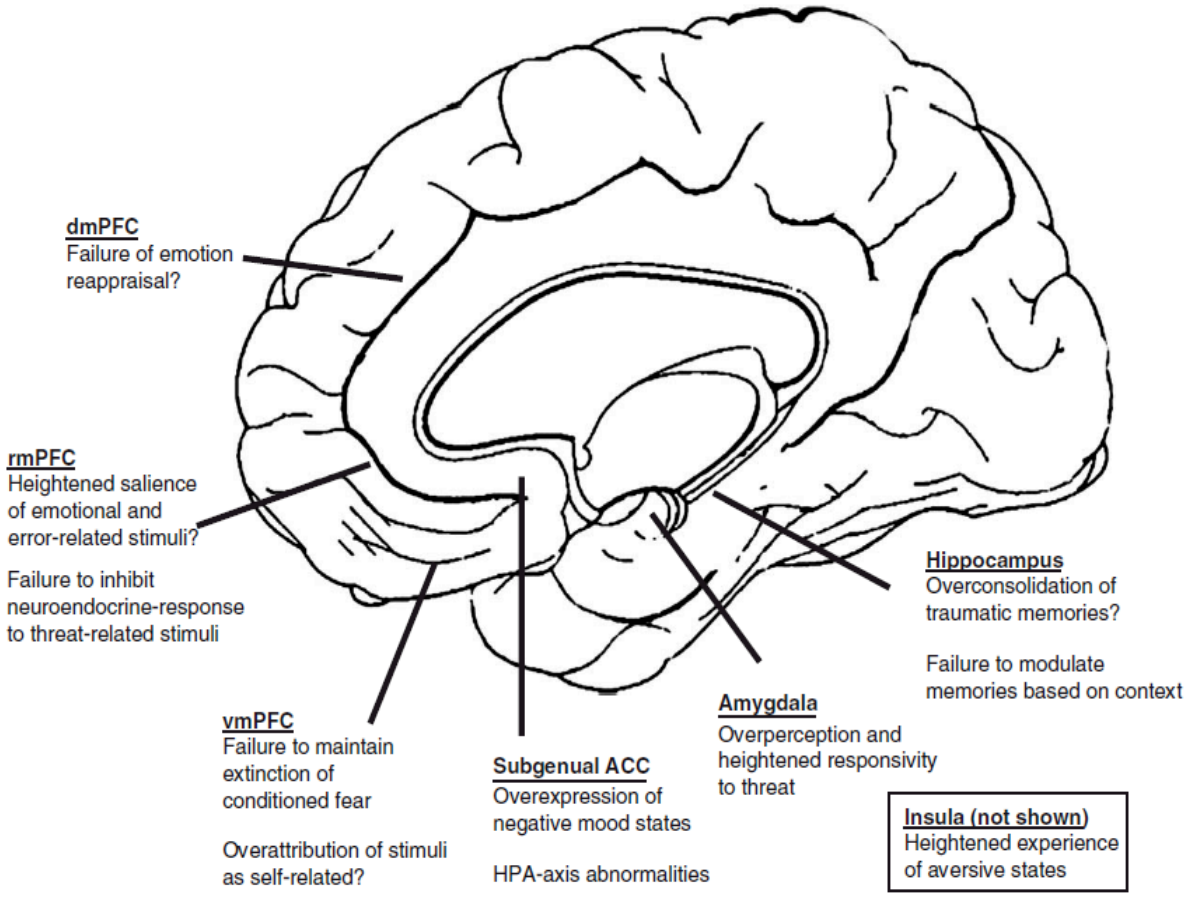

Fig. 5. Illustration of the neural regions implicated in PTSD (Liberzon et al., 2008)

Amygdala hyperactivity has been put forward in patients at rest (Protopescu et al., 2005) and in patients exposed to emotional fearful faces (Shin et al., 2005), to traumatic memories (Driessen et al., 2004), and to fear conditioning (Bremner et al., 2005; Milad et al., 2007). In parallel, a decreased vmPFC and ACC is described in the aforementioned paradigms in PTSD. Evidence from neuroimaging studies is also accumulating in favor of an altered connectivity between medial frontal and limbic regions in PTSD (Stein et al., 2007). This raises the question of an inadequate top-down or a deficient bottom-up interaction between limbic and cortical regions in PTSD.

The hypothesis of a central alteration of the fear pathway in the PTSD etiology is further supported by lesion studies showing amygdala resection abolishes PTSD (Koenigs & Grafman, 2009). Yet, some studies have shown increased activity in both PFC and amygdala in PTSD (Gilboa et al., 2004).

Given the controversies of the current literature, it is necessary to compare levels of frontal and limbic activation in our sample between PTSD patients and healthy controls. In accordance with most reviewed recent studies, our first hypothesis is that of a hyperactive amygdala and a hypoactive PFC in PTSD.

Based on Eysenck theoretical model of threat processing in anxiety, stipulating that anxiety would favor bottom-up effect of amygdala on PFC and weaken top-down regulatory control (Eysenck, Derakshan, Santos, & Calvo, 2007), we further hypothesized an altered fronto-limbic functional connectivity compared to healthy controls. If this alteration is a feature of anxious symptomatology, as suggested by some authors (Shin el al., 2010, review), we hypothesize it would correlate with symptoms severity and would subside after symptom elimination via successful treatment.

b) Peripheral alterations of fear conditioning

Consistent with conditioning theories of PTSD pertaining to an impaired fear processing at the central level, several studies have investigated its manifestation as physiological conditioning in patients using trauma-irrelevant aversive cues (painful electric stimulation) as US. Most revealed enhanced acquisition and slower extinction of the conditioned response in PTSD patients compared to healthy controls (Orr et al., 2000; Peri et al., 2000).

Studies have looked at bodily assessments in fear conditioning paradigm and have found increased electrodermal SC, HR and facial electromyographical (EMG) responses during acquisition and extinction phases when comparing CS types (paired with US (CS+) and unpaired (CS-)) on one hand, and on the other, when comparing PTSD patients, trauma exposed and healthy individuals (Peri et al, 2000, Blechert et al., 2007). Even when the US was no longer present and the expectation of danger was not met, the CR failed to extinguish in PTSD. This deficient extinction learning could explain why some patients maintain symptoms many years after the original trauma (Wessa & Flor, 2007) and fail to respond to exposure therapy, incorporating extinction procedures (Foa, 2000).

However, these findings have not always been replicated as PTSD and controls showed similar peripheral conditioning (Milad et al., 2008). It also remains controversial whether impaired conditioning was due to explicit fear cues (Orr et al., 2000) or rather to context conditioning and generalized fear responses. This later explanation implies that PTSD patients might have a difficulty learning safety cues (Grillon & Morgan, 1999) and becomes difficult to assess since studies have mostly failed at evaluating subjective verbal conditioning. Our second hypothesis is that central alterations in fear processing in PTSD are manifest via impaired physiological and verbal fear conditioning and extinction. PTSD patients would thus have increased fear sensitization to CS+ at acquisition compared to controls and would be slower in extinguishing conditioned fear. If again this altered fear conditioning is a marker of PTSD and develops after trauma exposure, then similarly to central mechanisms, peripheral modifications of fear acquisition and extinction should be restored after symptom removal in PTSD.

D. PTSD and global emotional deficit

Besides their major implication in the fear circuitry, amygdala and PFC are largely known to be involved in emotional processing. Fear processing deficits and inefficient fear extinction are in fact associated in PTSD with alterations in emotional processing. The DSM-IV diagnosis of PTSD includes symptoms directly reflecting difficulties in emotion generation and regulation, and defines emotional hyper-reactivity to trauma-related cues on the one hand and hypo-reactivity in the form of emotional numbing on the other (APA, 1994). Although emotional deficits are one of the main characteristics of PTSD; they remain the least understood and the most understudied aspects of the pathology (Litz et al., 2000).

a) Central Emotional Face Matching

As discussed earlier, an altered limbic and prefrontal processing of threat cues has been hypothesized as being part of the dysfunction causing aetiology and maintenance of PTSD. This view has been supported by experiments showing increased amygdala and decreased prefrontal activity in PTSD, not only in conditioning but also in fearful faces recognition. Evidence is converging to place the amygdala at the center of PTSD etiology (Rauch et al., 2000). It is consistent with the notion that, inspite of its central role, the amygdala is not all-important in PTSD; rather symtpoms are exaggerated by a default in PFC processing. This further implies that amygdala and PFC are reciprocally mediated (Garcia et al., 1999).

Facial expressions have been effective in probing amygdala response in healthy controls (Hariri et al., 2000) but more so in anxiety disorders such as GAD (Monk et al., 2008) and social phobia (Blair et al., 2008). A study on such task has shown that blood flow in vmPFC was inversely correlated with blood flow in amygdala in PTSD. Symptoms severity positively correlated with amygdala and negatively correlated with vmPFC (Shin et al., 2004). Even when masked fearful faces were used, Carlson et al., (2009) have evidenced amygdala response positively correlated with ACC in healthy subjects.

Although most recent findings in PTSD suggest there might be an abnormal connection between limbic and cortical structures, few studies have looked at the connectivity of the neural network. Neuroimaging studies suggest an alteration of the reciprocal amygdala-mPFC interaction in PTSD (Shin et al., 2005). With respect to connectivity, PTSD patients are shown to have less resting state than controls between amygdala and posterior cingulate (Lanius et al., 2009). A recent study has shown PTSD had decreased amygdala ACC connectivity when viewing angry faces (Fonzo et al., 2010).

Our third hypothes is concerned with the replication of previous findings of increased amygdala and decreased prefrontal activity in emotional face matching in PTSD. As such, studies thoroughly exploring fonto-limbic functional connectivity in emotional settings in PTSD are scarce. They seem to point toward a decreased connectivity between the amygdala and other frontal cornerstones of emotional processing. In this optic, we also hypothesize that a decreased fronto-limbic connectivity might exacerbate the overactive amygdala and account for PTSD symptomatology. This would be restored after symptom amelioration.

b) Peripheral Emotional Attending/Suppressing

Some of the frontal areas described as dysfunctional in PTSD have been shown to be involved in emotional processing. Using emotional attending/suppressing fMRI paradigm, Phan et al., (2005), has shown the existence of emotional regulatory (lateral PFC, dmPFC and ACC) and emotionally responsive brain regions (amygdala, insula, mOFC). These regions are also known to influence peripheral physiological responses of the ANS.

The ANS seem a fair marker of emotional processing deficits, and could reflect the upstream alterations of the central nervous system (CNS). In fact, ANS is regulated by or co-varies with measurements of the CNS, at limbic and frontal regions (Hagemann, Waldstein, Thayer, 2003). Considerable research has looked into the psychophysiology of PTSD patients at rest (Pole et al., 2007) and during their emotional response to films (Orsillo et al., 2004), pictures (Milad et al., 2000; Wagner et al., 2003), or trauma reminders (Litz et al., 2000), but few studies have explored thorough emotional response deficits in PTSD.

As previously mentioned, PTSD is associated with increased physiological measurements of ANS at rest, including HR, SC and facial EMG (Pole et al., 2007). HRV studies also demonstrated autonomic deregulation in PTSD, whereby patients had a higher ratio of sympathetic to parasympathetic influence than controls. These deregulations are similar to those monitored in healthy controls in stressful situations (Cohen et al., 1998). Despite the inherent relationship between anxiety disorders and emotion deficits in general, and more specifically in PTSD, there has been a relative lack of studies examining emotion generation and regulation within clinical samples of anxiety disorders.

Litz et al., (2000) have nonetheless shown that male combat-veterans PTSD and controls generally responded comparably and distinctly to three categories of emotional images (positive, neutral and negative), as assessed by self-report, peripheral autonomic responses, and expressive-motor activity. Patients were less responsive only to positively stimuli under trauma-priming conditions. However, a study in women with PTSD has shown that they exhibited higher levels of negative activation and expressed more negative emotion words to both positive and negative film stimuli, whereas no group differences emerged in facial expressivity (Orsillo et al., 2004).

Looking at another anxiety disorder, GAD, Mennin et al., (2002) have proposed that patients not only had emotion generative processes more intense than most, but also had deficiencies in altering their emotional experience. Subsequently, GAD patients

instigate more regulatory efforts, typically worry or suppression, leading to opposite of intended results (i.e., increases in anxiety rather than decreases). If similar mechanism were to operate in PTSD, an inadequate effortful emotional suppression may dampen their positive experiences thereby contributing to the increased risk of development of symptoms associated with this disorder (APA, 1994). In fact, among women exposed to traumatic events, those who reported infrequently and ineffectively regulating their emotions also reported higher anxiety and PTSD (Eftekhari et al., 2009). A study of 182 trauma-exposed individuals by North (1999) showed that 94% of participants with group C symptoms criteria (including avoidance and emotional detachment) subsequently developed PTSD. Interestingly, PTSD reported difficulties suppressing both positive and negative emotions than did non-PTSD participants.

Physiological impairments of the ANS during emotional processing are a fair reflection of CNS deficits. However measurements of emotional attending and suppressing are seldom studied in PTSD, and generally focus on the study of negative emotions solely. The literature is still controversial but clinical observations tend to support the fact that patients have modified evaluations of both positive and negative emotions and generally decreased control over their emotional experiences. In this perspective, our fourth hypothesis is that PTSD would have a hyperactive physiological responding when attending highly arousing emotions (both positive and negative), and would be less efficient than healthy individuals in controlling their emotions.

Along the line of thought stipulating that emotional disturbances result from upstream altered central processing and that these central mechanisms are correlated to symptomatology, we hypothesize that PTSD would have normal emotional attending and suppressing physiological measurements after symptom removal via successful treatment.

E. PTSD and attentional deficit

The brain centers implicated in fear and emotional processing that we have mentioned so far also intervene in attentional processes. In fact, amygdala and PFC are both shown to be activated in attentional bias tasks (Cisler et al., 2010). Attentional orientation towards threat cues might be associated in PTSD with emotional deficits and altered fear processing. Information processing models of PTSD suggest that this anxious pathology is characterized by attention orientation towards potentially threatening stimuli (Hayes et al., 2009).

A wealth of research has illustrated the presence of attention bias towards threat cues in PTSD (Bar Haim et al., 2007; MacNally et al., 1990; Pineles et al., 2007; Hayes, 2009). Hypervigilance to threatening information and avoidance of such cues seem to be important core features of PTSD, and are exacerbated most probably because PTSD patients have networks of cognitive representations of trauma-related stimuli that are altered or that are more readily activated than have controls (Litz et al., 1996).

From a cognitive point of view, one of the key factors of emotional distress and maintenance of anxiety disorder is the existence of non-adaptive attentional bias towards information with aversive value (Mogg &Bradley, 1998). One way to investigate such bias is via interference tasks which involves a central task to be performed while ignoring emotional distracters (MacLeod & Mathews, 2005). Two tasks are commonly used in the assessment of cognitive processing: the emotional stroop and detection of target.

1. Emotional Stroop task

The most common paradigm indexing sensitivity to threat-related events is the emotional stroop task (e-Stroop) (Williams et al., 1996), in which participants are explicitly asked to indicate the ink color of words they are shown and ignore their lexical content. In such paradigms, anxious people tend to be generally slower in responding to emotionally negative words than to neutral ones, implying the existence of selective attention to emotional cues (Fox et al., 2001).

2. Detection of target task

The specific selectivity in strategic cognitive processing seems to be best addressed by the detection of target (DOT) task in which participants are explicitly asked to identify a target that is presented adjacent to or distant from emotional words with negative valence (Posner & Petersen, 1990). In this task, anxious patients have shown a selective disengagement bias from emotionally negative words, exhibiting difficulty detaching their attention from threatening and aversive cues (Bar-Haim et al., 2007; Frewen et al., 2008).

a) Central Attention bias

To date, few studies have examined the neural pattern underlying attentional bias in anxious population in general and more precisely in PTSD. Even fewer have looked at attention and emotion in association with symptomatology. Evidence is accumulating that the threat detection mechanisms and orientation of attention are neutrally centered on the amygdala in anxious populations (Bar Haim et al., 2010). Decreased activity in the anterior cingulate cortex (ACC) was shown in an e-stroop in anxiety (Shin et al., 2010) and in women

with abuse-related PTSD (compared to abused non-PTSD women) (Shin et al., 2004) and in men war veterans (Hayes et al., 2008). Authors show that enhanced dorso-limbic responsiveness and decreased ACC function may be neural markers of attention bias in PTSD. In a task using masked fearful faces, a correlation was found between amygdala activity and attentional bias in generalized anxiety disorder (GAD) youth (Monk et al., 2008), suggesting that pre-conscious limbic involvement in automatic processing of emotional threat cues might influence attention orientation (Frewen et al., 2008).

b) Peripheral Attentional bias

These findings can be better understood in the light of Eysenck theoretical model of attentional bias towards threat in anxiety, stipulating that anxiety would favor bottom-up effect of amygdala on prefrontal cortex (PFC) and weaken top-down regulatory control which would manifest by difficulty in disengaging attention from distracting threatening stimuli (Eysenck, Derakshan, Santos, & Calvo, 2007). Central alterations of structures regulating attention to emotional information have been monitored behaviorally.

Behaviorally, PTSD patients are known to have an attentional bias towards trauma cues and general words with negative valence (MacNally et al., 1999, Pineles et al., 2007, Hayes et al., 2009). For instance in motor accidents, PTSD patients were significantly slower in color-naming accident related words in an e-stroop task (Beck et al., 2001). The e-Stroop task thus provides clear evidence of the presence of an attentional bias in PTSD, but gives no information on the underlying altered cognitive strategy.

Recent trauma victims were found to view trauma-related pictures longer than generally aversive pictures, unlike healthy control participants (Elsesser et al., 2004). Results for PTSD seem controversial. Some studies have shown that PTSD patients orient away from threat. For instance, Vietnam veterans with PTSD were found to have a vigilance bias; ‘escaping’ from the presentation of combat scenes when they were able to turn off the display (Blanchard et al., 1982; Malloy et al., 1983). PTSD patients were also shown to name targets faster when in close proximity to mild threat words (Bryant et al., 1997). Other studies, however, have shown that PTSD patients orient toward the threat cues, illustrating a disengagement bias (Pineles et al., 2009; Dalgeish et al., 2003). The need thus arise to reproduce e-Stroop robust findings in PTSD and then better address the cognitive strategy underlying attentional bias. We will also look at its relationships with central dysfunctions in PTSD.

Our fifth hypothesis thus favors a disengagement problem from threat cues in PTSD in a DOT task, rendering them slower in emotional trials. If this feature were to be correlated with symptomatology, it would be present before treatment but not after.

The merging literature suggests threat detection mechanisms might be neurally centered on the amygdala (Bar Haim et al., 2007), for instance in GAD (Monk et al., 2008). To extend GAD findings to PTSD, we looked at assaying the correlation between amygdala activation and disengagement difficulty in PTSD and symptomatology, bearing in mind the hypothesis of a positive correlation between them.

F. PTSD treatment by EMDR

In order to better understand the correlation between dysfunctions in PTSD and its symptomatology, we chose to study, central and physiological alterations before and after symptom removal. With the inclusion of a corresponding wait-list group, this paradigm would provide an alternative model to twin design to answerthe innate/acquired features of PTSD. Symptoms removal implies successful treatment. The APA recommends psychotherapies as the treatment of choice for trauma victims, superseding pharmacological approaches. According to its published reports in 2004, Eye Movement Desensitization and Reprocessing (EMDR) and Cognitive Behavioral Therapy (CBT) are the two empirically validated psychotherapies for PTSD. These reports are further supported by clinical practice guidelines issued by the UK department of Health, the French INSERM and other European health instances.

We chose to use EMDR as a treatment option of PTSD since it is a rapid therapy with validated effectiveness and a stable outcome demonstrated in a 35-month follow-up study (Hogberg et al., 2008). As such, it best suits our experimental design.

EMDR is an eight-step standardized protocol, based on an information-processing model (Shapiro & Maxfield, 2002). It includes associations of cognitive, emotional and physical assessments of actual distress to traumatic scenery, as well as imaginal exposure while attending to bilateral alternate stimulations. As the patient is asked to visualize the most salient aspect of a traumatic memory, the therapist induces bilateral stimulation (by means of ocular, sensory-motor or auditory left/right stimulation) (Shapiro, 1996). At the end of each session, patients are asked to evaluate their subjective index of distress.

The biological basis of EMDR remains unknown. Studies have shown that EMDR relieves traumatic symptoms and reduces the autonomic responsiveness of PTSD patients to aversive stimuli such as trauma recall (Aubert-Khalfa et al., 2008). EMDR is also associated with psychophysiological de-arousal for SC and HR over time sessions (Sack et al., 2008).

Additionally, two experiments using SPECT suggest that the anterior cingulate cortex (Levin et al., 1999) as well as the left medial ventral frontal gyrus (Lansing et al., 2005) would be more activated post than pre-EMDR treatment. A third study did not evidence any brain activity difference before and after EMDR (Pagani et al., 2007). Letizia et al., (2007) report a case study with restoration of hippocampal volume after successful EMDR.

Preliminary volumetric studies have shown that grey matter density in limbic and paralimbic cortices is associated with PTSD development after trauma (left posterior parahippocampal gyrus and posterior cingulate) and EMDR treatment outcome in PTSD (right amygdala, posterior cingulate and insula) (Nardo et al., 2009).

We used EMDR in PTSD to monitor potential restoration of emotional and attentional processing after symptom removal and provide evidence first and foremost for its correlation with PTSD symptomatology. On a second level, the paradigm could touch upon the fundamental question whether these factors are inherited characteristics of the disorder, resembling vulnerability factors present before trauma exposure or are rather acquired features of PTSD that develop after the trauma.

G. PTSD: other aspects

Although not within the scope of our research, PTSD is also associated with other pathological aspects such as cognitive impairments related to memory. The DSM-IV in fact defines PTSD with frequent distressing and intrusive memories and flashbacks, controversially with a potential amnesia of the details of the traumatic experience. Evidence suggests autobiographic memory is altered in PTSD similarly to depression; patients recall negative, traumatic memories with more ease than pleasurable ones (Buckley et al., 2000). Traumatic memory impairments could be induced by neuronal circuitry modifications in PTSD (Lanius et al., 2001). Those modifications involve the amygdala and PFC, alongside hippocampus.

Other memory processes altered in PTSD include working memory capacity (Brewin and Beaton, 2002) or memory of fear extinction (Garcia et al., 1999; Quirk et al., 2006). The effects of stress on reduced hippocampal volume have been described early on in animal models (Woolley et al., 1990). Bremner et al., (1995) first identified similar functional and structural alteration in the hippocampus in PTSD. Hippocampal failure in memory tasks has been extensively studied in PTSD (Shin et al., 2004) and plays a role in contextual fear conditioning (Milad et al., 2007). Preliminary evidence report hippocampal increased activity after trauma as acquired marker of PTSD correlated to symptom severity (Admon, 2009).

In such perspective, an important yet overlooked aspect of the fear/anxiety neurocircuitry is its overlap with the neurocircuitry that mediates stress response (Shin, 2010). Increased mPFC activity attenuated stress-induced HPA-axis activity in an animal model (Zeinberg et al., 2010); suggesting deregulation of mPFC activity associated with PTSD may contribute to impaired expression of stress-response adaptation and exacerbation of this disorder. A recent review of the neurocircuitry of anxiety has shown chronic stress decreased dendritic branching in the hippocampus and mPFC and increased it in the amygdala (Shin & Libertzon, 2010). Neurotransmitters like cortico-tropic releasing hormone are likely involved in the orchestration of HPA activity and fear response (Heim and Nemeroff, 2001).

Still, other aspects of the disorder include sex differences, as PTSD is more prevalent in women. Kessler et al., (1995 and 2000) reported that men and women might differ in trauma exposure type with women more exposed to sexual abuse and men to combat and road accidents. He had found that, in spite of this and even when faced with the same trauma type, women are twice as likely as men to develop PTSD after an extreme stress. In fact, combat exposure and war veterans do account for a large proportion of PTSD research in men populations, yet PTSD burden stems mostly from “common” events such as motor vehicle accidents, work-related accidents, aggressions, rapes and childhood abuse (Stein et al., 2002).

Gender difference in vulnerability to PTSD includes trauma exposure type (especially intimate partner violence in women), socio-cultural stigma (that might modulate response to trauma) and biological factors. Research in rats provides evidence that estrous cycle phase influence fear conditioning by influencing brain regions such as amygdala, vmPFC and hippocampus (sexually dimorphic and containing dense gonadal hormone receptors) involved in consolidation of fear extinction (Milad et al., 2009). Authors say the elevated fear observed in female relative to male rats during extinction recall suggests that gonadal hormones may in part play a role in the higher prevalence of anxiety disorders in women.

H. Aims of the Study

Most above-mentioned studies have suggested that central and peripheral impairments in PTSD revolve around altered neural fear processing network. These alterations involve mechanisms implicated in fear conditioning, as well as emotional and attentional processing, all of which are altered in PTSD. We hereby address PTSD as a pathological model of altered fronto-limbic processing after traumatic exposure, bearing in mind its correlation to anxious symptomatology.

The aim of our study is to investigate central and peripheral mechanisms involved in fear conditioning, emotional attending and cognitive processing of threat in PTSD and explore their correlation to symptomatology, by monitoring them before and after symptom removal by EMDR (Fig. 6). This might shed light on inherited v/s. acquired characteristics of PTSD and provide preliminary evidence for one alternative ove the other.

In practical terms, our study was divided in two main parts. We started using our pre- to post-treatment model at the physiological level by exploring the effect of EMDR on fear conditioning and extinction in PTSD. We monitored verbal and peripheral (SC, HR and EMG) markers. Similarly, we assayed PTSD performance on emotional and attentional tasks before and after symptom amelioration (Part I-peripheral).

Only when we found the EMDR model to be successful in restoring peripheral alterations, did we use the fMRI-based heavy and costly arsenal to better explore the deficient fear network involved in PTSD in emotional recognition task and its putative modulation after treatment (Part II-central), as well as its correlation with attentional bias and symptoms of PTSD.

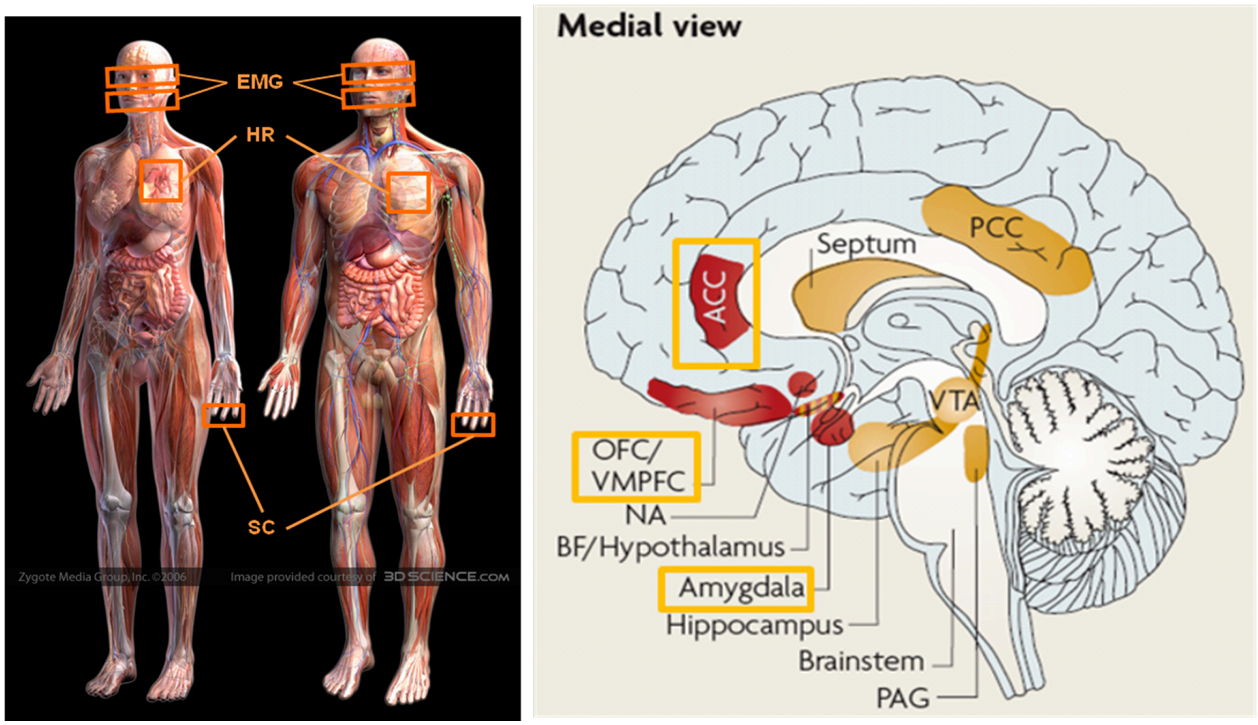

Fig. 6. Illustration of the peripheral (left panel) and central (right panel) mechanisms this study addresses in PTSD and its treatment by EMDR

I. Hypotheses

Based on the literature review, we hypothesize that PTSD patients would mainly suffer a hyperactive amygdala and a hypoactive PFC, functionally worsened by a decreased connectivity between the two. This would manifest first and foremost by altered fear conditioning and extinction, compared to healthy controls. This central deficit would also account for other aspects of PTSD symptomatology including emotional and attentional processing, and could be monitored at the neural, behavioral and physiological levels.

We hypothesize those altered mechanisms would be correlated to symptomatology. As such, we hypothesize PTSD would have neurally and behaviorally impaired fear processing, emotional and cognitive mechanisms, before treatment but not after. On the central level, PTSD would show malfunctions of underlying emotional processing hubs; including a hyperactive amygdala and a hypoactive PFC, accompanied by an altered connectivity of those 2 cornerstones of the fear circuitry. This would subsequently lead to initial heightened hypervigilance to negative or threat-related words, pictures and films in PTSD compared to the healthy group. In such terms, we hypothesized that the PTSD pathology would be associated with increased sensitization to fear conditioning and delayed extinction, exaggerated verbal and physiological activations in emotional attending and suppressing of highly arousing emotions (both positive and negative) and disengagement difficulties from threat cues. These emotional and attentional alterations would be restored after symptom amelioration by EMDR. If this was the case, then we hypothesize it would provide evidence that the features are acquired markers of PTSD.

These hypotheses are better addressed via various tasks:

1. Fear conditioning and extinction task: The aim of this task was to reproduce verbal and physiological fear processing deficits in the fear conditioning and extinction paradigm in PTSD and explore whether it is restored after a treatment ameliorating core PTSD symptoms (Article 1). We hypothesize that initially patients would have differential verbal assessment and increased SC, HR and EMG to CS+ images coupled with aversive stimulation at acquisition, compared to controls and would be slower in extinguishing the conditioned response. This would not generalize to CS-. After symptoms disappearance with EMDR, both populations would be comparable throughout all the conditioning phases.

2. Emotional attending/suppressing task: The aim of this task was to investigate how emotional generation and regulation are altered in PTSD, not only in fear conditioning but when volitionally attending or suppressing emotions with varying arousal and valence levels (happiness, peacefulness, fear and sadness), and whether they are restored after a treatment ameliorating core symptoms by EMDR (Article 2). We hypothesize that patients would have an exaggerated physiological responding to highly arousing emotions of fear and happiness. Unlike controls, they would be less efficient in suppressing highly arousing emotions, when instructed to control them. This would be valid, before EMDR but not after.

3. Attention bias task: Since one of the key factors of the emotional distress and maintenance of anxiety disorder is the existence of non-adaptive attentional bias towards information with aversive value, the aim of this task was to investigate the cognitive strategy underlying attentional bias to threat cues (disengagement v/s vigilance) in PTSD, and whether normal cognitive processing is restored after a treatment ameliorating core PTSD symptoms (Article 3). We hypothesize than PTSD patients would have attentional bias characterized by a disengagement difficulty from emotionally negative cues, as they would be slower than control in shifting their attention from aversive or negative cues compared to neutral one, before EMDR but not after.

4. Correlation of Attention bias and Face matching tasks: Stemming from the surprising lack of research investigating how different emotional and attentional components of PTSD interact, the aim of this study was to correlate threat-related amygdala hyperactivity, and other aspects of anxious responding such as self measures of distress and attentional bias (Article 4). We hypothesize that PTSD patients would atypically process threat cues. This would manifest on the cognitive edge in reproducibly larger disengagement index from emotional cues, and on the central level in increased amygdala activation to fearful and angry faces, in comparison with controls. Since amygdala is known to be involved in emotional processing as well as threat orientation, we tested correlation of its activity with symptom severity and attentional bias in PTSD.

5. Emotional Face matching task: After establishing the restoration of fear processing as well as emotional and cognitive alteration in PTSD after EMDR, the aim of this fMRI task was to assess the central alterations of the amygdala and prefrontal structures, known to underlie aforementioned deficits in PTSD (Article 5). We hypothesize that the BOLD signal and functional connectivity of those main key players involved in the fear circuitry is modulated in PTSD compared to healthy controls, only before treatment but not after. PTSD patients would initially have a hyper-activated amygdala and a hypo-activated PFC in processing emotional faces, compared to controls. PTSD would also be associated with altered amygdala-PFC connectivity. If cerebral functioning would be restored after EMDR, this would provide preliminary evidence that impairments of amygdala and PFC and their connectivity are acquired markers of the pathology after trauma exposure and would correlate with PTSD symptomatology.

CHAPTER II

MATERIALS AND METHODS

A. Participants

For each of the two parts of the study, adult outpatients were recruited by psychiatrists among trauma victims at the medico-psychological crisis cell (CUMP) at the Psychiatry Pole of the Conception Hospital in Marseille, France. They all met the DSM-IV criteria for PTSD following a single traumatic event (aggressions, motor vehicle accidents, work related accidents…). All of these events occurred in patients who had no previous history of neurologic or psychiatric disorders. Subsequent analysis included fewer patients as few of them voluntarily withdrew from the study and few of them left town. Some patients were on combined regimen of antidepressants and anxiolytics, some only took antidepressants and some only took anxiolytics.

Adult Healthy controls with no history of neurologic or psychiatric disorders were recruited via screening lists at the clinical investigation centre at the Timone Hospital (CIC-UPCET). They were matched to patients for age, sex and education. Detailed demographic data are on the next page.

All subjects were French-speaking, aged 18-50 years. Exclusion criteria were: pregnancy, substance abuse, current or previous neuropsychiatric disorders including dissociations (other than PTSD for patient group), claustrophobia and fMRI counter-indications (for the fMRI experiment).

B. Clinical Evaluations

A psychiatrist assessed all participants for PTSD and other mental health disorders using the structured Mini-Internal Neuropsychiatric Interview for DSM-IV (MINI) (Lecrubier, 1998). This allowed checking for the absence of psychiatric disorders prior to the trauma in PTSD (considered as exclusion criteria) and screen for potential comorbid psychiatric disorders. Participants responded to demographic questions and completed the Beck Depression inventory (BDI) (Cottraux, 1985) and the State-Trait Anxiety Inventory (STAI) (Schweitzer & Paulhan, 1990).

Patients completed trauma related scales: PTSD Check List Scale (PCL-S) (Ventureyra et al., 2002), Modified PTSD Symptoms Scale (MPSS) (Stephenson et al., 1999) and the revised Impact of Event Scale (IES-R) (Weiss & Marmar, 1996).

The validated French versions were used for all the scales.

a) PART - I- Peripheral assessment

Study initially included 23 patients, but 4 of them voluntarily withdrew. Analyses of results included data of 19 PTSD patients (12 aggressions, 4 motor vehicle accidents, 6 work related accidents, 1 grieving parent having witnessed the suicide of her son by hanging) and 20 matched healthy controls. Demographic data and scores on the various scales are displayed in Table 1.

- Patients were tested at T1 (inclusion), and T2 (after EMDR).
- Controls were tested at T1 (inclusion), and time lag matching patients' (T2-T1).
- Participants sat for the e-Stroop and DOT tasks
- Participants sat for the fear conditioning task (2 patients were reluctant to the electric stimulation so analysis included 17 patients and matched controls, Table 2).
- Participants sat for the emotional attending/suppressing task

	Healthy Controls (Time 1) (n=20)	**Healthy Controls (Time 2) (n=20)**	**PTSD Pre-EMDR (Time 1) (n=19)**	**PTSD Post-EMDR (Time 2) (n=19)**	**Statistics**
Age	37.8 (13.6)	-	44.5 (14.5)	-	t(1,37) = 1.5
Sex	9M – 11 F	-	7M – 12F	-	-
Education	9.0 (2.2)	-	7.5 (2.8)	-	t(1,37) = 1.94
BDI Depression	3.2 (2.6)	2.3 (2.6)	15.1 (8.3)	5.6 (3.9)	$F(1,37) = 24.20^{**}$
STAI Trait Anxiety	29.3 (8.0)	30.3 (8.0)	50.1 (11.6)	34.6 (8.7)	$F(1,37) = 20.47^{**}$
STAI State Anxiety	40.0 (8.4)	36.4 (9.4)	56.4 (10.8)	42.8 (7.6)	$F(1,37) = 14.43^{*}$
PCL-S	-	-	56.6 (22.4)	28.4 (6.6)	$t(1,18) = 5.48^{**}$
MPSS	-	-	67.1 (32.2)	21.9 (10.8)	$t(1,18) = 6.59^{**}$
IES	-	-	51.8 (21.8)	17.3 (10)	$t(1,18) = 6.48^{**}$

Table 1. Demographic Characteristics of all subjects included in part -I- of the study. Significant p-value: * $p < 0.05$ ** $p < 0.001$

In the PTSD group, 5 patients were on combined regimen of antidepressants and anxiolytics, 2 only took antidepressants and 2 only took anxiolytics.

Patients met the criteria for the following major current comorbid diagnoses (before/after EMDR): major depression (n=11/4), other anxiety disorders (n=16/8) and high-medium suicidal risk (n=6/0). Specific anxiety rates initially diagnosed in PTSD are as follow (out of the 19 patients): Social phobia (5), Generalized Anxiety Disorder (10), Panic disorder (5), Agoraphobia (8). Most patients had more than one comorbid anxiety disorder.

	Healthy Controls (Time 1) (n=18)	Healthy Controls (Time 2) (n=18)	PTSD Pre-treatment (Time 1) (n=17)	PTSD Post-treatment (Time 2) (n=17)	Statistics
BDI Depression	3.0 (2.6)	2.1 (2.3)	15.5 (8.1)	5.6 (4.1)	$F(1,33) = 21.72^{**}$
STAI Trait Anxiety	29.7 (8.3)	30.2 (8.1)	50.6 (11.3)	35.4 (8.6)	$F(1,33) = 15.33^{**}$
STAI State Anxiety	39.5 (7.9)	36.5 (9.0)	56.5 (10.5)	43.1 (7.5)	$F(1,33) = 10.76^{*}$
PCL-S	-	-	62.4 (11.7)	29.2 (6.3)	$t(1,16) = 10.82^{**}$
MPSS	-	-	73.5 (23.2)	22.1 (9.5)	$t(1,16) = 9.97^{**}$
IES	-	-	56.8 (13.8)	18.2 (9.9)	$t(1,16) = 8.81^{**}$
Stimulation level (mA)	1.9 (1.6)	1.4 (1.4)	10.4 (19)	8.9 (15.7)	NS
US aversiveness rating (0-100)	5.4 (16.8)	12.16 (14.5)	10.35 (26.9)	8.9 (16.5)	NS
UR: SCR (μS)	1.18 (0.84)	1.1 (0.86)	1.28 (1.03)	1.23 (0.87)	NS

Table 2. Demographic Characteristics of subjects included in the conditioning paradigm for part -I- of the study. Significant p-value: * $p < 0.05$ ** $p < 0.001$

b) PART - II- Central assessment

Study initially included 20 patients, but 2 voluntarily withdrew and data acquisition could not be analyzed for 2 others. Analyses included data of 16 PTSD patients (8 aggressions, 4 motor vehicle accidents, 4 others) and 16 matched controls. The wait-list PTSD group is still under construction and has thus far reached 6 patients.

Demographic data and scores on the various scales are displayed in Table 3.

- Patients were tested at T1 (inclusion), T2 (after EMDR) and T3 (6month after T2).
- Controls were tested at T1 (inclusion), time lag matching patients' (T2-T1) and T3 (6month after T2-T1).
- Wait-list PTSD were testes at T1 (Inclusion) and time lag matching (T2-T1)
- Participants sat for the DOT task
- Participants sat for the fMRI-based face matching task and anatomic fMRI acquisition

In the PTSD group, 4 patients were on combined regimen of antidepressants and 2 only took anxiolytics. Patients in the PTSD group met the criteria for the following major current comorbid diagnoses (before/after EMDR): major depression (n=10/2), other anxiety disorders (n=14/5) and high-medium suicidal risk (n=4/0).

	Healthy Controls (Time 1) (n=16)	Healthy Controls (Time 2) (n=16)	PTSD Pre-EMDR (Time 1) (n=16)	PTSD Post-EMDR (Time 2) (n=16)	PTSD wait-list (Time 1) (n=6)	PTSD wait-list (Time) (n=6)	Statistics
Age	33.1 (10.2)	-	33.6 (7.9)	-	37.1 (8.5)	-	t(1,30) = 0.13
Sex	10M – 6 F	-	13M – 12F	-	4M – 2F	-	-
Education	9.5 (1.2)	-	7.9 (2.6)	-	7.3 (4.0)	-	t(1,30) = 1.7
BDI Depression	1 (1.03)	0.8 (1.4)	16.6 (7.8)	4.8 (4.1)	15.3 (4.9)	15 (5.3)	F (1,30) = 24.20**
STAI Trait anxiety	27.1 (5.5)	26.7 (6.5)	50.3 (15.5)	31.4 (7.8)	48.2 (10.0)	49 (13.4)	F (1,30) = 16.68**
STAI State anxiety	31.4 (5.4)	31.4 (6.9)	55.3 (8.0)	40.6 (9.0)	58.7 (10.3)	56.5 (11.9)	F (1,30) = 26.97**
PCL-S	-	-	58.1 (11.4)	26.9 (7.2)	64 (13.3)	63 (14.5)	t (1,28)= 9.2**
MPSS	-	-	67.9 (19.8)	14.56 (13.2)	72 (23.7)	74.7 (34.3)	t (1,28)= 8.77**
IES	-	-	52.1 (16.6)	8.7 (8.2)	67 (11.7)	53 (24.26)	t (1,28)= 9.21**

Table 3. Demographic Characteristics of the subject included in part -II- of the study. Values between dotted brackets are still preliminary and not considered in statistical analyses. Significant p-value: * $p < 0.05$ **$p < 0.001$

C. EMDR Treatment

Patients were treated by one of 3 therapists, all trained by the French institute of EMDR. There was no fixed number of sessions. Sessions were planned every 7 to 15 days according to patients and therapists availabilities. The treatment was considered successful and complete when patients reported no more feelings of distress when thinking about their trauma. They were again interviewed by a psychiatrist, using the MINI.

They were retested when they no longer met PTSD classification according to DSM-IV criteria and their scores on PTSD scales were within normal ranges. The experimental protocol was thus administered twice for all participants: before treatment (T_1) and immediately after symptom amelioration (T_2) for the PTSD group, and in matching time lags (T_2-T_1) for the control group. The control group in fact served to monitor for potential habituation to repeated conditioning.

- For the study I, patients needed an average of 4.1 (1-7) sessions that lasted 2.5 months
- For the study II, patients needed an average of 4.3 (1-7) sessions that lasted 2.5 months

The wait-list patient group included in the part II of the study was tested at T1 and T2. After that patients were offered EMDR therapy (results are not the scope of this study).

D. Physiological Evaluations

Figure 7 illustrates placement of electrodes for the physiological evaluations (Fig. 7).

Skin conductance (SC) was measured in micro-siemens using two 5 mm inner diameter Ag/AgCl electrodes filled with isotonic paste. Electrodes were placed on the medial phalanges of the index and middle finger of the left hand in accordance with published guidelines (Fowles et al., 1981).

Since deep breathing and/or coughing may trigger artifacts on the SC responses; respiration pattern was recorded using a pneumagraphic belt with a respiration transducer at the rib cage, towards the end of the sternum.

Heart Rate (HR) was measured in beats per minute using 3 clip lead electrodes, attached in Type I EKG configuration; on the left flying rib, right collarbone and sternum.

Electromyogram (EMG) of the corrugator and zygomatic activity was measured in microvolts using three 4 mm Ag/AgCl shielded surface electrodes filled with electrolytic paste. The skin was locally abraded with alcohol imbibed cotton swab and two electrodes were attached with adhesive collars over the corrugator (frowning) and zygomatic (smiling) muscle site according to manufacturer specifications (Fridlund &Cacioppo, 1986). A ground electrode was placed on the lobe of the left ear.

Calculation of the physiological indices is detailed in later pages.

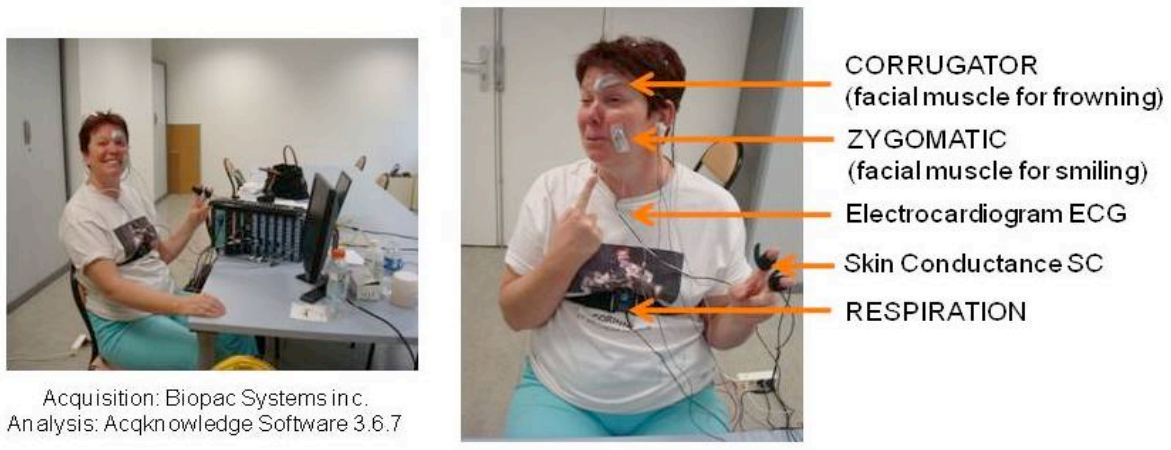

Fig. 7. Illustration of the electrodes placement for the physiological acquisitions

E. Emotional Stroop task

This is a task validated by Lanteaume et al., (2009) (Fig. 8).

It included 96 trials. Each trial consisted of a black fixation cross display of 500 ms at the center of a white screen, followed by a cue display in the middle of the screen until the response was given. The inter-trial interval was 500 ms. The cue display consisted of either an emotionally negative word (such as war, accident, raping…), or a neutral one (such as face, sphere, housing…). The type of word (emotional, neutral) and ink color (red, blue, green) were randomly counterbalanced across trials, with a new sequence for each participant. Participants were asked to fixate on the black cross. After it disappeared, they had to identify the color of the displayed word, as fast as they can and without scarifying accuracy.

An index of e-Stroop was calculated: e-Stroop Index = $RT_{\text{emotional words}} - RT_{\text{neutral}}$, where RT is the Reaction Time. When it differs from zero, it indicates the existence of an attentional bias; with a positive index meaning that attention is captured by emotional words and a negative index meaning that emotional words are avoided.

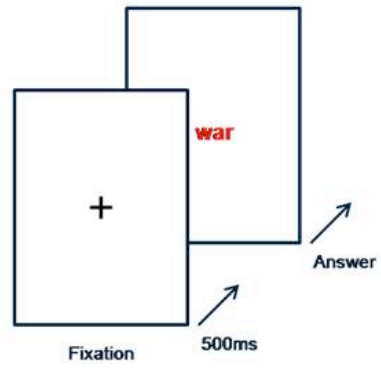

Fig. 8. Illustration of an emotional trial in the emotional STROOP task

F. Detection of target task

This is a task validated by Lanteaume et al., (2009) (Fig. 9).

It included 128 trials. Each trial consisted of a succession of three steps: a black fixation cross display of 500 ms at the center of a white screen, a cue display of 500 ms, and a dot display until the response was given. The inter-trial interval was 500 ms. The cues consisted of either an emotionally negative word (such as war, accident, …) and a matched neutral word (such as face, sphere, …), or a neutral word and another matched neutral word.

Words were vertically opposite at equal distance from the center of the computer screen, 4 cm apart. The probe display consisted of a black circle that appeared at the same spatial location as one of the two words. The emotional word position (lower, upper), the probe position (lower, upper), and the type of word pairs (emotional-neutral, neutral-neutral) were randomly counterbalanced across trials with a new sequence for each participant. For the emotional pairs, trials were said to be congruent when the dot replaced the emotional word and incongruent when the dot replaced the neutral word, as illustrated in Fig.1. Participants were asked to fixate on the black cross. They were told two words would appear immediately after the black cross and finally, that a dot target would appear after the words. They were asked to give the location of the target, as fast as they can and without scarifying accuracy. Three indices were calculated for the DOT:

- Congruency: $RT_{incongruent} - RT_{congruent}$. A positive index indicates a bias in threat detection, either on congruent or incongruent trials
- Disengagement: $RT_{incongruent} - RT_{neutral}$. A positive index indicates stronger attentional holding for negative cues; subjects are slower to respond to neutral cues in presence of emotional ones.
- Vigilance: $RT_{neutral} - RT_{congruent}$. A positive index indicates enhanced attention capture for negative cues; subjects are faster in responding to emotional cues in presence of neutral ones.

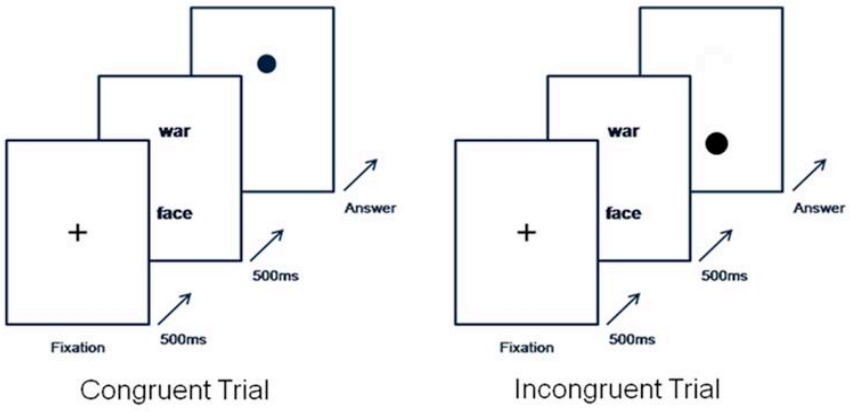

Fig. 9. Illustration of congruent and incongruent trials in the DOT task

Data and Statistical Analyses of e-Stroop and DOT tasks

A two-way repeated measures ANOVA was used on the calculated e-Stroop, disengagement and vigilance indices, with Group (2 levels: PTSD patients and Controls) as a between factor and Session (2 levels: for controls: session 1 and 2, and for patients: pre and post-EMDR) as a within factor. Significant main effects at 0.05 significance levels were followed by post-hoc tests using Bonferroni correction.

G. Fear conditioning task

This is a task validated by Blechert et al., (2007) (Fig. 10).

It consisted of three different phases: habituation, acquisition and extinction. The US was a 500-msec electric shock previously determined by the participant to be "highly annoying but not painful" using the up-down staircase method. This was done by gradually incrementing shock intensity, while participants rated its aversiveness using a digital analog scale (from "Not annoying"= 0; to "Highly annoying"= 100). Once the shock intensity determined, it was kept constant for the rest of the conditioning task.

The habituation phase started with written instructions telling participants that two pictures would be shown on the screen and that there will be no shock delivery. It consisted of 6 trials of each to-be-CS+ and to-be-CS-. CS+ and CS- images were used from the Rorschach inkblot test and were counterbalanced across participants. Images were presented for 8 s. The mean intertrial interval (ITI) was 18 s (range 16-20 s).

At the *acquisition phase*, instruction told participants that two pictures will be shown on the screen and that only one would be occasionally followed by the electric shock. It consisted of 6 trials of each CS type and each CS+ was followed by the US.

No instructions were shown at the *extinction phase* that consisted of 6 CS+ and 6 CS-.

Ratings of US expectancy and CS valence were repeatedly obtained. Six valence ratings were obtained for each CS in the middle and the end of each conditioning phase (every third CS was rated, yielding a total of 12 ratings).

US-expectancy ratings were obtained on the first and last presentation of each CS during the extinction phase. A baseline US-expectancy rating was obtained at the end of habituation. Following extinction training, contingency awareness was assessed by a screen presenting the CS+, the CS- and a control stimulus and asking which of the three pictures had previously been paired with the US.

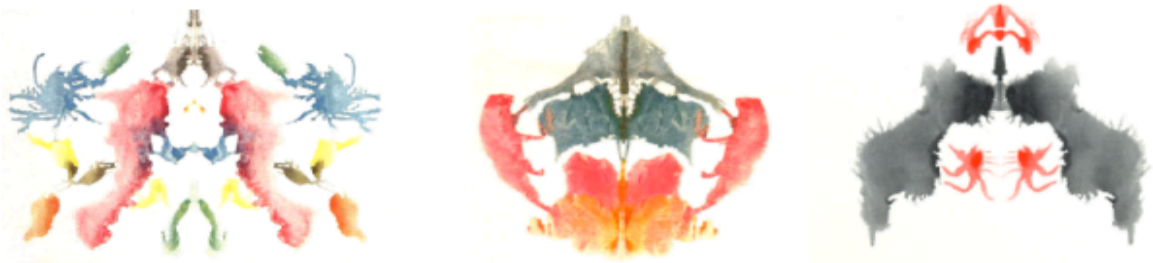

Fig. 10. Illustration of images used in the fear conditioning paradigm. Left to right images represents the distracter and CS+/- alternatively.

Data and Statistical Analyses

Similarly to Blechert et al., an SC response was calculated for each CS trial by subtracting the mean skin conductance level (SCL) during the 2 s immediately prior to CS onset from the highest SCL recorded during the 8 s CS presentation. This method has been documented to be more adapted in differential fear conditioning paradigm than the alternative scoring method that consisted of measuring either the First or the Second Interval Response (Pineles et al., 2009). The present scoring method allows for the detection of the maximal increase in skin conductance level at any point during the 8 s presentations.

In addition, the unconditioned response (UR) to the electric stimulation was calculated by subtracting the average SCL during the last 2 s of CS presentation from the highest SCL recorded during the 8 s following the US. SC below 0.01 μS were scored as zero and square root transformation was applied to normalize the SC distribution. SC to each CS-type (CS+, CS-) on three consecutive presentations was averaged, resulting in two blocks per conditioning phase (e.g. first and second half of habituation). Artifact correction for SCs consisted of a visual inspection of respiration and the manual exclusion of SC that appeared to be influenced by coughs, sights or deep breath (around 5% of either CS type was excluded).

Two participants in each group had no electrodermal conditioning i.e. they had no SC greater than 0.01 for any of the CS during acquisition. Their data have been removed from subsequent analyses.

Data for HR and EMG were similarly calculated for each CS trial.

A two-way repeated measures ANOVA was used on the SC, EMG and HR for each conditioning phase separately with Group (control, PTSD patients) as a between factor and Session (1, 2), CS-type (CS+, CS-) and Time (first half, second half) as within factors. Significant main effects at $p<0.05$ were followed by post-hoc t-tests using Bonferroni correction. All post-hoc were taken at a p level $p<0.05$.

H. Emotional Film viewing task: Attending and Suppressing

After a 10 min rest period, participants viewed a series of ten 45-second long color films. Two short clips were selected per emotion: happiness ("le Dîner de Cons" by Weber and a short film depicting a baby laughing), peacefulness ("Marche of the Penguins" by Jacquet and "le Grand Bleu" by Luc Besson), fear ("A Tale of Two Sisters" by Jee-Woon and "Perfect Murder" by Andrew Davis), and sadness (report on the famine in Biafra by INA and "Stepmom" by Chris Columbus). Films were fed into E-studio 2.2 software (E-Prime 2.2) and displayed on a 17" inch computer screen with 40W Yamaha NS10M Studio sound blasts, linked to a P2040 amplifier, at a sufficiently elevated and comfortable volume.

Films were presented in five pseudo-randomized sequences and the same film order was kept across both tasks for a given subject. In the attending task, subjects were instructed to watch the excerpts and feel the emotions it elicited the best they can. For the suppression task, they were told to control their emotions the best they can. We chose an ecological instruction, best mimicking real-life setting so participants would react as spontaneously as possible when faced with such situations. After each clip, subjects identified the most prominent emotion, rated its intensity, level arousal and valence (Fig. 11).

Films were previously validated (Reynaud el al., 2010 – Appendix 1) from a larger selection of clips based on the following criteria:

1/ identification percentage higher than 80 %

2/ intensity of induced emotion higher than 7 on the Intensity scale

3/ arousal level higher than 5 on Arousal scale for stimulating emotions (happiness, fear, disgust), & lower than 2 for the non-stimulating ones (peacefulness, sadness)

4/ valence level higher than 6 for pleasant emotions and lower than 4 for unpleasant

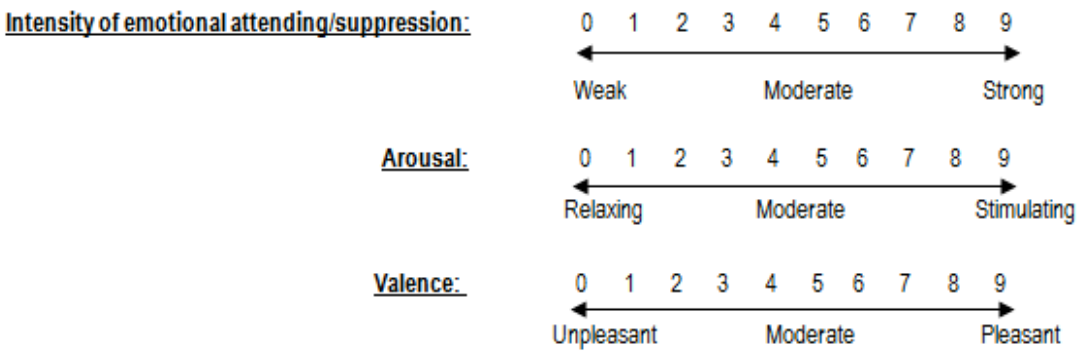

Fig. 11. Illustration of the Likert-like scale ued for the evaluation of the emotional attending/suppressing task. Participants had to complete it for each clip, after emotional identification.

Data and Statistical Analyses

Data of physiological parameters and verbal scoring were averaged for the 2 films per emotion.

Data for SC was obtained by averaging peak amplitudes of SC during the 45 s film excerpts SC below 0.01 μS were not considered. Artifact correction for SCs consisted of a visual inspection of respiration and the manual exclusion of SC that appeared to be influenced by coughs, sights or deep breath (around 5% of either film type was excluded).

Data for HR and EMG activity were calculated by subtracting the 15 s baselines before the film's onset from the mean level obtained during the 45 s film excerpt.

Data for heart rate variability (HRV) was reliably quantified using the 10 min rest period (Bernston et al., 1997). Three frequency bands are typically defined:

- High frequency (HF) (0.15 - 0.4 Hz), derived mainly from vagal activity.
- Low frequency (LF) (0.04 - 0.15 Hz) derived from sympathetic activity.
- Very low frequency (VLF) (0 - 0.04 Hz) reflecting physical activity.

HRV is calculated by the HF/LF ratio.

A two-way repeated measures ANOVA was used on the ratings of intensity, arousal and valence as well as SC, EMG and HR for each task separately with Group (control, PTSD) as a between factor and Session (1, 2) and Emotion (happiness, peacefulness, fear and sadness) as within factors. Significant main effects at $p < 0.05$ were followed by post-hoc t-tests using Bonferoni correction.

I. fMRI Acquisition

All data acquisition was performed on a 3-T MEDSPEC 30/80 AVANCE imager (Bruker, Ettlingen, Germany) at the fMRI center of Marseille, France. All stimuli were generated on a computer and back-projected onto a screen that subjects viewed through a mirror positioned above their eyes. fMRI scans were acquired using a T2*-weighted gradient-echoplanar sequence (TR/TE=2533.3/30 ms; FOV=19.2×19.2 cm, 64×64 matrix; flip angle= 82.4°). Thirty-eight interleaved axial slices, tilted -30^{o} to the intercommisural plane (to reduce artifacts in prefrontal regions), were obtained with a contiguous slice thickness of 3 mm.

Following the fMRI scans, a set of high-resolution T1-weighted images were acquired for the purpose of anatomical identification (sagittal MPRAGE Sequence, TE/TR = 4/10 ms, T1 = 800 ms, Flip Angle = 30°, Matrix=256×256×128).

J. Emotional Face matching task (fMRI)

This is the task is validated by Hariri et al., (2000), (Fig. 12).

In the emotional condition, subjects viewed a target face and had to select which one of two faces presented below it (on the same screen) expressed the same emotion (fear or anger). In control condition, they viewed a target shape, and chose which one of two shapes presented below it (on the same screen) matched the target (round or oval). The paradigm consisted of 12 experimental blocks of 44.5 s duration each, alternating emotional and control blocks. Each block contained 10 stimuli presented for 4 s with an inter-stimulus interval of 0.5 s. The inter-block interval was 2 s, giving a total scan length of 9 min.

We used four different sets of geometric forms for the control blocks, and sixty different images, ten per block, five of each gender, all derived from the Karolinska database (Lundqvist, Flykt & Vhman, 1998), for the emotional blocks.

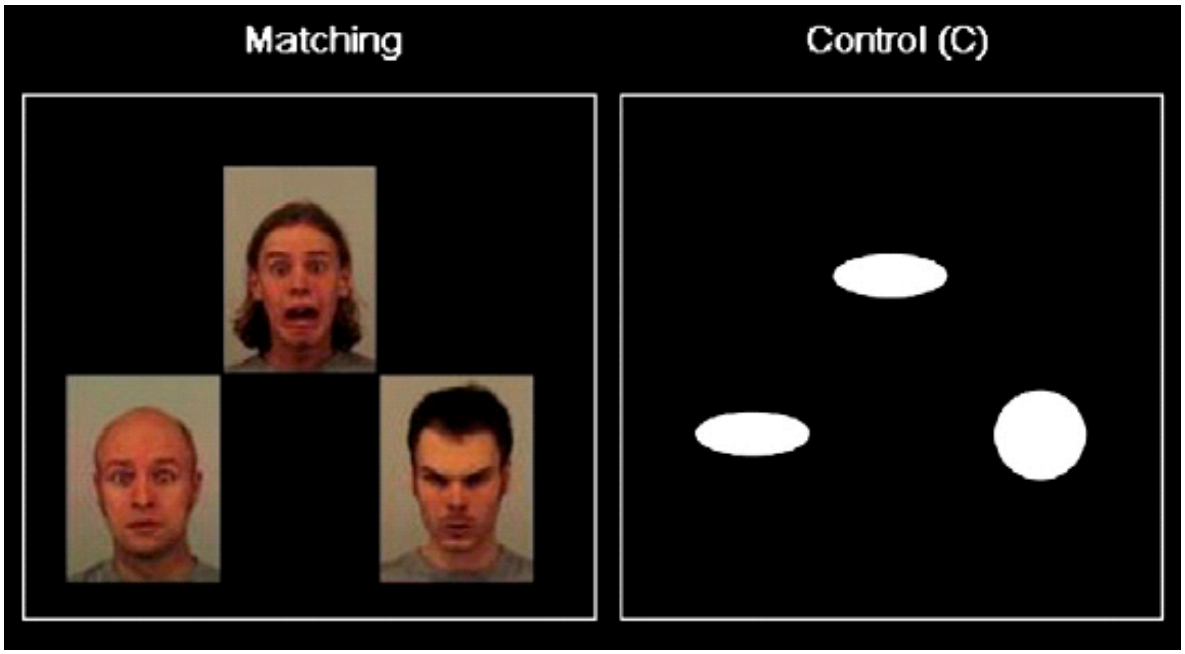

Fig. 12. Illustration of the emotional and control conditions in the face matching task

K. fMRI Data Analysis

a) Functional Analysis of BOLD signal

Data were processed using SPM5 software (Wellcome Department of Cognitive Neurology, University College London) implemented in Matlab 8.0 (Mathworks Sherborn, MA). For each subject, the first 4 scans, corresponding to a period of signal stabilization, were discarded. The remaining scans were corrected for differences in slice acquisition time. To remove the effects of head movement during scanning, the 234 scans of each session were realigned to the first scan of the session. Subject data were discarded if movement was larger than 3 mm on either x,y,z or 3^{o} rotations. All images were transformed into a standardized coordinate system corresponding to the MNI (Montreal Neurological Institute) space.

The normalized images were then spatially smoothed with an isotropic Gaussian kernel (full width at half maximum of 8 mm). Individual statistical maps were calculated for each subject to evaluate differences between the emotional versus control condition. Each condition was modeled by a box-car convolved with a canonical hemodynamic response function. The within-subject contrast images were then entered into a second-level t-test to examine both within- and between-group effect. We had a priori Regions of Interest (ROI) defined using an anatomical mask from the WFU Pickatlas (Version 2.4). These ROI were based on their validated functional implication in a similar face-matching task in healthy controls (Stein et al., 2007). ROI included the amygdala, ventromedial prefrontal cortex (vmPFC - BA25), anterior cingulate cortex (ACC - BA32) and orbitofrontal cortex (OFC - BA11).

b) Functional Connectivity Analysis

Similarly to Bettus et al., (2009), we assessed the functional connectivity between the amygdala and each of the vmPFC, ACC and OFC using an automated functional connectivity analysis. To do so, these ROIs were used as masks applied onto the residual images to extract the mean signal time-courses from each predefined ROI. To determine functional interactions between ROIs in each temporal lobe, correlation coefficients between pairs of signal time-courses were computed (JMP statistical software). Correlation coefficients were then normalized using the Fisher transformation ($rN=0.5*Log[(1+r)/(1-r)]$). The obtained z-scores reflected basal functional connectivity and allowed subsequent statistical analyses.

An ANOVA was performed on the extracted ROI peak maximum and z-scores separately, with Group (controls, treated patients) as a between factor and Session as a within factor. A significant level of $p<0.05$ was taken and post-hoc were Bonferoni corrected.

CHAPTER III

RESULTS

In the following section, all five articles are presented in the same order the hypotheses were formulated. They are always preceded by an abstract, typically as they were submitted.

They are presented as follows:

A. Article 1: Restoration of Normal Fear Conditioning and Extinction Following PTSD Symptom Amelioration

B. Article 2: Restoration of Emotional Attending and Suppressing in PTSD Following Symptom Amelioration by EMDR

C. Article 3: Attentional Bias in PTSD Vanishes After Symptom Amelioration

D. Article 4: Neurofunctional Alteration of Emotional Face Processing Correlates with Attentional Bias in PTSD

E. Article 5: Restoration of Decreased Functional Activity and Connectivity in PTSD Following Successful Treatment

Article 1: resubmitted to the Journal of Biological Psychology on December 2010

Restoration of Normal Fear Conditioning and Extinction Following PTSD Symptom Amelioration

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Objective: *Enhanced fear sensitization and failure of fear extinction have been hypothesized as being part of the dysfunction causing aetiology and maintenance of post-traumatic stress disorder (PTSD). The aim of the present study was to explore whether normal experimental fear conditioning and extinction is restored after a treatment suppressing PTSD symptoms.*

Methods: *Eighteen healthy controls and 17 PTSD patients were assessed on a classical fear conditioning and extinction paradigm, monitoring physiological markers and subjective evaluations. An electric stimulus served as the unconditioned stimulus (US) and two neutral pictures as conditioned stimuli being paired (CS+) or unpaired (CS-) with the US. Skin conductance, frowning and cardiac activity were recorded. The paradigm was performed before and after successful EMDR therapy.*

Results: *Fear conditioning was originally enhanced and fear extinction delayed in PTSD (vs. healthy individuals). After EMDR therapy and symptom amelioration, fear conditioning was no longer facilitated in patients, and extinction developed similarly to controls.*

Conclusions: *These results confirm the existence of an altered fear processing pathway in PTSD, which might be at the core of symptomatology. Mostly, we provide preliminary support for restoration of normal fear conditioning/extinction after symptom amelioration in PTSD.*

INTRODUCTION

Post traumatic stress disorder (PTSD) is an anxiety disorder that can occur after exposure to a traumatic event. According to the DSM-IV classification (American Psychiatric Association, 1994), it is characterized by hypervigilance, hyperactivity and persistent symptoms of re-experiencing. It also includes symptoms that persist for at least one month including intrusive memories, flashbacks, avoidance, signs of increased arousal (e.g., sleeplessness, irritability), as well as emotional indifference and social dysfunctions (Bremner et al, 1999a, Pole, 2007). Hypervigilance to reminders of a traumatic event is one of the main characteristics of PTSD. Interestingly, psycho-physiological studies have shown that external and internal reminders of traumatic events produce elevated heart-rate (HR), blood pressure, skin conductance (SC) and frowning electromyogram activity (EMG) in trauma survivors with PTSD (Blanchard et al, 1991; Malloy et al 1983; Orr et al., 1998a; Pitman et al., 1991).

Contemporary theories of PTSD describe it as a pathological fear sensitization and failure of extinction of learned fear associations (Charney, 2004). The presence of elevated physiologic responses to reminders of traumatic events has been interpreted as a result of altered fear conditioning (Pitman et al., 1989; Peri 2000). The prevailing hypothesis is that PTSD involves a defective fear processing pathway whereby patients have an increased propensity to learn associative fearful stimuli and, have a deficient capability to extinguish them afterwards (Francati et al., 2007; Blechert et al., 2007).

A much used way of looking at learned fear responses, both in animal and human models, is mimicking them in laboratory settings through the classical Pavlovian fear conditioning and extinction paradigm. Typically, a habituation phase accommodates subjects to stimuli presentation. Next, the acquisition phase consists of pairing the neutral conditioning stimulus (CS) (e.g. an image) to an aversive unconditioned stimulus (US) (e.g. an electric shock). After several trials, the CS, even presented alone, induces conditioned fear responses (CR) such as freezing in rats (Ledoux, 2000) and changes in autonomic nervous system such as HR and SC in humans (Orr et al., 2000). Ultimately during the extinction phase, repeatedly presenting the CS without the US extinguishes the learned fear CR (Berman & Dudai, 2001; Myers & Davis, 2002).

Consistent with conditioning theories of PTSD pertaining to an impaired fear processing pathway, several studies have investigated Pavlovian conditioning in PTSD

patients using trauma-irrelevant aversive cues (e.g., painful electric stimulation) as US. Most have revealed enhanced acquisition and slower extinction of the conditioned response in PTSD patients (Orr et al., 2000; Peri et al., 2000). Studies have looked at physiological assessments in differential conditioning in PTSD and found increased electrodermal SC, HR and facial electromyographical (EMG) responses during acquisition and extinction phases when comparing CS types (paired with US (CS+) and unpaired (CS-)) on one hand, and on the other, when comparing PTSD patients, trauma exposed individuals and healthy controls (Peri et al, 2000, Blechert et al., 2007). Even when the US was no longer present and the expectation of danger was not met, the CR failed to extinguish in PTSD. This deficient extinction learning could explain why some patients maintain symptoms many years after the original trauma (Wessa & Flor, 2007) and fail to respond to exposure therapy, incorporating extinction procedures (Foa, 2000).

However, these findings have not always been replicated (Milad et al., 2008). It also remains controversial whether impaired conditioning is due to explicit fear cues (Orr et al., 2000) or rather to context conditioning and generalized fear responses, arguing PTSD patients might have a difficulty learning safety cues (Grillon & Morgan, 1999). Studies have seldom looked at the subjective verbal conditioning.

Our first aim was thus to replicate previous findings on enhanced fear conditioning in PTSD while monitoring a more comprehensive set of measures to assess some of the major autonomic mechanisms involved; some of which are under voluntary control (corrugator EMG) while others are involuntary (electrodermal SC and heart rate HR). We also monitored affective (CS valence ratings) and verbal (US-expectancy ratings) responses throughout all the conditioning phases.

In a modified conditional discrimination procedure, Jovanovic et al. (2009) showed that the fear inhibition impairment in PTSD was inversely correlated to symptom severity as the high-symptoms PTSD group showed less fear inhibition and had greater fear potentiation as compared to low-symptoms PTSD patients and healthy controls. Studies have so far shown that one of the treatment option of PTSD; eye movement desensitization and reprocessing (EMDR) treatment, relieves traumatic symptoms and reduces the autonomic responsiveness of PTSD patients to aversive stimuli such as trauma recall (Aubert-Khalfa et al., 2008). EMDR is also associated with psychophysiological dearousal for SC and HR over time sessions (Sack et al., 2008). The biological basis of this quite fast process still remains unknown.

Our second aim was thus to explore the potential changes in PTSD conditioning after symptom amelioration by EMDR. We chose to explore fear processing in PTSD before and after treatment i.e. when patients are fully symptomatic versus when they are symptom-free. To the best of our knowledge, no study has so far looked at the modulation of fear conditioning/extinction in PTSD before and after successful therapy.

In the current study, we tested whether major psycho-physiological mechanisms altered in fear processing in PTSD are restored after a successful treatment suppressing core PTSD symptoms, using a validated fear conditioning and extinction paradigm (Blechert et al., 2007). We hypothesized that, similarly to anxious and PTSD populations previously studied on this task, PTSD patients would show increased fear sensitized and slower extinction of conditioned responses. Based on this premise; patients would have increased physiological reactivity (heightened HR, EMG and SC) and more aversive ratings to CS+ than healthy controls during fear acquisition and extinction. We also hypothesized that this heightened conditionability would be normalised after symptom removal and that after successful therapy PTSD patients would be similar to controls in terms of autonomic reactivity and valence ratings, throughout all conditioning phases.

METHODS

Subjects

A total of 23 adult outpatients (8 males and 15 females) were recruited by a psychiatrist among trauma victims at the medico-psychological crisis cell (CUMP) at the Psychiatry Pole of the Conception Hospital in Marseille, France. They all met the DSM-IV criteria for PTSD following a single traumatic event (12 aggressions, 4 road accidents, 6 work related accidents, 1 grief after witnessing of one's son commit suicide, by hanging) with no previous history of neurologic or psychiatric disorders. Subsequent analysis included 17 patients (7 males and 10 females, with mean age = 44 ± 15 years, mean education = 7.3 ± 2.7 years after grade 7 and mean trauma exposure = 18.4 months). Six patients were excluded from data analysis as 2 of them abandoned the study, 2 of them had symptom reduction but not disappearance and 2 of them were reluctant to the electric stimulation. Five patients were on combined regimen of antidepressants and anxiolytics, 2 patients only took antidepressants and 2 only took anxiolytics.

A total of 18 healthy adult controls (9 males and 9 females, with mean age = 37 ± 14 years and mean education = 8.9 ± 2 years after grade 7) with no history of neurologic or

psychiatric disorders, were recruited via screening lists at the clinical investigation centre at the Timone Hospital (CIC-UPCET). They were matched to patients for age, sex and education.

Psychological Assessment

All participants were assessed by a psychiatrist for PTSD and other mental health disorders using the structured Mini-Internal Neuropsychiatric Interview for DSM-IV (Lecrubier, 1998). This allowed us to check for the absence of psychiatric disorders prior to the trauma in PTSD and screen for potential comorbid psychiatric disorders. Participants responded to demographic questions and completed the Beck Depression inventory (BDI) (Cottraux, 1985) and the State-Trait Anxiety Inventory (STAI) (Schweitzer & Paulhan, 1990). Patients also completed trauma related scales: PTSD Check List Scale (PCL-S) (Ventureyra et al., 2002), Modified PTSD Symptoms Scale (MPSS) (Stephenson et al., 1999) and Impact of Event Scale (IES) (Weiss & Marmar, 1996). The validated French versions were used for all the scales.

EMDR Treatment

All PTSD patients underwent Eye Movement Desensitization and Reprocessing (EMDR) therapy (APA, 2004). According to the APA reports published in 2004, this eight-step standardized protocol is one of the validated treatments for PTSD. Based on an information processing model (Shapiro & Maxfield, 2002), EMDR includes associations of cognitive, emotional and physical assessments of actual distress to traumatic scenery, as well as imaginal exposure while attending to bilateral alternate stimulation. As the patient is asked to visualize the most salient aspect of a traumatic memory, the therapist induces bilateral stimulation (by means of ocular, sensory-motor or auditory left/right stimulation) (Shapiro, 1989). EMDR is an effective rapid therapy with stable outcome demonstrated in a 35-month follow-up study (Hogberg et al., 2008).

Patients were treated by one of 3 therapists, all trained by the French institute of EMDR. There was no fixed number of sessions. Sessions were planned every 7 to 15 days according to patients and therapists availabilities. The treatment was considered successful and complete when patients reported no more feelings of distress when thinking about their trauma. They were again interviewed by a psychiatrist, using the MINI. They were retested when they no longer met PTSD classification according to DSM-IV criteria and had no more pathological scores on PTSD scales.

Patients required an average of 4.3 ± 1.7 treatment sessions (ranging from 1 to 7 sessions), lasting on average for 2.5 ± 1.3 months.

Apparatus and physiological recordings

An electrical stimulator (constant current unit, Biopac Systems, Inc., Goleta, CA, USA) was used to deliver the US through a bar electrode with concave tin plated discs attached to participants left lower arm. This US was delivered was generated by varying the dial setting on a STMISOC stimulus isolation adapter, for a current ranging from 0.1 to 5.0 mA. It was isolated from line current and used a 9 V dry battery attached to an adjustable set-up transformer. Stimulus delivery and physiological data acquisition were controlled by two PCs running E-Prime (Psychology Software Tools, Inc., Pittsburgh, PA, USA) and Acqknowlege software (Biopac Systems, Inc., Goleta, CA, USA) respectively. Physiological channels and rating dial information were recorded at a rate of 1000 Hz in continuous mode using the Biopac MP150 system.

Skin conductance (SC) was measured in microsiemens using two 5 mm inner diameter Ag/AgCl electrodes filled with isotonic paste. Electrodes were placed on the medial phalanges of the index and middle finger of the left hand in accordance with published guidelines (Fowles et al., 1981). Since deep breathing and/or coughing may trigger artifacts on the SCRs; respiration pattern was recorded using a pneumagraphic belt with a respiration transducer at the rib cage, towards the end of the sternum.

Electromyogram (EMG) of the corrugator activity was measured in microvolts using three 4 mm Ag/AgCl shielded surface electrodes filled with electrolytic paste. The skin was locally abraded with alcohol imbibed cotton swab and two electrodes were attached with adhesive collars over the corrugator muscle site according to manufacturer specifications (Fridlund &Cacioppo, 1986). A ground electrode was placed on the lobe of the left ear.

Heart Rate (HR) was measured in beats per minute using 3 clip lead electrodes. Electrodes were attached in a Type I EKG configuration; on the left flying rib, right collarbone and sternum.

Procedure

The investigation was carried out in accordance with the latest version of the Declaration of Helsinki. Participants provided informed consent in accordance with local ethical committee guidelines set forth by the CPP committee South Mediterranean 2.

The experiment took place in a temperature-controlled, fully lit, and sound-attenuated room. Participants were comfortably seated at 60 cm viewing distance from a 17" computer screen, with a refresh rate of 100Hz. Electrodes were attached and the respiratory belt put in place. The conditioning task was the one developed by Blechert (for details see Blechert et al., 2007) and consisted of three different phases: habituation, acquisition and extinction.

The US was a 500-msec electric shock previously determined by the participant to be "highly annoying but not painful" using the up-down staircase method. This was done by gradually incrementing shock intensity, while participants rated its aversiveness using a digital analog scale (from "Not annoying"= 0; to "Highly annoying"= 100). Once the shock intensity determined, it was kept constant for the rest of the conditioning task.

The habituation phase started with written instructions telling participants that two pictures would be shown on the screen and that there will be no shock delivery. It consisted of 6 trials of each to-be-CS+ and to-be-CS-. CS+ and CS- images were used from the Rorschach inkblot test and were counterbalanced across participants. Images were presented for 8 s. The mean intertrial interval (ITI) was 18 s (range 16-20 s). At the *acquisition phase*, instruction told participants that two pictures will be shown on the screen and that only one would be occasionally followed by the electric shock. It consisted of 6 trials of each CS type and each CS+ was followed by the US. No instructions were shown at the *extinction phase* that consisted of 6 CS+ and 6 CS-.

Ratings of US expectancy and CS valence were repeatedly obtained. Six valence ratings were obtained for each CS in the middle and the end of each conditioning phase (every third CS was rated, yielding a total of 12 ratings). During these rating trials a visual analogue scale appeared on the screen, 4 s after CS offset, prompting participants to give retrospective valence ratings (''How did you find the last picture?'' ratings ranged from ''Pleasant'', 0; to ''Unpleasant'', 100). Upon completion of the rating, the ITI commenced. US-expectancy ratings were obtained on the first and last presentation of each CS during extinction phase. A baseline US-expectancy rating was obtained at the end of habituation. On these trials a visual analogue scale appeared on the screen immediately after CS offset (''Do you believe that this stimulus will be paired with an electric stimulation?'' No, Yes). Previous research established that these ratings do not influence the psychophysiological outcome variables in a differential aversive conditioning paradigm (Lipp et al., 2003).

Following extinction training, contingency awareness was assessed by a screen presenting the CS+, the CS- and a control stimulus and asking which of the three pictures had

previously been paired with the US. This recognition measure of contingency awareness is considered more sensitive than post-experimental questionnaires which require recall of contingency knowledge (Lovibond & Shanks, 2002).

The experimental protocol was administered twice for all participants: before treatment (P_1) and immediately after symptom amelioration (P_2) for the PTSD group, and in matching time lags (P_2-P_1) for the control group. The control group in fact served to monitor for potential habituation to repeated conditioning.

Data and Statistical Analyses

Similarly to Blechert et al., an SC response was calculated for each CS trial by subtracting the mean skin conductance level (SCL) during the 2 s immediately prior to CS onset from the highest SCL recorded during the 8 s CS presentation. This method has been documented to be more adapted in differential fear conditioning paradigm than the alternative scoring method that consisted of measuring either the First or the Second Interval Response (Pineles et al., 2009). The present scoring method allows for the detection of the maximal increase in skin level at any point during the 8 s presentation. In addition, the UR to the electric stimulation was calculated by subtracting the average SCL during the last 2 s of CS presentation from the highest SCL recorded during the 8 s following the US. SC below 0.01 μS were scored as zero and square root transformation was applied to normalize the SC distribution. SC to each CS-type (CS+, CS-) on three consecutive presentations was averaged, resulting in two blocks per conditioning phase (e.g. first and second half of habituation). Artifact correction for SCs consisted of a visual inspection of respiration and the manual exclusion of SC that appeared to be influenced by coughs, sights or deep breath (around 5% of either CS type was excluded).

Two participants in each group had no electrodermal conditioning i.e. they had no SC greater than 0.01 for any of the CS during acquisition. Their data have been removed from subsequent analyses.

Data for HR and EMG were similarly calculated for each CS trial.

A two-way repeated measures ANOVA was used on the SC, EMG and HR for each conditioning phase separately with Group (control, PTSD patients) as a between factor and Session (1, 2), CS-type (CS+, CS-) and Time (first half, second half) as within factors. Significant main effects at $p < 0.05$ were followed by post-hoc t-tests using Bonferroni correction. All post-hoc were taken at a p level $p<0.05$.

RESULTS

1- Clinical Data

Table 1 shows the psychometric measures for the two groups. Groups did not differ in age, sex and education. PTSD patients initially scored higher than healthy controls on BDI and STAI scales. After treatment, there was no significant difference between PTSD and control groups (Table 1).

Patients' scores on PTSD scales were initially higher than the cut-off for pathology and significantly dropped to normal levels after treatment termination.

Patients met the criteria for the following major current comorbid diagnoses (before/after EMDR): major depression (n=10/4), other anxiety disorders (n=15/8) and high-medium suicidal risk (n=6/0). Specific anxiety rates initially diagnosed in PTSD are as follow (out of the 17 patients): Social phobia (5), Generalized Anxiety Disorder (10), Panic disorder (5), Agoraphobia (7). Most patients had more than one comorbid anxiety disorder.

We found no difference between the two groups for selected intensity of stimulation, nor its aversiveness. We also found no differences between groups for the electrodermal reactivity to the US (Table 1).

	Healthy Controls (Time 1) (n=18)	Healthy Controls (Time 2) (n=18)	PTSD Pre-treatment (Time 1) (n=17)	PTSD Post-treatment (Time 2) (n=17)	Statistics
BDI Depression	3.0 (2.6)	2.1 (2.3)	15.5 (8.1)	5.6 (4.1)	$F(1,33) = 21.72^{**}$
STAI Trait Anxiety	29.7 (8.3)	30.2 (8.1)	50.6 (11.3)	35.4 (8.6)	$F(1,33) = 15.33^{**}$
STAI State Anxiety	39.5 (7.9)	36.5 (9.0)	56.5 (10.5)	43.1 (7.5)	$F(1,33) = 10.76^{*}$
PCL-S	-	-	62.4 (11.7)	29.2 (6.3)	$t(1,16) = 10.82^{**}$
MPSS	-	-	73.5 (23.2)	22.1 (9.5)	$t(1,16) = 9.97^{**}$
IES	-	-	56.8 (13.8)	18.2 (9.9)	$t(1,16) = 8.81^{**}$
Stimulation level (mA)	1.9 (1.6)	1.4 (1.4)	10.4 (19)	8.9 (15.7)	NS
US aversiveness rating (0-100)	5.4 (16.8)	12.16 (14.5)	10.35 (26.9)	8.9 (16.5)	NS
UR: SCR (μS)	1.18 (0.84)	1.1 (0.86)	1.28 (1.03)	1.23 (0.87)	NS

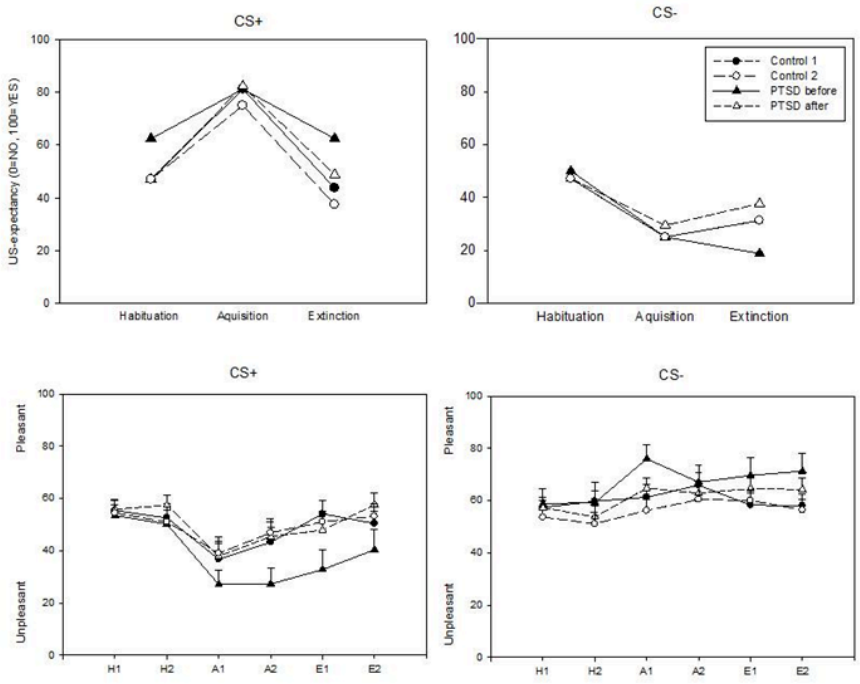
Table1. Characteristics of participants: Mean (SD) for Depression (BDI), Anxiety (STAI), and for the patients PTSD scales (PCL-S, MPSS, and IES). F values are the result of ANOVA for Group x Scale interaction and t values are the result of paired t-test for PTSD patients before and after EMDR. Significant p-value: $^{*}p < 0.05$, $^{**}p < 0.001$ and NS: not significant.

2- Conditioning procedure

Fig. 2 displays the means and standard errors of verbal assessment, and of the square root v values of SC, HR and EMG for both study groups, during first and second halves of the habituation (H1 and H2), acquisition (A1 and A2) and extinction (E1 and E2) phases. CS+ and CS- are shown separately for more clarity.

Verbal Evaluation and Contingency Awareness

Results for the contingency test of awareness after extinction revealed that one subject from the control group and 2 from the patient group failed to correctly identify the CS+. Since contingency awareness is frequently associated with differential conditioning (Lovibond & Shanks, 2002) unaware participants were excluded from the verbal ratings of valence and US expectancy. In subsequent analysis of physiological variables, including their data had no significant changes in the statistical interactions. We decided to keep their data for physiological assessments as we argue for the possibility of unaware, subtle conditioning in spite of conscious identification or not of CS+. In fact, conditional fear and differential amygdala activity were shown to develop on trials paired with aversive tones presented on both supra (perceived) and subconscious (unperceived) thresholds (Knight et al., 2009).



Article 1. Fig 1. Mean and Standard errors of verbal evaluations of CS+ and CS- of healthy controls and PTSD patients at test (1 and pre-treatment for groups respectively) and retest (2 and post-treatment for groups respectively), for expectancy ratings at habituation, acquisition and extinction and for valence ratings at each half of every phase: habituation (H1-H2), acquisition (A1-A2) and extinction (E1-E2). Ratings were done on a scale from 0-100.

US expectancy ratings

PTSD patients had higher US expectancy than controls at habituation and extinction. (Fig.1).

Valence Ratings and Physiological Responses

In all subsequent analyses, there was no effect of retest for the control group on either CS+ or CS- as we found no difference in ratings and physiological responses of controls at session 1 and 2 ($p > 0.05$).

Valence Ratings

During acquisition, a significant Session x Time x Group interaction was found ($F (1, 30) = 5.24$, $p<0.05$). Both groups showed lower ratings (less pleasurable) for CS+ than CS-. Before EMDR, the PTSD group has more aversive ratings of CS+ and more pleasant ratings of CS- than the control group, but not after treatment.

During extinction, a significant Session x CS-type x Group interaction was observed ($F (1, 28) = 4.31$, $p<0.05$). Only the PTSD group pre-EMDR had differential ratings of CS+ and CS-. Post hoc analyses showed that similarly to acquisition, the PTSD group initially has more aversive ratings of CS+ and more pleasant ratings of CS- than the control group, whereas after treatment groups had comparable evaluations for CS+ and CS- (Fig.1).

Skin conductance

During habituation, only the Time factor was significant ($F (1, 30) = 30.49$, $p<0.05$). Post hoc analyses showed greater SC for both groups at H1 compared to H2.

During acquisition, a significant Session x CS-type x Group interaction was found ($F (1, 28) = 4.18$, $p<0.05$). Both groups showed differential SC response and had higher electrodermal response to CS+ than CS-. The PTSD group also had higher SC to CS+ and CS- than controls, only before EMDR but not after.

During extinction, there was a significant Session x CS-type x Group interaction ($F (1, 28) = 4.07$, $p<0.05$), which reflected higher SC to the CS+ in the PTSD pre-EMDR group than in controls. After successful treatment, SC was comparable in patients and controls. No significant interactions were found for CS-.

Adjustment for SC: Analysis of differences at acquisition and extinction

At habituation, PTSD patients had slightly larger SC than controls. This difference was not significant and might relate to their general higher levels of anxiety or anticipation of an unknown task.

At acquisition, PTSD patients also had larger SC than controls for CS+. This significance indicates that PTSD patients seemed to exhibit physiologically more intense fear responses. To ensure that subsequently higher SC levels at extinction were not merely due to a generalized heightened reactivity at acquisition, we calculated the values of SC at extinction whereby E'1=E1-A2 and E'2= E2-A2. Statistical analysis revealed a significant Session x Group main effect at E'2 with $F(1, 27) = 5.24$ with $p<0.05$.

Adjustment of physiological indices for comorbid anxiety, depression and medications: Analysis of covariance (ANCOVA)

It had been suggested that comorbid disorders and/or medication can alter the fear conditioning pathway in PTSD (Harmer, 2008). We assayed the effect of anxiety, depression or medication on electrodermal activity and verbal evaluations at the acquisition and extinction stages. These factors were entered separately as covariates in the Session x CS-type x Time x Group ANOVAs. Statistical analysis revealed all three covariates; comorbid anxiety, depression and use of medication, were not significantly interfering with the main interaction we found for either SC or verbal responses. PTSD still showed significantly heightened SC responding to CS+ during acquisition ($F(1,28)= 4.29$, $p<0.05$ - $F(1,29)=5.51$, $p<0.05$ - $F(1,29)=5.61$, $p<0.05$) and extinction ($F(1,28)= 6.91$, $p<0.05$ - $F(1,29)=12.11$, $p<0.05$ - $F(1,29)=9.51$, $p<0.05$). PTSD also still showed significantly heightened verbal dislike to CS+ during acquisition ($F(1,25)= 7.44$, $p<0.05$ - $F(1,25)=8.53$, $p<0.05$ - $F(1,25)=4.20$, $p<0.05$) and extinction ($F(1,24)= 4.46$, $p<0.05$ - $F(1,24)=,4.07$ $p<0.05$ - $F(1,24)=4.96$, $p<0.05$).

Correlations analysis of PTSD scales, acquisition and extinction

Statistical analysis revealed significant correlation between the initial MPSS score of PTSD patients and their SC responses on CS+ for C1, C2, E1and E2 phases, with the respective Pearson Correlation index $r= 0.81$, $n=15$, $p<0.001$, $r= 0.60$, $n=15$, $p<0.05$, $r=0.57$, $n=15$, $p<0.05$ and $r=0.48$, $n=15$, $p<0.05$. Significant correlations were also found for C1 and E2 for the difference between the MPSS scores and the difference in SC levels before and after treatment with respective $r= 0.46$, $n=15$, $p<0.05$ and $r= 0.51$, $n=15$, $p<0.05$.

Heart Rate activity

During acquisition, statistical analysis revealed a significant Session x Time x Group ($F(1, 27) =5.33$, $p<0.05$). Only the PTSD group pre-treatment had higher HR in the presence

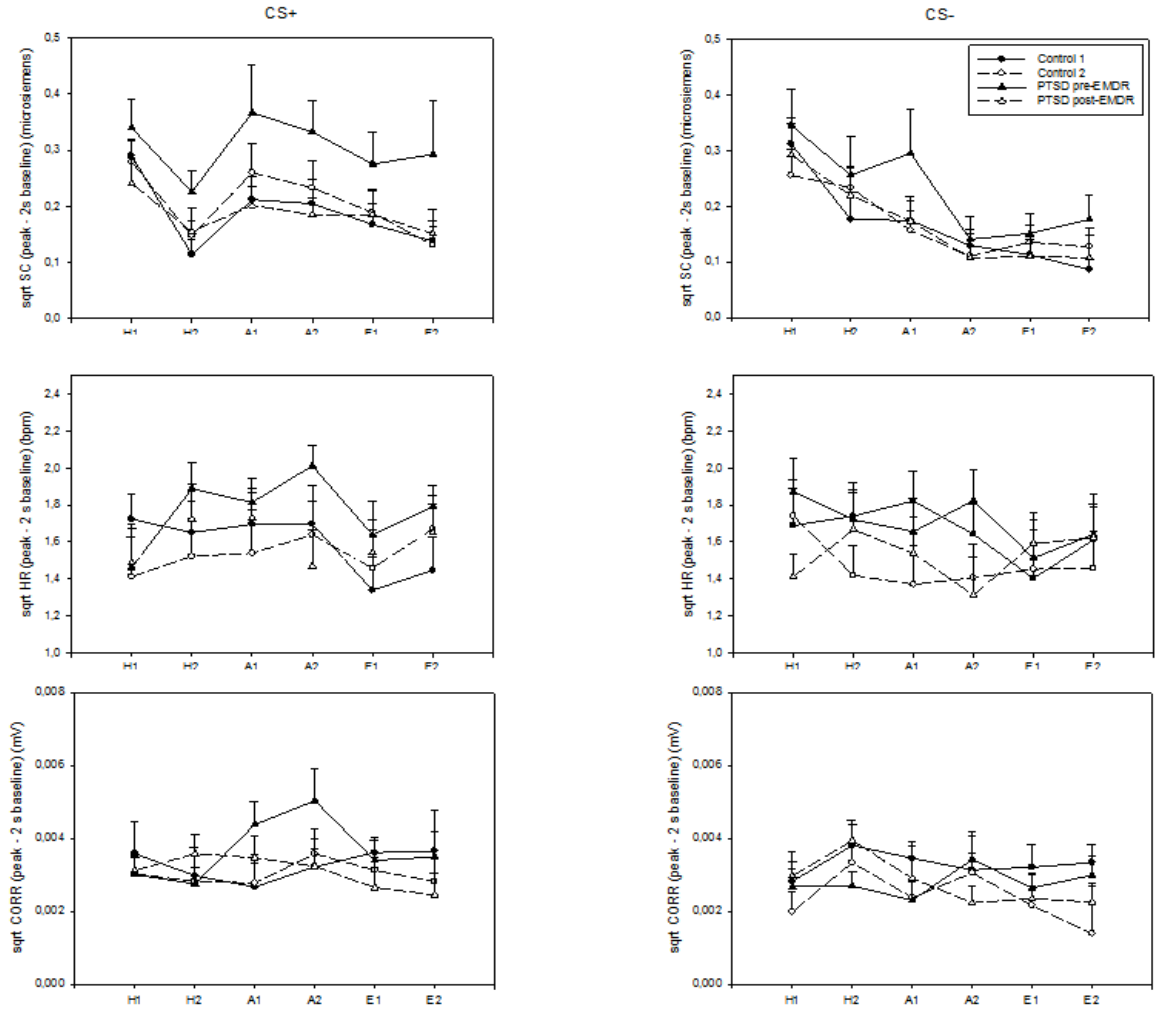
of CS+ and CS- compared to controls. After symptoms removal, the 2 groups had comparable cardiac activity for CS+ and CS-.

No significant interactions were found during extinction.

Corrugator activity

During acquisition, a significant Session x CS-type x Group interaction was found ($F(1, 30) = 6.11$, $p<0.05$). Post-hoc analysis showed that only PTSD pre-EMDR group had differential conditioning for EMG, meaning higher EMG to CS+ than CS-. Besides, PTSD patients initially had a stronger frowning activity to CS+ than controls have. After treatment however, there was no significant difference in corrugator activity between the 2 groups.

No significant interactions were found during extinction.



Article 1. Fig 2. Mean and Standard errors of physiological responses to CS+ and CS- of healthy controls and PTSD patients at test (1 and pre-treatment for groups respectively) and retest (2 and post-treatment for groups respectively), for the square root values of Skin Conductance (SC), Corrugator Activity (EMG) and Hear Rate (HR) at each half of every phase: habituation (H1-H2), acquisition (A1-A2) and extinction (E1-E2).

DISCUSSION

The present study illustrates major psycho-physiological deficiencies in PTSD pathology in the fear processing pathway via a classical fear conditioning procedure. First and foremost we have replicated the electrodermal and verbal results presented by Blechert et al. (2007) using the same conditioning paradigm, as well as HR and EMG results found by Orr et al. (2000), confirming increased fear conditioning and delayed extinction in PTSD (Pole, 2007; Wessa & Flor, 2007). Most importantly, we have shown that immediately after symptom amelioration by successful EMDR therapy, altered fear processing in PTSD (at acquisition and extinction) is restored.

EMDR therapy decreased patients' scores on anxiety, depression and PTSD scales, from pathological to normal levels. This result further validates the well established clinical and therapeutic effectiveness of EMDR and its rationale as a first-line treatment option for PTSD by APA health instances.

It is noteworthy that although PTSD patients chose higher US levels than controls, the difference was not statistically significant. We found no difference in the rating of US aversiveness between groups and we found no differences in groups SC to US. US aversiveness rating, relating to subjective perception of threat, would be a better indicator of subsequent induction of conditioned fear response than the mere US intensity. This further validated experimenter's observation that controls at session 1 and 2 as well as pre and post-EMDR patients similarly feared the electric stimulus.

The verbal ratings of image valence gave a fair overview of the fluctuation of increased perceived fear acquisition and delayed extinction towards a neutral stimulus as a function of its association with an aversive shock. Similarly to Lipp et al., (2003), we found that differential affective conditioning in PTSD is resisting to extinction. Unlike some of the previous studies (Blanchard et al 1982, 1991; Pitman et al 1987; Shalev et al., 1993), the stimuli we used were not direct reminders of traumatic events. The resulting responses, therefore, do not reflect trauma-related conditioning but somewhat express a newly acquired association between neutral and aversive stimuli (Peri, 2000).

Taken together, US aversiveness and verbal ratings are valuable indicators of the conditionability of subjects at both their first and second testing sessions. It quantitatively validates the experimenter's observations of equally elevated anxiousness and enhanced

emotionality during the 1st and 2nd session for patients and controls, which was not due to the novelty of the situation but rather to its aversive context involving an electric shock.

At habituation, PTSD patients initially had higher US anticipation compared to controls. They also had higher threat expectancy at extinction, even when the CS+ was no longer coupled to the aversive shock. PTSD patients indeed display a contingency bias in ambiguous situations (Blechert et al., 2007). This might account for instance for the tendency towards increased SC reactivity at the second half of extinction E2. This increased anxiety during anticipation of unpredictable stimuli is rather specific to PTSD and not to other anxiety disorders (Grillon et al., 2009), and might clinically relate to their generalized hypervigilance in the presence of aversive cues (Ehlers & Clark, 2000).

Our results replicate previous findings of enhanced fear conditioning and delayed extinction in PTSD patients. Patients have elevated SC at acquisition and delayed SC decrease at extinction (Blechert et al., 2007; Orr et al., 2000; Peri et al., 2000). At H1 both patients and controls had higher SC than at H2, indicating that all subjects were more reactive at the beginning of the experiment; SC being sensitive to novelty effect. Similarly to Orr et al., (2000) and Blechert et al. (2007), we found that PTSD patients have higher SC than controls to both CS+ and CS- at acquisition, and only to CS+ at extinction. Unlike some studies that failed to show PTSD differential conditioning (Grillon & Morgan, 1999), our finding argues with the sensitization of PTSD to an aversive context and the ensuing increased responding to either stimulus. PTSD patients can thus learn safety but have difficulty inhibiting the conditioned fear response.

We also found that similarly to SC activity, enhanced conditionability was found in PTSD for additional physiological (frowning EMG and HR) and verbal factors at acquisition, supporting previous findings (Orr et al., 2000). At extinction however, only SC and verbal evaluations differentiated both groups as patients still had high electrodermal activity and aversive verbal ratings to CS+ compared to controls. Along with verbal ratings, SC seems to be the most sensitive maker to differential fear conditioning in PTSD. It might be that higher brain centers regulating physiological factors are differently disturbed in PTSD. In fact, EMG and HR are known to be modified in PTSD in conditioning as well as other emotional tasks (Guthrie &Bryant, 2006; Miller & Litz, 2004; Orr et al., 1998b; Pitman et al., 2001). They seem to reflect patients' hyperactivity in aversive contexts rather to aversive cues, and are respectively under voluntary (EMG is a motor reactivity) and involuntary control (HR involve sympathetic and parasympathetic regulation) whereas SC is under sole sympathetic control.

Although inconclusive, our results support the emerging literature showing PTSD patients have high sympathetic activity and low parasympathetic control at rest (Blechert et al., 2007b).

Same groups have sometimes failed to replicate PTSD heightened conditionability at acquisition (Orr et al., 2006; Milad et al., 2008). They owe discrepancies in their results to the age of the studied population, the medications, the placebo-pill effect if any, or merely the interpersonal difference of PTSD with regards to the conditionability feature. In a meta-analysis, the conditioning procedure is also defined as a determining factor, with more consistent results of heightened differential conditionability in PTSD observed when using "simple" CS+ (Lissek et al., 2005).

The most important finding remains the restoration of normal fear conditioning and extinction following PTSD symptom amelioration by EMDR treatment. We have explored the implicit (physiological) and explicit (verbal) conditioning before and after successful EMDR therapy and found that after symptom amelioration, fear conditioning was no longer facilitated in PTSD and fear extinction developed similarly to controls. We thus provide preliminary evidence that fear processing alterations would be linked to PTSD symptomatology. This is further supported by significant correlations between intial MPSS scores (evaluating PTSD symptomatology) and SC at acquisition and extinction, as well as correlations between difference in MPSS scores and difference in SC before and after EMDR at A1 and E2. These data suggest that initially more severe symptoms correlate with larger SC to CS+ and that symptom amelioration quantitatively correlate with decreased in fear conditioning in PTSD.

At that stage, it remains unknown whether enhanced conditionability and fear processing deficits in PTSD are inherited vulnerability factors for developing PTSD upon traumatic exposure, or represent acquired signs of PTSD after traumatic exposure. Elegant studies have started looking at this using adequate designs of twins with one PTSD or trauma-exposed co-twin (Orr et al., 2003) or high trauma-exposed population such as policeman or fireman before and after trauma (Guthrie et al., 2006; Pole et al., 2009). They suggest that increased initial EMG startle reflex and SC to loud tone seems to be an inherited factor for increased PTSD symptom severity after trauma exposure whereas higher HR would be an acquired marker after trauma. Yet, caution should be taken as to monitor PTSD pathology development and not mere increased symptoms after traumatic exposure.

Our study design is limited in its ability to address acquired/inherited characteristic of altered fear conditioning, especially since we were unable to retest the drop-outs, who were mostly out of reach or refused to be retested. It also prevents the assessment of repeated sessions on fear conditioning and extinction in patients. On one hand, we argue against the mere effect of "passage of time", as patients have had PTSD symptoms for 18.4 months and showed no signs of spontaneous recovery. On the other hand, we argue against the effect of "learning" at the retest (i.e. by simply attending the paradigm twice). Controls do indeed show conditioned fear responses at their first and second testing as they have comparably high SC at acquisition each time. Moreover, at both session 1 and 2, the PTSD group had similarly increased SC at H1 and similarly elevated US aversiveness ratings indicating that repeating the paradigm did not seem to attenuate its induced anxiety. One could improve the procedure by including a wait-list group of PTSD patients. Alternatively one could include a group of patients who would sit for the conditioning paradigm only after treatment.

The second limitation arises from the comorbid disorders and medications of the patients included. Although comorbidity profiles of PTSD in this study are similar to those reported in most published studies dealing with PTSD, and although patients were on stable medical regimen, our PTSD group was too small to distinguish subgroups of medicated vs. non-medicated and pure vs. heterogeneous PTSD diagnosis. Still, our results are consistent with those of non-medicated samples (Orr et al., 2000), and unlikely affected explicit evaluations (US expectancy, valence) of conditioning. The most frequent comorbidities in PTSD patients (depression, generalized anxiety disorder, social anxiety and panic disorder) did not seem to account for the results as we found no significant changes in the main interactions when they were entered as covariables. Nonetheless, their alterations of physiological markers cannot be totally ruled out (Lissek et al., 2005, 2008). It would be useful in future studies, to explore drug and comorbidity interaction with larger PTSD subpopulations with or without medication, and with or without comorbidities.

Other limitations include reduced sample size, homogeneous PTSD population of a single trauma without prior psychiatric disorder and and use of multiple testing.

Finally, it is surprising to observe that after only an average 4.3 EMDR sessions (about 2.5 months of therapy), the elevated psychophysiological responses to fear conditioning and extinction in PTSD immediately decreased. The biological basis of this quite fast process

remains unknown although two experiments using SPECT suggest that the anterior cingulate cortex (Levin et al., 1999) as well as the left medial ventral frontal gyrus (Lansing et al., 2005) would be more activated post than pre-EMDR treatment. SC and HR are associated with medial prefrontal cortex (mPFC) and amygdala activities among others (Critchley et al., 2000). In PTSD patients, neuroimaging studies have shown structural and functional alterations in homologous brain regions (Bremner et al., 2004; Milad et al., 2006). The brain mechanisms involved in this paradigm might thus be at the core of PTSD symptoms. They could be targeted by EMDR and should be further explored by functional neuroimaging techniques, both before and after treatment.

Our results indicate that psycho-physiological impairments in patients with PTSD might be represented as such by altered fear sensitization at the acquisition and extinction phases of fear conditioning and would subsequently be restored after EMDR. This abnormal conditioning implies stronger physiological responding and more aversive verbal rating of a learned feared stimulus (during acquisition) and slower extinction of those feared associations' responses (at extinction). This abnormal conditioning seems to relate to PTSD symptom severity, and might be playing a causal role in the development and or maintenance of the pathology in trauma exposed individuals. Effect of symptom elimination in fear conditioning in PTSD should be monitored in future paradigms by treatment options focused on extinction of learned fear responses such as the Cognitive Behavioral Therapy. It should also be monitored in design better addressing the acquired/inherited characteristics of the fear circuitry.

Shin and Handwerger (2009) strongly argue for characterizing PTSD as a stress-induced fear circuitry disorder (Shin & Handwerger, 2009). Thus the move toward forming diagnostic categories (such as length of treatment or different treatment responders and non-responders) based on validated biological central and peripheral markers is a useful endeavor that deserves attention in future research.

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Conflict of Interest Statement

The authors have no actual or potential conflict of interest including financial or personal relationships that could influence or could be perceived the work in this manuscript.

Article 2: currently being edited before submission to the Journal of Psychophysiology

Restoration of Emotional Attending and Suppressing in PTSD Following Symptom Amelioration by EMDR

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Objective: *Enhanced emotional sensitization and emotional numbing are controversial facets of the emotional deficits frequently described as being part of the dysfunction causing aetiology and maintenance of post-traumatic stress disorder (PTSD). The aim of the present study was to explore how emotional attending and suppressing are altered in PTSD and whether they are restored after a treatment suppressing core symptoms.*

Methods: *Twenty healthy controls and 20 PTSD patients were assessed on an emotional attending and suppressing tasks using film excerpts inducing happiness, peacefulness, fear, and sadness. Skin conductance, frowning, smiling and cardiac activity were recorded. The paradigm was performed before and after successful Eye Movement Desensitization and Reprocessing (EMDR) therapy.*

Results: *Attending fearful clips had a strong tendency to enhance SC and frowning in PTSD whereas happy ones tended to decrease HR and smiling (v/s. healthy individuals). In the suppression task, PTSD had similar altered physiological processing of studied emotions. After EMDR therapy and symptom amelioration, fear attending was no longer modified in patients, and suppression developed similarly to controls.*

Conclusions: *These results confirm the existence of an altered emotional processing pathway for highly arousing emotions in PTSD, and generalized deficient emotional suppression which might be at the core of symptomatology. Mostly, we provide preliminary support for restoration of normal processing after symptom removal in PTSD.*

INTRODUCTION

Humans are emotional in part because of their cognitive capacities (Oschner et al.,2009).

Post traumatic stress disorder (PTSD) is an anxiety disorder that can occur after exposure to a traumatic event. According to the DSM-IV classification (American Psychiatric Association, 1994), it is characterized by hypervigilance, hyperactivity and persistent symptoms of re-experiencing. It also includes symptoms that persist for at least one month including intrusive memories, flashbacks, avoidance, signs of increased arousal, as well as emotional indifference and social dysfunctions (Bremner et al, 1999a; Pole, 2007). Although emotional deficits are one of the main characteristics of PTSD; they remain the least understood and the most understudied aspects of the pathology (Litz et al., 2000).

The DSM-IV diagnosis of PTSD includes symptoms directly reflecting difficulties in emotion regulation, with emotional hyper-reactivity to trauma-related cues on the one hand and hypo-reactivity in the form of emotional numbing on the other (APA, 1994). In such terms, difficulties in emotion regulation are a risk factor for the development and/or maintenance of the disorder (Ehring et al., 2010). Based on theoretical assumptions a number of authors suggest that emotion regulation difficulties are one of the complex symptoms that specifically develop after interpersonal trauma (van der Kolk et al., 2005).

Research has looked into the emotional response of PTSD patients to films (Orsillo et al., 2004), pictures (Milad et al., 2000; Wagner et al., 2003), or trauma reminders (Litz et al., 2000), but few studies have explored thorough emotional response deficits in PTSD, in terms of exploring physiological and verbal responses of patients to various emotions such as fear, happiness, anger etc... For instance, Litz et al., (2000) have shown that male combat-veterans PTSD and controls generally responded comparably and distinctly to three categories of emotional images (positive, neutral and negative), assessed by self-report, peripheral autonomic responses, and expressive-motor activity. In that study, patients were less responsive to positive stimuli only under trauma-priming conditions. Another study in women with PTSD has shown that they exhibited higher levels of negative activation and expressed more negative emotion words i.e to both positive and negative film stimuli, whereas no group differences emerged in facial expressivity (Orsillo et al., 2004).

Peripheral physiological responses can provide fair information about emotional states, and its modulation in pathological conditions, independent of self-reports. Studies of emotional responding to date have conceptualized emotional stimuli as dichotomous on valence and arousal scales (Gross, 2002). For instance, facial expressive motor activity more directly relates to emotional valence with frowning associated with negative emotions and smiling with positive ones. Bradley and Lang (2000) review electrodermal skin conductance (SC) as fair marker of emotional arousal, increasing for more stimulating emotions. They describe heart rate (HR) as controversially reflecting arousal in some studies and valence in others, whereby it mostly seems to increase for positive emotions but decrease for negative ones (Ohira et al., 2006). Additionally, individual differences in heart rate variability (HRV) have predictive value in defining emotional and clinical profiles (Appelhans & Luecken, 2006). Research is needed to determine whether or not there are emotion-specific peripheral disruptions associated with PTSD.

Our first aim was thus to examine emotional deficit in PTSD by monitoring physiological parameters while attending a panoply of emotions with varying intensity and valence of presented clips; including happiness, peacefulness, fear and sadness.

Despite the inherent relationship between anxiety disorders and emotion deficits in general, and more specifically in PTSD, there is a relative lack of studies examining emotion generation and regulation within clinical samples of anxiety disorders. Mennin, Heimberg, Turk, and Fresco (2002) have proposed that individuals with generalized anxiety disorder (GAD) not only have emotion generative processes more intense than most, but also have deficiencies in altering their emotional experience. They instigate more regulatory efforts, typically worry or suppression, leading to opposite of intended results (i.e., increases in anxiety rather than decreases).

Similar mechanism seem to operate in PTSD, as inadequate effortful emotional suppression may dampen their positive experiences thereby contributing to the increased risk of development of symptoms associated with this disorder (APA, 1994). In fact, among women exposed to traumatic events, those who reported infrequently and ineffectively regulating their emotions also reported higher anxiety and PTSD. Interestingly, PTSD participants reported difficulties suppressing both positive and emotions than did non-PTSD participants (Eftekhari et al., 2009).

Our second aim was thus to extend GAD findings and examine emotional suppression in PTSD while monitoring their physiological parameters using the aforementioned panoply of emotions.

The inefficient allocation of resources to generate adequate emotions, suppress traumatic material, conceal emotions, avoid events that might elicit unwanted feelings, and regulate responses to trauma cues are expected to have a compound effect on other goal directed behavior (Ehlers & Clark, 2000). This is because people have a limited amount of energy, attention, and self-control at any given time (Kashdan et al., 2010). Evidence was found suggesting that the degree to which veterans orient their lives around attending emotions or avoiding them moderates the relation between PTSD and well-being (Kashdan et al., 2010), and efficiency of suppression was related to PTSD symptoms severity (Roemer et al., 2009). Studies have so far shown that one of the treatment option of PTSD; eye movement desensitization and reprocessing (EMDR) treatment, relieves traumatic symptoms and reduces the autonomic responsiveness of PTSD patients to aversive stimuli such as trauma recall (Aubert-Khalfa et al., 2008). EMDR is also associated with psycho-physiological de-arousal for SC and HR over time sessions (Sack et al., 2008).

Our third aim was thus to explore the potential changes in PTSD emotional attending and suppressing after symptom amelioration by EMDR. We chose to explore emotional processing in PTSD before and after treatment i.e. when patients are fully symptomatic versus when they are symptom-free. To the best of our knowledge, no study has so far looked at the modulation of emotional attending/suppressing in PTSD before and after successful therapy.

In the current study, we tested whether major psycho-physiological mechanisms altered in emotional processing in PTSD are restored after a successful treatment ameliorating core PTSD symptoms, using 45 sec clips inducing happiness, peacefulness, fear and sadness. We hypothesized that, similarly to anxious and PTSD populations previously studied on this task, PTSD patients would show increased hyper-arousal while attending to highly arousing emotions. Based on this premise; patients would have increased physiological reactivity (heightened HR, EMG and SC). Patients would also be less efficient suppressing those emotions. We finally hypothesized that this altered processing would be normalised after symptom amelioration; and that after successful EMDR, PTSD would be similar to controls in terms of autonomic reactivity and valence ratings, throughout both tasks.

MATERIALS AND METHODS

Subjects

A total of 23 adult outpatients (8 males and 15 females) were recruited by a psychiatrist among trauma victims at the medico-psychological crisis cell (CUMP) at the Psychiatry Pole of the Conception Hospital in Marseille, France. They all met the DSM-IV criteria for PTSD following a single traumatic event (12 aggressions, 4 motor vehicle accidents, 6 work related accidents, 1 grieving parent having witnessed the suicide of her son by hanging) with no previous history of neurologic or psychiatric disorders. Subsequent analysis included 19 patients (7 males and 12 females, with mean age = 44.5 ± 14.5 years, mean education = 7.5 ± 3 years after grade 7 and mean trauma exposure = 17.0 months). Four patients were excluded from data analysis as they abandoned the study. Five patients were on combined regimen of antidepressants and anxiolytics, 2 patients only took antidepressants and 2 only took anxiolytics.

A total of 20 healthy adult controls (9 males and 11 females, with mean age = 37.8 ± 13.5 years and mean education = 9.2 ± 2.3 years after grade 7) with no history of neurologic or psychiatric disorders, were recruited via screening lists at the clinical investigation centre at the Timone Hospital (CIC-UPCET). They were matched to patients for age, sex and education.

Psychological Assessment

All participants were assessed by a psychiatrist for PTSD and other mental health disorders using the structured Mini-Internal Neuropsychiatric Interview for DSM-IV (Lecrubier, 1998). This allowed us to check for the absence of psychiatric disorders prior to the trauma in PTSD and screen for potential comorbid psychiatric disorders. Participants responded to demographic questions and completed the Beck Depression inventory (BDI) (Cottraux, 1985) and the State-Trait Anxiety Inventory (STAI) (Schweitzer & Paulhan, 1990). Patients also completed a trauma related scale: PTSD Check List Scale (PCL-S) (Ventureyra et al., 2002). The validated French versions were used for all the scales.

EMDR Treatment

All PTSD patients underwent Eye Movement Desensitization and Reprocessing (EMDR) therapy (APA, 2004). According to the APA reports published in 2004, this eight-

step standardized protocol is one of the validated treatments for PTSD. Based on an information processing model (Shapiro & Maxfield, 2002), EMDR includes associations of cognitive, emotional and physical assessments of actual distress to traumatic scenery, as well as imaginal exposure while attending to bilateral alternate stimulation. As the patient is asked to visualize the most salient aspect of a traumatic memory, the therapist induces bilateral stimulation (by means of ocular, sensory-motor or auditory left/right stimulation) (Shapiro, 1989).

EMDR is an effective rapid therapy with stable outcome demonstrated in a 35-month follow-up study (Hogberg et al., 2008). Patients were treated by one of 3 therapists, all trained by the French institute of EMDR. There was no fixed number of sessions. Sessions were planned every 7 to 15 days according to patients and therapists availabilities. The treatment was considered successful and complete when patients reported no more feelings of distress when thinking about their trauma. They were again interviewed by a psychiatrist, using the MINI. They were retested when they no longer met PTSD classification according to DSM-IV criteria and had no more pathological scores on PTSD scales.

Patients required an average of 4.1 ± 1.7 treatment sessions (ranging from 1 to 7 sessions), lasting on average for 2.5 months.

Apparatus and physiological recordings

Physiological data acquisition was controlled by two PCs running E-Prime (Psychology Software Tools, Inc., Pittsburgh, PA, USA) and Acqknowlege software (Biopac Systems, Inc., Goleta, CA, USA) respectively. Physiological channels and rating dial info were recorded at a rate of 1000 Hz in continuous mode using Biopac MP150 system.

Skin conductance (SC) was measured in microsiemens using two 5 mm inner diameter Ag/AgCl electrodes filled with isotonic paste. Electrodes were placed on the medial phalanges of the index and middle finger of the left hand in accordance with published guidelines (Fowles et al., 1981). Since deep breathing and/or coughing may trigger artifacts on the SCs; respiration pattern was recorded using a pneumagraphic belt with a respiration transducer at the rib cage, towards the end of the sternum.

Electromyogram (EMG) of the corrugator and zygomatic activity was measured in microvolts using three 4 mm Ag/AgCl shielded surface electrodes filled with electrolytic paste. The skin was locally abraded with alcohol imbibed cotton swab and two electrodes were attached with adhesive collars over the corrugator and zygomatic muscle site according

to manufacturer specifications (Fridlund &Cacioppo, 1986). A ground electrode was placed for each measurement on the lobe of the left ear.

Heart Rate (HR) was measured in beats per minute using 3 clip lead electrodes. Electrodes were attached in a Type I EKG configuration; on the left flying rib, right collarbone and sternum.

Films and validation

After a 10 min rest period, participants viewed a series of ten 45-second long color films. Two short clips were selected per emotion: happiness ("le Dîner de Cons" by Weber and a short film depicting a baby laughing), peacefulness ("Marche of the Penguins" by Jacquet and "le Grand Bleu" by Luc Besson), fear ("A Tale of Two Sisters" by Jee-Woon and "Perfect Murder" by Andrew Davis), and sadness (report on the famine in Biafra by INA and "Stepmom" by Chris Columbus)

Films were fed into E-studio 2.2 software (E-Prime 2.2) and displayed on a 17" inch computer screen with 40W Yamaha NS10M Studio sound blasts, linked to a P2040 amplifier, at a sufficiently elevated and comfortable volume. Films were previously validated from a larger selection of clips (Reynaud et al., 2010)

Procedure

The investigation was carried out in accordance with the latest version of the Declaration of Helsinki. Participants provided informed consent in accordance with local ethical committee guidelines set forth by the CPP committee South Mediterranean 2.

The experiment took place in a temperature-controlled, fully lit, and sound-attenuated room. Participants were comfortably seated at 60 cm viewing distance from a 17" computer screen, with a refresh rate of 100Hz. They completed the scales and then electrodes were attached and the respiratory belt put in place. They performed an attending and a suppression tasks. Task order was counterbalanced between subjects. Films were presented in five pseudo-randomized sequences and the same film order was kept across both tasks for a given subject. In the attending task, subjects were instructed to watch the excerpts and feel the emotions it elicited the best they can. For the suppression task, they were told to control their emotions the best they can. We chose an ecological instruction, best mimicking real-life setting so participants would react as spontaneously as possible when faced with such situations. We refrained from indicating specific control strategies (reappraisal or expressive suppression), based on studies of Ohira et al., (2006), merely asking subjects to decrease their emotional

response by voluntary suppressing emotional responses while viewing the film. PTSD are in fact a heterogeneous group in terms of their emotional reactivity and reliance on particular self-regulatory strategies (Ehlers & Clark, 2000).

Films were separated by a break during which participants completed film related ratings. After each film, in the attending task, subjects first identified the emotion. Then they rated its intensity of emotional attending, arousal and valence using Likert-type scales, each comprising a line with equally spaced numbered tick marks labeled 0 to 10 (Fig. 1). In the suppression task, subjects first identified the emotion. Then they similarly rated the intensity of emotional control arousal and valence. These evaluations indicated subjective emotional experience during film-viewing. After each film, the verbal evaluation was completed and the following film started when physiological parameters returned to baseline.

Data and Statistical Analyses

Data of physiological parameters and verbal scoring was averaged for the 2 films per emotion.

Data for SC was obtained by averaging peak amplitudes of SC during the 45 sec film excerpts SC below 0.01 μS were not considered. Artifact correction for SCs consisted of a visual inspection of respiration and the manual exclusion of SC that appeared to be influenced by coughs, sights or deep breath (around 5% of either film type was excluded).

Data for HR and EMG activity were calculated by subtracting the 15 sec baseline before the film's onset from the mean level obtained during the 45 sec film excerpt.

Data for heart rate variability (HRV) was reliably quantified using a 10 min rest period before the films, at the beginning of the experiment (Bernston et al., 1997). Three frequency bands are typically defined:

- High frequency (HF) (0.15 - 0.4 Hz), derived mainly from vagal activity.
- Low frequency (LF) (0.04 - 0.15 Hz) derived from sympathetic activity.
- Very low frequency (VLF) (0 - 0.04 Hz) reflecting physical activity.

HRV is calculated by the HF/LF ratio, programmed in Matlab.

A two-way repeated measures ANOVA was used on the ratings of intensity, arousal and valence as well as SC, EMG and HR for each task separately with Group (control, PTSD) as a between factor and Session (1, 2) and Emotion (happiness, peacefulness, fear and sadness) as within factors. Significant main effects at $p < 0.05$ were followed by post-hoc t-tests using Bonferoni correction.

RESULTS

1- Clinical Data

Table 1 shows the psychometric measures for the two groups. Groups did not differ in age, sex and education. PTSD patients initially scored higher than healthy controls on BDI and STAI scales. After treatment, there was no significant difference between PTSD and control groups (Table 1).

Individual patients' scores on PCL-S were initially higher than the cut-off for pathology and significantly dropped to normal levels after treatment termination.

Patients met the criteria for the following major current comorbid diagnoses (before/after EMDR): major depression (n=11/4), other anxiety disorders (n=16/8) and high-medium suicidal risk (n=6/0). Specific anxiety rates initially diagnosed in PTSD are as follow (out of the 19 patients): Social phobia (5), Generalized Anxiety Disorder (10), Panic disorder (5), Agoraphobia (8). Most patients had more than one comorbid anxiety disorder.

	Healthy Controls (Time 1) (n=20)	Healthy Controls (Time 2) (n=20)	PTSD Pre-EMDR (Time 1) (n=19)	PTSD Post-EMDR (Time 2) (n=19)	Statistics
BDI Depression	3.2 (2.6)	2.3 (2.6)	15.1 (8.3)	5.6 (3.9)	$F(1,37) = 24.20^{**}$
STAI Trait Anxiety	29.3 (8.0)	30.3 (8.0)	50.1 (11.6)	34.6 (8.7)	$F(1,37) = 20.47^{**}$
STAI State Anxiety	40.0 (8.4)	36.4 (9.4)	56.4 (10.8)	42.8 (7.6)	$F(1,37) = 14.43^{*}$
PCL-S	-	-			$t(1,18) = 5.48^{**}$
MPSS	-	-			$t(1,18) = 6.59^{**}$
IES	-	-			$t(1,18) = 6.48^{**}$

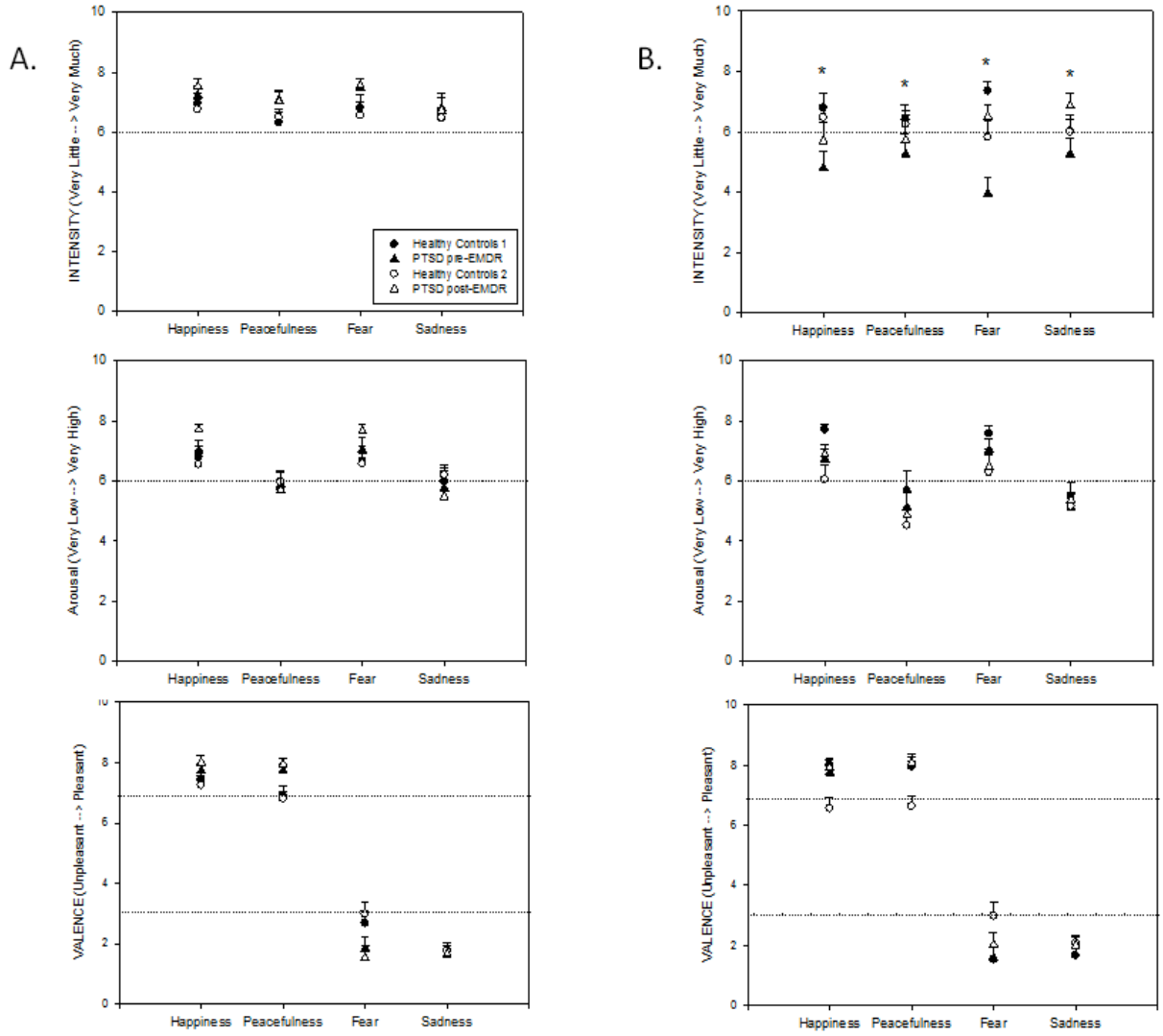
Table 1. Demographic Data of the Control and Patients group and their scores on the various scales of Depression (BDI), anxiety (STAI) and PTSD (PCL-S, MPSS and IES).

2-Attending Task

2.1 Verbal Evaluation: Intensity, Arousal and Valence

There were no differences between groups for the evaluation of emotional intensity (Fig. 1A). There was a significant effect of Emotion ($(F(3, 105) = 8.28$; $p<0.001$) on the evaluation of emotional arousal, as happiness and fear were considered more arousing than peacefulness and sadness (Fig. 1A).

There was a significant Emotion X Group interaction on the evaluation of emotional valence (F (3, 105) = 5.65; p<0.001). Post-hoc analyses showed that the PTSD group had more pleasant rating of happiness and more unpleasant ratings of fear (p<0.05) (Fig 1A).



Article 2. Fig 1. Illustration of the subjective ratings on the scales of intensity, arousal and valence of controls at testing times 1 and 2 and patients pre- and post-EMDR, during the A. Attending and B. Suppressing task, for the four emotions studied. *p<0.05

2.2 Physiological Evaluations:

Skin Conductance

There were no differences between groups on the electrodermal activity.

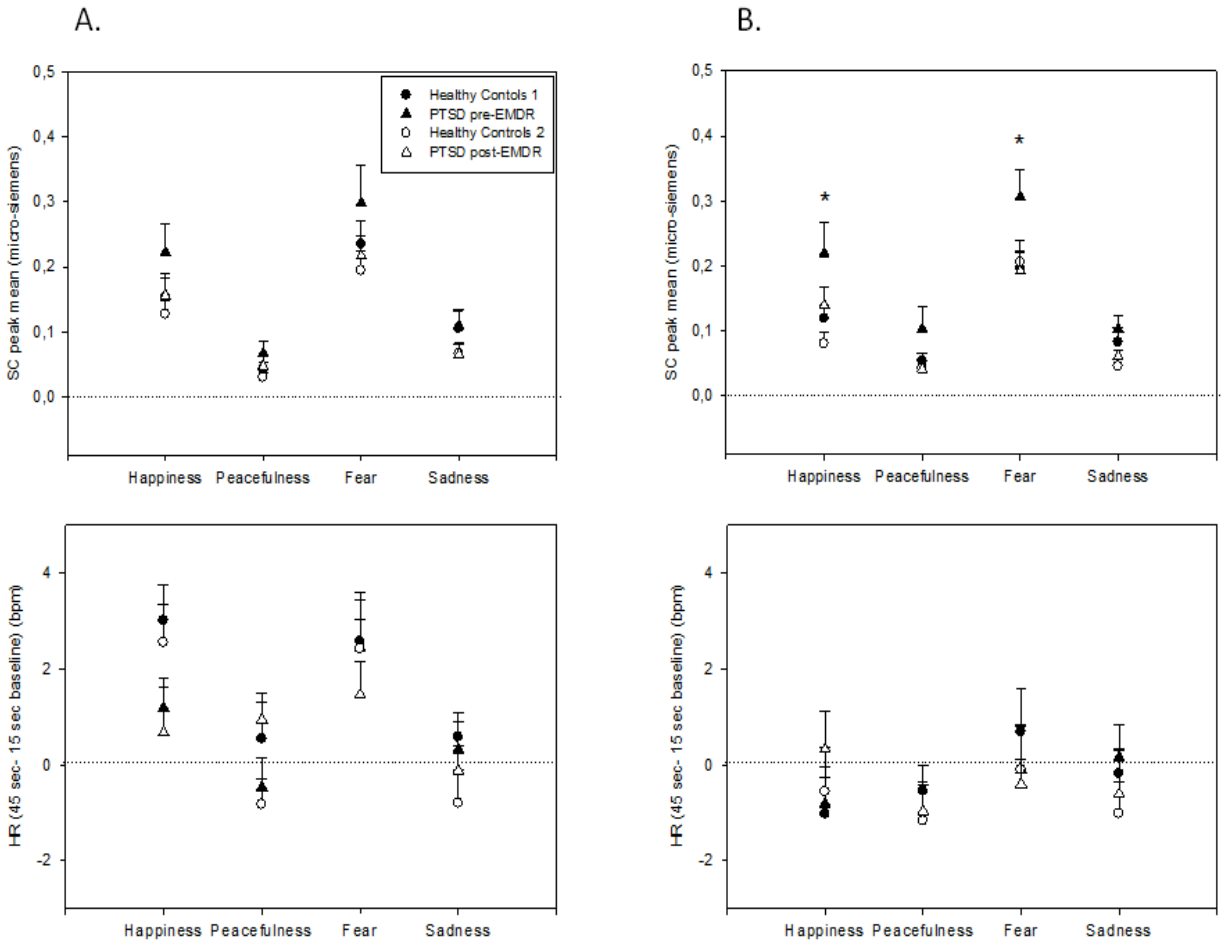
There was a significant effect of Emotion (F (3, 93) = 39.77; p<0.001) as happy and fearful clips induced higher SC than peaceful and sad ones (p<0.05).

Heart Rate

There were no differences between groups on the cardiac activity.

There was a significant effect of Emotion ($F(3, 87) = 8.61$; $p<0.01$) as happy and fearful clips induced higher HR than peaceful and sad ones ($p<0.05$).

We found a significant Session X Group interaction for basal HR (i.e. at rest) ($F(1, 25) = 4.84$ $p<0.05$. Only before therapy did patients have higher resting HR state than controls. After successful EMDR, patients and controls no longer had different basal HR ($p<0.05$) (Fig. 2A).



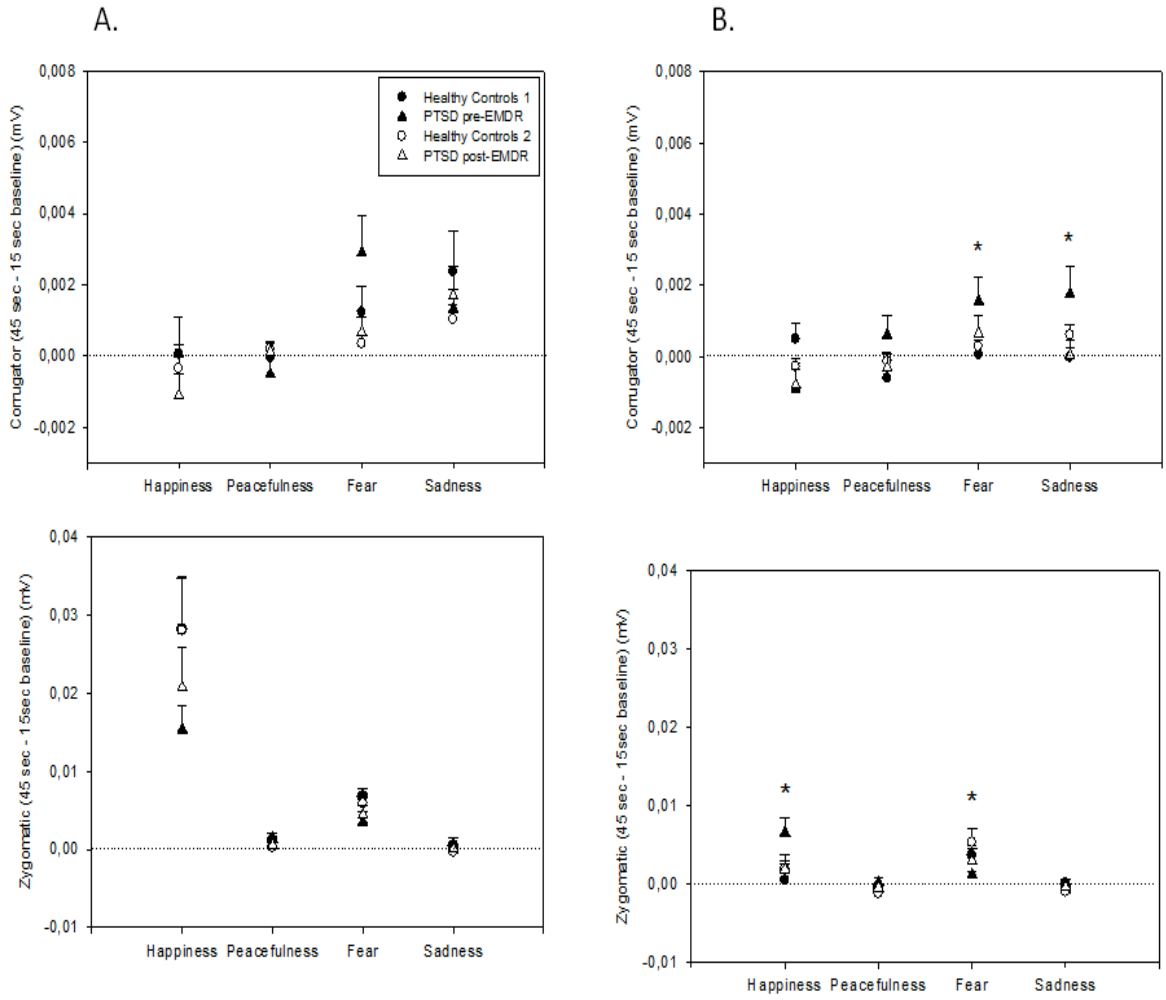
Article 2. Fig 2. Illustration of the skin conductance (SC) and heart rate (HR) of controls at testing times 1 and 2 and patients pre- and post-EMDR, during the A. Attending and B. Suppressing task, for the four emotions studied. *p<0.05

Further HRV analyses showed a significant Session X Group interaction for HF (parasympathetic input) ($F(1, 23) = 6.12$, $p<0.05$). PTSD patients initially had lower HF than controls, before EMDR but not after ($p<0.05$) (Fig. 4).

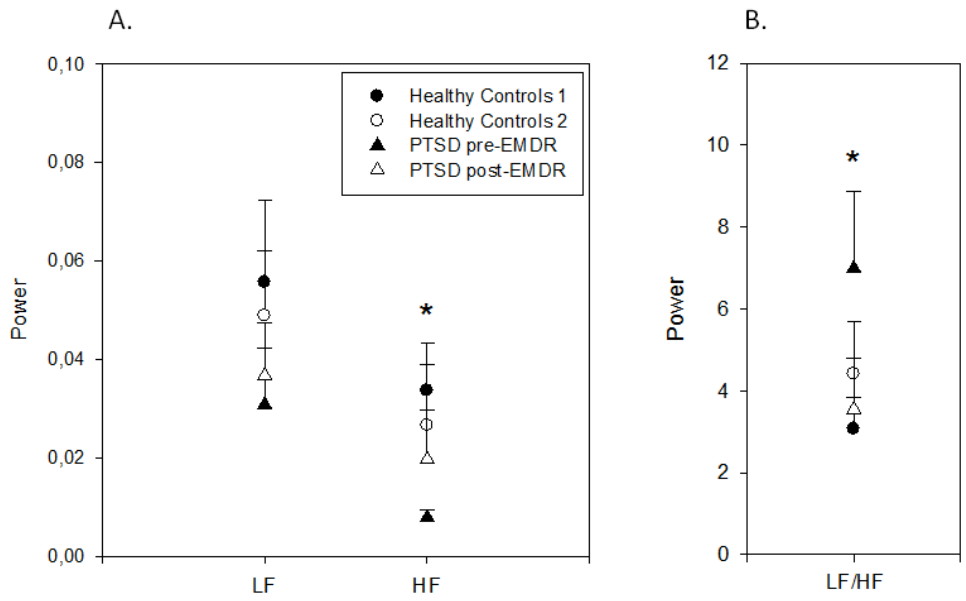
EMG: Corrugator &Zygomatic

There were no differences between groups on the either corrugators or zygomatic activity.

There was a significant effect of Emotion for both EMG measurements ($F(3, 102) = 8.8$; $p<0.001$ and $F(3, 96) = 29.76$; $p<0.001$ respectively) as fear and sadness induced more frowning than happiness and peacefulness, and happy excerpts increased smiling activity more than the other 3 emotions ($p<0.05$) (Fig. 3A).



Article 2. Fig 3. Illustration of the Facial Muscle activity of Corrugator (frowning) and Zygomatic (smiling) of the controls at testing times 1 and 2 and patients pre- and post-EMDR, during the A. Attending and B. Suppressing task, for the four emotions studied. *p<0.05



Article 2. Fig 4. Illustration of the HRV variables of the controls at testing times 1 and 2 and patients pre- and post-EMDR, showing A. Low Frequency (LF) and High Frequency (HF) power spectrum and B. LF/HF ratio during the 5min rest period. *p<0.05

3-Suppressing Task

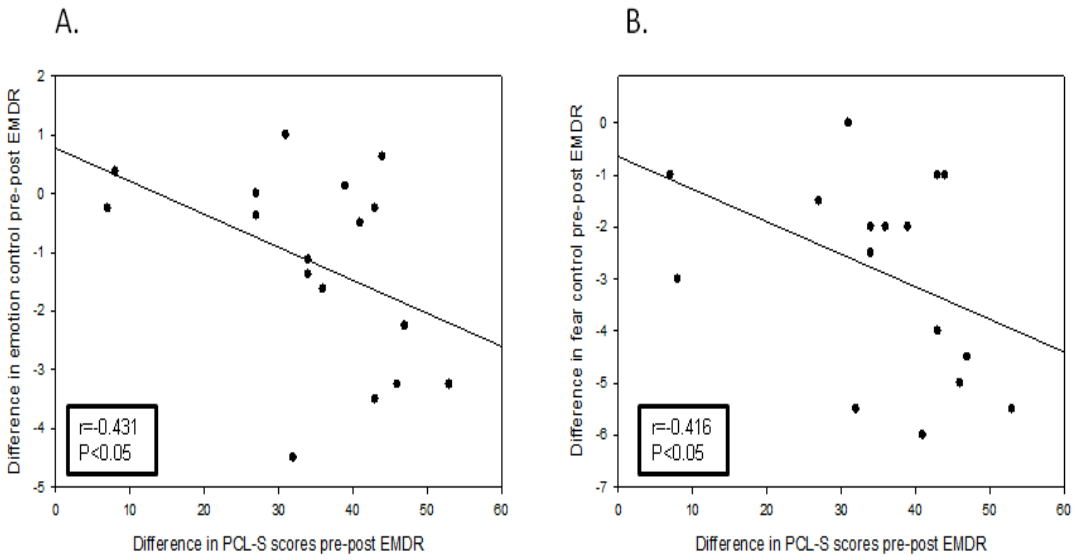
3.1 Verbal Evaluation: Intensity, Arousal and Valence

There was a significant Emotion X Session X Group interaction for the evaluation of emotional control (F (3, 105) =5.27, p<0.01). Post hoc showed patients initially were less efficient at controlling all four emotions than healthy controls (p<0.05). After EMDR, both groups rated this scale similarly (Fig. 1B).

There was a significant effect of Emotion (F (3, 105) = 18.4; p<0.001) on the evaluation of emotional arousal, as happiness and fear were considered more arousing than peacefulness and sadness (p<0.05) (Fig. 1B).

There was a significant effect of Emotion (F (3, 105) = 43.8; p<0.001) on the evaluation of emotional valence, as happiness and peacefulness were considered as pleasant whereas fear and sadness were rated unpleasant (p<0.05) (Fig. 1B).

Based on Ehring et al., (2010) who found a positive correlation between PTSD symptom severity and difficulties of emotional regulation, we decided to look at the correlation between PCL-S scale and intensity of control for the PTSD group. Pearson correlation showed intensity of control for when averaged for all emotion negatively correlated with PTSD symptoms (N=19, r= -0,431 and p<0.05), with similar significance for fear (least well controlled emotion) (N=19, r= -0,416 and p<0.05) (Fig 5).



Article 2. Fig 5. Illustration of the Pearson correlations between the difference in control intensity and scores of PTSD in the PCLS in patients pre- to post-EMDR, for A. all emotions averaged and B. fear alone. Values of r and p are also displayed.

3.2 Physiological Evaluations:

Skin Conductance

We found a significant Emotion X Session X Group interaction for the SC ($F(3, 96) = 2.49$, $p<0.05$) (Fig. 2B). Patients initially had larger SC for films evoking happiness and fear as compared to controls when trying to control their emotions ($p<0.05$). This hyper-reactivity was restored after EMDR.

Heart Rate

There were no differences between groups on the HR during the suppressing task (Fig. 2B).

EMG: Corrugator &Zygomatic

We found a significant Emotion X Session X Group interaction for both corrugator ($F(3, 108) = 2.9$, $p<0.05$) and zygomatic activity ($F(3, 99) = 4.49$, $p<0.01$) (Fig. 3B).

Post-hoc showed that patients indeed had higher frowning activity on fearful and sad clips compared to controls before EMDR ($p<0.05$). This was no longer the case after treatment as both groups had similar EMG levels.

Patients also had lower smiling activity on fearful clips but higher activity on happy clips compared to controls only before EMDR but not after ($p<0.05$).

DISCUSSION

The present study illustrates major psycho-physiological deficiencies in PTSD pathology in emotional attending and suppressing pathway via clips with varying valence and arousal. First and foremost we have shown PTSD emotional suppressing is exaggerated for highly arousing emotions. Most importantly, we have shown that immediately after symptom amelioration by successful EMDR therapy, altered emotional processing in PTSD (at attending and suppressing) is restored.

It is noteworthy that the 4 studied emotions were well differentiated on valence and arousal scales, allowing their bi-dimensional categorization (in accordance with Lang et al., 1998). Emotion generation and its volitional regulation are associated with changes in verbal assessment of emotion, in displaying facial expressions and autonomic responsiveness, differentially expressed in the two groups, depending on the task performed.

ATTENDING

In the attending task, we found no differences on intensity and arousal dimensions of emotional evaluations between patients and controls. On verbal scales, groups only differed in valence ratings of positive and negative clips, with PTSD having more extreme ratings of pleasantness.

Fear processing is frequently described as altered in anxiety in general and more specifically in PTSD (Ledoux, 2000). In our study we expected it to manifest in patients by higher SC and increased frowning, this was not the case. The lack of striking difference on verbal evaluations between patients and healthy controls and the lack of significance of their physiological differences could be mostly due to a ceiling effect of emotional subjective and peripheral expression; as the clips were chosen to be quite intense (7/10 on the corresponding scales in the validation phase with other controls). This result is similar to previous findings of Litz et al., whereby patients and controls had similar physiological measurements, except in priming condition.

Additionally, lower HR for happiness in patients as compared to healthy subjects is most likely be accounted for by their higher basal HR, as HR for happiness is calculated by the value of HR during the clip minus the value of HR at rest. This might alternatively be explained to some extent by experimenter observations of patients' comments after happy

clips. They report feeling sorry about themselves not being entitled to feel happy, or not deserving pleasant experiences after their trauma; this conscious blockade could be reflected by lower HR.

SUPPRESSING

In the suppressing task, patients did significantly less well than others in controlling the intensity of their emotions. This parallels other studies on PTSD showing for example the reported level of perceived controllability over intrusive rape-related thoughts for the PTSD participants was significantly lower during the suppression phase (as compared with the expression phase) relative to the non-PTSD participants (Shipherd &Beck; 1999). This cognitive perception in patients is in adequacy of their physiological hyper-arousal when viewing films (Orsillo et al., 2004), which is more exacerbated in comparison with controls in our paradigm during the suppressing than attending tasks.

Recent work has shown that emotional suppression leads to increases in sympathetic activation (Gross & Levenson, 1993). Indeed, the sympathetic nervous system is involved in the preparation of intellectual activity and may reflect the cognitive effort directed towards fear control. This could be an additional byproduct of the imbalance of sympathetic over parasympathetic ratio in PTSD, which we found to be higher than in controls. Low vagal tone (parasympathetic) observed in PTSD is related to poor emotion regulation (Thayer & Lane, 2000). Conversely, they suggest that high vagal tone can allow the individual to better selectively attend to aspects of situations, and enables adaptable responses.

Patients decreased suppression efficacy would thus manifest in higher sympathetic activity, especially for arousing emotions. This is manifest by higher SC, which is a direct reflexion of sympathetic activity. Controls verbal evaluations indicate that the emotion of fear is the most difficult to suppress and this is typically where patients fail the most. Alongside happiness, PTSD have increased SC for fear. However, we found no group differences in HR for those 2 emotions. This seemingly discrepancy in autonomic measures could be explained by the fact that SC and HR are regulated by different branches of the nervous system, since SC is solely under sympathetic control whereas HR is modulated by both the sympathetic and parasympathetic branches. The parasympathetic system may compensate for the enhanced sympathetic activity observed in PTSD patients when controlling for emotions.

Additionally, the differential facial expression was annihilated between emotions for the control group. PTSD on the contrary could not blunt their facial expressions as they still had more frowning for fearful and sad clips, and more smiling for happy ones. Once more our results illustrate a congruency between facial expressions and self-report in both tasks, similarly to other studies on PTSD (Wagner et al., 2003). These results indicate that on verbal and physiological scales, patients do less well than controls in controlling their emotions and their expression.

Studies had previously described increased basal HR in PTSD (Litz et al., 2000). Together with the aforementioned imbalanced sympathetic and parasympathetic outputs, it could represent an automatic preparation for threat in PTSD, in uncertain emotional contexts. In such perspective, highly arousing emotions that stimulate the sympathetic alarm system are shyly counterbalanced by the parasympathetic in PTSD and would explain the hyperactivity of patients. Their emotional deficits are also worsened by inefficient control mechanisms to down regulate unwanted increased physiological arousal, at the HR, SC and EMG levels. Contrary to what is implied by emotional numbing in PTSD, patients might subsequently voluntarily avoid emotional situations since they are aware of their uncontrollability. It also appears like PTSD participants' attempts at emotion regulation would ironically further their symptoms.

EMDR

To the best of our knowledge this is the first study monitoring emotional attending/suppressing in PTSD before and after therapy. Few studies had described correlations between severity of emotional deficits and PTSD symptomatology. In fact, Tull et al., (2007) and Ehring et al., (2010) had shown that overall difficulties in emotion attending and regulation were associated with PTSD symptom severity.

Yet no previous studies had looked at modifications of emotional processing after PTSD symptom amelioration. We showed that the physiological attending of highly arousing emotions is restored and general suppressing of emotions, verbal and peripheral, is rendered more efficient after successful EMDR in patients. Interestingly we found a positive correlation between intensity of emotional suppression and PTSD symptoms; the more efficiently patients perceive their control of emotions, the less symptomatic and anxious they are.

The regulation of physiological parameters coincides on one hand with more efficient emotional control (verbal evaluations) and other dimensions of well-being (clinical evaluation, BDI, STAI and PCL-S). This finding becomes valuable when confronted to a study of veterans with PTSD who were shown to expend greater effort when striving to regulate emotions but having no better well-being (Kashdan et al., 2010). On the other hand, this restored emotional processing goes along basal HR levels and HRV comparable to controls. Barlow et al., (2004) suggests the pre to post-treatment restoration of HRV in clinical populations could be a marker of successful outcome.

LIMITATIONS

Our study design is limited in its ability to address acquired/inherited characteristic of altered emotional processing in PTSD, especially since we were unable to retest the drop-outs, who were mostly out of reach or refused to be retested. It also prevents the assessment of repeated sessions on emotional attending/suppressing in patients. One could improve the procedure by including a wait-list group of PTSD patients. Alternatively one could include a group of patients who would sit for the paradigm only after treatment.

The second limitation arises from the comorbid disorders and medications of the patients included. Comorbidity profiles of PTSD in this study are similar to those reported in most published studies dealing with PTSD, and although patients were on stable medical regimen, our PTSD group was too small to distinguish subgroups of medicated vs. non-medicated and pure vs. heterogeneous PTSD diagnosis. Still, those variables unlikely affected explicit verbal evaluations. Nonetheless, their alterations of physiological markers cannot be totally ruled out (Lissek et al., 2005). It would be useful in future studies, to explore drug and comorbidity interaction with larger PTSD subpopulations with or without medication, and with or without comorbidities.

Other limitations include adopting a specific strategy for emotional regulation, reduced sample size, homogeneous PTSD population of a single trauma without prior psychiatric disorder and use of multiple testing.

Recent studies have shown that restoring the parasympathetic/sympathetic balance seems of cardinal importance for individuals' cognitive and affect regulation (McCraty et al., 1998). Reduced cardiac coherence (an indicator of HRV and thus autonomic imbalance) co-occurred with deficient attention and affect regulation in PTSD veterans (Ginsberg et al.,

2010). These authors have also shown that improving HRV post-training in patients was accompanied by significant improvements in the information processing. As such, this was the concept behind the adaptation of a cardiac coherence therapy aimed to better manage stress and boost emotional control (O'Hare, book). Meditation is a conscious mental process that induces a set of integrated physiologic changes termed the relaxation response. A study has shown that the practice of meditation activates neural structures involved in attention and control of the autonomic nervous system (Lazar et al., 2000).

Another aspect of sympathetic/parasympathetic balance might stem from its central counterparts. In fact, SC and HR are associated with medial prefrontal cortex (mPFC) and amygdala activities among others (Critchley et al., 2000). CC is differentially involved in emotion generation (Vogt, 2005) together with the amygdala whereas suppression solicits lateral PFC and orbito-frontal cortex (OFC) (Ohira et al., 2006). In PTSD patients, neuroimaging studies have shown structural and functional alterations in homologous brain regions (Milad et al., 2006). Those altered structures have direct projection to the midbrain where centers of physiological functions exist (Ledoux, 2000). The brain mechanisms involved in this paradigm might thus be at the core of PTSD symptoms. They could be targeted by EMDR and should be further explored by functional neuroimaging techniques, both before and after treatment.

CONCLUSION

Our results indicate that psycho-physiological emotional impairments in patients with PTSD might be represented as such by altered suppressing of highly arousing emotions, and would subsequently be restored after EMDR. This abnormal emotional processing implies stronger physiological responding and more aversive verbal rating of fear and happiness and lower efficiency controlling emotions during the suppressing task. This abnormal processing seems to relate to PTSD symptom severity, and might be playing a causal role in the development and or maintenance of the pathology in trauma-exposed individuals. Effect of symptom elimination in emotional processing in PTSD should be monitored in future paradigms by treatment options focused on restoring altered central processing using cognitive methods that restructure one's consciously accessible appraisals and control strategies (Ochsner & Gross, 2005) such as the Cognitive Behavioral Therapy, and options focused on regulating sympathetic/parasympathetic balance such as relaxation or cardiac coherence.

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Attentional Bias in PTSD Diminishes after Symptom Amelioration

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Background*: Avoidance and hypervigilance to reminders of a traumatic event are among the main characteristics of post traumatic stress disorder (PTSD). Attentional bias toward aversive cues in PTSD has been hypothesized as being part of the dysfunction causing etiology and maintenance of PTSD. The aim of the present study was to investigate the cognitive strategy underlying attentional bias in PTSD and whether normal cognitive processing is restored after a treatment suppressing core PTSD symptoms.*

Methods*: Nineteen healthy controls were matched for age, sex and education to 19 PTSD patients. We used the emotional Stroop and Detection of Target tasks, before and after an average of 4.1 sessions of Eye Movement Desensitization and Reprocessing (EMDR) therapy.*

Results*: We found that on both tasks, patients were slower than controls in responding in the presence of emotionally negative words compared to neutral ones. After symptoms removal, patients no longer had attentional bias, and responded similarly to controls.*

Conclusion*: These results support the existence of an attentional bias in PTSD patients due to a disengagement difficulty. There was also preliminary evidence that the disengagement was linked to PTSD symptomatology. It should be further explored whether attentional bias and PTSD involve common brain mechanisms.*

1-INTRODUCTION

The Post-Traumatic Stress Disorder (PTSD) is an anxiety disorder that occurs in the aftermath of a traumatic event. According to the DSM-IV (American Psychiatric Association, 1994), it is a non-adaptive reaction to stress characterized by intrusive memories, avoidance, hypervigilance and social dysfunctions, persisting at least one month after the trauma. From a cognitive point of view, one of the key factors of the emotional distress and maintenance of anxiety disorder is the existence of non-adaptive attentional bias towards information with aversive value (Mogg &Bradley, 1998). One way to investigate such bias is via interference tasks, such as the emotional stroop (e-Stroop) and detection of target (DOT), which involves a central task to be performed while ignoring emotional distracters (Mathews & MacLeod, 2005).

The most common paradigm indexing sensitivity to threat-related events is the e-Stroop (Williams et al., 1996). In such tasks, anxious people tend to be generally slower in responding to emotionally negative words than to neutral ones, implying the existence of selective attention to emotional cues (Fox et al., 2001). More specifically, PTSD patients are known to have an attentional bias on e-Stroop tasks with trauma cues and general words with negative valence (MacNally et al., 1999, Hayes et al., 2009). For instance in motor accidents, PTSD patients were slower in color-naming accident related words than neutral ones (Beck et al., 2001). The e-Stroop task thus provides clear evidence of the presence of attentional bias in PTSD, but gives no information on its underlying altered processing strategy.

The specific selectivity in strategic cognitive processing seems to be best addressed by the DOT (Posner &Petersen, 1990). In this task, anxious patients have shown a selective disengagement bias from trauma-related material, with a difficulty detaching their attention from threatening cues (Bar-Haim et al., 2007). Recent trauma victims behave alike, as they were found to view trauma-related pictures longer than generally aversive pictures, unlike healthy control participants (Elsesser et al., 2004). Results for PTSD seem controversial. Some studies have shown that PTSD patients orient away from threat. For instance, Vietnam veterans with PTSD were found to ‘escape’ the presentation of combat scenes when they were able to turn off the display (Blanchard et al., 1982). PTSD patients were also shown to name targets faster when in close proximity to mild threat words (Bryant & Subbiah, 1999). Recent studies, however, have shown that PTSD patients orient toward the threat cues (Pineles et al., 2009, Browning et al., 2010).

Too many stimuli modalities, intensities and durations were used to settle discrepant results as to whether PTSD have decreased reaction time (RT) on emotional trials due to vigilance (facilitation by threat cues) or increased RT due to disengagement (interference of threat cues). Our first aim was to replicate PTSD attentional bias on an e-Stroop task, and further refine the altered cognitive strategy on a DOT task, including generally negative and neutral words.

The PTSD model has been implemented by recent observations of altered brain structures involved in attentional bias tasks such as the amygdala (Cisler et al., 2010). Evidence is accumulating in PTSD that attentional bias and symptomatology are both positively correlated with limbic functioning and negatively correlated with prefrontal activity (Cisler et al., 2010). These findings can be understood in the light of Eysenck theoretical model of attentional bias toward threat in anxiety, stipulating that anxiety would favor bottom-up effect of amygdala on prefrontal cortex (PFC) and weaken top-down regulatory control which would manifest by difficulty in disengaging attention from distracting threat cues (Eysenck, Derakshan, Santos, & Calvo, 2007).

At that stage, it remains unknown whether cerebral alterations and attentional bias is initially present in PTSD patients before traumatic exposure or only occurs after it, alongside symptom development. Yet, the causal relationship between attentional bias and symptomatology seems bidirectional in anxiety disorders. On one hand, attentional biases are reduced following interventions using for instance implementation intentions in social anxiety (Webb et al., 2010). On the other hand, interventions designed to modify attentional biases are associated with reductions in anxiety (Hakamata et al, 2010), in non-clinical as well as pathologically anxious populations (Koster et al., 2009). These interventions have not been studied in PTSD; though studies show that initially attentional bias and symptom severity are correlated in PTSD (Pineles et al., 2009).

Our second aim was thus to monitor potential changes in attentional bias in PTSD using e-Stroop and DOT tasks when the symptoms were relieved i.e. before and after successful treatment. The Eye Movement Desensitization and Reprocessing (EMDR) therapy was chosen as a validated treatment option for PTSD, rapidly and efficiently relieving PTSD symptoms (APA, 2004). So far, only one study had looked at the effect of psychotherapy (Cognitive Behavioral Therapy (CBT)), on PTSD attentional bias on a Stroop task (Devieni et al., 2004). It had failed to evidence initial e-Stroop interference effect in patients and thus

found no relation between changes in color-naming delays and treatment response or modality.

Our study explored the attentional bias in PTSD on e-Stroop and DOT tasks, before and after symptom amelioration. This was done to better define the impaired cognitive processing in PTSD and further examine its modulation with symptom amelioration. We hypothesized that, similarly to anxious populations previously studied on these tasks; PTSD patients would have a difficulty disengaging their attention from aversive cues and would be slower in the presence of emotionally negative stimuli. Based on aforementioned premise, we also hypothesized that this bias would be initially observed in PTSD but not after EMDR.

METHODS

Subjects

A total of 23 adult outpatients were recruited among trauma victims at the medico-psychological crisis cell (CUMP) at the Psychiatry Pole of the Conception Hospital in Marseille, France. They all met the DSM-IV criteria for PTSD following a single traumatic event (12 aggressions including hold-ups and rapes, 5 road accidents, 6 work related accidents) with no previous history of neurologic or psychiatric disorders. Four patients were excluded from data analyses as they abandoned the study. Subsequent analysis included 19 patients (7 males and 12 females, with mean age = 45 ± 15 years and mean education = 7.6 ± 2.7 years after grade 7). Patients had been exposed to their traumatic event for an average of 17.2 months. Seven patients were on antidepressants and 7 took anxiolytics.

A total of 19 healthy adult controls (9 males and 10 females, with mean age = 38 ± 14 years and mean education = 9.0 ± 2 years after grade 7) with no history of neurologic or psychiatric disorders, were recruited via screening lists at the clinical investigation centre at the Timone Hospital (CIC-UPCET).

Groups were matched for age, sex and education level. Patients and controls were also individually matched. As such, each patient was matched with a control having the same age (plus or minus 5years) and education (plus or minus 2years).

Psychological Assessment

All participants were assessed by a psychiatrist, using the structured Mini-Internal Neuropsychiatric Interview for DSM-IV (Lecrubier et al., 1998).

This was done to check for the absence of psychiatric disorders prior to the trauma and screen for PTSD and potential comorbid disorders. Participants responded to demographic questions and completed the Beck Depression Inventory (BDI) (Cottraux, 1985) and the State-Trait Anxiety Inventory (STAI-Y) (Schweitzer & Paulhan, 1990). Patients also completed trauma related scales: PTSD Check List Scale (PCL-S) (Ventureyra et al., 2002) and Modified PTSD Symptoms Scale (MPSS) (Stephenson et al., 1999). The validated French version was used for all the scales.

EMDR Treatment

All PTSD patients underwent EMDR. According to the APA reports published in 2004, this eight-step standardized protocol is one of the validated treatments for PTSD. It is based on an information processing model (Shapiro & Maxfield, 2002). EMDR is an effective rapid therapy with stable outcome demonstrated in a 35-month follow-up study (Hogberg et al., 2008).

Patients were treated by one of 3 therapists, all trained by the French Institute of EMDR. There was no fixed number of sessions. Sessions were planned every 7 to 15 days according to patients and therapists availability. The treatment was considered successful and complete when patients no longer reported distress when thinking about their trauma. They were interviewed again by the psychiatrist using the MINI. Patients required an average of 4.1 treatment sessions (ranging from 1 to 9 sessions) lasting for an average of 2.5 month (0.5 to 4months). They were retested when they no longer met PTSD classification according to DSM-IV criteria and had no more pathological scores on PTSD scales.

Healthy controls were also tested twice, at time points matching the interval between patients testing sessions.

Cognitive tests

The cognitive tasks we used were the ones validated by Lanteaume et al. (2009). They were administered by the experimenters.

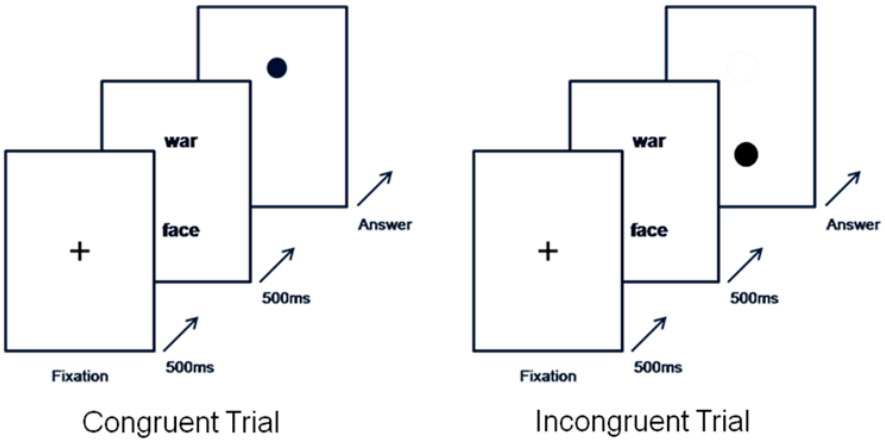
1.Emotional Stroop task

This task included 96 trials. Each trial consisted of a black fixation cross display of 500 ms at the center of a white screen, followed by a cue display in the middle of the screen until the response was given. The inter-trial interval was 500 ms. The cue display consisted of either an emotionally negative word (such as accident, raping…), or a neutral one (such as

sphere, housing…). The type of word (emotional, neutral) and ink color (red, blue, green) were randomly counterbalanced across trials, with a new sequence for each participant. Participants were asked to fixate on the black cross. After it disappeared, they had to identify the colour of the displayed word.

2.Detection of Target task

This task included 128 trials. Each trial consisted of a succession of three steps: a black fixation cross display of 500 ms at the center of a white screen, a cue display of 500 ms, and a dot display until the response was given. The inter-trial interval was 500 ms. The cues consisted of either an emotionally negative word (such as accident, raping…) and a matched neutral word (such as sphere, housing…), or a neutral word and another matched neutral word. Words were vertically opposite at equal distance from the center of the screen, 4 cm apart. The probe display consisted of a black circle that appeared at the same spatial location as one of the two words. The emotional word position (lower, upper), the probe position (lower, upper), and the type of word pair (emotional-neutral, neutral-neutral) were randomly counterbalanced across trials with a new sequence for each participant. For the emotional pairs, trials were said to be congruent when the dot replaced the emotional word and incongruent when the dot replaced the neutral word (Fig.1). Participants were asked to fixate on the black cross. They were told two words would appear immediately after the black cross and, that a dot target would appear after the words. They were asked to give the location of the target.



Article 3. Fig 1. Illustration of the succession of the three steps for the DOT task for a congruent and incongruent trial.

Procedure

The investigation was carried out in accordance to the latest version of the Declaration of Helsinki. After receiving clear explanations of the procedure, participants provided informed consent in accordance with the guidelines set forth by the local ethical committee CPP South Mediterranean 2. Before starting the experiment, all participants completed the BDI and STAI-Y and PTSD patients completed additional PTSD scales.

Participants were comfortably seated at 60 cm viewing distance from a 17" computer screen. In both tasks, participants were asked to answer as quickly as possible without sacrificing accuracy, by pressing corresponding color or location keys on an AZERTY keyboard.

Data and Statistical Analyses

Trials in which participants gave the wrong word color (%error rate for controls at session 1, 2, PTSD patients pre, post-EMDR is 1.3, 1.2, 1.1 and 0.8% respectively), the wrong dot target location (0.6, 0.8, 0.5 and 0.5% respectively) or in which reaction time (RT) was above or below 2 standard deviations of their mean RT were considered error trials and removed from subsequent analysis, similarly to Lanteaume et al. (2009).

An e-Stroop index was calculated: RT emotional words – RT neutral

When it differs from zero, it indicates the existence of an attentional bias; with a positive index meaning that attention is captured by emotional words and a negative index meaning that emotional words are avoided.

Three indices were calculated for the DOT:

- Congruence: RTincongruent – RTcongruent. A positive index indicates a bias in threat detection, either on congruent or incongruent trials
- Disengagement: RTincongruent – RTneutral. A positive index indicates stronger attentional holding for negative cues; subjects are slower to respond to neutral cues in presence of emotional ones.
- Vigilance: RTneutral – RTcongruent. A positive index indicates enhanced attention capture for negative cues; subjects are faster in responding to emotional cues in presence of neutral ones (Salemink et al., 2007).

A two-way repeated measures ANOVA was used for the e-Stroop, disengagement and vigilance indices, with Group (2 levels: PTSD patients and Controls) as a between factor and Session (2 levels: for controls: session 1 and 2, and for patients: pre and post-EMDR) as a

within factor. Significant main effects at 0.05 significance levels were followed by post-hoc tests using Bonferroni correction.

RESULTS

3.1 Clinical Data

Groups did not differ in terms of age, sex and education levels.

Data for the various scales used was found to conform the normal distribution according to the Kolmogorov-Smirnov test results.

Table 1 shows the psychometric measures of controls and patients. In accordance with clinical evaluations, PTSD patients initially scored higher than controls on BDI and STAI scales. After treatment, both groups had comparable scores (Table 1).

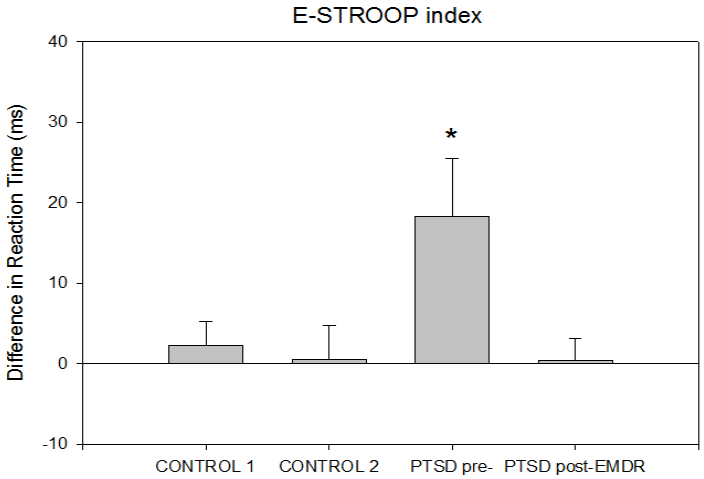
Patients also scored higher than the cut-off for pathology on PTSD scales pre but not post-EMDR. They met the criteria for the following major current comorbid diagnoses (pre/post-EMDR): major depression (n=12/4), other anxiety disorders (n=15/7) and high-medium suicidal risk (n=7/0).

	CONTROL 1 (n=19)	CONTROL 2 (n=19)	PTSD pre-EMDR (n=19)	PTSD post-EMDR (n=19)	
BDI Depression	3.2 (2.6)	2.3 (2.6)	15. (8.1)	5.6 (3.9)	$F(1,36) = 22.61^{*}$
STAI State Anxiety	29.5 (8.1)	30.5 (8.4)	50.1 (11.3)	34.6 (8.7)	$F(1,36) = 20.47^{*}$
STAI Trait Anxiety	40.3 (7.9)	37 (8.9)	56.5 (10.5)	42.8 (7.6)	$F(1,36) = 14.43^{*}$
PCL-S	-	-	62.3 (11.4)	28.4 (6.6)	$t(1,18) = 11.28^{**}$
MPSS	-	-	74.2 (22.7)	21.9 (10.8)	$t(1,18) = 9.93^{**}$
IES	-	-	56.7 (13.4)	17.3 (10.0)	$t(1,18) = 9.89^{**}$

Table 1. Characteristics of participants: Mean (SD) for Beck Depression Inventory (BDI), State Trait Anxiety Inventory STAI-Y, and for the patients PTSD Check List Scale PCL-S, Modified PTSD Symptoms Scale MPSS, and Impact of Event Scale IES. Significant p-value: $^{*}p < 0.05$ $^{**}p < 0.001$.

3.2 Emotional Stroop

Statistical analysis revealed a significant Group x Session interaction for the e-Stroop index ($F(1, 36) = 4.604$, $p < 0.05$) (Fig 2). Post-hoc tests showed that unlike controls who had a null e-Stroop index, patients had a positive one ($p < 0.05$) with longer RT to emotional words than neutral ones.



Article 3. Fig 2. Mean and error bars of the e-Stroop index. For the control group, digits 1 and 2 indicate testing sessions. *$p < 0.05$ according to Session x Group interaction.

After treatment, we found no significant difference in e-Stroop index between groups. There was no effect of test/retest as we found no difference in controls RT at their $1^{st}/2^{nd}$ session. Session had a significant effect only for the PTSD group with larger e-Stroop index pre than post-EMDR ($p < 0.05$)

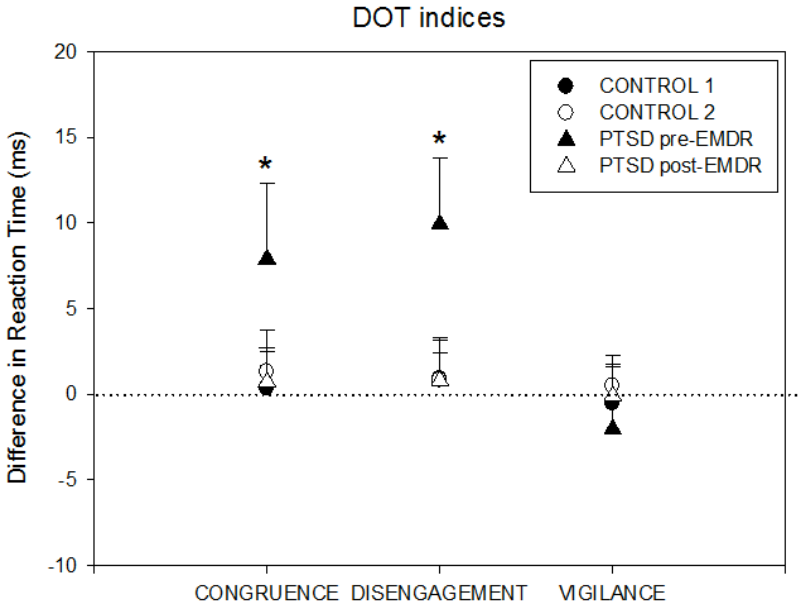
3.3 DOT

Only the PTSD group had a Congruency index different from zero ($t(1, 18) = 2.32$, $p < 0.05$).

Group x Session interaction was significant for the Disengagement ($F(1, 36) = 4.94$, $p < 0.05$) but not for the Vigilance index ($F(1, 36) = 0.028$, $p > 0.05$) (Fig 3). Post-hoc tests showed that patients had larger disengagement index than controls ($p < 0.05$). Patients indeed had longer RT compared to controls on incongruent compared to neutral trials; they were slower to respond to a neutral word in the presence of an emotional one than when they responded to a neutral pair.

After treatment, there was no significant difference between PTSD and control groups on either index. There was also no effect of test/retest as we found no difference in controls'

indices at their 1st/2nd session. Session had a major effect for the PTSD patients, with significantly larger Disengagement index before EMDR than after ($p < 0.05$).



Article 3. Fig 3. DOT effect. Mean and error bars of Congruence, Disengagement and Vigilance indices. For the control group, digits 1 and 2 indicate testing sessions. *$p < 0.05$ according to null hypothesis for the Congruence index and according to the Session x Group interaction for the disengagement and vigilance indices.

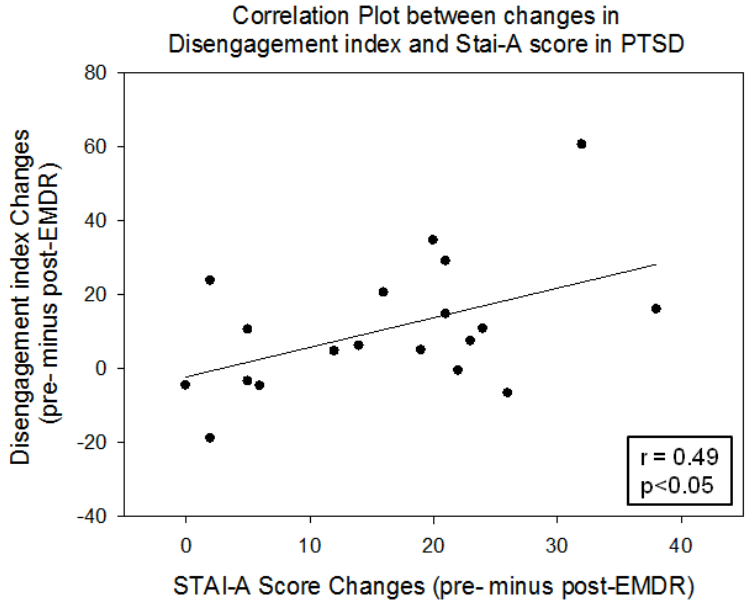
3.4 Adjustment for RT differences: Analysis of covariance (ANCOVA)

It had been suggested that comorbid disorders and/or medication can alter the cognitive processing in PTSD (Harmer, 2008). To examine if comorbid anxiety, depression or use of medication would explain the effects found for PTSD on e-Stroop and disengagement index, they were entered separately as covariates in the Group x Session ANOVA.

Statistical analysis revealed all three covariates; anxiety, depression and use of medication, were not significantly related to any of patients' attentional bias with the following ANCOVA values respectively for anxiety, depression and medication:
- for the Stroop index ($F(1,33) = 7.535$, $p < 0.05$, $F(1,34) = 4.25$, $p < 0.05$ and $F(1,34) = 5.29$, $p < 0.05$),
- for the disengagement Index ($F(1,33) = 4.89$, $p < 0.05$, $F(1,33) = 7.15$, $p < 0.05$ and $F(1,34) = 5.69$, $p < 0.05$).

3.5 Correlation analysis

To test for correlation of cognitive bias modulation with changes in anxiety levels and in PTSD symptomatology, correlation analyses were conducted with the Pearson correlation between the calculated disengagement index pre minus post-EMDR and the score of STAI-A (state anxiety) and MPSS (symptom severity) pre minus post-EMDR, at a 0.05 significance level, with the Bonferroni correction. Statistical analysis revealed significant correlation between changes in Disengagement index and changes in STAI-A scores with r= 0.49, n=18, and p<0.05 (Fig 4). We also found a strong tendency for a significant correlation between changes in Disengagement index and changes in MPSS scores.



Article 3. Fig 4. Correlation. Plot of correlation between difference in disengagement index and difference in Stai-A (state anxiety) scores in PTSD patients. r = 0.493, n=18 and p < 0.05.

DISCUSSION

The present study illustrates a deficiency in PTSD's cognitive processing of emotional cues. It first and foremost replicates results on the attentional bias in PTSD reflecting patients' difficulty in disengaging their attention from aversive cues. The most important result is that the disengagement bias in PTSD diminishes after symptom amelioration post-EMDR.

We have shown that altered threat processing in PTSD is evidenced on attentional bias tasks. Our results showed that patients were slower than controls in indicating the color of emotional words on the e-Stroop and had difficulty orienting their attention away from

negatively-valenced words on the DOT task. This result supports previous findings reviewed by Bush (2000) on the existence of attentional bias in PTSD towards aversive words. It also points to the existence of a disengagement problem in PTSD (Browning et al., 2010), rather than a vigilance one (Bryant et al., 1999). It is noteworthy that further investigations are still needed to better define time course changes of attentional bias in PTSD in shorter (50, 100 and 200 ms) and longer (1250 ms) presentations, and its putative modulation by other somewhat more ecologic stimuli; such as sounds and IAPS images or faces (Koster, 2005).

Taken together, our results indicate that emotional information seems to reduce processing efficiency in PTSD. These findings of patients delayed RT in the presence of aversive cues go along the lines of what is generally held about patients with other anxiety disorders such as generalized anxiety disorder, social phobia, panic disorder and non-clinical high trait anxiety subjects (Cisler &Koster, 2010).

The major finding here remains that the attentional bias in PTSD diminishes after symptom removal by EMDR. We found that similarly to controls, EMDR treated patients who were symptom-free had null e-Stroop and disengagement indices. To the best of our knowledge, no studies have so far looked at the modulation of attentional bias in PTSD before and after symptom removal. Restoration of processing bias has been found after CBT in patients with chronic pain (Dehghani et al., 2004) and alcohol dependence (Fadaradi &Cox, 2009).

Moreover, changes in disengagement index after EMDR positively correlated with changes in state anxiety, with larger disengagement bias found in highly anxious patients. This result suggests that such bias could be corrected by decreasing state anxiety. It goes along recent findings that modification of attention bias reduces anxious symptomatology (Browning et al., 2010; See et al., 2009). EMDR is thus one more approach to modify attentional bias, alongside expressive writing for instance (Vedhara et al., 2010).

We additionally found a strong tendency for a correlation between attentional bias and PTSD symptom severity. The failure to reach significant levels might be due to the non-specificity of the words used and/or to the heterogeneity of the PTSD population in terms of trauma-type. A study by Sveen et al., (2009) had indeed evidenced a correlation between burn-specific e-Stroop bias in PTSD and symptom severity. We now provide preliminary evidence that attentional bias in PTSD would be linked to symptomatology, and could be a marker of the pathology.

A first limitation of the study arises from our inability to retest the 4 drop-outs, which were mostly out of reach or refused to be retested. Their inclusion, alternatively to the presence of a wait-list group of PTSD patients, would have provided more reliable evidence in correlating attentional bias to acquired symptomatology rather than inherited factors or mere practice effect.

Another limitation is to assess for the effect of the delay between testing sessions. It is however not likely for mere passage of time to account for the restoration of cognitive processing of aversive cues in our tasks, as patients were diagnosed with symptoms lasting for an average of 17.2 months and showed no signs of spontaneous recovery. It has been shown that treatment gain by EMDR is stable over 35 months (Hogberg et al., 2008). Further studies should address the current limitation to better assess treatment benefit in attentional bias in follow-up studies.

A third limitation is that some of the patients evaluated were on stable medical regimen for antidepressants and/or anxiolytics (9 of 19 patients) and had other comorbid anxiety and/or mood disorders. The meta-anaysis by Bar-Haim et al., (2007) suggested that co-occurrence of mood disorders with anxiety does not play a major role in the threat-related bias of anxious individuals. Also, attentional bias appears to occur in equal magnitudes in all anxiety disorders (Cisler &Kostler, 2010). We can also argue that our findings are relevant to anxiety, which is associated with perturbations of early attentional deployment (10-500ms), rather than being relevant to depression, which is associated with later effects (500-1000ms) (Browning et al., 2010).

None of the comorbid factors co-varied with our results; however, their alteration of cognitive processing cannot be totally ruled out. It would be useful in future studies, to further explore drug and comorbidity interaction with attentional and cognitive bias in larger PTSD subpopulations with or without medication and with or without comorbidities.

Inasmuch as the inherited and/or acquired facets of attentional bias are yet undefined, it is important in clinical terms to better outline the altered strategy underlying attentional bias in PTSD, its association to symptoms and its modulation by treatment; to better optimize therapeutic options. Our design addresses the correlation between attentional bias and anxious symptomatology in PTSD, before and after symptom removal. Our results do suggest biases are reduced following interventions that reduce anxiety.

Our group has previously explored causal relationship between limbic functioning, attention orientation and PTSD symptomatology. We had shown that in symptomatic patients, amygdala over-activation causes disengagement problems, which subsequently drives anxious symptomatology (El-Khoury et al., 2011). We have currently shown that the attentional bias diminishes after symptom removal by EMDR in PTSD. Further studies should monitor whether such therapeutic interventions relieving symptoms severity and correcting attentional bias also re-establishes proper cerebral functioning in PTSD.

To conclude, our study has shown that cognitive impairment in PTSD is substantiated via e-Stroop and DOT indices, as patients have a disengagement bias toward emotionally negative words. More importantly, we have demonstrated for the first time that this pathologically impaired attentional bias diminishes after symptom amelioration by successful EMDR. These indices could constitute cognitive markers of PTSD. In order to verify that symptom removal also restores normal functioning of the cerebral structures known to be involved in PTSD and attentional bias, similar paradigms should be further explored at the brain level using fMRI.

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The CNRS had no further role in study design; in the collection, analysis and interpretation of data; in writing the report nor in the decision to submit the paper for publication

Conflict of Interest Statement

The authors have no actual or potential conflict of interest including financial or personal relationships that could influence or could be perceived the work in this manuscript.

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Neurofunctional Alteration of Emotional Face Processing Correlates with Attentional Bias in PTSD

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Post-traumatic stress disorder (PTSD) is an anxiety disorder that arises in the aftermath of a traumatic event. The most prevalent hypothesis is that of an increased amygdala activity to threat cues. The amygdala has also shown an implication in orienting attention toward threat. The aim of the present study was to explore the correlations between amygdala activity and symptom severity on one hand and attentional bias to threat on the other. PTSD patients and healthy controls were assayed on an fMRI emotional face matching task and an attentional detection of target (DOT) task. The amygdala showed enhanced activity in PTSD patients (vs. healthy individuals). It positively correlated with anxiety scores and PTSD symptoms. It also positively correlated with the disengagement index during the attentional DOT task. Mostly, these results provide preliminary support for an implication of the amygdala in attention orientation to threat in PTSD. These results are further discussed in light of recent theories concerned with cortico-limbic functioning.

AMYGDALA ACTIVITY CORRELATES WITH ATTENTIONAL BIAS IN PTSD

Post traumatic stress disorder (PTSD) is characterized by panoply of symptoms in the aftermath of a traumatic event (APA, 1994), including re-experiencing of the aversive event, avoidance of its reminders and generalized hypervigilance (DSM-IV). Although PTSD pathophysiology remains largely unknown, the most prevailing hypothesis is that of a hyperactive amygdala (Rauch et al., 2000). This hypothesis is also supported by lesion studies showing amygdala resection abolishes PTSD (Koenigs &Grafman, 2009).

Shin & Liberzon (2010) review numerous studies consistently evidencing increased amygdala activity in PTSD v/s. healthy controls. Amygdala has generally shown activation in response to emotionally arousing and/or salient stimuli, and its overactivation in PTSD might account for exaggerated fear responses and is thought to support immediate threat processing (Anderson, Christoff, Panitz, De Rosa & Gabrieli, 2003).

Facial expressions have been effective in probing increased amygdala response in anxiety disorders (Monk et al., 2008). In such task, amygdala activity positively correlates with anxiety scores in healthy individuals (Fakra et al., 2009). To better assess if amygdala over-activation of is a marker of PTSD pathology or a mere reflection of patients anxiousness, our first aim was to assay amygdala activity to emotional cues in PTSD and to explore the correlation between amygdala activity, anxiety scales and PTSD symptoms. We used a validated face matching task known for its robust amygdala activation (Hariri, Bookheimer & Mazziotta, 2000).

Moreover, a wealth of research illustrates the presence of attentional bias toward threat in anxiety disorders and notably in PTSD (Bar-Haim et al., 2007), and suggests threat detection mechanisms might be neurally centered on the amygdala. Amygdala activity was found to correlate with attentional bias in generalized anxiety disorder (GAD) youth (Monk et al., 2008), suggesting that limbic involvement in automatic processing of emotional threat cues might influence attention orientation (Frewen et al., 2008). To extend the GAD findings to PTSD, our second aim was thus to assay attentional bias in PTSD and to explore the correlation between amygdala activation and disengagement difficulty in PTSD. We used a validated DOT task known to evidence disengagement bias (Lanteaume, Bartolomei & Bastien-Toniazzo, 2009).

Consistent with previous findings, we hypothesized that traumatized subjects with PTSD would exhibit increased amygdala activation compared to controls in response to angry and fearful faces, which would correlate with their symptomatology, and would positively correlate with disengagement bias on a DOT task.

Materials and Methods

Subjects

Seventeen adult outpatients (9 males and 8 females, aged 31.7 ± 6.7 years (mean ± standard deviation), with 7.8 ± 1.9 years of education after grade 7) were recruited by a psychiatrist among trauma victims at the Psychiatry Pole of the Conception Hospital in Marseille, France. They all met the DSM-IV criteria for PTSD following a single traumatic event (mean exposure = 28 month). Six patients took antidepressants and/or anxiolytics. A total of 17 healthy adult controls (10 males and 7 females, aged 34.8 ± 9.8 years, with 8.9 ± 1.5 years of education after grade 7) were recruited via screening lists at the clinical investigation center at the Timone Hospital.

Psychological Assessment

Participants were assessed by a psychiatrist to ensure they had no previous history of neurologic or psychiatric disorders, and screen for PTSD and potential comorbid psychiatric disorders in patients, using the structured Mini-Internal Neuropsychiatric Interview for DSM-IV (Lecrubier et al., 1998). Participants responded to demographic questions and completed the State-Trait Anxiety Inventory (STAI) (Schweitzer & Paulhan, 1990). Patients also completed a trauma related PTSD Check List Scale (PCL-S) (Ventureyra, Yao & Cottraux, 2002). The validated French version was used for all the scales.

DOT Task

This task included 128 trials, each consisting of three successive steps: a fixation cross display of 500 ms at the center of a screen, a cue display of 500 ms, and a dot display until the response was given (Fig 1A).

Participants were asked to fixate on the cross and indicate the position of the dot (high or low) as quickly as possible without sacrificing accuracy.

Matching Task

In the emotional condition, subjects viewed a target face and had to select which one of 2 faces presented below it (on the same screen) expressed the same emotion (fear or anger). In neutral/control condition, they viewed a target shape, and chose which of two shapes presented below it (on the same screen) matched the target (round or oval). The paradigm consisted of 12 experimental blocks of 44.5 s each, alternating emotional and control blocks. Each block contained 10 stimuli presented for 4 s with an inter-stimulus interval of 0.5 s. The inter-block interval was 2 s, giving a total scan length of 9 min. We used four different sets of geometric forms for the control blocks, and sixty different images, ten per block, five of each gender for the emotional blocks.

fMRI specification. All data acquisition was performed on a 3-T MEDSPEC 30/80 AVANCE imager (Bruker, Ettlingen, Germany) at the fMRI center of Marseille, France. All stimuli were generated on a computer and back-projected onto a screen that subjects viewed through a mirror positioned above their eyes. fMRI scans were acquired using a T2*-weighted gradient-echoplanar sequence (TR/TE=2533.3/30 ms; FOV=19.2×19.2 cm, 64×64 matrix; flip angle= 82.4°. Thirty-eight interleaved axial slices, tilted -30o to the intercommisural plane to reduce artifacts in prefrontal regions, were obtained with a contiguous slice thickness of 3 mm. Following the fMRI scans, a set of high-resolution T1- weighted images were acquired for the purpose of anatomical identification (sagittal MPRAGE Sequence, TE/TR = 4/10 ms, TI = 800 ms, Flip Angle = 30°, Matrix=256×256×128).

Procedure

The investigation was carried out in accordance with the latest version of the Declaration of Helsinki. Participants provided informed consent in accordance with the guidelines set forth by the local ethical committee CPP South Mediterranean 2.

Data and Statistical Analyses

DOT data. Three indices were calculated: congruence, vigilance and disengagement (Fig.1). A t-test was used on the calculated disengagement and vigilance indices with $p<0.05$.

Behavioral Matching data. On trials in which the subjects answered within the 4 sec time-frame of image presentation, differences in task performance (accuracy score and reaction time (RT)) between control and patient groups were compared using a t-test with a significant $p <0.05$.

fMRI Matching data. Data were processed using SPM5 software (Wellcome Department of Cognitive Neurology, UCL). A standard preprocessing of data was performed (slice timing, movement correction, spatial normalization and smoothing with a kernel of 6 mm). Individual statistical maps were calculated for each subject to evaluate differences between emotional versus control conditions. Each condition was modeled by a box-car convolved with a canonical hemodynamic response function. The within-subject contrast images were then entered into a second-level t-test to examine both within- and between-group effects, with an a priori Region of Interest (ROI) with an anatomical mask selected using the WFU Pickatlas (Version 2.4). Between-group comparisons at the ROI were taken at an uncorrected $p<0.001$ and outside the ROI at pFDR corrected < 0.05 and a min of 5 voxels.

Correlation analyses. To assess the potential correlation between amygdala activity, anxiety, PTSD scores and disengagement index, a Pearson correlation analysis was performed between ROI extracted activity (emotional v/s. control condition) and various scores, with a significant $p<0.05$, subject to Bonferoni corrections for multiple comparisons. Alternatively scores were entered respectively as covariates to assay whole-brain analysis with pFDR corrected < 0.05 and a minimum of 5 voxels. For additional causal analyses of those factors, the Sobel test and multiple regression tests were used.

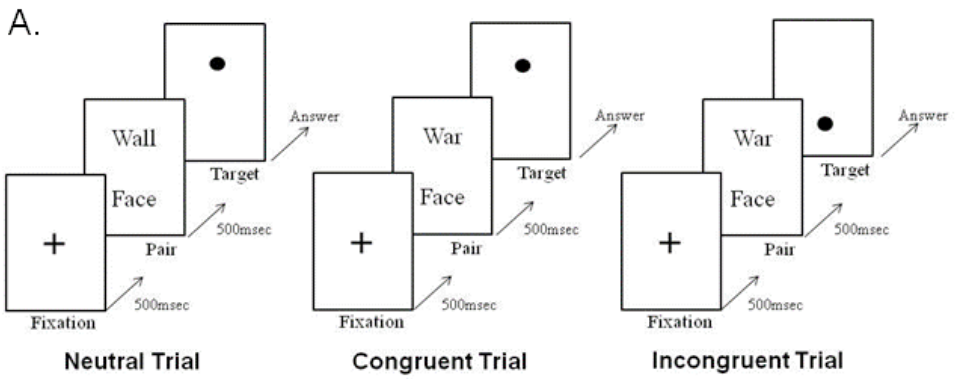
Results

Clinical Data

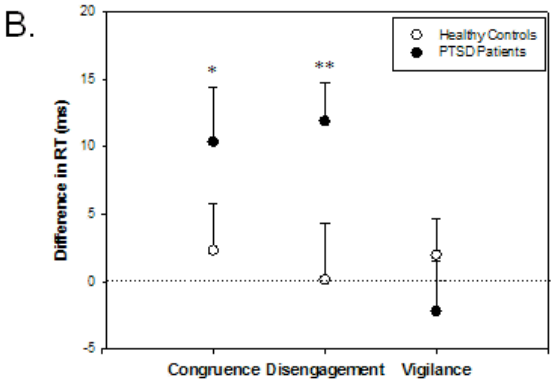
Groups did not differ in age, sex and education. PTSD scored significantly higher than controls on STAI-trait (mean ± standard deviation) (controls 29.9 ±8.8; PTSD 51.7 ±14.9; $t(32) =5.19$; $p<0.001$) and STAI-state (controls 35.1 ±7.9; PTSD 54.6 ±10.8; $t(32) =6.01$; $p<0.001$). Patients' individual score on PCL-S scale were higher than the cut-off for pathology (58.4 ±12.8).

Cognitive Bias on the DOT task

The congruence index was significantly different from zero only for PTSD ($t(16)=2.55$; $p<0.05$). Furthermore, PTSD and controls significantly differed for the disengagement ($t (32) = 2.32$; $p < 0.05$) but not the vigilance index (Fig. 1B.).



Congruence	•Reaction time for: Congruent – Incongruent trials •If <0; subjects have an attentional bias towards threatening words
Disengagement	•Reaction time for: Incongruent – Neutral trials •If >0; subjects are slower at responding to neutral words in presence of threatening ones
Vigilance	•Reaction time for: Congruent – Neutral trials •If <0; subjects are faster at responding to threatening words in presence of neutral ones



Article 4. Fig 1. A. Illustration of the DOT task with neutral congruent and incongruent trials and subsequent calculation of indices. B. Plot of Congruence, Disengagement and Vigilance indices (mean and standard error) for controls and patients. * significant difference from zero; ** significant t-test with $p < 0.05$

Behavioral Performance on the Matching task

There was no significant difference between groups in terms of accuracy and RT for the matching task on either emotional or control conditions.

fMRI data on the Matching task

Areas of activation of activation for emotional v/s. control contrast are shown for each group in Table 1. Activations are similarly localized between groups and results show a significantly increased BOLD activity in right amygdala in patients (compared to controls) (x,y,z = 27,-3,-21) ($t(34) = 2.56$; $pFDR < 0.05$) (Fig. 2).

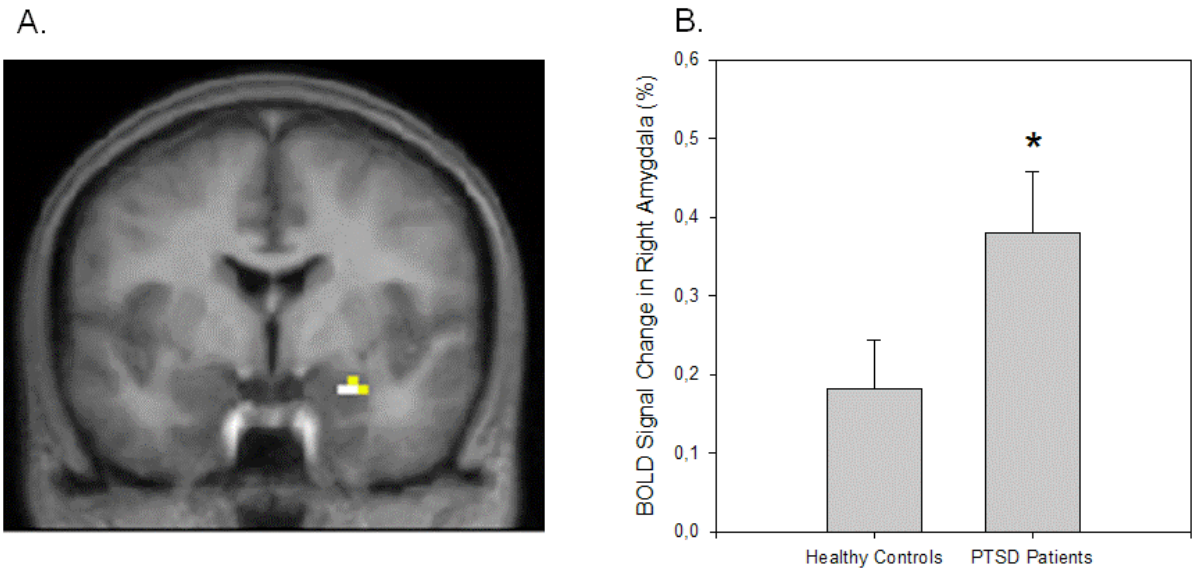
Control group

AREA	COORDINATE	t-VALUE
Anterior Cingulate [BA 32]	0 12 48	6.09
Bilateral Amygdala	-21 -3 -18	5.89
	24 -6 -21	5.58
Bilateral Inferior Frontal Gyrus	-36 18 21	5.38
[BA 45,47]	45 15 21	7.12
Dorsolateral Prefrontal cortex	-39 -3 39	7.53
[BA 6,9]	48 3 33	7.42
Temporal lobe	-24 -81 21	8.18
	36 -78 27	6.27
Thalamus	-9 -18 9	5.78
	18 -21 12	5.78
Parahippocampus	-21 -33 0	8.1
	27 -30 0	6.8
Precuneus	-27 -66 48	6.91
	30 -69 48	7.22
Right Putamen	21 0 3	4.65

PTSD group

AREA	COORDINATE	t-VALUE
Anterior Cingulate [BA 32]	9 18 42	5.44
Bilateral Amygdala	-21 -6 -21	5.01
	24 -6 -21	7.17
Bilateral Inferior Frontal Gyrus	-36 20 20	5.08
[BA 45,47]	48 24 21	8.14
Dorsolateral Prefrontal cortex	-36 3 30	6.88
[BA 6,9]	51 0 42	6.64
Temporal lobe	-27 -57 45	5.63
	39 -60 45	5.36
Thalamus	-6 -9 9	4.19
	15 -15 9	5.12
Parahippocampus	-18 -30 -9	4.42
	30 -24 -9	6.53
Precuneus	-9 -69 48	4.69
	10 -65 37	6.02
Right Putamen	21 3 0	4.29

Table 1. Areas activated for the emotional versus neutral contrast in Control and PTSD groups separately. All activations are observed in whole-brain analyses with p_{FWR} <0.05



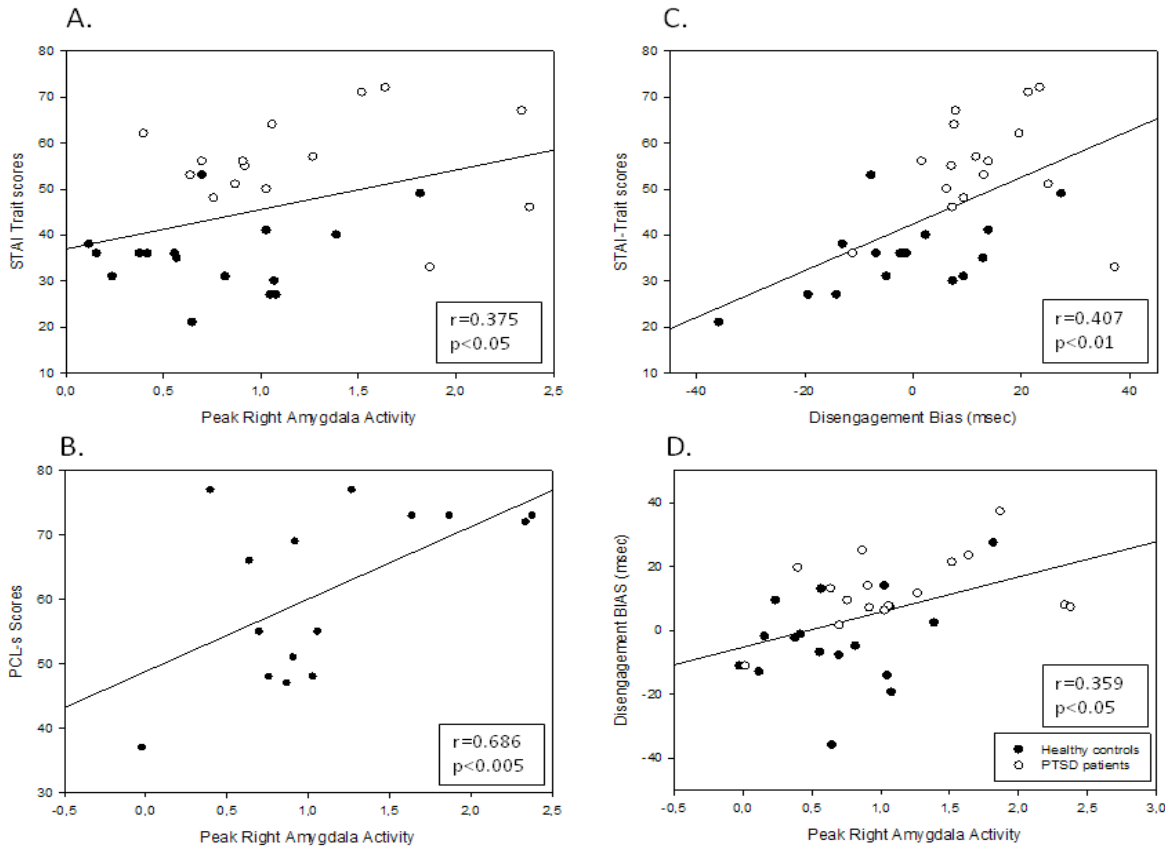
Article 4. Fig 2. Statistical parametric maps illustrating patterns of activation of the emotional vs. neutral contrast in PTSD v/s controls. Compared to controls, patients showed increased right amygdala activation. Voxels reaching significance at the $p_{uncorrected}$ <0.001 are rendered onto a normalized averaged T1-anatomical image. B. Bar graphs representing mean activation within the right amygdala cluster for the contrast in both groups separately. *p<0.05.

Correlation between amygdala activation, anxiety scores & PTSD symptomatology

There was a positive correlation between the peak activation within the right amygdala cluster and STAI-trait and PCL-S scale, with N= 33, r= 0.375; p <0.05 and N=16, r= 0.686; p <0.005 respectively. This indicates that higher amygdala activation corresponds to larger anxiety scores and to more severe symptoms (Fig. 3A. and 3B.). Considering control and patient groups separately gave no significant correlation between amygdala activation and anxiety scores.

Correlation between disengagement bias, anxiety scores & PTSD symptomatology

There was a positive correlation between the disengagement bias and STAI-trait scores, with N=33 and r= 0.407; p < 0.01, indicating that larger attentional bias corresponded to more anxious subjects (Fig. 3C.). No significant correlation was found between disengagement index and PCL-S scores.



Article 4. Fig 3. Plot of the correlation indices between A. increasing amygdala activity and higher trait anxiety on STAI, B. increasing amygdala activity and higher PTSD symptoms severity on PCL-S, C. larger disengagement bias and higher trait anxiety and D. increasing amygdala activity and larger disengagement index, with corresponding values of Pearson correlation r and p for all participants (except for B).

Correlation between amygdala activation & disengagement bias

There was a positive correlation between the right amygdala activation and disengagement index, with N=33 and r= 0.359; $p < 0.05$, indicating that amygdala over-activation corresponds to larger disengagement bias (Fig. 3D.). When groups were considered separately, no such correlation was found for controls, however it was still significant for patients; with N=17, r= 0.483 and $p< 0.05$, with $r^2 = 0.23$. Additionally, combining disengagement bias and anxiety scores better predicted amygdala activity in PTSD (r=0.776, N=16, $p<0.005$). Similarly, combining amygdala activity and disengagement bias better predicted PTSD symptoms (r=0.692, N=16 and $p<0.05$).

Alternatively, using whole-brain analysis with disengagement as covariate showed that the attentional bias positively correlated with amygdala activity at p=0.05, when considering all participants. The correlation was significant for the PTSD patients but not controls when groups were considered independently. It also correlated with hippocampal and PFC activity.
Given the triad of correlations between amygdala activation, attentional bias and anxiety scores, we used a mediation analysis (Sobel test) to examine their causal relationship. We found that amygdala hyperactivation leads to disengagement which in turn leads to increased anxious symptoms (S=1.65 and $p<0.05$).

Adjustment for comorbidities and medication

To evaluate the effect of comorbid mood, anxiety disorders and medication use on the amygdala activity and disengagement bias, we compared their means in PTSD subpopulations with/without depression, with/without other anxiety disorder and with/without medications. We found no significant difference in PTSD subgroups for any of the aforementioned factors. For a given factor both subgroups had comparable means of amygdala activation and disengagement.

Discussion

Stemming from the surprising lack of research investigating how different emotional and attentional components of PTSD interact, we studied the correlation between threat-related amygdala hyperactivity, and other aspects of anxious responding such as self measures of distress and attentional bias. Our results indicate that PTSD atypically process threat whereby a correlation between amygdala overactivation and attentional bias might account for aspects of the disorder concerned with a state of increased responsivity to threat cues.

Functional analyses differentiate the groups on the matching task although groups show no difference in behavioral RT. Compared to controls; patients have increased amygdala activity to emotional faces. This supports findings of overactive amygdala in PTSD (Shin &Libertzon, 2010), and further establishes a correlation with PTSD symptom severity using prolonged faces exposures, similarly to previous studies using masked faces (Rauch et al., 2000). Taken together, these findings suggest that more prominent PTSD symptoms relate to increased amygdala activity, in both pre-conscious and conscious stages of threat processing.

The right sided amygdala overactivity in patients might relate to volumetric asymmetry defined in a meta-analysis, with PTSD patients having smaller left than right amygdalae (Woon & Hedges, 2009) and/or might relate to predominantly right sided amygdala overactivation in PTSD (Shin et al., 2005) and other anxiety disorder (Monk et al., 2008). One case study had indeed reported PTSD diagnosis in a trauma-exposed individual with prior left amygdala resection (Smith et al., 2008).

Altered threat processing in PTSD is also evidenced on the DOT task. We found that patients are slower than controls in disengaging their attention from negative emotional words, similarly to established work on PTSD (Cisler &Koster 2010).

Both attentional and brain alterations in PTSD positively correlate. Larger disengagement index (slower attention shifting from threat cues) correlate with increased amygdala activity. This correlation in only true for the patient group, suggesting it could be a differential pathological factor and could be subsequently considered as a clinical diagnosis. Moreover these alterations positively correlate with trait anxiety, suggesting an enhanced perceptual sensitivity to salient threatening events, whereby we found amygdala hyperactivation induces disengagement difficulty which in turn leads to anxious symptomatology.

To the best of our knowledge, this is the first study causally correlating amygdala activity and attentional bias in PTSD. These findings can be understood in the light of Eysenck theoretical model of attentional bias towards threat in anxiety, stipulating that anxiety would favor bottom-up effect of amygdala on prefrontal cortex (PFC) and weaken top-down regulatory control which would manifest by difficulty in disengaging attention from distracting threat cues (Eysenck, Derakshan, Santos, & Calvo, 2007).

In such terms, we found amygdala activity accounts for 20% of attentional bias variability in PTSD. It could imply that both markers are influenced by common neural cross-

links involved in both tasks. Evidence is piling up on the involvement of the PFC in control of attention to emotional information and disengagement from threat (Cisler &Koster, 2010).

One limitation is that some patients were on stable medical regimen and had other comorbid disorders. None of those factors significantly co-varied with our results. We argue that co-occurrence of mood disorders with anxiety does not play a major role in the threat-related bias of anxious individuals (Bar-Haim et al., 2007). However, their alteration of cognitive and neural processing cannot be totally ruled out. It would be useful in future studies, to explore such interactions in larger PTSD subpopulations with/ without medication and comorbidities. Another limitation is dictated by experimental conditions since the DOT was not conducted in the scanner, somewhat restricting the interpretation of amygdala implication in the attentional task. This design was based on the choice of a validated fMRI paradigm that robustly activates the amygdala, allows the measurement of its threat-related activation, and most importantly is behaviorally insensitive, as stipulated by Browning et al., (2010b) to obtain neural findings unconfounded by behavioral differences between groups.

We have shown that amygdala hyperactivity correlates with PTSD symptom severity and disengagement bias. Threat detection and fear processing depend to a large extent on amygdala and its associated functional outputs; such as the ventromedial PFC. Recent studies have shown differential implication of ventral and limbic regions in other anxiety disorders in attention bias (Van den Heuvel et al., 2005), and should be monitored in PTSD.

On the therapeutic edge, the correlations between amygdala activity, disengagement and symptom severity in PTSD would have valuable clinical implications. First, attentional bias, STAI and PCL-S could constitute a rapidly accessible diagnostic tool to infer on patients' amygdala activation, and its evolution after therapeutic interventions. Second, one would predict that consciously modifying attention orientation would allow modulation of amygdala activity and subsequent ease pathological symptoms. In fact, amygdala activity has been recently modulated by trainings directly involving prefrontal processing (Etkin & Wager, 2007). Approaches such as Cognitive Bias Modification have shown successful outcomes in modifying anxious symptomatology by inducing selective changes in information processing, in non-clinical as well as pathologically anxious populations (Koster, Fox & MacLeod, 2009). They seem to modify the neural systems involved in the control of attention to emotional stimuli (Browning et al., 2010a).

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Restoration of Decreased Functional Activity and Connectivity in PTSD Following Successful Treatment

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Objective: *Post-traumatic stress disorder (PTSD) is an anxiety disorder that arises in the aftermath of a traumatic event. An altered limbic and prefrontal processing of threat cues has been hypothesized as being part of the dysfunction causing aetiology and maintenance of PTSD. This view has been supported by experiments showing increased amygdala and decreased prefrontal activity in PTSD, for instance in fearful faces recognition. The aim of the present study was to assess the BOLD signal and functional connectivity of these structures in PTSD and to explore whether it is inherited or acquired by monitoring PTSD patients before and after successful EMDR treatment and symptom removal.*

Methods: *Sixteen controls were matched in age, sex and education to 16 PTSD patients. 6 PTSD patients were included in a wait-list group. We used an fMRI emotional face matching task with fearful and angry faces.*

Results: *PTSD patients had enhanced amygdala activity and decreased anterior cingulate cortex (ACC) and orbito-frontal cortex (OFC) activity for fearful and angry faces (vs. healthy individuals). Initially, they had less between and amygdala-vmPFC and amygdala-ACC functional connectivity. Both BOLD and connectivity alterations were restored to normal level after successful EMDR but remained unchanged in the wait-list group.*

Conclusions: *These results confirm the existence of an altered fear processing pathway in PTSD mediated by a hyperactivated amygdala and a hypoactivated frontal cortex, which might be at the core of symptomatology. Mostly, these results provide preliminary support for the definition of acquired markers of the pathology.*

1- INTRODUCTION

PTSD is characterized by panoply of symptoms in the aftermath of a traumatic event (American Psychology Association, 1994). These typically include re-experiencing of the aversive event (e.g. flashbacks, nightmares), avoidance of its reminders and generalized hypervigilance (DSM-IV). Although the pathophysiology of PTSD remains largely unknown, the most prevailing hypothesis in PTSD is that of a modified fear processing pathway (Milad et al., 2006). This pathway mainly relies on amygdala and prefrontal cortex (PFC) (Ledoux 1998; Damasio 1994), and these structures are shown to be altered in PTSD.

Extensive animal and human research point to the orchestrating role of amygdala in the acquisition of associative fear learning in classical conditioning tasks (LeDoux et al., 1996, Orr et al, 2000, Milad et al., 2007). Evidence is converging to place the amygdala at the center of PTSD etiology whereby amygdala overactivation might account for exaggerated fear responses and persistence of traumatic memories as well as altered emotional regulation (Rauch et al., 2000). This hypothesis is supported by lesion studies showing its resection abolishes PTSD (Koenigs &Grafman, 2009).

In their recent work, Shin & Liberzon (2010) review numerous studies that have consistently evidenced amygdala hyperactivity in PTSD compared to healthy controls at rest, but also in response to trauma-related imagery, to emotion-inducing photographs or words and to fear conditioning. According to these authors, increased bilateral amygdala activation remains the most robust finding in PTSD while processing emotional cues,

While amygdala is a central part of the neural circuitry of emotion, it does not operate in isolation (Stein et al., 2007). Anatomically, the amygdala is highly interconnected with the ventral portion of the prefrontal cortex (PFC) including the anterior cingulate cortex (ACC) and the orbitofrontal cortex (OFC) in primates (Carmichael &Price, 1995) and humans (Bracht et al., 2009, Johansen-Berg et al., 2008). These 3 frontal areas have consistently shown decreased activation in PTSD (Nardo et al., 2010). In a brief functional overview, the PFC has a role in emotion integration and subsequent guidance of adapted behaviors and decisions (Bechara et al., 1999) and its ventral part is involved in attention and cognitive-emotional association (Bush et al., 2000). Its implication in fear extinction in animal and human studies has led to the elaboration of its role in top-down regulation of the amygdala (Garcia et al., 1999; Milad et al., 2007; Phelps et al., 2004). Deficits in its ability to modulate the activity of the amygdala have been hypothesized to be instrumental in PTSD development

(Hariri et al., 2000). OFC's role in emotional processing is still under investigation (Frodl et al., 2009), and is largely involved in control of behavioral and emotional responses and seems altered in anxiety disorders (de Marco et al., 2006).

Facial expressions have been especially effective in probing increased amygdala response in healthy controls (Hariri et al., 2000) but more so in anxiety disorders such as social phobia (Blair et al., 2010), generalized anxiety disorder (Monk et al., 2008) and PTSD (Cisler et al., 2009). In PTSD, presentation of masked stimuli overstimulates amygdala response in the absence of frontal cortical activation, in comparison to trauma exposed healthy controls (Armony et al., 2005; Rauch et al., 2000). However when faces are overtly presented, their conscious processing additionally implicates prefrontal regions (Shin et al., 2005; Williams et al., 2006). Blood flow in vmPFC inversely correlates to blood flow in amygdala in PTSD on such task, with symptoms' severity positively correlated to amygdala and negatively correlated to vmPFC (Shin et al., 2004 and 2005).

As such, neurocircuitry model of PTSD emphasizes amygdala hyperactivation and frontal hypoactivation, suggesting abnormal fronto-limbic regulation. Researchers have started looking at the functional relationship between those cornerstones of the fear circuit. Neuroimaging studies suggest an alteration of the reciprocal amygdala-mPFC interaction in PTSD (Rauch et al., 2006). PTSD patients are shown to have less resting state amygdala-posterior cingulate connectivity than controls (Lanius et al., 2009). PTSD was shown to have decreased amygdala-ACC connectivity when viewing angry faces (Fonzo et al., 2010). The focus on amygdala-ACC might be incomplete in assessing the altered emotional network in PTSD, and should encompass other nodes of this network. Since amygdala, ACC, OFC and vmPFC are altogether involved in emotion processing (Liberzon et al., 2007) and are known to be altered in PTSD (Nardo et al., 2010), the first aim of our study was to assay neurofunctional alteration of those 4 regions of interest (ROI) in PTSD, to examine both their functional activity and connectivity.

Moreover, controversy exists over the nature and origin of those central fear processing deficits in PTSD. It remains unknown whether these are innate pre-existing vulnerability factors for developing PTSD upon trauma exposure or rather acquired markers of the PTSD after the trauma. Volumetric studies have addressed this issue by using for instance a design with monozygotic twin pairs discordant for trauma exposure. Kasai et al., (2008) included PTSD and non-PTSD combat exposed twins with their combat-unexposed co-twins. They have put forward gray matter density decrease in ACC as an acquired sign of

PTSD, consistent with stress induced loss. An alternative more affordable design to look at inherited v/s. acquired origins of brain alterations in PTSD is to monitor them after symptom removal. Seldom functional neuroimaging studies have addressed the issue by looking at the effect of a treatment on brain alterations in PTSD, and yet have included too few patients (Shin &Libertzon, 2010). They tend to show that pharmacotherapy targets the amygdala decreasing its activity, whereas psychotherapy impacts the PFC conversely increasing its activity (Cisler et al., 2009). The second aim of our study was thus to explore the origins of neurofunctional and connectivity alterations of the 4 ROI in PTSD, before and after symptom removal.

Consistent with previous findings, we hypothesized that traumatized subjects with PTSD would exhibit initial exaggerated amygdala responsivity and decreased prefrontal activation compared to healthy controls in response to emotional faces. Furthermore, we predicted that amygdala-prefrontal functional connectivity would be altered in PTSD. If those alterations are restored after symptom removal, it would evidence their acquired origins as signs of PTSD.

2-MATERIALS and METHODS

2.1 Subjects

Our study included 3 groups: PTSD patients that will receive treatment, PTSD patients on a wait-list and healthy controls.

A total of 20 adult outpatients (12 males and 8 females) were recruited by a psychiatrist among trauma exposed victims at the medico-psychological crisis cell (CUMP) at the Psychiatry Pole of the Conception Hospital in Marseille, France. They all met the DSM-IV criteria for PTSD following a single traumatic event with no previous history of neurologic or psychiatric disorders. Subsequent analysis included 16 patients (10 males and 6 females, with mean age = 33.6 ± 7.9 years and mean education = 7.9 ± 2.6 years after grade 7). Patients had been exposed to their traumatic event for an average of 32.9 months. Four patients took an association of antidepressants and anxiolytics and 2 patients took only anxiolytics.

To control for re-testing effects, 6 patients (4 males and 2 females, mean age = 37.1 ± 8.5 years and mean education = 7.3 ± 4.0 years after grade 7) were similarly recruited at the CUMP and included in a wait-list group.

A total of 16 healthy adult controls 10 males and 6 females (with mean age = 33.1 ± 10.2 years and mean education = 9.6 ± 1.2 years after grade 7), with no history of neurologic or psychiatric disorders, were recruited via screening lists at the clinical investigation centre at the Timone Hospital. Groups were matched for age, sex and education level.

2.2 Psychological Assessment

All participants were assessd by a psychiatrist, using the structured Mini-Internal Neuropsychiatric Interview for DSM-IV (Lecrubier et al., 1998), to check for the absence of psychiatric disorders prior to the trauma in PTSD and screen for PTSD and potential comorbid psychiatric disorders. Participants responded to demographic questionnaires and completed the State-Trait Anxiety Inventory (STAI) (Schweitzer & Paulhan, 1990). Patients also completed trauma related Modified PTSD Symptoms Scale (MPSS) (Weiss & Marmar, 1996). The validated French version was used for all the scales.

2.3 *EMDR Therpay*

All PTSD patients underwent Eye Movement Desensitization and Reprocessing (EMDR) therapy (APA, 2004). According to the APA reports published in 2004, this eight-step standardized protocol is one of the validated treatments for PTSD. Based on an information processing model (Shapiro & Maxfield, 2002), EMDR includes associations of cognitive, emotional and physical assessments of actual distress to traumatic scenery, as well as imaginal exposure while attending to bilateral alternate stimulations. As the patient is asked to visualize the most salient aspect of a traumatic memory, the therapist induces bilateral stimulation (by means of ocular, sensory-motor or auditory stimulations). This results in a change of cognitive processing of memory and cessation of trauma-related distress, while eliminating physical discomfort associated with the initial memory and establishing a positive cognition about the self (Shapiro, 1989). Patients were treated by one of 3 therapists, trained by the French institute of EMDR. There was no fixed number of sessions. Sessions were planned every 7 to 15 days according to patients and therapists availabilities.

The treatment was considered successful and complete when patients reported no more feelings of distress when thinking about their trauma. They were again interviewed by a psychiatrist, using the MINI. They were retested when they no longer met PTSD classification according to DSM-IV criteria. Patients required an average of 4.3 ± 1.7 treatment sessions (ranging from 1 to 7 sessions), lasting on average for 2.5 ± 1.4 months.

2.4 Central Activity: Emotional Face Matching Task

The Matching task used was the one validated by Hariri et al. (2000).

In the emotional condition, subjects viewed a target face and had to select which one of 2 faces presented below it on the same screen expressed the same emotion (fear or anger). In control condition, they viewed a target shape, and chose which of two shapes presented below it on the same screen matched the target (round or oval). The paradigm consisted of 12 experimental blocks of 44.5 s duration each, alternating emotional and control blocks. Each block contained 10 stimuli presented for 4 s with an inter-stimulus interval of 0.5 s. The inter-block interval was 2 s, giving a total scan length of 9 min. We used four different sets of geometric forms for the control blocks, and sixty different images, ten per block, five of each gender, all derived from the Karolinska database (Lundqvist, Flykt & Vhman, 1998), for the emotional blocks.

2.5 fMRI specification

All data acquisition was performed on a 3-T MEDSPEC 30/80 AVANCE imager (Bruker, Ettlingen, Germany) at the fMRI center of Marseille, France. All stimuli were generated on a computer and back-projected onto a screen that subjects viewed though a mirror positioned above their eyes. After an initial localizing scout scan to place image slices, fMRI scans were acquired using a T2*-weighted gradient-echoplanar sequence (TR/TE=2533/30 ms; FOV=19.2×19.2 cm, 64×64 matrix; flip angle= 82.4°. Thirty-eight interleaved axial slices, tilted -30^{o} to the intercommissural plane, in order to reduce artifacts in prefrontal regions, were obtained with a contiguous slice thickness of 3 mm. Following the fMRI scans, a set of high-resolution T1- weighted images were acquired for the purpose of anatomical identification (sagittal MPRAGE Sequence, TE/TR = 4/10 ms, TI = 800 ms, Flip Angle = 30°, Matrix=256×256×128).

2.6 Procedure

The investigation was carried out in accordance to the latest version of the Declaration of Helsinki. After receiving clear explanations of the procedure, participants provided informed consent in accordance with the guidelines set forth by the local ethical committee CPP South Mediterranean 2. Before starting the experiment, all participants completed the STAI and patients additionally completed the MPSS. They were then installed in the fMRI scanner and performed the face matching task.

2.7 Data and Statistical Analyses

2.7.1 Behavioral data Analysis on the Matching task

Differences in task performance (accuracy score and reaction time) between control and patient groups were compared using an ANOVA with a significant $p < 0.05$. Only trials in which the subjects answered within the 4 sec time-frame of image presentation were recorded and considered for analysis.

2.7.2 fMRI data analysis of the Matching task

Data were processed using SPM5 software (Wellcome Department of Cognitive Neurology, University College London) implemented in Matlab 8.0 (Mathworks Sherborn, MA). The first 4 scans, corresponding to a period of signal stabilization, were discarded. The remaining scans were corrected for differences in slice acquisition time. To remove the effects of head movement during scanning, the 234 scans of each session were realigned to the first scan of the session. All images were transformed into a standardized coordinate system corresponding to the MNI (Montreal Neurological Institute) space. The normalized images were then spatially smoothed with an isotropic Gaussian kernel (full width at half maximum of 8 mm).

Individual statistical maps were calculated for each subject to evaluate differences between the emotional versus control conditions. Each condition was modeled by a box-car convolved with a canonical hemodynamic response function.

The within-subject contrast images were then entered into a second-level t-test to examine both within- and between-group effects, with an a priori Region of Interest (ROI) with an anatomical mask selected using the WFU Pickatlas (Version 2.4). Between-group comparisons were constrained to the amygdala ROI; using a Small Volume Correction (SVC) approach ($p < 0.001$ with a minimum of 5 voxels). The choice and definition of the ROI is based on a validated functional model on an identical face matching task in healthy controls (Stein et al., 2007). Authors have used a bootstrapping approach in a large data set of participants They have defined amygdala, vmPFC (BA25), ACC (BA32) and OFC (BA11) based on prior knowledge of their interaction in emotional processing and activation or functional connectivity to the amygdala.

An ANOVA was performed on the extracted ROI peak maximum, with Group (controls, treated patients) as a between factor and Session as a within factor. A significant level of $p<0.05$ was taken and post-hoc were Bonferoni corrected. A separate ANOVA was

similarly performed with the treated and wait-list patients groups to directly compare the second testing session in treated (a-symptomatic) and untreated (symptomatic) PTSD patients.

2.7.3 Functional Connectivity Analysis

Similarly to Bettus et al., (20029), we assessed the functional connectivity between the amygdala and each of the vmPFC, ACC and OFC. An automated functional connectivity analysis was performed that correlated extracted peak activity from aforementioned ROI. These ROIs were used as masks applied onto the residual images to extract the mean signal time-courses from each predefined ROI. To determine functional interactions between ROIs in each temporal lobe, correlation coefficients between pairs of signal time-courses were computed (JMP statistical software). Correlation coefficients were then normalized using the Fisher transformation (rN=0.5*Log[(1+r)/(1-r)]) to reflect basal functional connectivity and to perform subsequent statistical analyses.

This returned a correlation r-value that was transformed into z-score. Z-scores for populations were then compared using an ANOVA with a significant p value<0.05 and post-hoc comparisons subjected to Bonferoni corrections.

3- RESULTS

3.1 Clinical Data

Groups did not differ in terms of age, sex and education. In accordance with the clinical evaluations, PTSD patients initially scored significantly higher than healthy controls on STAI-trait and STAI-state, and individual scores on the PCL-S scale were higher than the cut-off for pathology. After treatment there was no difference between the 2 groups (Table 1).

A.	Healthy CONTROL (Time 1) (n=16)	Healthy CONTROL (Time 2) (n=16)	PTSD Pre-EMDR (Time 1) (n=16)	PTSD Post-EMDR (Time 2) (n=16)	STATISTICS
STAI Trait	27.1 (5.5)	26.7 (6.5)	50.3 (15.5)	31.4 (7.8)	$F(1,30) = 16.68^{**}$
STAI State	31.4 (5.4)	31.4 (6.9)	55.3 (8.0)	40.6 (9.0)	$F(1,30) = 26.97^{**}$
PCL-S	-	-	58.1 (11.4)	26.9 (7.2)	t(1,28)= 9.2**
MPSS	-	-	67.9 (19.8)	14.56 (13.2)	$t(1,28) = 8.77^{**}$

B.	PTSD Pre-EMDR (Time 1) (n=16)	PTSD Post-EMDR (Time 2) (n=16)	PTSD wait-list (Time 1) (n=6)	PTSD wait-list (Time 2) (n=6)	STATISTICS
STAI Trait	50.3 (15.5)	31.4 (7.8)	48.2 (10.0)	49 (13.2)	
STAI State	55.3 (8.0)	40.6 (9.0)	58.7 (10.3)	56.5 (11.9)	
PCL-S	58.1 (11.4)	26.9 (7.2)	64 (13.3)	63 (14.5)	
MPSS	67.9 (19.8)	14.56 (13.2)	72 (23.7)	74 (34.3)	

Table 1. Characteristics of participants: Mean (SD) for Beck State Trait Anxiety Inventory STAI-Y, and for the patients Modified PTSD Symptoms Scale MPSS. Significant p-value: * $p < 0.05$ $^{**}p < 0.001$
A. compares PTSD patients and healthy controls and B. PTSD patients treated and on a wait-list.

3.2 Results on the Behavioral Performance in the Matching task

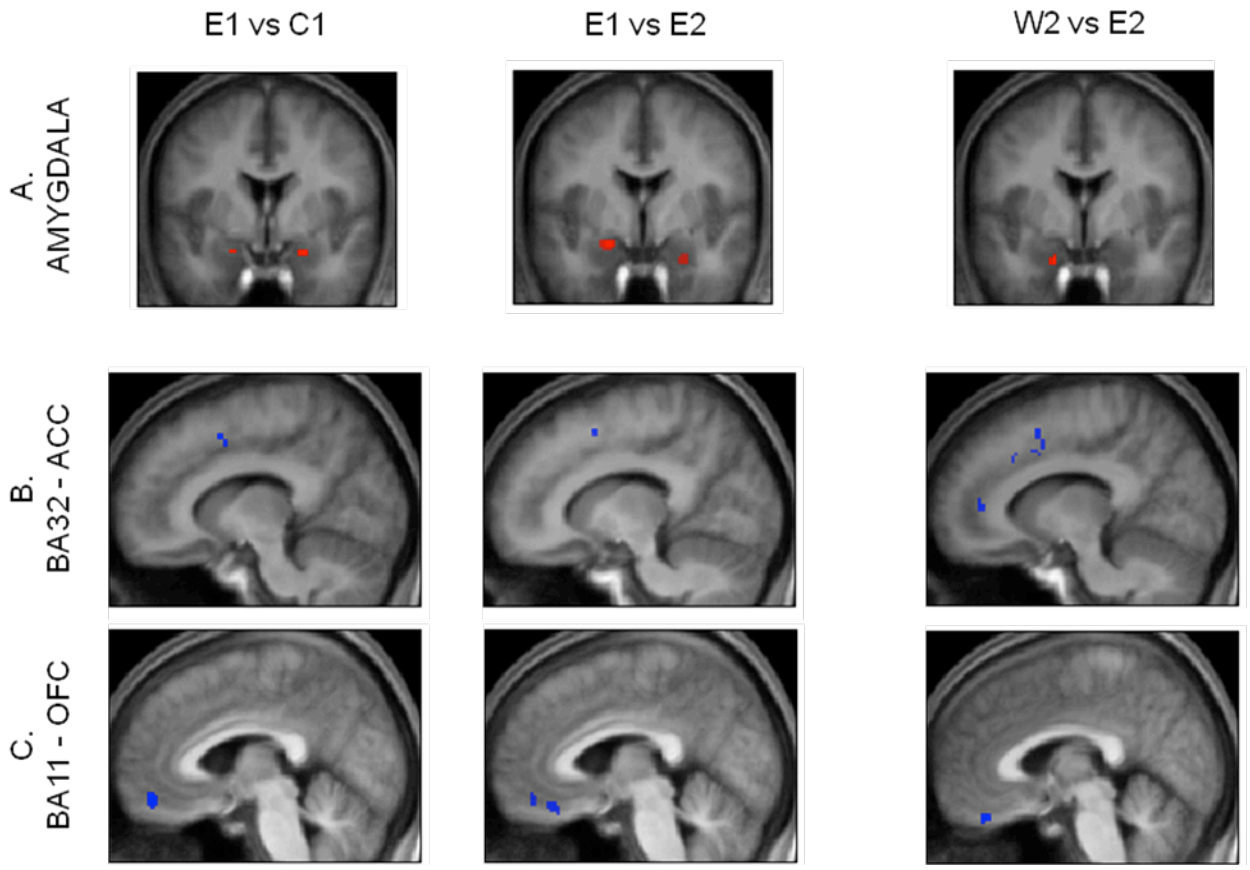
There was no significant difference between groups in terms of accuracy and reaction time for the emotional matching and control conditions.

3.3 *Results on the fMRI BOLD data for the Matching task*

There was a significant Group X Session interaction for left ($F(1,30) = 6.17$; $p<0.05$) and right amygdala activity ($F(1,30) = 4.89$; $p<0.05$), BA32 activity ($F(1,30) = 4.74$; $p<0.05$) and BA11 activity ($F(1,30) = 3.749$ and $p<0.05$) in the emotional matching vs. control contrast (Fig.1).

Results show an initially increased BOLD activity in the right (x,y,z = 22,9,-18) and left amygdala (x,y,z = -27,0,-16), in the PTSD group compared to the control group before treatment but not after.

Patients also had initial decreased blood flow activity in the BA32 (x,y,z = 12,10, 48), and BA11 (x,y,z = -46,40,-12) compared to healthy controls. After successful EMDR, there was no difference between patients and control groups in BA32, BA11 or BA25 BOLD activations.



Article 5. Fig 1. BOLD matching effect. Statistical parametric maps illustrating differential patterns of activation of the matching contrast for each ROI A. Amygdala, B. BA32 and C. BA11. C1 Healthy Controls (Time 1), E1 PTSD patients pre-EMDR(Time 1), E2 PTSD patients post-EMDR (Time 2) W2 wait-list patients (Time 2). ACC is for anterior cingulate cortex, and OFC for orbitofrontal cortex.

Compared to controls, PTSD patients initially showed increased amygdala activation and decreased BA32 and BA11 activity in the emotional versus the control condition. This was true for W1 and W2 but not after successful EMDR. Post-treatment, patients had similar BA32 and BA11 activations as controls. Voxels reaching significance at $p_{FDR}<0.05$ level are rendered onto a normalized averaged T1-anatomical image.

3.4 Functional Connectivity analysis

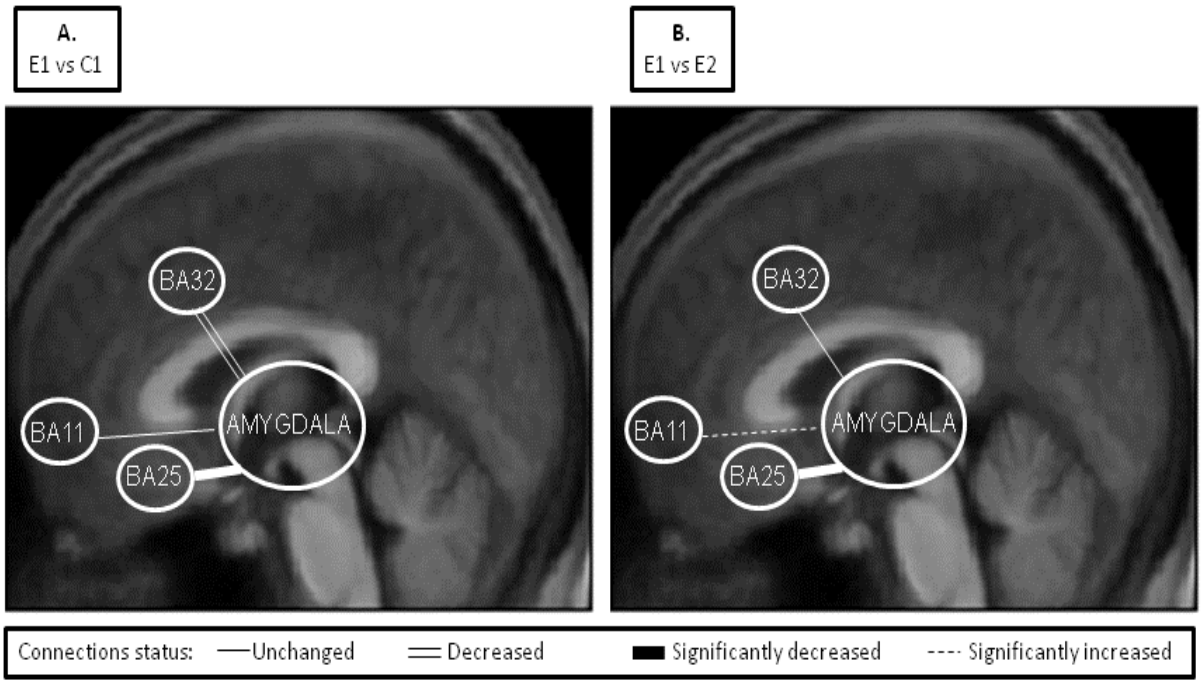
Functional analysis was assayed between amygdala-BA11, amygdala-BA25 and amygdala-BA32.

There was a significant Group X Session interaction in the functional connectivity for amygdala-BA25 with $F(1,29) = 4.26$ and $p< 0.05$, (Fig.2). Post hoc analysis show an initial decrease in functional connectivity for the amygdala-BA25 in PTSD patients compared to controls. After EMDR, there was no difference in amygdala-BA25 connectivity between patients and controls.

We found a trend of a decrease in functional connectivity for the amygdala-BA32 relative to controls (F (1,29)= 3.60 and p= 0.068). This trend remained in PTSD patients after EMDR.

Initial amygdala-BA11 was similar in patients and controls. Post-EMDR patients however had a significant decrease in amygdala-BA11 connectivity compared to both pre-EMDR patients and controls.

For the wait-list group, there was a significant Group X Session interaction for the amygdala-BA25 with F= and p<0.05. The decreased amygdala-BA25 initially present at W1 was not restored at W2.



Article 5. Fig 2. Functional Connectivity. Illustration of differences in functional connectivity between groups for the emotional v/s. neutral matching contrast within the 4 ROI: amygdala, BA32, BA25 and BA11. A. E1 versus C1 and B. E1 versus E2 C1 Healthy Controls (Time 1), E1 PTSD patients pre-EMDR(Time 1) and E2 PTSD patients post-EMDR (Time 2).

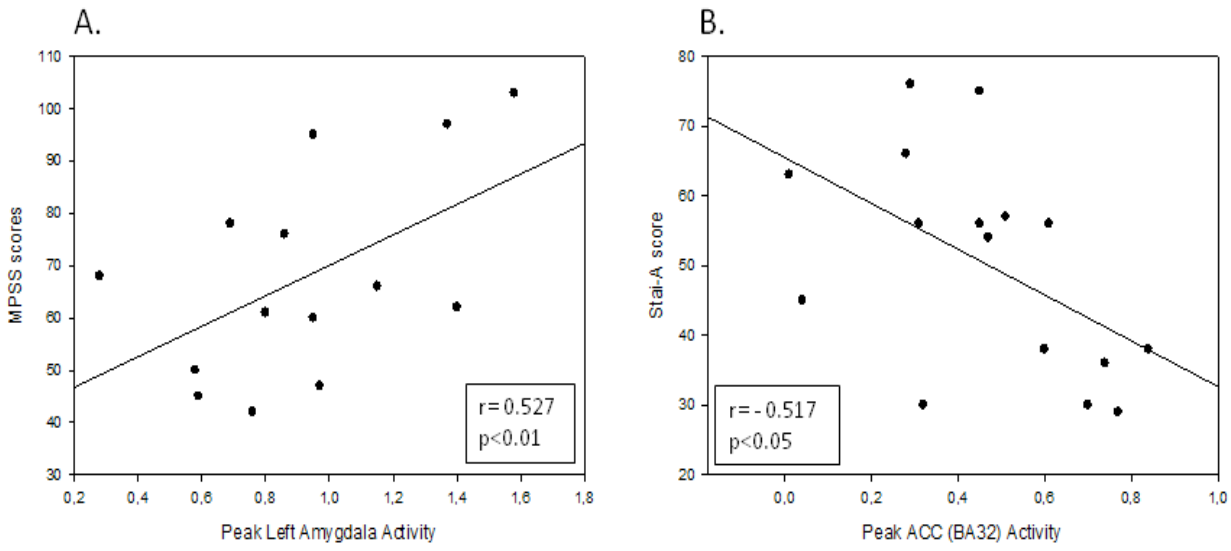
Compared to controls, PTSD patients initially showed decreased amygdala connectivity to BA32 and significantly decreased BA25connectivty in the emotional versus control condition. Post-EMDR, patients recover amygdala-BA25 connectivity but not amygdala-BA32. They have decreased amygdala-BA11 connectivity compared to pre-EMDR condition.

3.5 Correlation analysis

This analysis showed a positive correlation between the left amygdala activation and PTSD scores for the matching v/s. control contrast, with n= 14, r= 0.527; p <0.05 and a negative correlation between the BA32 activation and state anxiety with n=16, r= -0.517; p <0.05. This indicates that higher amygdala activation corresponded to more severe symptoms on the MPSS scale and that lower BA32 activation corresponded to larger anxiety scores on the STAI state (Fig. 3A and 3B). This was only true for the PTSD patient group.

3.5 Adjustment for comorbidities and medication.

To evaluate the effect of comorbid mood and anxiety disorders as well as medication use on the amygdala activity and on the other ROI, we compared the mean of the PTSD subpopulations with and without depression, with and without other anxiety disorder and with and without medications. We found no significant difference in PTSD subgroups for any of the aforementioned factors with corresponding measures of either ROI activation; meaning that for a given factor both subgroups of PTSD patients had comparable means.



Article 5. Fig 3. Correlation results. Illustration of the Pearson correlations index and p-value of correlation between A. Increased amygdala activity and higher PTSD symptoms severity measured by MPSS, B. Increased BA32 activity and lower state anxiety measured by STAI-A in the treated patient group.

4- DISCUSSION

Stemming from the lack of research investigating functional connectivity of the neurocircuitry of fear processing in PTSD, we chose to study the functional activity and connectivity of the amygdala, ACC, OFC and vmPFC, before and after symptom amelioration. Consistent with current neural models of PTSD, our results suggest exaggerated amygdala responsivity and deficient top-down governance of the amygdala by vmPFC. We provide evidence for acquired neural alterations of PTSD as symptom amelioration restores some of their normal functioning.

Neurofunctional analysis clearly differentiated patients and controls, although groups show no behavioral differences in reaction times in the matching task. Consistently with the massive literature showing amygdala, ACC and OFC deficits in PTSD when processing threat cues (vs. healthy controls) (Nomura et al., 2004; Shin &Libertzon, 2010), we found that PTSD patients initially had hyperactivated amygdala and hypoactivated ACC and OFC during processing of emotional faces. This prefrontal deficit, on top of exaggerated amygdala, is a feature that distinguishes PTSD from other anxiety disorders, also marked by excessive fear (Shin &Libertzon, 2010). The hypoactivation of rostral ACC (BA32) and adjacent OFC (BA11) mostly involved in emotional experience and regulation could subsequently underlie the PTSD characteristic symptoms of emotional numbing, re-experiencing, intrusiveness of traumatic material and constant state of anxiousness and hyperactivity (Etkin &Wager, 2006). We did not find vmPFC hypoactivation in PTSD as this task is not a suitable probe for its activation. Other studies using the matching task do not report vmPFC involvement either (Hariri et al., 2000; Fakra et al., 2009 and Salgado et al., 2010).

We support recent findings associating fronto-limbic impairments with PTSD symptomatology. We found that increased amygdala activity correlates with more severe symptoms on MPSS in patients and ACC inversely correlated with state anxiety in the patients group only. In such regards, we further show that symptom amelioration restores altered functioning in PTSD. In fact, after successful EMDR, amygdala, ACC and OFC activity in PTSD was similar to controls, whereas their activity remained altered in the wait-list symptomatic group. To the best of our knowledge, this is the first time amygdala, ACC and OFC are monitored in PTSD after symptom removal. Their modification after EMDR suggests that they could be considered as acquired markers of the pathology.

Besides functional alterations of limbic (amygdala) and frontal areas (ACC, OFC) in PTSD, we also found altered fronto-limbic connectivity.

First, as hypothesized, our observations illustrate initial diminished amygdala-ACC and significantly decreased amygdala-vmPFC connectivity in PTSD compared to controls. vmPFC projects directly to the amygdala and modulates its output in fear conditioning (Koenings et al., 2008). According to theses authors, amygdala can also modulate prefrontal activity, either directly or via connection with the ACC. This goes along findings of trauma altering emotional processing at the amygdala and mPFC sites, whereby PTSD patients were characterized by an uncoupling of the amygdala-ACC during the time-course of emotional face attending (Williams et al., 2006). This same study suggests that loss of prefrontal regulation in PTSD is exacerbated with increasing symptom severity. We have shown that Symptom removal via EMDR restored amygdala-vmPFC connectivity in the treated patients but not the wait-list group, providing evidence that symptomatology in PTSD is most likely exacerbated because amygdala overactivation is inadequately regulated by the prefrontal. This decreased connectivity is a reversible acquired marker of PTSD.

Second, post-EMDR patients had decreased amygdala-OFC connectivity. This bi-directional connection might provide a route by which emotions affect attentional system. is thought to be critical for stimulus-reinforcement association learning (Pears et al., 2003). Additionally, diminished amygdala-OFC after symptom amelioration might imply weakening of amygdala bottom effect on ACC processing. In fact, attending to fearful faces causes a flow of information generated in the amygdala to reach the ACC through OFC (de Marco et al., 2006), whereby the amygdala sends ventral projection to the OFC through the uncinate fascicle (Bracht et al., 2009). Alternatively, Stein et al., (2007) have shown a positive path coefficient directed from PFC to amygdala, interpreted as the degree to which increase activity in the OFC predicts increased activity in the amygdala. Interpretation of BOLD in terms of excitatory or inhibitory terms remains limited, and this diminished OFC-amygdala connection could suggest increased inhibition of amygdala. Conversely, this diminished connection could represent a neural “scar” of PTSD symptomatology that might leave room for risks of relapse.

Although the directionality of the interaction remains to be tested, these findings can be better understood in the light of Eysenck theoretical model of anxiety, stipulating that anxiety would favor bottom-up effect of amygdala on prefrontal cortex (Eysenck et al., 2007) and weaken top-down regulatory control of vmPFC (Milad &Rauch, 2006). Treating anxiety by EMDR therefore resulted in favoring the opposite trend; that is for instance weakening the bottom-up effect of amygdala on OFC, and restoring normal top down control of vmPFC on amygdala to hamper its hyperactivity.

Locations of cortico-limibic alterations in PTSD vary across studies. Functional and anatomical definition of areas remain approximate and study-dependant as the OFC is sometimes defined to include areas of the vmPFC and ACC (Milad &Rauch, 2006), and the ACC is to include parts of BA 24, 32 and 25 (Bush et al., 2000). Characterizing the neurocircuitry of PTSD becomes a tedious task and is additionally complexified by the fact that a given area seems to have specialized heterogeneous subfunctions that might be interdependent in a given task.

Overall literature on processing emotional stimuli tends to consider ventral and dorsal routes of processing. A ventral emotional generation, affect-oriented flow and a dorsal emotional regulation, more prone to cognitive control one. Ventral and dorsal parts of each area might differentially contribute to this network.

For instance, OFC, thought to integrate sensory modalities and influence behavioral outcome to emotional experience, is anatomically subdivided to lateral and medial sub-domains (Milad &Rauch, 2006). The lateral network receives input from different sensory modalities and in turn, projects to central and dorsal striatum influencing reward/punishment behaviors and negative affect processing. The medial part projects to the amygdala, hypothalamus, and hippocampus and influence positive emotional expression. This model suggests that lOFC would be hyperactivated whereas mOFC would be deficient in anxious individuals and PTSD patients. Similarly ACC can be subdivided based on anatomical connectivity: a pregenual region strongly connected to medial prefrontal and anterior midcingulate cortex and a subgenual region with strongest connections to nucleus accumbens, amygdala, hypothalamus, and orbitofrontal cortex (Johansen-Berg et al., 2008)

Bottom-up amygdala effect on cortical structures can be direct (for e.g. on ACC through thalamic peduncle) or indirect (via the OFC). Similarly top-down control of limbic functioning can be directly or indirectly exerted by each cortical structure.

In addition to exaggerated amygdala activation and diminished ACC and OFC activity in PTSD, abnormalities in functional connectivity in amygdala-ACC and amygdala-vmPFC seem to pave the way to PTSD psychopathology. It is therefore important that future fMRI studies involve, not only assessment of functional activation, but also functional connectivity of involved brain structures. Genotype-related alterations in anatomy and function of the amygdala-cingulate feedback circuit critical for emotion regulation were shown to be correlated to increased anxiety-related traits and increased amygdala reactivity (Pezawas et al., 2005). Furthermore, the magnitude of coupling inversely predicted almost 30% of variation in anxiety.

Compared to cognitive and behavioral therapy, it is surprising to observe that after only an average 4.3 EMDR sessions (about 2.5 months of therapy), the altered brain processing and connectivity in PTSD is immediately restored. Studies have so far shown that successful EMDR treatment reduces the autonomic responsiveness of patients to aversive stimuli such as trauma recall (Aubert-Khalfa et al., 2008; Sack et al., 2008).

The biological basis of this quite fast process and its neuroanatomic correlates have been seldom studied although two experiments using SPECT suggest that the anterior cingulate cortex (Levin et al., 1999) as well as the left medial ventral frontal gyrus (Lansing et al., 2005) would be more activated post than pre-EMDR treatment. A third study did not evidence any brain activity difference before and after EMDR (Pagani et al., 2007). We show that symptom elimination following successful EMDR seems to restore limbic and cortical activation as well as their functional interactions. It could be that EMDR therapy targets functionality of amygdala decreasing its hyperactivation and prefrontal areas such as ACC and OFC increasing their input on one hand and on the other modulates the connectivity of areas involved in the fear circuitry, strengthening for instance the amygdala-vmPFC interaction.

One limitation of the study is that some of the patients evaluated were on stable medical regimen for antidepressants and/or anxiolytics (6 of 16 patients) and had other comorbid anxiety and/or mood disorders (13 of 16 patients). None of the aforementioned factors (comorbidities and medication) significantly influenced our results. However, their alteration of cognitive and neural processing cannot be totally ruled out. It would be useful in

future studies, to further explore drug and comorbidity interaction with neurofunctional processing in larger PTSD subpopulations with or without medication and with or without comorbidities.

Additionally, effect of symptom elimination in PTSD should be monitored in future paradigms by other treatment options such as Cognitive Behavioral Therapy, to better define acquired or inherited physiological markers in PTSD.

Convergent, with these functional imaging results, structural neuroimaging studies of PTSD have shown selectively reduced vmPFC and hippocampal volumes (Rauch et al., 2006). An elegant twin study of Vietnam veterans showing that ACC gray matter (GM) loss seems to be predominantly acquired impairment of the PTSD pathology rather than merely traumatic exposure or inherited vulnerability (Kasai et al., 2008). Similarly, a recent study evidences reduced GM density in posterior cingulate in PTSD patients compared to trauma-exposed individuals (Nardo et al., 2010). This group also puts forward diminished CC and amygdala GM in EMDR treatment responders v/s non responders. Further studies are needed to assess potential volumetric alterations and gray matter volume loss within the amygdala, ACC, OFC and vmPFC in PTSD, before and after EMDR.

Our study of emotional face matching in PTSD shows that exaggerated amygdala activity, deficient top-down governance by vmPFC, as well as hypoactive ACC and OFC are all acquired markers of the pathology. They are restored after symptom removal by EDMR. Other aspects of the PTSD Further follow-up studies are to better define potential structural modifications in PTSD induced by EMDR.

CHAPTER IV

DISCUSSION

There are about 450 million people who suffer at a certain point of a neurological, psychiatric or behavior related disease; that is about 25% of all the inhabitants in the world. Mental disorders contribute significantly to the Global Burden of Disease. The WHO (WHO, 2001) states that four out of the ten diseases with the highest burden are psychiatric: depression, alcohol abuse, schizophrenia and bipolar disorder, two of which are highly comorbid in PTSD.

Three decades of research on PTSD has significantly improved our understanding of this highly prevalent subtype of anxiety disorders, but have also revealed that much remains to be done to properly investigate the billions of neurons and trillion of connection that make up the brain networking, which alterations could be innate or acquired by trauma exposure.

We have addressed this challenge by:

- First, examining central, cognitive and peripheral alterations in PTSD. Based on the most prevalent hypothesis of a central deficit in the fear processing network in PTSD, we have assayed this deficit in four validated paradigms:
  - Fear conditioning and extinction
  - Attentional bias
  - Emotional attending and suppression
  - Emotion recognition
- Second, monitoring changes of those neural, cognitive and psycho-physiological alterations with symptom amelioration, before and after EMDR, to address the correlation to symptomatology and infer on innate/acquired features of PTSD.

For clarity issues, we articulate the discussion around:

- An overview of the major findings detailed in our 5 articles previously presented
- A general discussion of our findings
- A special focus on PTSD's sensory modality and comparison with other anxiety disorders
- An overview of the advancement this study brings to PTSD treatment and diagnosis
- A listing of the major limitations of our study
- A suggestion of future directions in continuation of this work.

1. Major Findings

Based on the literature review, we hypothesized that PTSD patients would mainly suffer a central malfunction of emotional processing hubs; including a hyperactive amygdala and a hypoactive PFC, accompanied by an altered connectivity of those two cornerstones of the fear circuitry. This would manifest by altered fear conditioning and extinction, compared to healthy controls. The central deficit would also account for other aspects of PTSD symptomatology including emotional and attentional processing, and could be monitored at the neural, behavioral and physiological levels. In such terms, PTSD would subsequently have initial heightened hypervigilance to negative or threat-related words, pictures and films compared to the healthy group. The PTSD pathology would be associated with increased sensitization to fear conditioning and delayed extinction, exaggerated verbal and physiological activations in emotional attending and suppressing of highly arousing emotions and disengagement difficulties from threat cues.

We hypothesized those altered mechanisms in PTSD would be positively correlated to symptomatology. As such, we hypothesize PTSD would have neurally and behaviorally impaired fear processing, emotional and cognitive mechanisms, that would be restored after symptom removal by successful EMDR. If these emotional and attentional alterations are indeed restored after symptom amelioration by EMDR, this would provide preliminary evidence of them being acquired features of PTSD.

The first part (part I-) of our study included the monitoring of peripheral impairments in PTSD before and after EMDR by monitoring their physiological markers on three main tasks: fear conditioning, emotional regulation and attention orientation. Significant results for each task are detailed in an article submitted for publication and summerized below.

ARTICLE 1. The aim of the fear conditioning and extinction task was to monitor physiological manifestation of central deficits in PTSD via reproducing verbal and physiological fear processing deficits in this paradigm, and further explore whether it is restored after a treatment ameliorating core PTSD symptoms.

This paradigm illustrated major psycho-physiological deficiencies in PTSD pathology in the fear-processing pathway. First and foremost we replicated the electrodermal results of Blechert et al. (2007) using the same classical fear conditioning and extinction paradigm. We found that PTSD had higher SC at acquisition and slower SC decrease at extinction compared to controls. We also found that patients have elevated SC to both CS+ and CS- at acquisition,

and only to CS+ at extinction. This differential conditioning in PTSD is similar to some of the previous findings (Orr et al., 2000 and Blechert et al., 2007), and contradicts others (Grillon & Morgan, 1999).

Our finding argues with the sensitization of PTSD to an aversive context and the ensuing increased responding to either stimulus. PTSD patients can learn safety but have difficulty inhibiting the conditioned fear response. Similarly to SC activity, PTSD showed enhanced conditionability for additional physiological (frowning EMG and HR) and verbal factors at acquisition, supporting previous findings (Orr et al., 2000). At extinction however, only SC and verbal evaluations differentiated both groups as patients still had high electrodermal activity and aversive verbal ratings to CS+ compared to controls (Fig. 13). Along with verbal ratings, SC seems to be the most sensitive maker to differential fear conditioning in PTSD. It might be that higher brain centers regulating physiological factors are differently disturbed in PTSD. In fact, EMG and HR are known to be modified in PTSD in other emotional tasks than conditioning (Guthrie &Bryant, 2006; Miller & Litz, 2004; Orr et al., 1998b). They seem to reflect patients' hyperactivity in aversive contexts rather than to aversive cues. Additionally EMG is under voluntary control and HR involve sympathetic and parasympathetic regulation, whereas SC is under sole sympathetic control.

Our results indicate that psycho-physiological impairments in patients with PTSD might be represented as such by increased fear sensitization at acquisition and delayed extinction of conditioned fear. Although inconclusive at that stage, our results support the emerging literature showing PTSD patients have high sympathetic activity and low parasympathetic control at rest (Blechert et al., 2007b).

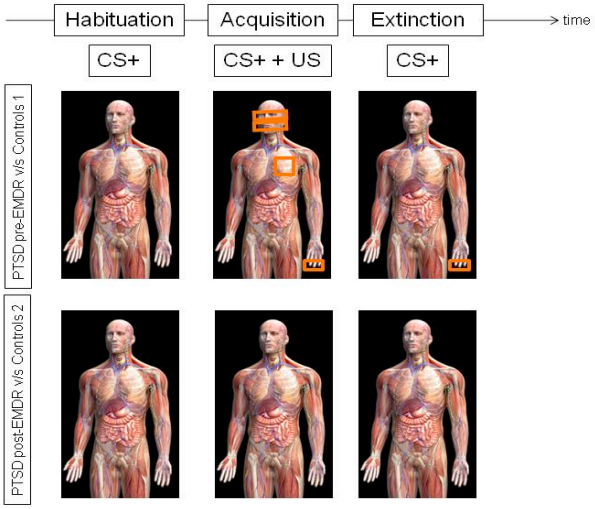


Fig. 13. Illustration of initially increased conditionability in PTSD at acquisition and delayed extinction compared to controls. Facial muscles, heart rate and electrodermal conductance are restored after EMDR.

ARTICLE 2. We then investigated how emotional generation and regulation are altered in PTSD, not only in fear conditioning but when volitionally attending or suppressing emotions with varying arousal and valence levels (happiness, peacefulness, fear and sadness), and whether they are restored after a treatment ameliorating core symptoms by EMDR.

The study illustrated psycho-physiological deficiencies in PTSD in emotional attending and suppressing using clips with varying valence and arousal. It included assessment of HRV in PTSD during a 5 min rest period. This has provided further evidence that PTSD have lesser parasympathetic tone than controls. This low vagal tone (parasympathetic) observed in PTSD is associated with poor emotion regulation (Thayer & Lane, 2000). Conversely, authors suggest that high vagal tone can allow the individual to better selectively attend to aspects of situations, thus enabling adaptable responses. Studies have previously described increased basal HR in PTSD (Litz et al., 2000). Together with decreased parasympathetic outputs, it could represent an automatic preparation for threat in PTSD, in uncertain emotional contexts.

We have failed to show intergroup differences at the physiological level for the attending task. Even though there was a tendency for higher SC in patients than controls when watching highly arousing clips, significant threshold were not reached most probably due to a ceiling effect as we chose the films to be quite intense. This goes along other studies showing groups only differed under priming conditions (Litz et al., 2000).

In a study led by our team using the same emotional attending paradigm in controls (Reynaud et al. - Appendix 2), we evaluated the same physiological parameters and found that they could be markers of personality trait. Personality traits are generally divided to five schemes: extroverted, neurotics, agreeable, open and conscious. Since neurotics are reported to behave differently than others in emotional settings, controls were divided in high v/s. low neurotics group. Groups did differ in emotional processing whereby the high neurotics resembled the PTSD group in having increased SC for fear and happiness. Knowing that neuroticism is a risk factor for mental health disorders, emotional disturbances could be markers of subsequent anxious symptomatology. Besides, central mechanisms underlying emotional regulation could be similarly altered in PTSD and neuroticism, as for instance mOFC thickness is shown to be inversely correlated with neuroticism scores (Rauch et al., 2005).

In our current study, PTSD emotional dysfunctions stemmed mainly from deficient suppression. Patients did less well than others in controlling their emotions (Fig. 14).

This results parallels other studies on PTSD showing the reported level of perceived controllability over intrusive thoughts for PTSD participants was significantly lower during the suppression (as compared with the expression) phase relative to non-PTSD (Shipherd &Beck; 1999). This cognitive perception in patients is in adequacy of their physiological hyper-arousal when viewing films (Orsillo et al., 2004), which is more exacerbated in comparison with controls in our paradigm during the suppressing than attending tasks. Emotional suppression leads to increased sympathetic activation (Gross & Levenson, 1993). This system is involved in the preparation of intellectual activity and may reflect the cognitive effort directed towards emotion control. The autonomic responses enhanced in PTSD v/s controls during suppression could be an additional byproduct of the imbalance of sympathetic over parasympathetic ratio in PTSD. Highly arousing emotions that stimulate sympathetic alarm system are shyly counterbalanced by the parasympathetic system, accounting for the hyperactivity of patients. Additionally brain structures such as amygdala, vmPFC, OFC and ACC, known to be altered in PTSD (Shin et al., 2010), have direct projection to the midbrain where centers of physiological functions exists (Ledoux, 2000).

The emotional deficits in PTSD seem worsened by inefficient control mechanisms to down regulate unwanted physiological arousal. Contrary to what is implied by emotional numbing in PTSD, patients might subsequently voluntarily avoid emotional situations since they are aware of their uncontrollability. It also appears like PTSD participants' attempts at regulating emotion were ironically furthering their symptoms. The DSM-V suggests a revised version of PTSD separating emotional hyperactivity and numbing in two diagnostic clusters.

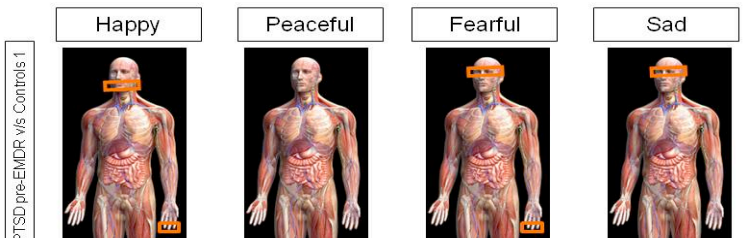


Fig. 14. Illustration of the physiological results in patients and controls during the suppression task when viewing emotional films inducing happiness, peacefulness, fear and sadness. Orange squares indicate increased activity in the corrugator, zygomatic or electrodermal conductance.

ARTICLE 3. Since one of the key factors of the emotional distress and maintenance of anxiety disorder is the existence of non-adaptive attentional bias towards information with aversive value, the aim of this task was to investigate the cognitive strategy underlying attentional bias to threat cues (disengagement v/s vigilance) in PTSD, and to assess whether normal cognitive processing is restored after a treatment ameliorating core PTSD symptoms.

We have replicated previous results on the PTSD patients' attention bias towards emotionally negative cues in an e-Stoop task (Bush et al., 2000) and further refined that the underlying cognitive strategy stems from difficulty disengaging their attention from threat in a DOT task. Patients are initially slower than controls in responding in the presence of an emotionally negative word. They behave similarly to patients diagnosed with other anxiety disorders such as generalized anxiety disorder (Bradley et al., 1995), social phobia (Amir et al., 2003), panic disorder (Buckley et al., 2002) and non-clinical high trait anxiety subjects (Cisler &Koster, 2010). Anxious patients are in fact characterized by disengagement bias from threat cues (Bar-Haim et al., 2007), as opposed to patients with depressive disorder having a vigilance bias and being faster in the presence of threat cues (Dalgeish et al., 2003).

The PTSD model is implemented by recent observations of brain structures involved in attentional bias tasks such as the amygdala and anterior cingulate cortex (Whalen et al., 2006; Frewen et al., 2008). These same structures are known to be to be altered in PTSD (Shin et al., 2001). They seem implicated in threat detection, orientation of attention and its maintenance in anxiety disorders (Cisler et al., 2010 and Browning et al., 2010) and fairly account for impairments of cognitive processing in PTSD, as shown by fMRI when assessing a rather small-sized group of patients (Bremner et al., 2004). It takes 12 msec for the nervous impulse generated in the thalamus to reach the amygdala and twice as long for the cortical input (Ledoux, 2000). In our study, the 15 msec delay causing disengagement bias in patients could result from a slowed capacity of PFC to encode emotional information and direct attention.

In the part –I- of our study we have come to replicate certain deficits previously described in PTSD at the physiological, emotional and cognitive level. We have shown all physiological impairments are related to anxious and traumatized symptomatology. To the best of our knowledge this is the first time such impairments are tested in parallel and are further monitored after symptom amelioration in PTSD.

We have shown that altered fear processing (at acquisition and extinction), emotional suppressing and attention orientation are restored in PTSD post-EMDR. We have explored the implicit (physiological) and explicit (verbal) conditioning pre- to post-EMDR and found that after symptom amelioration, fear conditioning was no longer facilitated in PTSD and fear extinction developed similarly to controls. We have also shown that the improved regulation of emotions corresponds to normalized physiological parameters. This restored emotional processing is associated with restored basal HR and HRV. Barlow et al., (2004) suggests the pre to post-treatment restoration of HRV in clinical populations could be a marker of successful outcome.

The disengagement bias evidenced in PTSD also vanished post-EMDR. Symptom-free patients had null e-Stroop and disengagement indices, similarly to controls. Restoration of processing bias has been found after CBT in patients with chronic pain (Dehghani et al., 2004) and alcohol dependence (Fadaradi &Cox, 2009).

Most importantly, changes in SC pre- to post-EDMR during fear conditioning, changes in perceived emotional control intensity and changes in disengagement index altogether positively correlated with changes in state anxiety and PTSD symptomatology. The larger the PTSD symptoms and anxiety the higher SC to conditioned fear acquisition, the lower the control of emotion and the larger the disengagement difficulty from threat cues.

Taken together, these results would provide preliminary evidence that those alterations are acquired markers of PTSD. They can be perceived as developping after traumatic exposure, are correlated to symptom severity and are restored after symptom amelioration. Yet our design lacks a wait-list PTSD group to settle the inherited v/s. acquired question. We had initially planned to test the symptomatic patients twice (notably the EMDR non-responders) to rule out the repeated testing and/or learning explanation to the normalized physiological measures, especially during the conditioning paradigm. Unfortunately, the few non-cured patients voluntarily withdrew from the experiment or had exclusion criteria. Elegant studies have looked at this question of inherited/acquired features in different ways, using veterans twin designs with one PTSD or trauma-exposed co-twin (Orr et al., 2003) or high trauma-exposed population such as policemen or firemen before and after trauma exposure (Guthrie et al., 2006; Pole et al., 2009). Data published so far suggest that increased initial EMG startle reflex and initial SC to loud tones seems to be inherited factors for increased PTSD symptom severity after trauma exposure whereas higher HR would be an acquired marker after trauma.

Nonetheless it is worthwhile mentioning that restored peripheral mechanisms involved in fear conditionning; efficient emotional control and attention orientation correlate with dimensions of improved well-being in patients (clinical evaluation, STAI and PCL-S).

Further studies should verify the putative alterations of the underlying brain structures in PTSD and its modulation post-EMDR. Bearing this in mind, we have conducted the part – II- of our study to examine central deficits in PTSD.

ARTICLE 4. Stemming from the surprising lack of research investigating how different emotional and attentional components of PTSD interact, the aim of this study was to correlate threat-related amygdala hyperactivity, and other aspects of anxious responding such as self-measures of distress and attentional bias.

We have replicated the extensively described literature on overactive amygdala in PTSD compared to controls in emotional settings (Shin et al., 2010). The breakthrough finding was that this increased amygdala activity positively correlated with disengagement index in PTSD. In our study, larger disengagement index (slower attention shifting from threat cues) correlated with increased amygdala activity in the patient group only and not the controls. This could be a differential pathological factor and could be subsequently considered as a clinical diagnosis. It also provides preliminary support for an implication of the amygdala in attention orientation to threat in PTSD (Cisler et al., 2010).

Moreover amygdala activity and disengagement index positively correlated with trait anxiety. This triad of correlations implied an enhanced perceptual sensitivity to salient threatening events. We ran the Sobel mediation test and the model that fit allowed us to draw a causal relationship between the 3 entities. Accordingly, we found that amygdala hyperactivation induces disengagement difficulty that in turn leads to anxious symptomatology (Fig. 15). This supports Eysenk's theoretical model of anxiety, stipulating that favoring bottom-up effect of amygdala on prefrontal cortex (PFC) and weaken top-down regulatory control would manifest by difficulty in disengaging attention from distracting threat cues in anxiety disorders (Eysenck, Derakshan, Santos, & Calvo, 2007).

On the therapeutic edge, the correlations between amygdala activity, disengagement and symptom severity in PTSD would have valuable clinical implications.

First, attentional bias, STAI and PCL-S could constitute a rapidly accessible diagnostic tool to infer on patients' amygdala activation, and its evolution after therapeutic interventions. Second, one would predict that consciously modifying attention orientation would allow modulation of amygdala activity and subsequent ease pathological symptoms. Approaches such as Cognitive Bias Modification have shown successful outcomes in modifying anxious symptomatology by inducing selective changes in information processing, in non-clinical as well as pathologically anxious populations (Koster, Fox & MacLeod, 2009). They seem to modify the neural systems involved in the control of attention to emotional stimuli (Browning et al., 2010a) and could thus indirectly regulate amygdala activity. In fact, amygdala activity has been recently modulated by trainings directly involving prefrontal processing (Etkin & Wager, 2007).

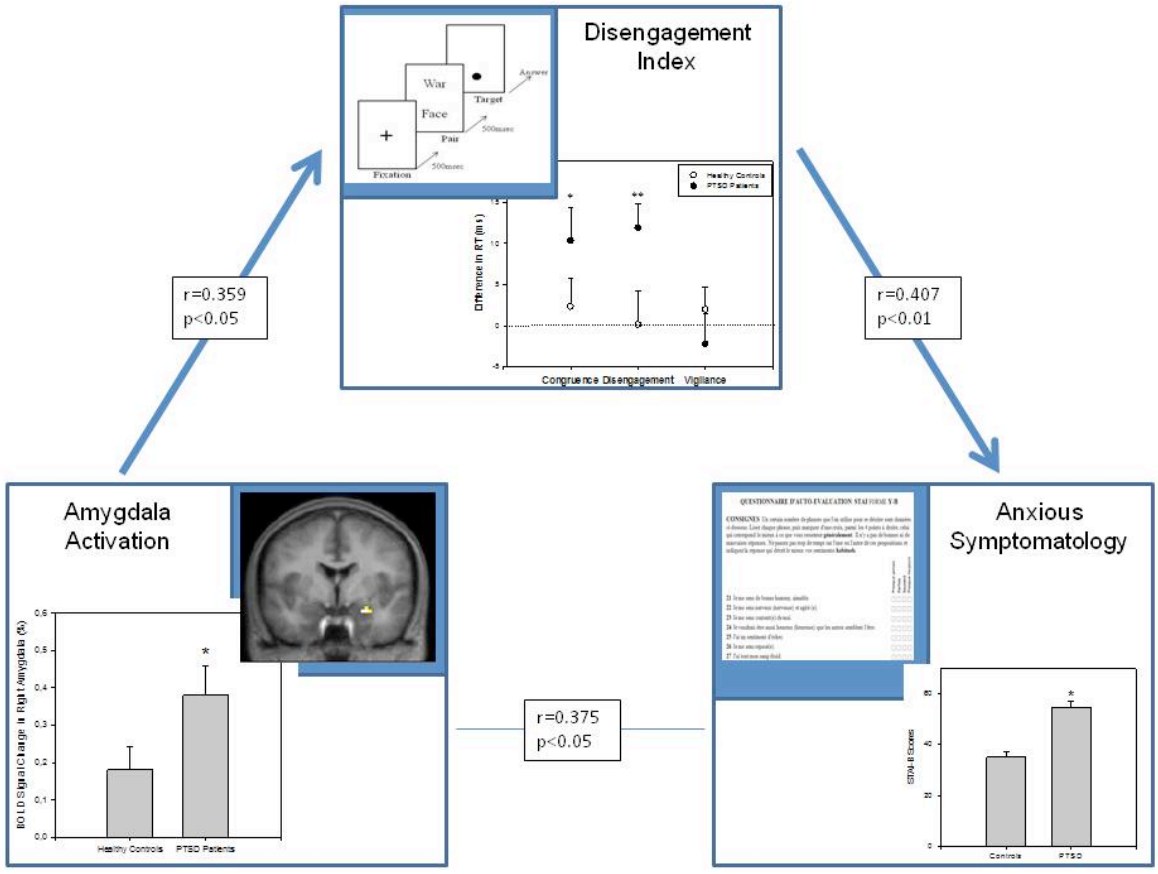


Fig. 15. Schematic representation of the triad of correlations between amygdala overactivity (in emotional face matching), disengagement index (in the attentional DOT) and the anxiety scores (on the STAI-B scale) for symptomatic patients and healthy controls. *p<0.0

ARTICLE 5. In order to assess central alterations, including amygdala but also other prefrontal structures, known to underlie fear processing as well as emotional and cognitive deficits in PTSD, we studied a face matching fMRI task.

Consistent with current neural models of PTSD, our results suggest exaggerated amygdala responsivity and deficient top-down governance of the amygdala by vmPFC. Consistently with the massive literature showing amygdala, ACC and OFC deficits in PTSD when processing threat cues (vs. healthy controls) (Nomura et al., 2004; Shin &Libertzon, 2010), we found that PTSD patients initially had hyperactivated amygdala and hypoactivated ACC and OFC during processing of emotional faces. Prefrontal deficit, on top of exaggerated amygdala, is a feature that distinguishes PTSD from other anxiety disorders, similarly marked by excessive fear (Shin &Libertzon, 2010).

The hypoactivation of rostral ACC (BA32) and adjacent OFC (BA11) mostly involved in emotional experience and regulation could subsequently underlie the PTSD characteristic symptoms of emotional numbing, re-experiencing, intrusiveness of traumatic material and constant state of anxiousness and hyperactivity (Etkin &Wager, 2006). Frontal lesions, specifically in the mPFC and OFC, have also been associated with alterations in social functioning of primates and humans. Healthy social functioning is primordial to human well-being and serves as a protective factor in regards to stressors. It could be that functionally decreased mPFC and OFC contribute to PTSD interpersonal difficulties and impaired social functioning (Libertzon &Sripada, 2008).

We support recent findings associating fronto-limbic impairments with PTSD symptomatology (Garcia et al., 1999) as we found that increased amygdala activity correlates with more severe symptoms on MPSS in patients and ACC inversely correlated with state anxiety in the patients group only.

Besides functional alterations of limbic (amygdala) and frontal areas (ACC, OFC) in PTSD, we also found altered fronto-limbic connectivity. Our observations illustrate initial diminished amygdala-ACC and significantly decreased amygdala-vmPFC connectivity in PTSD compared to controls. We found no difference in amygdala-OFC connectivity between patients and healthy controls. Figure 16 represents an illustration of so far defined anatomic connection of the amygdala, ACC, OFC and vmPFC. Anatomically, vmPFC projects directly to the amygdala and can modulate its output in fear conditioning (Koenings et al., 2008).

Amygdala can also modulate prefrontal activity, either directly or via connection with the ACC (Bracht et al., 2009), and have its activity modulated by PFC connections.

It is important to keep in mind that localization of cortico-limibic alterations in PTSD vary across studies (Milad &Rauch, 2006; Bush et al., 2000). Characterizing the neurocircuitry of PTSD becomes a tedious task and is additionally complexified by the fact that a given area seems to have specialized heterogeneous subfunctions that might be interdependent in a given task (Milad et al., 2006). Overall literature on processing emotional stimuli tends to consider ventral and dorsal routes of processing. A ventral emotional generation, affect-oriented flow and a dorsal emotional regulation, more prone to cognitive control one. Ventral and dorsal parts of each area might differentially contribute to this network. Anatomical refinements are important in the advancement of studies of the brain.

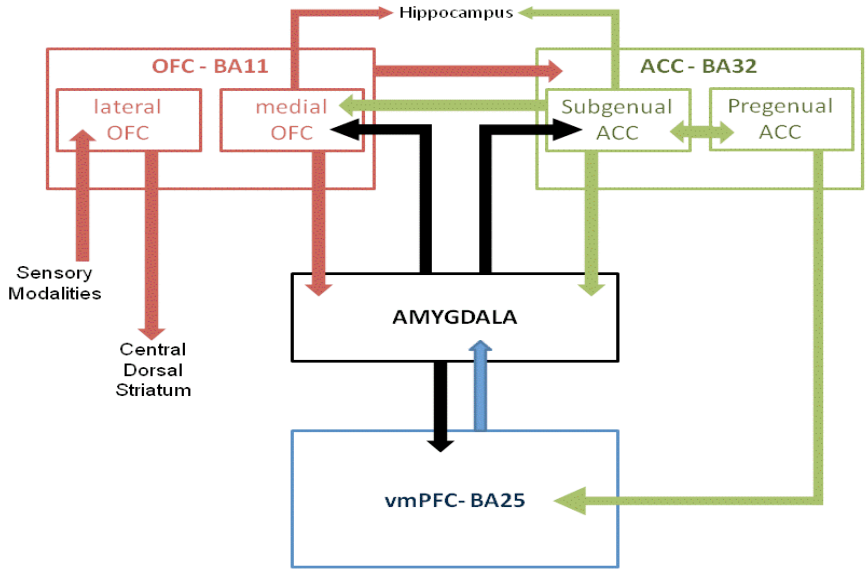


Fig. 16. Illustration of various anatomic connections between amygdala, anterior cingulate cortex (ACC), orbitofrontal cortex (OFC) and ventromedial prefrontal cortex (vmPFC).

Our pre- to post-treatment model of PTSD allows to best address acquired and inherited features of PTSD than the physiological part of the study. In such regards, we show that symptom amelioration restores altered functioning in PTSD. Exaggerated amygdala activity, hypoactive ACC and OFC, as well as deficient top-down governance by vmPFC, all seem acquired markers of the pathology. In fact, they are restored directly after symptom removal and are maintained 6 month after EMDR. Figure 17 illustrates that amygdala, ACC and OFC are initially altered in PTSD compared to control. After successful symptom removal, treated patients and controls had comparable functional activation in those regions, whreas untreated patients retain dysfunctional cerebral processing. This provides strong

evidence that these structures acquire functional alterations after trauma exposure and symptom development.

On the connectivity edge, amygdala-vmPFC is decreased in symptomatic trauma-exposed individuals, but is restored after EMDR. Once again this makes sense considering Eysenck model. Decreased vmPFC-amygdala would represent initial weakened top-down inhibition in anxiety that is restored after EMDR.

Post-EMDR, patients had decreased amygdala-OFC connectivity, compared to both symptomatic patients and controls. This might imply weakening of amygdala bottom-up effect on ACC processing, since de Marco et al., (2006) showed that attending to fearful faces causes a flow of information generated in the amygdala to reach the ACC through OFC. Besides, interpretation of BOLD in terms of excitatory or inhibitory terms remains limited, although this diminished OFC-amygdala connection could suggest less excitation of amygdala. Conversely, this diminished connection could represent a neural "scar" of PTSD symptomatology that might leave room for risks of relapse.

Convergent with these functional imaging results, structural neuroimaging using an elegant twin study of Vietnam veterans showed that ACC gray matter (GM) loss seems to be predominantly acquired impairment of the PTSD pathology rather than inherited vulnerability (Kasai et al., 2008). Similarly, a recent study evidences reduced GM density in patients' CC compared to trauma-exposed (Nardo et al., 2010). Alongside those acquired markers (restored after EMDR), certain unchanged connectivity suggest the existence of innate mechanisms that could be considered as vulnerability factors. For instance, amygdala-ACC shows a strong tendency for a decreased connectivity in patients compared to controls and remains as such after symptom amelioration.

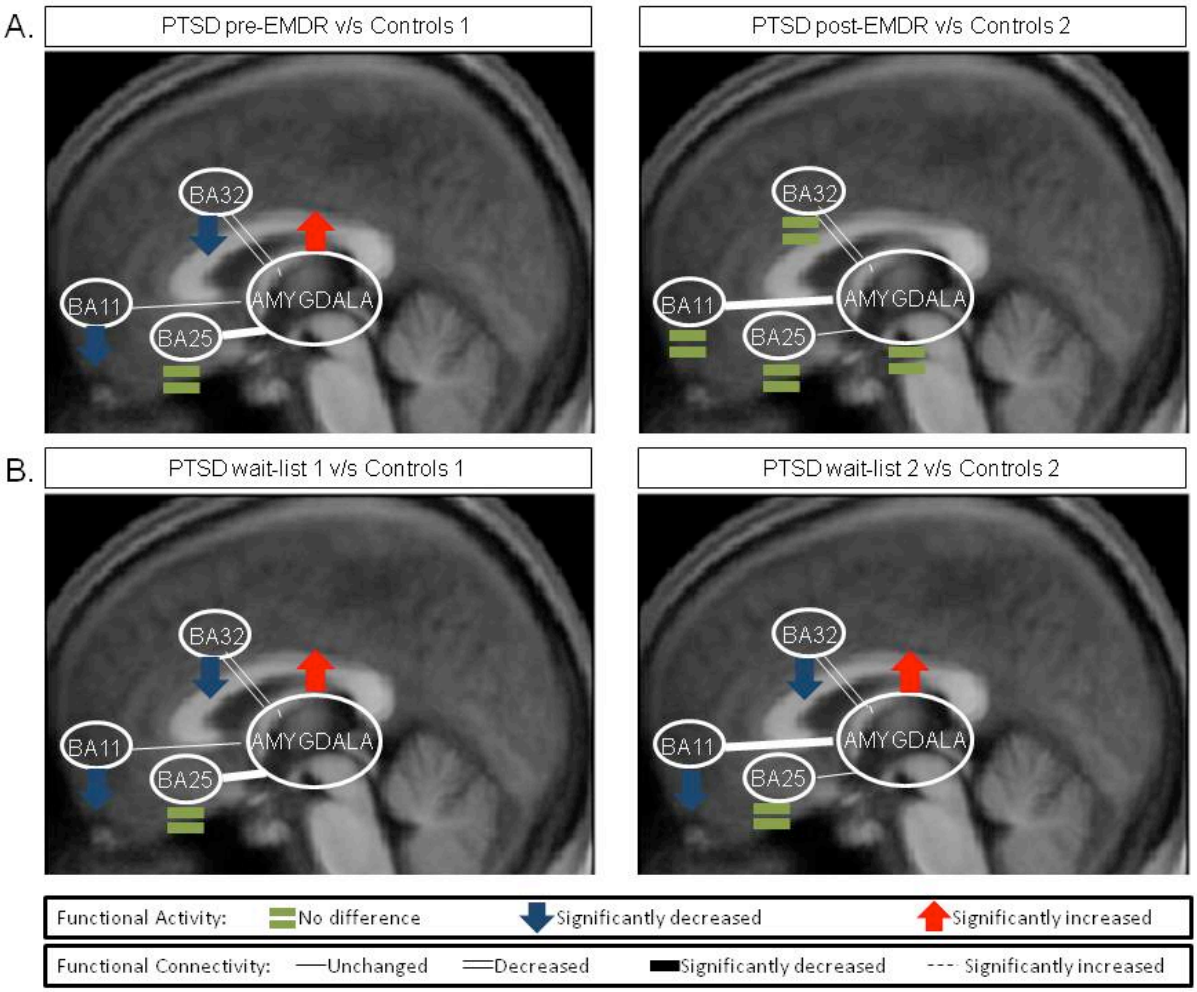


Fig. 17. Illustration of functional activity and connectivity in A. patients pre- and post-EMDR compared to controls at testing time 1 and 2 and B. wait –list patients compared to controls at testing time 1 and 2 during the emotional face matching task for the emotional v/s neutral contrast.

2. General Discussion

Anxiety and fear are closely related. Both are normal adaptive response to potential harmful situations that allows an organism to better cope with its environment. Anxiety is an essential innate defense mechanism (Ursin & Eriksen, 2004). It is distinguished from fear by the lack of external stimuli. They are not themselves pathological conditions but can become so when recurrent in the absence of threat cues.

Our work has shown that in emotionaly aversive situations, the amygdala is first recruited and subsequently drives the ANS system to respond adequately to the fearful situation, increasing for instance HR, SC and EMG. The PFC is involved at a later stage to analyse the situation and once the original threat cue vanishes, it participates in extinguishing the learned fear. As such, PTSD appears as a maladaptive and persistent response to stress that prevents the organism from coping with one's environment. We found that in PTSD this

happens because of an atypical processing of fearful and threatening stimuli. Patients suffer an imbalanced brain functioning inasmuch as the fear circuitry is concerned with increased amygdala activity, decreased prefrontal activity and decreased amygdala-prefrontal connectivity. The increased amygdala functioning seems to best account for the attentional bias in PTSD as patients have a difficulty disengaging their attention from aversive cues. The decreased OFC and ACC fairly account for patients' emotional disturbances and their inability to regulate their emotions. Symptoms are worsened by a decreased vmPFC-amygdala connectivity rhyming with an inefficient top-down control over the amygdala that might account for higher physiological responding in PTSD, even in the absence of danger. EMDR seems to work in PTSD by restoring cerebral activity and connectivity. In fact amygdala and PFC are set back to normal activity levels as compared to healthy controls. Increased vmPFC-amygdala allows the correct extinction of learned fear and decreased OFC-amygdala releaves bottom-up inhibition of limbic alert zones. Patients subsequently exhibit "normalized" physiological, attentional and emotional responding.

Our results covered a number of seemingly heterogeneous processes potentially involved in PTSD, including attentional bias, fear conditioning and emotional processing. All these processes have been linked to activation of fear circuitry regions in prefrontal and limbic regions. While it is possible that all these functions solicit cortico-limbic structures independently, it might be speculated these disparate processes interact.

Amygdala and vmPFC are largely involved in fear conditioning and extinction (Garcia et al., 1999; Milad et al., 2009). Along with ACC and OFC, they have a large role in emotional generation and regulation (Ohira et al., 2006; Oschner et al., 2009). Besides their role in attention orientation and threat detection is being elucidated (Ohman, 2005). Fearful and angry faces trigger amygdala activity, even when presented at subconscious levels (Armony et al., 2005). Amygdala seems to orchestrate rapid automatic evaluation of stimuli's valence (Ohman, 2005).

Studies suggest this automatic threat detection is highly correlated to cognitive attention orientation strategies monitored by prefrontal cortex (Cisler et al., 2009) and the ACC has been implicated in attention bias tasks (Shin et al., 2004). As such, bottom-up mechanisms by amygdala over prefrontal cortex would direct attention to emotional cues. Cortical structures in turn can regulate amygdala activity by top-down inhibitory action (Taylor et al., 2005).

In any case, our fronto-limbic findings of amygdala, ACC and vmPFC involvement in PTSD are further supported by a large-scale lesion study on 193 war veterans (Koenings et al., 2008). Imaging data cannot determine whether the neuroanatomical findings reflect a cause of the disease (either inherited risk factors or acquired impairments) or secondary effect of the disorder (due to alteration of other primary regions). Lesion studies better address this issue, and could in principle elucidate causal contributions of vmPFC and amygdala, by monitoring the impact of brain damage in either region on PTSD symptom development. These studies show that unilateral amygdala lesions eliminated occurrence of PTSD and vmPFC lesions independently reduced its incidence. According to authors, their study first implies that vmPFC and amygdala are causally involved in PTSD pathogenesis. This also means that amygdala is central to PTSD and also implies that vmPFC-amygdala interaction is not uniformly inhibitory, since vmPFC damage would have otherwise increased PTSD occurrence. This could be because projections from the vmPFC may excite neurons in the basolateral amygdala and inhibit others in the central amygdala (Quirk et al., 2003), and that other prefrontal structures additionally regulate amygdala activity.

On the etiological aspect of PTSD, we have shown that increased amygdala activity would lead to attentional bias and subsequently to anxious symptomatology. In other words, exaggerated amygdala functioning seems to direct patients' attention towards threatening or fearful stimuli, thus accounting for aspects of the disorder concerned with a state of increased hyperactivity and hypervigilance. Although the etiology of PTSD is defined in terms of the traumatic event, evolving models of pathogenesis should take into account the potential interaction between the identified traumatic event (or events), past experiences, and intrinsic individual vulnerabilities (Rauch et al., 2006). Jovanovic & Ressler (2010) in fact state "exposure to traumatic events that produce extreme fear and horror is all too common in both military and civilian populations, but not all individuals develop PTSD as a result of the exposure. What mediates risk and resilience in the development of PTSD and other stress-related psychopathology is of paramount importance to our further understanding of trauma-related psychopathology as well as the development of new treatment approaches. Biological factors, such as genotype and neurobiology, interact with environmental factors, such as childhood background and trauma load, to affect vulnerability and resilience in the aftermath of trauma exposure".

On the clinical edge, the causal relationship we found between amygdala, disengagement problem and symptomatology provides on one hand a diagnostic tool inferring on patients amygdala activity. In fact, we have shown in a multiple regression approach that taken together disengagement bias and PCL-S scale highly correlate with limbic functioning, explaining around 60% of its variability. Simply asking patients to rapidly fill a trauma-related scale and complete a 3 min somewhat entertaining attentional task, clinicians and health care providers could infer on PTSD patients' amygdala activity. By doing so, diagnosis of PTSD can be refined, and treatment option better customized. This correlation between amygdala overactivity and treatment efficacy has been touched upon in a preliminary study by Nardo et al., (2010), showing that among a group of PTSD patients, EMDR treatment responders had larger amygdala and ACC grey matter density that non-responders.

On the other hand, the causal route from amygdala overactivity, to attentional bias to anxiety provides a therapeutic tool by ultimately aiming at decreasing amygdala activity. One would predict this could be done with approaches consciously modifying attention orientation, allowing modulation of amygdala activity and subsequently easing pathological symptoms. In fact, amygdala activity has been recently modulated by trainings like Cognitive Bias Modification (CBM) directly involving prefrontal processing (Etkin & Wager, 2007), or alternatively by much more fun approaches such as the tetris game (Holmes et al., 2010).

CBM has shown successful outcomes in modifying anxious symptomatology by inducing selective changes in information processing, in non-clinical as well as pathologically anxious populations (Koster, Fox & MacLeod, 2009). They seem to modify the neural systems involved in the control of attention to emotional stimuli (Browning et al., 2010a). PTSD patients would thus benefit largely from treatments that can function similarly to CBM.

3. Special Focus

PTSD and SENSORY MODALITY. Evidence is piling up on the implication of amygdala in sensory processing in PTSD. The amygdala is well positioned to modulate perceptual processing, because its sends and receives rich inputs from all sensory modalities, in addition to sending projections to cortical and sub-cortical regions (Vuilleumier, 2005). This direct pathway to perceptual pathways allows emotional influence to operate in parallel with fronto-parietal influence and may account for environment-related deficiencies in PTSD.

In a side study, we have shown that auditory perception is altered in PTSD patients (Aubert-Khalfa et al., 2010 - Appendix 3). Hearing thresholds were found to be significantly poorer in patients than in controls for frequencies from 2.75 Hz to 8 kHz in bone conduction, and for 0.5, 0.75, 0.875 and 2.0–8.0 kHz pure-tone frequencies in air conduction. The serotoninergic system could be a key element in the explanation of perceptual differences. Its terminal neurons innervate various anatomical structures involved in hearing mechanisms (Thompson et al., 1994; 1998). This system is known to be altered in PTSD (Krystal & Neumeister, 2009). Amygdala could alternatively influence sensory systems via cholinergic routes. These modulate parietal and OFC response to emotional distracters. This shows that the central alterations in PTSD studied in the scope of fear processing could additionally influence sensory modalities, and account for their personal sufferings. Moreover, sensory modalities, such as auditory thresholds, could be monitored as markers of the pathology.

PTSD and OTHER ANXIETY DISORDERS. It is not clear which of the amygdala or PFC deregulation drives the overall outcome in PTSD. Yet, a hyperresponsive amygdala alongside a hyporesponsive prefrontal cortex may lead to a series of deficiencies of fear extinction, emotional regulation and attention (Libertzon &Stripada, 2008). Similarly to other anxiety disorders, such as GAD, OCD, and phobias, PTSD is characterized by heightened amygdala activation in response to emotionally aversive stimuli (Etkin et al., 2007). Amygdala has a say in emotional, attentional, cognitive, memory and even sensory perception. Interestingly, resilience to PTSD was shown to be associated with decreased amygdala activation (Britton et al., 2005). As such amygdala seems important in PTSD but not all-important (Ledoux, 2002). That is, amygdala alone cannot account for PTSD but rather its projections to cortical structures better represent pathological neural functioning.

Unlike the other anxiety disorders, PTDS is associated with diminished responsivity in cortical regions: ACC and vmPFC (Shin &Libertzon, 2010). In PTSD, mPFC showed decreased activity compared to controls at rest, but also in e-Stroop (Shin et al., 2001), traumatic scripts (Lanius et al., 2001), presentation of trauma-related stimuli (Yang et al., 2004) and negative non-trauma related stimuli (Shin et al., 2005) and especially during extinction of conditioned fear (Quirk et al., 2006; Bremner et al., 2005). Studiying common features and discordant ones in anxiey disorders would allow a better comprehension of thoses pathologies and a better diagnostic tool.

4. PTSD Treatment and Diagnosis

EMDR. In terms of treatment effectiveness and recommendations, EMDR remains a validated recommended therapy for clinical and biological post traumatic symptom elimination. In spite over 200 recent papers on EMDR and PTSD, its mechanisms of actions are still unknown. Whether it is via inter-hemispheric integration (Engel &Konig, 1991), bilateral stimulation (Servan-Schreiber, 2006), REM-like mechanisms (Stickgold, 2002), double attention, cognitive restructuring (Teasdale et al., 2003), exposure or all of the above, the exact mechanisms of EMDR effectiveness in trauma are still unknown. It could be that EMDR is working through cognitive reappraisal of one's emotions. Cognitive reappraisal is a form of emotional regulation that involves volitionally reinterpreting the meaning of a stimulus to change one's emotional response to it. This ultimately reflects in changes in activity in emotional processing areas. Studies have shown that merely labeling the emotion decreases amygdala response (Hariri et al., 2000). Yet, the therapy posits dysfunctional memory networks in PTSD that would inappropriately stock the traumatic information. These networks would be subsequently reactivated and regulate patients' altered perceptions, attitudes and behaviors. EMDR is thought to access the traumatic information, stimulate memory-processing systems to "move" its neuronal coding towards a more adaptive one.

Compared to CBT, EMDR is quite fast; it could be that the two therapies target different brain structures. Either way, the use of EMDR in our study, all in all, has required an average of 4.3 sessions (almost 2.5 month) to be around 90% efficient in PTSD patients who have been trauma exposed for an average of 17 month (part I) and 32 month (part II). Treatment effectiveness was measured by corresponding PTSD scales, personal evaluation of decreased level of distress when thinking about the traumatic event and finally psychiatrist's clinical diagnosis using the DSM-IV criteria.

EMDR therapy has decreased patients' scores on anxiety, depression and PTSD scales, from pathological to normal levels. This result further validates the well-established clinical and therapeutic effectiveness of EMDR and its rationale as a first-line treatment option for PTSD by APA health instances. EMDR has also shown a sustainable effect in PTSD treatment in a follow up study at 6 months post-EMDR.

The biological basis of this quite fast process and its neuroanatomic correlates has been seldom studied. Our findings resemble those of recent neuroimaging studies providing preliminary evidence that post- to pre-EMDR patients have increased ACC activity (Levin et al., 1999; Lansing et al., 2005) and decreased amygdala activity. We provide further understanding in the restoration of fronto-limbic connectivity after successful treatment. Other teams of researcher have preliminarily started looking at structural changes induced by EMDR in PTSD and have shown that it restores initially diminished hippocampal volume (Letizia et al., 2007). Additional preliminary volumetric studies have described that grey matter density in limbic and paralimbic cortices is associated with PTSD development after trauma (posterior parahippocampal gyrus, posterior cingulate) and with EMDR treatment outcome in PTSD (amygdala, posterior cingulate, insula) (Nardo et al., 2009).

Our protocol supports previous findings in EMDR and PTSD, yet it does not bring conclusive evidence to EMDR mechanism of action. Accordingly, it could be that EMDR directly tackles amygdala and PFC, or it could be that it interferes with a third structure, in turn regulating amygdala and PFC. To best address this issue, one would have to monitor real-time EMDR conducted inside the fMRI scanner. One would then be face with ethical considerations on one hand since the scanner does not provide the comfort and safe zone for suffering patients to interact with their therapist. On the other, one would be faced with technical limitations since EMDR lacks inter-patients reproducibility. Besides, sessions last for 45-90 min on average and are often accompanied with emotional outburst (crying, shouting…). As such the use of fMRI in understanding EMDR mechanism per se remains questionable and quite challenging.

An additional central question at the heart of the debate surrounding EMDR is whether the effectiveness of the treatment is due solely to the exposure to the trauma memory during the exercise, thereby rendering the treatment merely a disguised exposure therapy, or whether there is in fact added benefit to the dual stimulation.

Studies have so far shown that eye-movements (compared to static eye fixation) diminished arousal produced by auditory stimuli (Barowcliff et al., 2003) and decreased image vividness, emotional valence and electrodermal arousal associated with negative autobiographical memories in healthy controls (Van der Hout etl al., 2001). Servan-Schreiber et al., (2006) had modulated EMDR stimulation type (visual, auditory and kinesthetic) and found all sensory modalities were clinically useful in decreasing distress of PTSD patients. Due to the nature of the EMDR treatment, it would be difficult to separate the elements to evaluate their independent contributions (Cukor et al., 2010).

It could also be that the eye movement component in EMDR contributes in restoring normal conditioning in PTSD somewhat resembles REM sleep effect. Although the function of sleep remains elusive, accumulating evidence suggests that it plays an important role in post-learning processing of hippocampus dependent memories (Stickgold, 2002). A study by Dechaux et al., (2010) has shown that immediate post-learning REM sleep deprivation can result in impairments of spatial memory-related hippocampal processing, and is associated with deficits in the retrieval of spatial memories. In fact, fear conditioning reduced time spent in REM sleep, which was restored with fear extinction. Hippocampal low frequency stimulation, applied immediately following extinction training, abolished the restorative effect of fear extinction on REM sleep and impaired extinction retrieval. Authors suggest bidirectional interactions between hippocampal functioning and REM sleep for successful extinction retrieval. The general postulate is that fear conditioning memory and fear extinction memory are two separate entities (Fig. 18) that compete in a context-dependent manner involving the hippocampus.

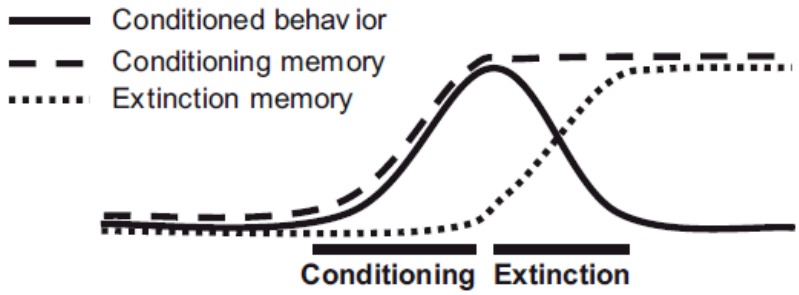


Fig. 18. Schematic relating conditioned behavior to memory for conditioning and extinction (Quirk et al., 2006).

Sleep regulation could thus offer two possibilities with important clinical value for anxiety disorders such as PTSD:

1) Disrupting the consolidation of the fear conditioning memory after trauma exposure via sleep deprivation (Quirk & Milad, 2010)

2) Enhancing the consolidation of the fear extinction memory after therapy, via a good sleep (Pace-Schott et al., 2009, Spoormaker et al., 2010).

As such, with the bilateral eye-movement aspect and its subsequently induced physiological and central modifications, EMDR could play a role in reactivating REM sleep-like functions. Recent news and views attribute EMDR's success to a shift in waking brain states to facilitate the resolution of emotional memories, otherwise resistant to normal sleep dependant processing (Stickgold, 2007). According to his review, contrary to synaptic homeostasis theory, reactivating memories in short-wave sleep enhances learning and hippocampal activation. By decreasing autonomic activation and using inductions similar to REM-slepe, EMDR would accelerate the processing of trauma memories and subsequent recovery from PTSD.

DIAGNOSIS. Our work could contribute to improve the diagnosis of PTSD and its treatment by EMDR. The DSM-V manual expected in May 2013 reflects new advancements in the mental health field, in terms of conceptualization of mental health disorders and needs of patients. Perhaps a major step forward should be the inclusion of biological markers for instance in the PTSD diagnosis.

In fact, even when patients and controls have comparable behavioral performances, we have shown that the underlying cerebral functioning remains differential. For example, PTSD and healthy participants had similar reactions times in matching fearful faces, yet the PTSD group had increased amygdala activity that correlated to symptoms severity.

This suggests that it would be beneficial to include subtle biological markers with the above-mentioned simultaneous use of questionnaires and attention bias task in the diagnostic criteria, and this could be easily infer on amygdala functioning for instance.

Shin and Handwerger (2009) strongly argue for characterizing PTSD as a stress-induced fear circuitry disorder, revolving around amygdala dysfunction (Shin & Handwerger, 2009).

The move toward forming diagnostic categories (length of treatment or different treatment responders/non-responders and even risk of relapse) based on this validated central marker is a useful endeavor that deserves attention in future research. According to Rauch et al., (1998) and Bremner et al., (1995), amygdala hyperresponsivity mediates symptoms of hyperarousal and explains the indelible quality of the emotional memory for the traumatic event; inadequate influence by PFC underlies deficits of extinction as well as the capacity to suppress attention and response to trauma-related stimuli; and decreased hippocampal function underlies deficits in identifying safe contexts, and explicit memory difficulties.

In addition to exaggerated amygdala activation, abnormalities in functional connectivity in amygdala-ACC and amygdala-vmPFC could also be markers of the PTSD psychopathology. Pezawas et al., (2005) have shown that the magnitude of fronto-limbic coupling inversely predicted almost 30% of variation in anxiety. It is therefore important that future fMRI studies involve, not only assessment of functional activation, but also functional connectivity of involved brain structures. This would allow better characterization of prefrontal structures with altered connectivity to the amygdala v/s those with intact connectivity.

Amygdala activity should be one of the factors best informing about central processing of emotion and attention. Other markers are to be tested. For instance, a study in healthy controls has shown that genetic variation, notably of the serotonin autoreceptor (5-HTTLTR) indirectly affects emergent behavioral processes related to anxiousness and psychiatric disease risk by biasing the response of underlying neural circuitries and affecting amygdala activity (Fakra et al., 2009). Yet, authors suggest that social environment modifies the effect of the 5-HTTLTR genotype on PTSD risk, as its polymorphism is associated with low PTSD risk in low-risk environment and high PTSD risks in high-risk environment (Koenan et al., 2009). Integrative, multidimensional analyses are thus of utmost importance in the field.

5. Major Limitations of the study

COMORBIDITY. The interpretation of PTSD pathology being linked to impaired processing is somewhat limited by the high prevalence of comorbid disorders in our PTSD sample, notably mood and other anxiety disorders. A major limitation thus arises with the presence of comorbid disorders and the use of different classes of medication, even though patients were on stable regimen although the experimental duration.

Since it was impossible to recruit a sufficiently large population of non-medicated patients with clean PTSD diagnosis (i.e. without other comorbid disorders), we have entered those parameters as covariables in our statistical analyses and found no changes in the results. We have also compared subgroups of patients with/without comorbidities and with/without medication. Although these subgroups were not large enough to allow parametric testing, yet we found the subgroups behaved similarly at central and peripheral levels.

Clinical wisdom and recent evidence suggest that anxiety and depression share common neural cross-links, and are targeted by common effective treatments such as SSRI and dual serotonin-norepinephrine reuptake inhibitors (Nemeroff et al., 2006). According to authors, a great deal can be learned from the extensive "matured" depression literature, conceptualized as a end-product of failed adaptation to chronic emotional stress. A such, successful treatment seems not a matter of absolute changes of given structures, but rather a more complex adaptation of multiple brain regions to a new homeostatic balance and the maintenance of this balance.

LACK OF WAIT-LIST PTSD GROUP. Our physiological study design is limited in its ability to address acquired/inherited characteristics of altered fear conditioning, emotional responding and attentional bias, especially since we were unable to retest the drop-outs, who were mostly out of reach or refused to be retested. It also prevents the assessment of repeated sessions in patients. On one hand, we argue against the mere effect of "passage of time", as patients have had PTSD symptoms for 18.4 months and showed no signs of spontaneous recovery. On the other hand, we argue against the effect of learning at the retest (i.e. by simply attending the paradigm twice). Controls do indeed show physiological responding at their first and second testing as they have comparably high SC each time. One could improve the procedure by including a wait-list group of PTSD patients. Alternatively one could include a group of patients who would sit for the experimental paradigm only after treatment.

OTHER. Other limitations include reduced sample size, especially for the physiological part of study. This prevents us from subdividing groups to compare for instance males and females, or medicated and non-medicated, etc…

Our sample is a homogeneous PTSD population of a single trauma without prior psychiatric disorder, so generalizing our findings to the PTSD population as a whole should be done with caution, as one would think for example that memories of childhood abuse might have differentially consolidated than car accident memories.

An additional limitation stems from the task used in cerebral assessment. In fact the face matching task might not necessarily indicate patients' brain functioning when processing trauma-related. It is a mere reflection of the altered functional activity and connectivity of given brain regions, known to be altered in PTSD.

Last but not least, since our study was quite intense in terms of use multiple testing, it might have left some space for statistically exaggerated results on one hand or statistically diminished significance on the other.

6. Suggestions for future directions

FEAR CONDITIONING AND EXTINCTION IN fMRI. Our study provides promising findings in the advancement of the understanding of peripheral and central mechanisms involved in fear conditioning, emotional suppressing and attentional bias in PTSD, and their restoration after successful symptom removal by EMDR. Further studies are in progress within our team to better explore other aspects of PTSD and palliate the limitations we have mentioned. First, we have replicated extensively documented results that amygdala and PFC are functionally and connectively altered in PTSD. Their involvement in fear conditioning and extinction is validated, with patients having increased amygdala activity at fear acquisition and decreased vmPFC at extinction (Milad et al., 2009; Shin &Libertzon, 2010).

The core symptom of PTSD being the inability to control fear has lead investigator to conceptualize it as a disorder of fear and most particularly its inhibition and thus they orient treatment strategies on enhancing fear inhibition (Jovanovic &Ressler, 2010). It thus comes as a continuation of this thesis and as a further exploration of central mechanisms involved in fear processing in PTSD pre- and post-symptom removal, to study inherited v/s. acquired fronto-limbic impairments in fear conditioning and extinction in PTSD using an fMRI adapted paradigm. This would allow us to monitor vmPFC activity (not activated in emotional face matching task), and better define its inherited/acquired deficiency in PTSD.

USE OF CBT. Another option envisaged is to monitor the altered neuronal network in PTSD, involving amygdala, ACC, OFC and vmPFC, before and after CBT. The general idea behind this is addressing the central mechanisms involved in PTSD symptom removal. Given the altered central processing in PTSD, the question arising is formulated as such: are the treatment mechanisms common for different therapeutic alternatives or are there treatment-specific cerebral targets?

A review by Bar-Haim et al., (2010) seemed to indicate that CBT targets the PFC whereas pharmacological drugs mainly modulate the amygdala. Further studies are also in progress in our team to compare EMDR and CBT in treating PTSD symptomatology.

VOLUMETRIC ANALYSES. After dwelling in functional alteration of central structures in PTSD, it would be all the most interesting to monitor their structural modulations, as neuroanatomic alterations could impact neuronal functioning. Volumetric studies have started describing alteration in many structures of the fear circuitry in PTSD. Recent reviews by (Koenings at al., 2008 and 2009) have described a trend toward decreased amygdala and ACC volume in PTSD. Grey matter loss has been documented in ACC (Chen et al., 2006), with smaller ACC volumes associated with greater PTSD symptoms severity. In an elegant study of monozygotic twins discordant for PTSD diagnosis, ACC was shown to be an acquired sign of the disorder (Kasai et al., 2008). Conversely, diminished hippocampal volume appears to be an inherited vulnerability factor in PTSD (Gilbertson et al., 2002). The hippocampus is becoming particularly studied in PTSD, with deceased size correlated with symptom severity (Rauch &Shin, 2002; Francati et al., 2007).

In part –II- of our study, we have tested patients pre- and post-EMDR as well as 6 months after symptom removal. This has allowed us to monitor consolidation of the functional restoration of amygdala and prefrontal cerebral centers altered in PTSD. Furhter analyses are in progress in our team to monitor structural changes within those same centers using the VBM approach, and additionally examine other regions such as the hippocampus.

HIPPOCAMPUS. Inasmuch as the hippocampus is concerned, its consolidation of negative-valence stimuli is modulated by the amygdala (McGaugh, 2004). Our study has focused on amygdala and prefrontal processing in PTSD, in emotional and attentional tasks, but we did not include memory related paradigms.

Abnormal hippocampal function and structure contribute to deficits in contextual processing and memory impairments, described in PTSD. In fact, hippocampal implication in PTSD is extensively concerned in recall of fear extinction studies (Milad et al., 2009).

On a different note, path analysis has shown that extinction retention mediates the relationship between OFC thickness and extraversion, illustrating one of the means by which brain structure influences personality (Rauch et al., 2005).

It would thus be more exhaustive to include hippocampal processing in future studies.

For the 15 million suffering PTSD worldwide (WHO, 2001), our work brings scientific answers and explanations to certain major facets of the disorder and therapeutic perspective for efficient symptom amelioration. We have shown that in PTSD, central deficits are mostly concerned with increased amygdala activity and decreased frontal inputs, fairly accounting for the altered fear processing. PTSD symptoms would be worsened, not only by exaggerated amygdala activity as well as diminished prefrontal inputs but also by inadequate amygdala-PFC connectivity. These central deficits are physiologically manifest in fear conditioning, emotional responding and attentional impairments in PTSD, all of which manifestations we have monitored.

These impairements are far from being independent. To the best of our knowledge, this is the first study establishing a correlation between emotional, cognitive and central impairments in PTSD and most typically their restoration after symptom elimination. We have also established a causal relationship between those cornerstones. Following traumatic exposure PTSD, the amygdala overactivity would cause attention orientation towards threat cues, inducing a disengagement bias. This would subsequently leed to increased anxious symptomatology, as patients would be more alert to aversive cues. This relationship from biological markers to psychological ones vias behavioral assement is of utmost importance in clinical settings and research purpose.

Our findings provide preliminary evidence that most features of PTSD are restored after EMDR and therefore can be considered as acquired markers. They would develop with symptomatology after trauma exposure. Some mechanisms seem however innate, and can be rather viewed as vulnerability factors for pathological reactions after a traumatic event.

In this perspective, reliable diagnosis could be simply established after trauma, using the STAI, PCL-S and DOT, to better assess central functioning and define those at higher risk of developing PTSD.

At that stage, we have shown that central, emotional and attentional features in PTSD are correlated with its symptomatology and restored after symptom removal, thus suiting the criteria for acquired characteristics. It would be a potential advancement to assay whether those same markers could allow the prediction of risks of relapse that remains quite frequent in PTSD.

"People do not come preassembled; they are glued together by life. Nature and Nurture make up the synaptic self: a channel for information storage and transmission. Learning and processing live events involves the nurturing of nature. In a way, Nature and Nurture are the same; they are 2 different ways of making deposits to the brain's synapses" (Ledoux, 2002).

CHAPTER V

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CHAPTER VI

APPENDICES

A. Appendix 1 - Article 6: Voluntary Emotion Suppression Modifies Psycho-physiological Responses To Films

B. Appendix 2 - Article 7: Neuroticism Modifies Psycho-physiological Responses To Films

C. Appendix 3 - Article 8: Pure-tone auditory thresholds are decreased in depressed people with post-traumatic stress disorder

Appendix 1: Submitted the Journal of

Voluntary Emotion Suppression Modifies Psycho-physiological Responses To Films

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Background: *When an emotion is induced, it drives bodily modifications of both physiological indices and facial expressions. This is an adaptive faculty to acclimatize to changing life circumstances. A fundamental aspect of personal well-being and successful social interaction is that emotions are not always expressed to their fullest extent but rather are controlled in a context-dependent manner. The process by which we influence the kind of emotions we have, when we have them, and how we experience and express them is referred to as emotion regulation. Emotion regulation involves changes in one or more aspects of the emotion, including the eliciting situation, subjective experience, behavior or physiology. Various works have studied different autonomic nervous system (ANS) responses during emotion regulation using positive and negative stimuli without clear separation between emotions.*

Our study thus aims at comparing the physiological activity (ANS and facial muscles activity) during emotion attending and emotion suppression but using specific categories of emotion.

Methods: *Fifty healthy adult volunteers were presented with five 45-seconds duration color films in an attending and a suppression tasks. The five clips intended to elicit five different emotions (happiness, sadness, fear, peacefulness and disgust). We evaluated various physiological measures such as the galvanic skin response (GSR), as an index of electrodermal activity, heart rate (HR) and the respiration rate, the activity of zygomatic (smiling) and corrugator (frowning) muscles as an index of emotional expression.*

Results: *Performing a suppression task as compared to an attending task results in diminishing the HR and the activity of the zygomatic muscle during the happy film. Moreover, voluntary attempts to control emotion also results in an increased GSR for the fearful film.*

Conclusion: *The observed emotion effects on autonomic responses contribute to a growing literature on the physiological effects of regulating pleasant and unpleasant emotions, indicating that the conscious and volitional regulation of emotion has selective effects on psychophysiological parameters which differ according to the presented emotion.*

1. Introduction

Body, mind and emotions are interlinked by intricate neuronal networks, and function as an entity to build up our knowledge; our intellectual capacities are constantly influenced by our emotional state. In such a perspective, emotions are more than just a part of our daily lives; they are the natural mean by which the brain can evaluate and adapt to the outward and inward environments (Damasio, 1994).

Consequently, emotions play a crucial role in behavioral responses, decision making and in facilitating interpersonal interactions (Baumeister et al., 2007). Contemporary conceptions of emotion rely on a multi-factorial definition of the phenomenon (Scherer, 2000). As such, the word "emotion" refers to diversified states sharing the common property of being associated to emotional experience on the one hand (cerebral and visceral response to emotions), and to somatic manifestations on the other (such as facial expressions). One's emotional experience and one's emotional expression are considered two of the major manifestations of emotion (Phillips et al., 2003). Emotions thus allow the coordination of behavioral and physiological responses that define to a large extent how we respond to changes perceived as threats or opportunities.

Peripheral physiological responses of the autonomic nervous system (ANS) provide a fair amount of information about emotional states i.e. our emotional experience, independently of self reports. Activation levels of the ANS can be measured by recording various physiological indices. One of the mostly used indices is the heart rate (HR) as it gives information on both branches of the ANS: the sympathetic and parasympathetic systems. Indeed, studies have shown that the HR is accelerated during the presentation of positive images and decelerated during the presentation of negative images (Lang et al., 1993; Bradley and Lang, 2000; Palomba et al., 1997). It has been also found that viewing arousing musical excerpts results in an increase in HR (Witvliet, 1998; Etzel et al., 2006). The HR seems to be modulated by the emotional valence of stimuli as well by their arousal levels.

Another index is the electrodermal conductance or skin conductance response (SCR) used as an indicator of the sweat glands activity. SCR amplitudes fluctuate with acetylcholine release upon sympathetic activation and are evidenced upon presentation of emotional stimuli and in cognitive tasks (Critchley, 2002). In 2002, Khalfa et al. studied SCR modulation when

presenting subjects with musical excerpts with varying valence and arousal dimensions. Larger SCRs were observed for fearful and joyful excerpts i.e. for the most stimulating emotions. Other studies have also looked at ANS activation upon the presentation of positive and negative film extracts. They found increase of SCR activity, as well as HR and respiration rate, for highly stimulating extracts, in particular for negative emotions like anger, fear and disgust (Palomba et al., 2000; Williams et al., 2001; Kreibig et al., 2007).

These studies clearly show that the ANS is differentially stimulated by the presentation of emotionally loaded stimuli according to the arousal and valence levels. The HR seems to allow the differentiation of emotions by valence and arousal levels whereas electrodermal activity is more dependent upon the arousal level (Bartlett, 1996).

Therefore, when an emotion is induced, it drives bodily modifications: physiological manifestations and specific facial expressions (Lang et al., 1998). This is an adaptive faculty to adapt to changing life circumstances. However, a fundamental aspect of personal well-being and successful social interaction is the fact that emotions are not always expressed to their fullest extent but rather are controlled in a context-dependent manner (Gross, 2002). The process by which we influence which emotions we have, when we have them, and how we experience and expressed them is referred to as emotion regulation (Gross, 1998b). Emotion regulation involves modifications to one or more aspects of the emotion, including the eliciting situation, subjective experience, behavior or physiology (Bargh and Williams, 2007; Gross and Thompson, 2007).

A number of studies have tried to characterize the psychophysiological correlates of voluntary emotion regulation. For example, attempts to decrease negative emotion like disgust through **the suppression of emotion** (defined as the conscious inhibition of ongoing emotion-expressive behavior) should decrease the expressive behavior of disgust and has been associated with increased sympathetic activity leading to increase SCR and less consistently with decreased HR (Gross and Levenson, 1993 ; Gross, 1998), whereas emotion reduction through **reappraisal** (the cognitive reinterpretation of an event so as to change its emotional impact) generally decrease the extent to which emotion response tendencies are activated, leading to lesser subjective and expressive signs of negative emotion than otherwise would have been evident (Gross, 1998 ; Ray et al., 2010).

More recent studies have chosen to refrain from indicating specific control strategies (reappraisal or suppression); simply asking subjects to decrease their emotional response. They gave an ecological instruction, best mimicing real-life setting, so participants control their emotions as they would spontaneously do if faced with such situations. These studies have shown antagonistic effects. In fact, when comparing this emotion suppression task to an emotion attending task, larger SCR mean amplitudes and diminution of HR was observed in the suppression task for positive and negative emotions (Ohira et al., 2006). Yet, a recent review failed to reproduce such findings and instead found decreased SCR and no effect of emotion suppression on HR during pleasant and unpleasant film watching in emotion suppression versus attending task (Driscoll et al., 2009).

Thus, the results of these studies are controversial. One explanation could be that various works monitoring the effect of emotional control on psychophysiological responses used pleasant and unpleasant stimuli without a clear separation between emotions (Driscoll et al., 2009; Ohira et al., 2006). Yet, many studies have shown that unpleasant emotions with different arousal level such as sadness, disgust or fear produced different patterns of physiological activation (Palomba et al., 2000; Kreibig et al., 2007), hence the importance of distinguishing between emotions with specific arousal and valence levels. Moreover, another possible reason for such discrepancies is the large variability in interpersonal emotional reactivity and the small sample size of subjects in some studies (approximately 10 subjects).

Given these heterogeneous results it still remains unclear whether there are patterns of physiological activation in the emotional control that are specific to the emotion presented. Our study thus aims at comparing the ANS physiological activity during emotion attending and ecological emotion suppression in a larger sample of subjects, using five specific categories of emotion (happiness, fear, disgust, sadness and peacefulness), considering both their arousal and valence dimensions. To address this aim, we evaluated five measures of ANS: the SCR, HR, and respiration rate. We also assessed emotional expression by measuring the facial muscles activity of zygomatic (smiling) and corrugator (frowning).

Based on the aforementioned review (Witvliet, 1998; Palomba et al., 2000; Khalfa et al., 2002), we predicted that the emotion attending task would result in an increased SCR, HR and respiratory frequency when viewing emotions with a high level of arousal such as happiness, disgust, or fear, compared to emotion with a low level of arousal.

Additionally, based on the principal studies on emotional control, we predicted that the emotion suppression task would result in an increased SCR (Gross, 1998; Gross & Levenson, 1993; Ohira et al., 2006) and a decreased HR and respiratory frequency for pleasant and unpleasant emotions (Gross & Levenson, 1993; Driscoll et al., 2009) as compared to the attending task.

2. Materials and Methods

2.1. Participants

Fifty healthy adult volunteers (42 women, 8 men) with a mean age of 26.9 years (SD=9.5) were recruited. Participants were recruited via screening lists at the clinical investigation unit at the Timone Hospital in Marseille, France. Subjects were instructed not to eat or drink for two hours before the study. They had no history of neurological illnesses. Subjects' self-reports on the Beck Depression Inventory (BDI) (Beck et al., 1961) confirmed that they were not currently depressed (scores below 7). Nine subjects were excluded from data analysis because they had scores on the BDI higher than 7.

Participants provided informed consent in accordance with the guidelines set forth by the CPP committee South Mediterranean 2.

2.2. Materials and design:

2.2.1. Validation of stimuli

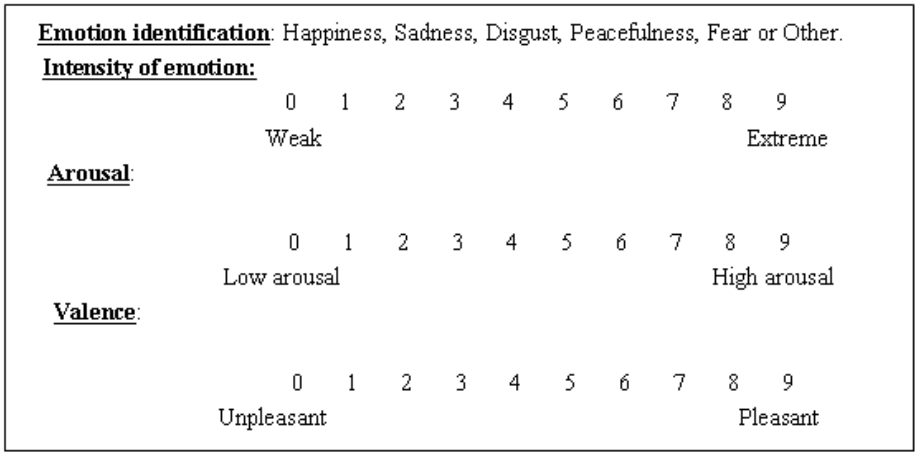
In the present study, participants viewed a series of five 45-second long color films. The five clips were chosen to elicit five different emotions (happiness, sadness, fear, peacefulness and disgust).

The excerpts had been previously validated by 15 healthy controls (8 men and 7 women) with mean age of 45.6 years (± 16.7). This validation allowed the selection of clips that would strongly induce the presented emotions. The first selection included 26 short films from the national audiovisual institute and full-length movies inducing the five aforementioned emotions: 9 films for disgust, 4 for happiness, 5 for sadness, 4 for fear and 4 for peacefulness.

Films were shown individually. They were fed into E-studio 2.2 software (E-Prime 2.2) and displayed on a 17 inch screen computer with 40W Yamaha NS10M Studio sound blasts, linked to a P2040 amplifier, at a sufficiently elevated and comfortable volume.

Participants were asked to watch the 45-second movies and to be aware of the resulting emotional experience to the best of their ability. At the end of each short movie presentation they had to fill a cognitive evaluation sheet by dictating their scores to the experimenter. The evaluation was explained at the beginning of the experiment and participants had to first identify the emotion by choosing between happiness, sadness, fear, peacefulness, disgust or other emotion, and then identify its intensity, arousal and valence on a scale from 0 to 10 (see Fig.1).

Figure 1
Cognitive evaluation scales used when validating the short films.



2.2.2. Results of stimuli validation

Among the 26 tested short films we selected the best 5, one for each category of emotion, according to the following criteria:

1/ identification percentage higher than 80 %;

2/ intensity of induced emotion higher than 7 on the Intensity of Emotion Feeling scale;

3/ arousal level higher than 5 on the Arousal scale for stimulating emotions (happiness, fear, digust) and lower than 2 for non-stimulating ones (peacefulness and sadness);

4/ valence level higher than 6 for pleasant emotions and lower than 4 for unpleasant ones.

We have kept the excerpts that best fit these four criteria thus allowing us to select stimuli that intensely induce the studied emotions and that are well differenciated on arousal and valence scales.

One short film was selected per emotion: for happiness (excerpt of the movie "le Dîner de Cons" by Weber), for sadness (report on the famine in Biafra by INA), for fear (excerpt of "A Tale of Two Sisters" by Jee-Woon), for disgust (excerpt of "Accro" by Mettling, a short film depicting a cannibalism scene), and for peacefulness (excerpt of "Marche of the Penguins" by Jacquet).

2.3. Task procedure

Participants were comfortably seated at 50 cm viewing distance from a 17" computer screen and informed that the experiment was designed to study emotions using short films. Participants filled out the self-reported Beck Depression Inventory. Physiological sensors were attached to capture physiological activity. They performed the attending and suppression tasks. The order of the tasks was counterbalanced between subjects and films were presented in five pseudo-randomized sequences across subjects. The same film order was kept across both tasks for a given subject. In the attending task, subjects were instructed to watch the excerpts and to feel the emotions elicited by each extract to the best of their ability. For the suppression task, we have chosen to give an ecological instruction, best mimicing real-life setting so participants control their emotions as they would spontaneously do if faced with such situations. We have chosen to refrain from indicating specific control strategies (reappraisal or expressive suppression). For the task instruction, we have thus relied on studies of Ohira et al. (2006) and Driscoll et al. (2009); asking subjects to decrease their emotional response by voluntary suppressing any emotional responses while viewing film.

To assess subjective feelings and to verify that the films elicited the targeted emotional states, there was a 1-min post-film break, during which participants completed emotion-rating scales. In the attending task, subjects had to identify the emotion induced, to rate intensity of emotional feeling on a 10-point scale (0 = very low emotional feeling, 10 = very high emotional feeling). In the suppression task they had to rate intensity of emotional suppression on a 10-point scale (0 = very low emotional control, 10 = very high emotional control). They were then asked to assess levels of arousal and valence (see Fig 1). These evaluations assess their emotional experience during the film. After verbal evaluation, and after physiological parameters returned to baselines levels, the following film was displayed.

2.4. Measures

The experiment took place in a temperature-controlled, fully lit, and sound-attenuated room. Physiological data acquisition was controlled by two PCs running E-Prime (Psychology Software Tools, Inc., Pittsburgh, PA, USA) and Acqknowlege software (Biopac Systems, Inc., Goleta, CA, USA). Physiological channels and rating dial information were recorded at a rate of 1000 Hz in continuous mode using the Biopac MP150 system.

SCR was monitored for each subject using 5-mm inner diameter Ag/AgCl filled with isotonic electrode paste. Electrodes were attached to the volar surface of the second phalanx of the second and third right fingers (Fowles et al., 1981).

Cardiac activity was monitored with an electrocardiogram (ECG). The mean HR was measured using three electrocardiograph electrodes, with one placed on the right collarbone and two on the left side of the body, on the torso and below the rib cage. Respiration patterns were recorded using a pneumagraphic belt with a respiration transducer at the rib cage placed towards the end of the sternum to capture the breath.

Electromyogram (EMG) activity of facial muscles was recorded to verify induction of emotions. Facial smiling and frowning behavior were respectively measured by monitoring the Zygomaticus Major muscle and Corrugator Supercilii muscle on the left side of the face using surface Ag/AgCl electrodes (4mm diameter; 10mm distance between the two electrode centers) filled with conductive paste (Fridlund and Cacioppo, 1986). Sensor placement, followed recommendations by Fridlund and Cacioppo (1986). A ground electrode was placed on the lobe of the left ear.

2.5. Statistical analysis of self-report and physiological data

Statistical analysis was conducted using the 11.5.1 version of SPSS. A two-way repeated measures ANOVA was used with Emotion (happiness, sadness, fear, peacefulness and disgust) as a between factor, and Task (attending and suppressing) as a within factor. Significant main effects were followed by post hoc tests using Bonferroni correction with p values set to 0.05.

Data for HR, respiration rate and EMG activity was calculated by subtracting the mean level of the measurements for the 45 sec of each movie from the basal level that was obtained

while recording a 15 sec baseline before the film's onset, when subjects were told to relax, i.e. when physiological parameters were at baseline levels. The mean SCR was measured only during the movie. We manually counted the number of peaks for this parameter. SCR was obtained by averaging peaks amplitude for each film. SCR below 0.01 µS was not considered. Artifact correction for SCR consisted of a visual inspection of respiration and subsequent exclusion of SCR that seemed influenced by deep breath.

3. Results

3.1. Emotional Identification

There was neither significant Emotion X Task interaction nor main effects of these factors on the percentage of emotional identification. All emotions in each task were correctly identified in more than 85% of the trials (see Table 1 and Table 2).

Table 1
Selected ANOVA effects for Emotional Effect, Task Effect, and Emotion X Task.

	Emotional Effect	Task Effect	Emotion X Task
Emotional Identification	Non Significant	Non Significant	Non Significant
Post-hoc			
Intensity of Emotional Attending	(F(4,168)=9.3)***	Non Significant	Non Significant
Post-hoc	H,D,F,S > P and D > S,P		
Intensity of Emotional Control	(F(4,196)=12.4)***	Non Significant	Non Significant
Post-hoc	H,D,P,S > F and P,S > D		
Arousal	F (4,196) = 14.8)***	(F(1,49) = 10.8)**	Non Significant
Post-hoc	F > H,P,S and D > P,S and H > P	A > Su	
Valence	(F (4,196) = 281.9)*	Tendency P=0.059	Non Significant
Post-hoc	H,P > D,F,S and F > D,S	A > Su	
zygomatic	(F (4,188) = 4.2)**	Non Significant	(F(4,188) = 3.6)**
Post-hoc	H > S,F,D,P		HA > HSu
corrugator	(F (4,176) = 4.2)**	Non Significant	Tendency p< 0.09
Post-hoc	S,F,D > H,P		DA > DSu
Skin Conductance Response	(F (4,204) = 29.3)***	Non Significant	(F(4,204) = 6.3)***
Post-hoc	F > H,S,D,P and H > S,D,P S,D > P		FSu > FA
Heart Rate	(F (4,188) = 14.9)***	Non Significant	(F(4,188) = 3.9) **
Post-hoc	H > S,F,D,P and F,S,P > D		HA > HSu
Respiration	(F (4,112) = 3.5)**	Non Significant	Non Significant
Post-hoc	H > S,F,D		

Note. * p<.05, **p<.01, ***p<.001, H: happiness, F: fear, S: sadness, D: disgust, p: peacefulness, A: attending task, Su: suppression task.

Table 2
Mean percentage of emotional identification for the two tasks

Emotions	Happiness	Disgust	Peacefulness	Fear	Sadness
% identification	97,1%	96,1%	87,4%	100%	95,2%

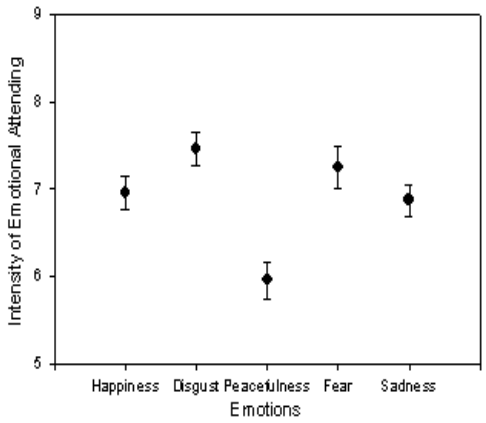
3.2. Intensity of Emotional Attending

There was a significant effect of Emotion on the emotional intensity rating ($F(4,168) = 9.3$; $p < 0.0001$) in the attending task. Post-hoc analysis revealed emotions of happiness, fear, disgust and sadness were more intensely rated than emotions of peacefulness ($p<0.001$), as displayed in Figure 2.

3.3. Intensity of Emotional suppression

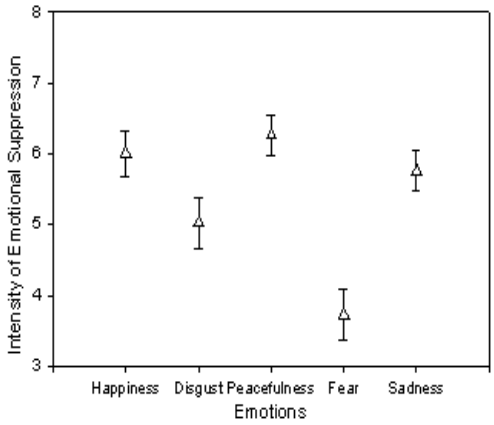
There was a significant effect of Emotion on the emotional suppression rating ($F(4,196) = 12.4$; $p<0.0001$). Post-hoc analysis reveal emotions of happiness, disgust, sadness and peacefulness were more readily controlled than emotion of fear ($p<0.01$), as illustrated in Figure 3.

Figure 2
Plot of the intensity of Emotional Attending
as a function of the various emotions



Note. Means and Standard Errors bars are reported.

Figure 3
Plot of the intensity of Emotional Suppression
as a function of the various emotions



Note. Means and Standard Errors bars are reported.

3.4. Arousal

There was a significant main effect of Task on arousal ratings since emotions presented during the attending task were evaluated as more stimulating than when presented in the suppression task ($F(1,49)=10.8$; $p<0.01$).

There was also a significant main effect of Emotion on the arousal evaluation ($F(4.196) = 14.8$; $p < 0.001$). Post hoc analysis revealed that happy, fear and disgust excerpts were more stimulating than peaceful extracts, as also shown in Figure 4.

3.5. Valence

There was a tendency of Task effect since emotions presented during the attending task were evaluated as more pleasant than when presented in the suppression task, ($p=0.059$).

There is also a significant main Emotion effect on the evaluation of valence ($F(4.196) = 281.9$); $p < 0.0001$). Post-hoc analysis reveal that happy and peaceful films are considered as more pleasant than fearful, disgusting and sad movies as shown in Fig 5 ($p < 0.0001$).

3.6. EMG zygomatic muscle activity

There was a significant effect of Task X Emotion interaction with the zygomatic activity during the happy film being greater in the attending than in the suppressing task ($F(4, 188) = 3.6$; $p<0.01$) (Fig 6).

There was also a significant effect of Emotion on the zygomatic muscle activity ($F(4,188) = 4.2$; $p < 0.01$). Post hoc analysis revealed this muscle is significantly more activated for films inducing happiness than in those of fear, disgust, sadness and peacefulness ($p<0.05$).

3.7. EMG corrugator muscle

There was a significant Emotion effect on the left corrugator muscle activity ($F(4,176) = 4.2$; $p < 0.01$). Corrugator's activity was larger for emotions of disgust, fear and sadness versus emotions of happiness and peacefulness ($p<0.05$) (see Fig 7).

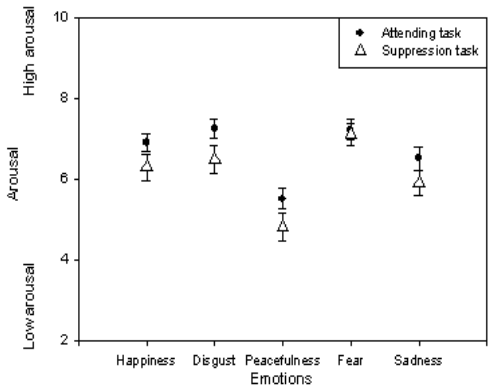
Moreover, there is a tendency of Task X Emotion interaction ($F(4,164)=2$; $p < 0.095$). Post hoc analysis revealed the corrugator activity is greater during the disgust film in the attending than in the suppressing task ($p<0.002$).

3.8. Electrodermal Conductance

There was a significant Task X Emotion interaction. The SCR was larger in suppressing v/s. attending task, for the fear-inducing film ($F(4,204) = 6.3$; $p <0.0001$) as represented in Figure 8.

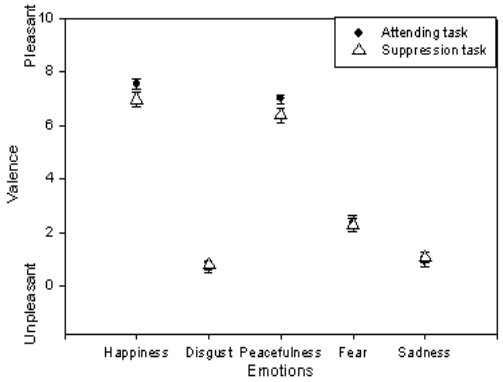
There was also a significant main effect of Emotion on the mean amplitude of the electrodermal response ($F(4, 204) = 29.3$; $p < 0.0001$). Post-hoc analysis revealed an increased amplitude of the SCR for emotions of fear and happiness as compared to emotions of disgust, sadness and peacefulness ($p < 0.05$).

Figure 4
Plot of the activity of the Arousal level
as a function of the various emotions in the
attending and suppression task



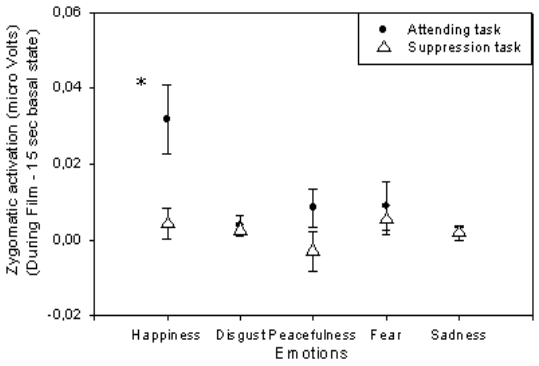
Note. Means and Standard Errors bars are reported.

Figure 5
Plot of the activity of the Valence level
as a function of the various emotions in the
attending and suppression task



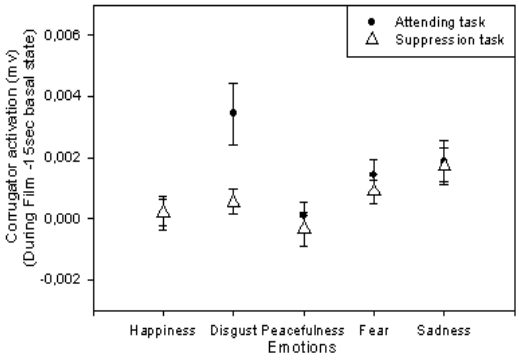
Note. Means and Standard Errors bars are reported.

Figure 6
Plot of the activity of the left zygomatic smiling muscle
as a function of the various emotions
in the attending and suppression task



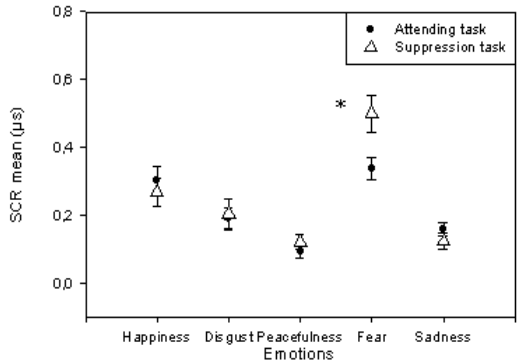
Note. Means and Standard Errors bars are reported. * p<.05

Figure 7
Plot of the activity of the left corrugator frowning muscle
as a function of the various emotions
in the attending and suppression task



Note. Means and Standard Errors bars are reported.

Figure 8
Plot of the mean amplitude of the electrodermal response as a function of the various emotions in the attending and suppression task



Note. SCR = Skin conductance responses. Means and Standard Errors bars are reported. * p<.05

3.9. Heart Rate

There was a significant Task X Emotion interaction. HR for happiness was indeed larger in attending v/s. suppressing task ($F(4,188) = 3.9$; $p<0.01$) (Fig9).

There was also a significant effect of Emotion on the HR ($F(4, 188) = 14.9$; $p < 0.0001$). Post hoc analysis revealed an increased HR for films eliciting happiness as compared to those that induce fear, sadness, peacefulness or disgust ($p<0.01$). Moreover, HR was significantly greater for scary movie as compared to one eliciting disgust ($p<0.001$).

Figure 9
Plot of the Heart Rate as a function of the various emotions in the attending and suppression task

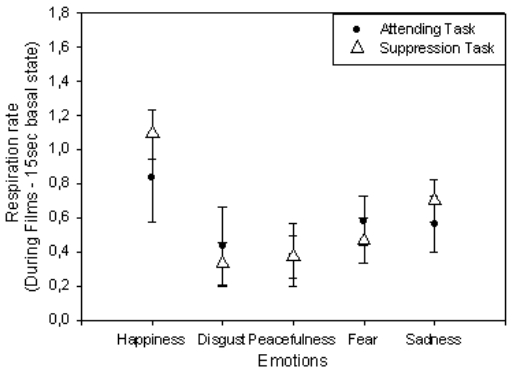
Note. BPM = Beats per minute. Means and Standard Errors bars are reported. * p<.05

3.10. Respiration

There was a significant Emotion effect on respiration rate ($F(4,112) = 3.5$; $p < 0.01$).

Post hoc analysis indicated higher number of respirations for the happy films as compared to films representing sadness, disgust and fear ($p <0.05$) (Fig 10).

Figure 10
Plot of the respiration rate as a function of the various emotions in the attending and suppression task



4. Discussion

Our aim was to study the differences between an emotional attending task and an emotional suppression task on the behavioral, verbal and physiologic responses during film viewing.

4.1. The attending task

We verified the effects of emotion induction on various behavioral and physiological measurements, confirming by the same token the efficiency of our experimental protocol in inducing specific emotions to given excerpts. We presented film excerpts to induce intense emotions through the means of audiovisual stimuli.

Considering the emotional assessments, we first observed a higher arousal level for films conveying happiness, fear and disgust as compared to peaceful films. The valence scale allows labeling emotions of happiness and peacefulness as pleasant, whereas emotions of sadness, fear and disgust are considered as unpleasant. This effect of emotions on verbal scales further supports the emotional categorization suggested by Lang et al. (1998), defining emotions according to bidirectional dimensions, i.e valence and arousal.

Second, considering the facial expressions, our results have shown a modulation of EMG activity by emotional valence. The zygomatic muscle activity was increased in response to the happy, pleasant movie. This finding corroborates the study of Lang et al. (1998) using visual stimuli and the study of Hubert et al. (1990), using audiovisual stimuli. Both had previously established that the more an emotion tends to be pleasurable, the more it induces zygomatic activation. Our results of corrugator activaty revealed an increased amplitude for emotions of disgust, fear and sadness as compared to positive emotions and are also consistent with a recent study (De Wied et al., 2009), showing that negative clips significantly increased corrugator muscle activity.

Third, considering the autonomic responses to emotions, we found that emotions differentially modulate physiological parameters. The electrodermal activity, the HR and the respiratory frequency are increased when viewing happy excerpts as compared to disgust- and sadness-inducing ones. Furthermore, we observed a stronger electrodermal activity when viewing fearful sequences as compared to disgusting, sad or peaceful ones. An increased HR was also found for fear-inducing films as compared to ones that induced disgust.

Therefore, among the studied emotions, happiness seems to stand out as the one with the most notable influence on physiologic measures. It is clearly distinguishable from disgust, sadness and peacefulness and to a lesser extent from fear. This could be explained by the fact that emotions of happiness and fear are both stimulating emotions. Bartlett (1996) had come to similar conclusions, showing that recordings such as SCR and HR can differentiate emotions based on their arousal ratings. Palomba et al. (2000) and Williams et al. (2001) had similarly found the greatest electrodermal activity in response to stimulating audiovisual or visual emotions. However, we did not find an increase in SCR or HR for the emotion of disgust, which is considered as stimulating on the arousal scale compared to low stimulating emotions. The physiological response for this emotion does not seem to depend merely on its arousing level. In fact, Ekman et al. (1983) had already found a decrease in HR when inducing disgust as compared with emotions provoking anger and fear.

More recent studies also confirmed a decrease in HR when disgust is induced (Johnsen et al., 1995; Rohrmann et al., 2008). The HR measurement seems thus able to differentiate disgust significantly from other negative emotions (Levenson et al., 1990, 1991). To better understand the physiological mechanisms specifically involved in the processing of disgust, one should bear in mind that HR reflects inputs from both the sympathetic and the parasympathetic nervous systems. In line with this system, our results most likely indicate that the emotion of disgust most importantly causes an HR decrease through an inhibition of sympathetic system and/or an activation of the parasympathetic system. The enhancement of the parasympathetic tone is known to be mediated via the insular cortex (Saleh et al., 1998) which is also activated by disgust rather than by other negative emotions (Murphy et al., 2003; Olatunji et al., 2010; Stark et al., 2007; Sambataro et al., 2006; Phillips et al., 1998). The specific involvement of the insular cortex in disgust and its influence on the parasympathetic system may indicate why this emotion differs from other negative arousing emotions at psychophysiological level.

Finally, we did not find the overall effect of stimulating emotions on the respiratory rhythm previously described by Gomez et al. (2005). Our results showed that only the happiness-inducing film resulted in a significant increase in the respiratory frequency as compared to films displaying fear, sadness and disgust. This discrepancy could be explained by the differences between time duration of the stimuli. In Gomez et al. (2005), participants

viewed films for at least an average of 10 minutes each whereas they viewed films for 45 seconds, in the present experiment. Still, our results match findings of other authors such Krumhansl et al. (1997) who have used musical stimuli of three minutes. In their study on musical induction of emotion, they found an increased respiratory frequency during happy excerpts. These results might indicate that it takes time for the respiratory frequency to change according to a given emotion state.

Thus, the results of the attending task replicate previous finding indicating that there are physiological markers of emotions. We thus wonder wether these markers are the same when emotions have to be suppressed.

4.2. Comparison between the two tasks

The major aim of this study was to compare verbal responses, facial expressions, as well as psychophysiological responses during emotion attending vs suppression.

In the suppression task, in the same manner as in the attending, we observed a higher arousal level for films conveying happiness, fear and disgust as compared to peaceful films. The valence scale allows labeling emotions of happiness and peacefulness as pleasant, whereas emotions of sadness, fear and disgust are considered as unpleasant. Moreover, the results from verbal ratings when comparing the two tasks showed that instructions given to subjects to limit their emotional responses had no specific influence on the emotion presented but resulted in global reductions of arousal and valence assessments since the emotions altogether were considered as less stimulating and less pleasant than during the attending task. This suggests the efficacy of the emotion regulatory strategy that subjects used in our study and corroborate various studies using reappraisal to regulate one's emotions (Gross, 1998; Ray et al., 2010; Giuliani et al., 2008). It also confirms finding that emotional control should decrease the extent to which emotion response tendencies are activated, leading to lesser subjective responses.

Furthermore, at the EMG level, the suppression instruction annihilates the differences between emotions. This corresponds to the decrease of zygomatic muscle activation for the emotion of happiness and the decrease of corrugator muscle activation for the emotion of

disgust during emotion suppression as compared to attending. Consistent with previous works, (Gross and Levenson, 1993; Gross, 1998) analysis of facial expressivity revealed the expected decrease in expressive behavior. Indeed, according to studies conducted in EMG in the emotional experience (Hu et al., 2003; Wolf et al., 2005), these two emotions are generating the most significant activation of these facial muscles which explains the specific influence of emotional regulation on these emotions.

At the physiological level, we did not find a greater in HR in response to happy films as compared to all other emotions as was observed in the attending task since the suppression task leads to a lower HR for the emotion of happiness. Regarding verbal assessment, participants indicate that they manage to control this emotion; this self-restraint is reflected at the physiological level by a decrease in HR. Gross et al. (1993) explained this decrease by suggesting that HR deceleration during emotional control was due to a reduction of general somatic activity, which is known, in turn, to decrease HR. However, Gross et al. (1993) observed this deceleration for disgust which is not the case in our study. The discrepant HR finding for this emotion could be explained by the fact that in their study and contrary to ours, disgust had strong effect on HR in the attending task. This could be the reason why we find no effect of emotional control for emotions of sadness, and peacefulness.

This suggests that effects of suppression on physiological parameters in relation to attending task, depends on the precise pattern of somatic activity generated by the target emotion in the non-control setting (Gross, 1998).

On the contrary, and consistent with previous findings (Gross, 1998; Gross and Levenson, 1993; Ohira et al., 2006), mean amplitudes of SCR were larger during the suppression task than during the attending task for the emotion of fear specifically. This means that the control of fear increases an activation of the sympathetic nervous system. It is not surprising since this system is involved in the preparation of intellectual activity (Gross et al., 1997). In our study, the cognitive effort is greater towards fear control since our verbal evaluations indicate that the emotion of fear is the most difficult to control. Fear is indeed the most archaic emotion, because it ensures the survival of the individual. It is the only emotion that puts us on alert, increasing ANS activation and subsequently SCR. The SCR might be increased by the cognitive effort during emotion regulation since this task involves a particularly genuine effort. Physiological changes associated with suppression may also reflect the additional metabolic demands caused by that effort (Gross, 1998b).

More recently, neuroimaging and physiological studies have tried to understand the association of neural and physiological responses during voluntary emotion suppression. These works have documented that the orbitofrontal cortex (OFC) activity (Mak et al., 2009) is positively correlated with SCR in a suppression task (Ohira et al., 2006). This region mediates sympathetic activation reflected by enhancement of SCR during emotion suppression. Moreover, the OFC has projections to the periaqueductal gray which is deeply involved in stress reactivity, such as elevation of sympathetic activity and fear behaviors (Bandler et al., 1991, 2000). The OFC's connections may indicate why we found that fear is the only emotion creating an elevation of SCR during emotion regulation.

4.3. Limitations

There is evidence (Gross, 1998) that the psychophysiological effects of voluntary emotional control are determined in part by the regulation strategy adopted (suppression or cognitive reappraisal). In our study, the physiological effects of different regulation approaches were not examined. In fact, we have not asked subjects to adopt a clear strategy of regulation in order to let them control their emotions the most naturally. While the outcomes of the present study suggest that regulating emotions may have different physiological effects depending on the emotion presented, further work is needed to clarify the effectiveness of different approaches to regulate emotion. Finally, this study included eight men and fourty two women. It should be important to study a homogeneous population of subjects because previous laboratory study had shown that women responded more strongly to the emotional stimuli than men (Ito et al., 1998).

4.4. Conclusion

To conclude, our study provides further evidence that voluntary intention to regulate emotion is associated with a global reduction of verbal assessment of emotion. Moreover, at the expressive level, the suppression instruction results in a decrease of zygomatic muscle activation for the emotion of happiness and a decrease of corrugator muscle activation for the emotion of disgust. At the physiological level, voluntary attempts to control emotion leads to a lower HR for the emotion of happiness and a higher SCR for the fear-inducing film. These emotional effects are observed on the emotions that drive the most important physiological changes in the non-control setting.

This study sheds new light on the topic because it is the first time that specific categories of emotions are compared during the emotional suppression. The observed effects on autonomic responses indicate that the conscious and volitional regulation of emotion has selective effects on psychophysiological parameters which differ according to the presented emotion.

One of the main motivations that drive people to regulate their emotions is motivational hedonism whose goal is to reduce or avoid painful, stress or unpleasant emotions (Krauth-Gruber, 2009). What people want to express or regulate is thus determined by the negative consequences of emotions that can affect them and their relationships with others. In this perspective, the inability to regulate emotions through the ANS becomes a key component in understanding many forms of psychopathology and maladaptive behaviors as depression, post traumatic stress disorders (PTSD) or personality disorders (Davidson, 2000; Machado and Bachevalier, 2003). Given these considerations, our task could be used as a clinical test to assess the skills to control emotional state. Specifically, further work should be conducted to investigate the response to films inducing fear which is disturbed in psychopathologies such as PTSD (Charney, 2004).

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Appendix 2: pending for submission to the Journal

Neuroticism Modifies Psycho-physiological Responses To Films

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Background: *High neurotic people tend to be more psychologically reactive to stressors. This emotional reactivity might be assumed to have physiological correlates, and some studies have shown that neurotics might be more physiologically reactive to emotional negative events. However, to our knowledge, we have little information on the influence of positive emotions on physiological responses in neurotic subjects. To better understand the physiological bases of neuroticism, and to go further than previous researches, our study will focus on studying the influence of neuroticism on verbal and physiological responses during the presentation of positive and negative emotions using measures of ANS (autonomic nervous system).*

Methods: *Fourteen low neurotic subjects and eighteen neurotics were assessed on an emotional attending task using film excerpts inducing happiness, peacefulness, fear, disgust and sadness. We evaluated physiological measures such as the skin conductance response (SCR), heart rate (HR) and the activity of zygomatic (smiling) and corrugator (frowning) muscles as an index of emotional expression.*

Results: *Neuroticism increased corrugator activity and SCR during the fear-inducing film. Also, we found a decrease in HR during the happy and peaceful films in neurotics subjects.*

Conclusion: *Following decades of evidence that individual higher in neuroticism experience more intense emotional reactions to even minor stressors (Larsen & Ketelaar, 1991),our results indicate that these individuals also show greater expressive and SCR reactivity to aversive stimuli. We also show for the first time that they have less HR reactivity to positive stimuli. The fact that personality is a significant factor in physiological reactivity to emotional stimuli highlights the importance of individual differences in the study of the biological basis of emotion*

Key Words : *Neuroticism, Autonomic nervous system, Heart rate, Skin conductance response.*

1. Introduction :

When a person feels an emotion, it produces changes in her body, including the level of activity of the ANS, facial expressions, and of course in brain structures (Oatley and Jenkins, 1992). One's emotional experience and one's emotional expression are considered two of the major manifestations of emotion (Phillips et al., 2003). However, many authors have broadened the field of components needed to define a particular emotion to include interindividual factors of variability such as personality traits. In fact, they can influence and condition our way to experiment and respond to emotions (authors). Most accepted models of personality include the dimension of neuroticism (Eysenck's 1967, 1981 in Kumari). Characteristics of this traits include a tendency to worry and to be anxious (Canli et al. 2001), and is related to the experience of negative affect (Larsen and Ketelaar, 1991; Robinson et al., 2007, Zelenski and Larsen, 1999 in Cremers). In fact, studies shown that persons with high neuroticism scores were more distressed by negative mood induction than participants with low neuroticism scores (Larsen and Ketelaar, 1989, 1991). These researches reported that persons scoring high in neuroticism tend to be more psychologically reactive to stressors. When we think of someone who experiments a situation of distress, we can assume that this distress leads to physiological reactivity. The emotional reactivity reported by people high in neuroticism might be assumed to have physiological correlates, and some studies have tried to characterize the psychophysiological correlates of neuroticism.

Initial studies on the subject have used cardiovascular measures, recording the heart rate (HR) of subjects during different stressful paradigms (Fredrikson & Georgiades, 1992; Kirkcaldy, 1984 ; Hinton & Craske, 1977 ; Schwebel & Suls, 1999). All results suggested that neurotic and nonneurotic subjects showed similar HR elevations. They found no evidence for differences in cardiovascular reactivity to emotional stressors as a function of neuroticism. Moreover, another cardiac index largely studied is the heart rate variability (HRV). There is now a reasonable size literature showing that individual differences in HRV have predictive value in understanding outcomes important to the personality. Clinical and emotional literatures (Appelhans & Luecken, 2006) and a recent study have shown that higher HRV that has been consistently associated with greater capacities to regulate stress should be inversely correlated with neuroticism trait (Ode et al., 2010).

More recent topics have shown that skin conductance reactivity (SCR) is higher among neurotics than emotionally stables individuals. Neurotic individuals exhibit both

greater reactivity and more sustained responses to emotional unpleasant stimuli than do nonneurotic subjects (Norris et al., 2007). Moreover, as compared to control samples, patients with schizophrenia, who tend to be high in neuroticism (Horan et al., 2005), show greater skin conductance activity at rest.

In sum, neurotics might be more physiologically reactive to emotional negative events, but to our knowledge, we have little information on the influence of positive emotions on physiological responses. Moreover, studies on personality traits have used physiological parameters isolated. To better understand the physiological bases of neuroticism, and to go further than previous researches, our study will focus on studying the influence of neuroticism on verbal and physiological responses during the presentation of positive and negative emotions. To address this aim, we evaluated four measures of ANS: the SCR, HR, we also assessed emotional expression by measuring the facial muscles activity of zygomatic (smiling) and corrugator (frowning).

Based on the aforementioned literature (Robinson et al., 2007, Zelenski and Larsen, 1999 in Cremers ; Larsen and Ketelaar, 1989, 1991), we predicted that neurotic subjects might report subjective responses more intense for negative emotions.
Additionally, based on the principal studies on physiological responses, we predicted that individuals higher in neuroticism should show strong SCR to emotional stimuli, in particular for negative mood (Norris et al., 2007). Moreover, no influence of neuroticism on HR should be found (Fredrikson & Georgiades, 1992; Kirkcaldy, 1984) in emotional condition.

2. Methods :

2.1. Participants

Two hundred subjects were recruited via screening lists at the clinical investigation unit at the Timone Hospital in Marseille, France. To assess personality traits, all participants completed the NEO PI-R (Costa and McGrae, 1992). We have selectioned for our study, Thirty two subjects (29 women and 3 men) with a mean age of 27,5 years (SD=10,7) ; fourteen subjects with low neurotic scores (71,1 ± 8,2) and eighteen subjects with high neurotic scores (125,4 ± 9,6) defined by the rating scale of the NEO PI-R.
Participants provided informed consent in accordance with the guidelines set forth by the CPP committee South Mediterranean 2.

2.2. Materials and design:

2.2.1 Validation of stimuli

In the present study, participants viewed a series of ten 45-second long color films. The ten clips were chosen to elicit different emotions (happiness, sadness, fear, peacefulness and disgust).

The excerpts had been previously validated by 15 healthy controls (8 men and 7 women) with mean age of 45.6 years (± 16.7). This validation allowed the selection of clips that would induce strong emotions. The first selection included 26 short films from the national audiovisual institute and full-length movies inducing the five aforementioned emotions: 9 films for disgust, 4 for happiness, 5 for sadness, 4 for fear and 4 for peacefulness.

Films were shown individually. They were fed into E-studio 2.2 software (E-Prime 2.2) and displayed on a 17 inch screen computer with 40W Yamaha NS10M Studio sound blasts, linked to a P2040 amplifier, at a sufficiently elevated and comfortable volume.

Participants were asked to watch the 45-second movies and to be aware of the resulting emotional experience to the best of their ability. At the end of each short movie presentation they had to fill a cognitive evaluation sheet by dictating their scores to the experimenter. The evaluation was explained at the beginning of the experiment and participants had to first identify the emotion by choosing between happiness, sadness, fear, peacefulness, disgust or other emotion, and then identify its intensity, arousal and valence on a scale from 0 to 10 (see Fig.1).

2.2.2. Results of stimuli validation

Among the 26 tested short films we selected the best 5, one for each category of emotion, according to the following criteria:

1/ identification percentage higher than 80 %;

2/ intensity of induced emotion higher than 7 on the Intensity of Emotion Feeling scale;

3/ arousal level higher than 5 on the Arousal scale for stimulating emotions (happiness, fear, digust) and lower than 2 for non-stimulating ones (peacefulness and sadness);

4/ valence level higher than 6 for pleasant emotions and lower than 4 for unpleasant ones.

We have kept the excerpts that best fit these four criteria thus allowing us to select stimuli that intensely induce the studied emotions and that are well differenciated on arousal and valence scales.

Two short films were selected per emotion: for happiness (excerpt of the movie "le Dîner de Cons" by Weber and excerpt of a video of a young child laughing selected from the website "youtube"), for sadness (report on the famine in Biafra by INA and excerpt of the movie "Stepmom" by Columbus), for fear (excerpt of "A Tale of Two Sisters" by Jee-Woon and excerpt of "A perfect murder" by Davis), for disgust (excerpt of "Accro" by Mettling, a short film depicting a cannibalism scene and a surgery performed in the hospital of la Timone in Marseille), and for peacefulness (excerpt of "Marche of the Penguins" by Jacquet and an excerpt of "Le gran bleu" by Besson).

2.3. Task procedure:

Participants were seated at 50 cm from a 17' computer screen with a refresh rate of 100Hz. They were informed that the experiment was designed to study emotions using short films. Physiological sensors were attached to capture physiological activity. Subjects were instructed to watch the excerpts and to feel the emotions elicited by each extract to the best of their ability. To assess subjective feelings and to verify that the films elicited the targeted emotional states, there was a 1-min post-film break, during which participants completed emotion-rating scales. Subjects had to make an emotional identification, to rate intensity of emotional feeling, and to assess levels of arousal and valence (see Fig 1). These evaluations assess their emotional experience during the film. After this verbal evaluation, and after physiological parameters returned to baselines levels, the following film was displayed.

Emotion identification: Happiness, Sadness, Disgust, Peacefulness, Fear or Other.

Intensity of emotional feeling

0 1 2 3 4 5 6 7 8 9

Weak Extreme

Arousal:

0 1 2 3 4 5 6 7 8 9

Low arousal High arousal

Valence:

0 1 2 3 4 5 6 7 8 9

Unpleasant Pleasant

Figure 1: Cognitive Evaluation scales used when validating the short films.

2.4 Measures:

Physiological data acquisition was controlled by two PCs running E-Prime (Psychology Software Tools, Inc., Pittsburgh, PA, USA) and Acqknowlege software (Biopac Systems, Inc., Goleta, CA, USA) respectively. Physiological channels and rating dial information were recorded at a rate of 1000 Hz in continuous mode using the Biopac MP150.

SCR was monitored for each subject using 5-mm inner diameter Ag/AgCl filled with isotonic electrode paste. Electrodes were attached to the volar surface of the second phalanx of the second and third right fingers in accordance with published guidelines (Fowles et al., 1981).

Cardiac activity was monitored with an electrocardiogram (ECG) and measured in beats per minute. The mean HR was measured using three electrocardiograph electrodes attached in a Type I EKG configuration; on the left flying rib, right collarbone and sternum.

Electromyogram (EMG) activity of facial muscles was also recorded in to verify successful induction of emotions. Facial smiling and frowning behavior were respectively measured in microvolts by monitoring the activity of Zygomaticus Major muscle and Corrugator Supercilii muscle on the left side of the face using surface Ag/AgCl electrodes (4mm diameter; 10mm distance between the two electrode centers) filled with conductive paste (Fridlund & Cacioppo, 1986). Electrodes were placed on the left cheek in the middle of the mouth-to-ear tip line for Zygomaticus Major activity, and above the left eyebrow for assessment of Corrugator Supercilii muscle activity. Sensor placement, followed recommendations by Fridlund and Cacioppo (1986). A ground electrode was placed for each measurement on the lobe of the left ear.

2.5. Statistical analysis of self-report and physiological data

Statistical analysis was conducted using the 11.5.1 version of SPSS.

A two-way repeated measures ANOVA was used with Emotion (happiness, sadness, fear, peacefulness and disgust) as a between factor, and Personality (neurotics subjects and non neurotics subjects) as a within factor. Significant main effects were followed by post hoc tests using Bonferroni correction. A significant level of 0.05 was adopted in all tests.

Data of physiological parameters and verbal scoring was averaged for 2 films per emotion.

Data for HR and EMG activity were calculated by subtracting the mean level of the measurements for the 45 sec of each movie from the basal level that was obtained while

recording a 15 sec baseline before the film's onset, when subjects were told to relax, i.e, when physiological parameters were at baseline levels.

Data for heart rate variability (HRV) was reliably quantified using the 10 min rest period (Bernston et al., 1997). Three frequency bands are typically defined:

- High frequency (HF) (0.15 - 0.4 Hz), derived mainly from vagal activity.
- Low frequency (LF) (0.04 - 0.15 Hz) derived from sympathetic activity.
- Very low frequency (VLF) (0 - 0.04 Hz) reflecting physical activity.

HRV is calculated by the HF/LF ratio.

Data for SCR was measured by averaging peak amplitudes of SCR during the 45 sec film excerpts SC below 0.01 μS were not considered. Artifact correction for SCs consisted of a visual inspection of respiration and the manual exclusion of SCR that appeared to be influenced by coughs, sights or deep breath.

3. Results

3.1 Intensity of Emotional Attending

There was a significant effect of Emotion on the emotional intensity rating ($F(4,124) = 8.7$; $p < 0.0001$) in the attending task. Post-hoc analysis revealed emotions of happiness, fear, disgust were more intensely rated than emotions of sadness and peacefulness ($p<0.001$), as displayed in Figure 2.

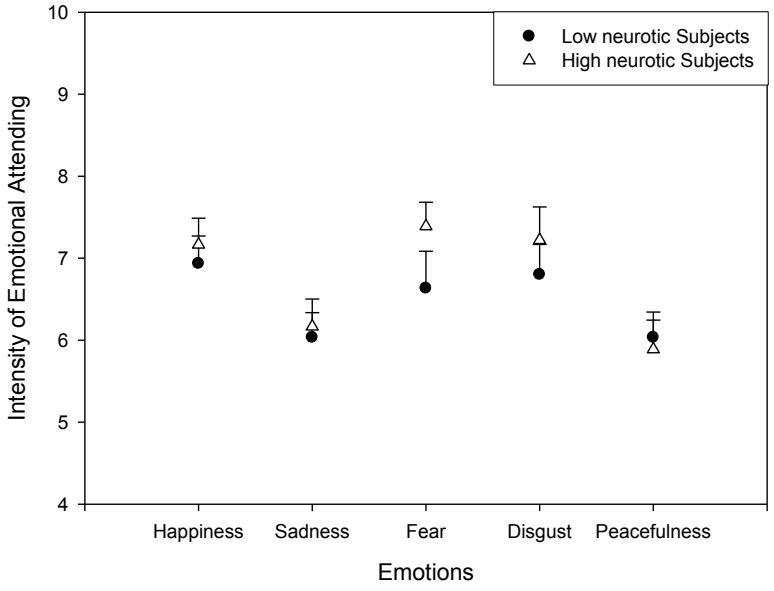

Figure 2: Plot of the intensity of Emotional Attending of the two populations as a function of the various emotions (mean and Standard Error bars)

3.2 Arousal

There was a significant main effect of Emotion on the arousal evaluation ($F(4.124) = 9.4$; $p < 0.0001$). Post hoc analysis revealed that happy, fear and disgust excerpts were more stimulating than sad and peaceful extracts, as also shown in Figure 3.

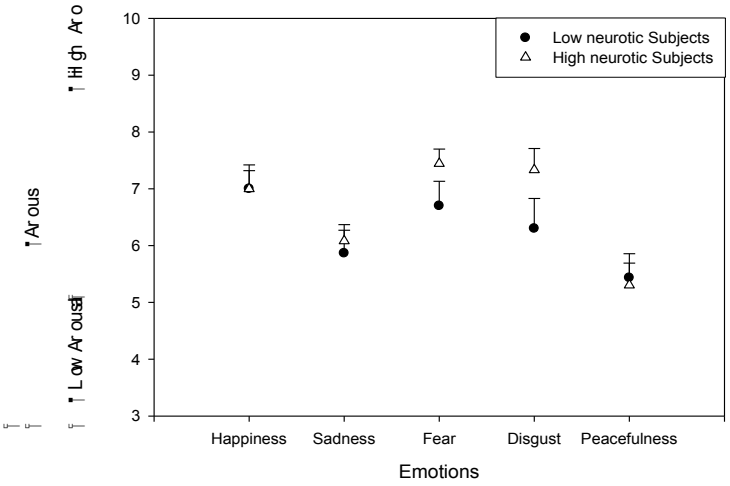

Figure 3: Plot of the activity of the Arousal level of the two populations as a function of the various emotions in the attending task (mean and Standard Error bars).

3.3 Valence

There is a significant main Emotion effect on the evaluation of valence ($F(4.124) = 208.1$); $p < 0.0001$). Post-hoc analysis reveal that happy and peaceful films are considered as more pleasant than fearful, disgusting and sad movies as shown in Fig 4 ($p < 0.0001$). There was also a tendency of Population effect since high neurotic subjects evaluated emotions as more unpleasant than low neurotic subjects ($p=0.1$).

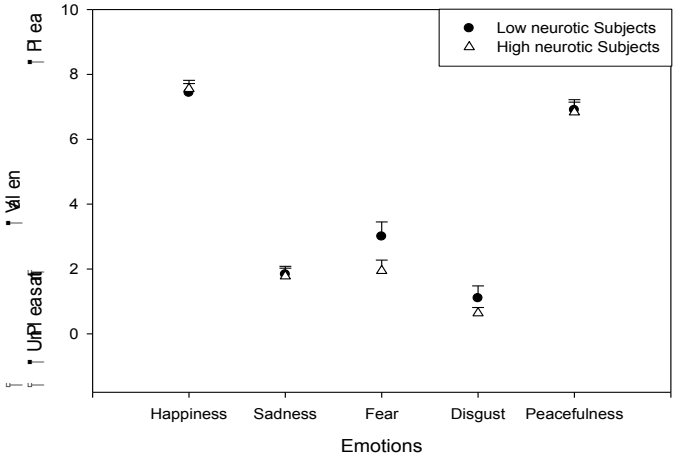

Figure 4: Plot of the activity of the Valence level of the two populations as a function of the various emotions in the attending task (mean and Standard Error bars)

3.4 EMG zygomatic muscle activity

There was a significant effect of Emotion on the zygomatic muscle activity ($F(4,120) = 21.7$; $p < 0.0001$). Post hoc analysis revealed this muscle is significantly more activated for films inducing happiness than in those of fear, disgust, sadness and peacefulness ($p<0.0001$). (Fig 5).

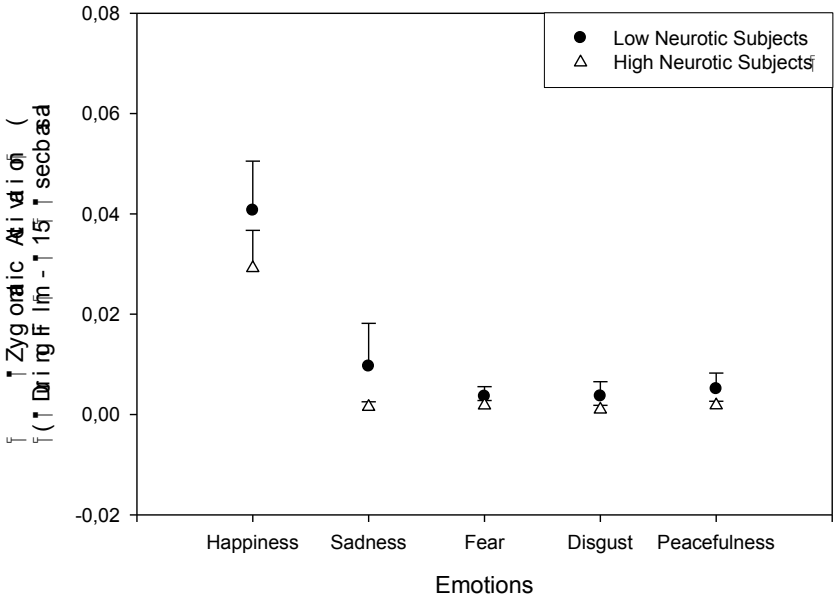

Figure 5: Plot of the activity of the left zygomatic smiling muscle of the two populations as a function of the various emotions in the attending task (mean and Standard Error bars)

3.5 EMG corrugator muscle

There was a significant Population X Emotion interaction with the corrugator activity ($F(4, 120) = 2.6$; $p<0.05$). The corrugator muscle activity was larger for high neurotic subjects when emotion of fear was induced than for non neurotic subjects ($p <0.05$).

There was also a significant Emotion effect on the left corrugator muscle activity ($F(4,120) = 7.1$; $p < 0.001$). Moreover, corrugator's activity was larger for emotions of disgust, versus emotions of happiness, peacefulness, fear and sadness ($p<0.05$) (see Fig 6).

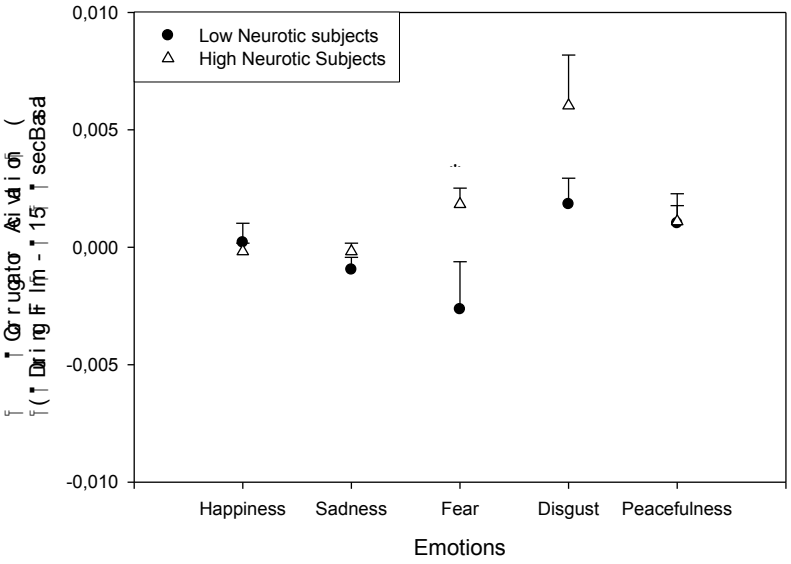

Figure 6: Plot of the activity of the left corrugator frowning muscle of the two populatiobs as a function of the various emotions in the attending sion task (mean and Standard Error bars)

3.6 Electrodermal Conductance

There was a significant Population X Emotion interaction (F (4,120) = 3.8 ; p <0.01). The SCR was larger for high neurotic subjects when emotion of fear was induced than for non neurotic subjects (p <0.01).

There was also a significant main effect of Emotion on the mean amplitude of the electrodermal response (F (4,120) = 11.9; p < 0.0001). Post-hoc analysis revealed an increased amplitude of the SCR for emotions of fear and happiness as compared to emotions of sadness and peacefulness (p < 0.05).

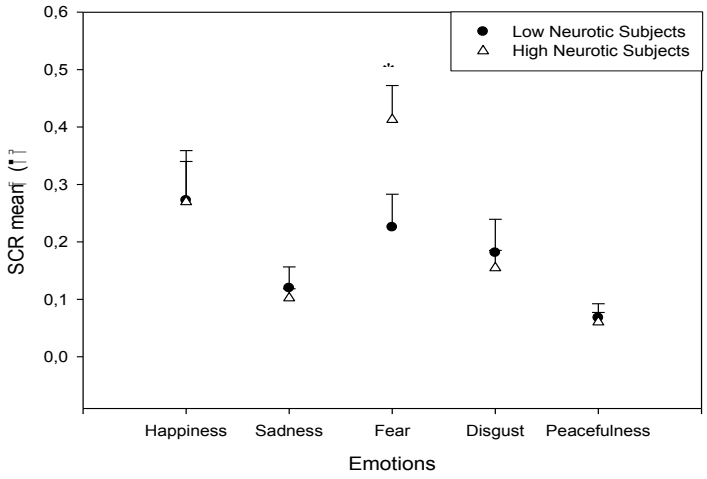

Figure 7: Plot of the mean amplitude of the electrodermal responseof the two populations as a function of the various emotions in the attending and suppression task (mean and Standard Error bars)

3.7 Heart Rate

There was a significant Population X Emotion interaction (F (4,120)=3 ; p<0.05) (Fig8). Post hoc analysis revealed that there is a tendency for the HR which was lower for high neurotic subjects when emotion of happiness (p=0.06) and peacefulness (p=0.07) were induced than for non neurotic subjects.

There was also a significant effect of Emotion on the HR (F (4,120) = 16.9; p < 0.0001). Post hoc analysis revealed an increased HR for films eliciting happiness as compared to those that induce fear, sadness, peacefulness or disgust (p<0.0001). HR was significantly greater for scary movie as compared to ones eliciting disgust and peacefulness (p<0.01).

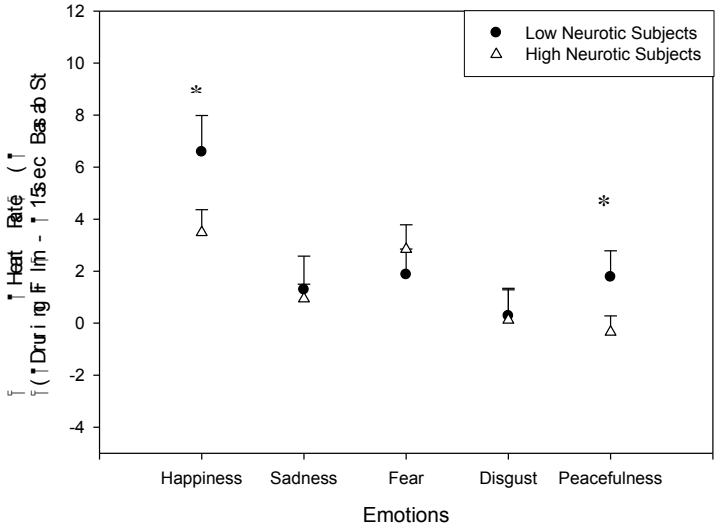

Figure 8: Plot of the HR of the two populations as a function of the various emotions in the attending task (mean and Standard Error)

3.8 Heart Rate variability

The t-test revealed a tendency in a decrease in HF for high neurotics compared to low neurotic subjects subjects (F (39, X) = 12.3 ; p < 0.055).

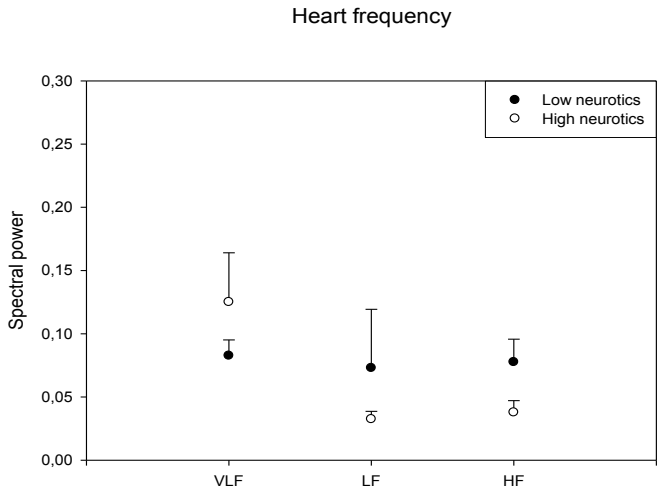

Figure 9: Plot of the HRV of the two populations as a function of the various emotions in the attending task (mean and Standard Error)

4. Discussion:

Our aim was to study the differences between high neurotic subjects and low neurotic subjects on the behavioral, and physiologic responses during film viewing.

We verified the effects of emotion induction on various behavioral and physiological measurements, confirming by the same token the efficiency of our experimental protocol in inducing specific emotions to given excerpts. We presented film excerpts to induce intense emotions through the means of audiovisual stimuli.

Considering the emotional assessments, we first observed higher arousal for emotions of happiness, fear and disgust as compared to emotions of peacefulness and sadness. The valence scale effectively allows the labeling emotions of happiness and peacefulness as pleasant, whereas emotions of sadness, fear and disgust are considered as unpleasant. Our results suggest that there is only an effect of emotion on arousal and valence responses. Unlike previous behavioural studies (Gross et al., 1998; Watson & Clark, 1984), we did not find differences between the two populations in verbal evaluations, . This discrepancy might be related to the fact that the other studies have used a population larger than ours and have characterized their two groups by using correlations between neuroticism scores and the various scales. In our study, we have chosen to include individuals with extreme scores in neuroticism and this has limited the number of subjects included.

Although low and high neurotic subjects did not differ on verbal evaluations; they were distinct in terms of physiological responses. This suggests the existence of dissociation between the way people rate their subjective experience and their inner physiological responses.

First and foremost, physiological assessments showed greater SCR and corrugator muscle activity for neurotics than for non neurotics when the fear emotion is induced. This is in line with the finding that neurotic individuals exhibit both greater SCR and more sustained responses to emotional unpleasant stimuli than do emotionally stable individuals (Norris et al., 2007). A large number of studies have indeed shown that there are clear associations between neuroticism and measures of negative affect (Costa & Mc Crae, 1980; Larsen & Ketelaar, 1991), whereby they easily experience feeling such as anxiety, stress, depression and fear (Watson & Clark, 1984). This sensitivity to negative affects is reflected in our study at both physiological and expressive levels. Yet, among the negative emotions we studied, the difference between the two groups was specific to fear. This is consistent with Eysenck's hypothesis (1967) that neuroticism involves hyperactivation of the limbic system and a consequently low tolerance for stressors or aversive stimuli. At the cerebral level, functional magnetic resonance imaging (fMRI) studies have provided evidence that regions associated with neuroticism include the amygdala (Haas et al., 2007; Reuter et al., 2004; Stein et al., 2007) the anterior cingular cortex (ACC) (Eisenberger et al., 2005; Reuter et al., 2004) and the medial prefrontal cortex (Britton et al., 2007; Haas et al., 2007), known to be involved in the fear circuitry. Moreover, a recent study in functional connectivity has shown that high neurotic participants display diminished ACC control over the amygdala when processing fearful faces (Cremers et al., 2010). Results of these neuroimaging studies converge in suggesting that the mechanisms involved in the overall response to fear are altered in neuroticism. Neuroticism seems to have a particular characteristic of being associated with activity modification in the amygdala and ACC. These structures are known to modulate the autonomic nervous system or rather covariate with measures of this peripheral nervous system (review Hagemann, Waldstein, Thayer, 2003). This interaction between the central nervous system and autonomic nervous system may therefore explain the increase in SCR and corrugator activity for neurotic subjects when fear emotion is induced.

If the results of the SCR and corrugator are in line with those obtained in previous studies, the HR observation is quite innovative because to the best of our knowledge, this is

the first time that the effect of HR on positive emotions is demonstrated. In fact, studies evaluating the influence of neuroticism on cardiovascular reactivity used aversive or stressful stimuli, and found little support for the hypothesis that high neurotic individuals exhibit differential HR responses to stressful situations (Fredrikson & Georgiades, 1992; Kirkcaldy, 1984; Hinton & Craske, 1977; Schwebel & Suls, 1999). Similarly to these studies, we did not observe any difference between subjects scoring high versus low in neuroticism in HR responses when inducing negative emotions. However, we found a decreased HR when positive emotions of happiness and peacefulness are induced. This seemingly incoherent result makes sense insofar as neurotic subjects are responsive to negative emotions (as indicated by SCR and corrugator responses), they would also be less sensitive to positive emotions (as demonstrated by HR). Moreover, our findings of HRV indicated a diminished HF in high compared to low neurotics. This supports the hypothesis of a decrease in the parasympathetic system in neurotics at rest. Still, during the emotional task, the HR results indicated an increase in the parasympathetic system in neurotics, subsequently resulting in a decreased HR when positive emotions of happiness and peacefulness are induced. HR outcomes suggest that different mechanisms would be involved in resting v/s. emotional conditions.

Following decades of evidence that individual higher in neuroticism experience more intense emotional reactions to even minor stressors (Larsen & Ketelaar, 1991),our results indicate that these individuals also show greater expressive and SCR reactivity to aversive stimuli. We also show for the first time that they have less HR reactivity to positive stimuli.

The fact that personality is a significant factor in physiological reactivity to emotional stimuli highlights the importance of individual differences in the study of the biological basis of emotion. In this line of work, further studies should aim to observe the effects of individual differences on verbal and physiological responses to emotional stimuli using a large sample of subjects. They could also verify the effect we observed on HR as well as the dissociation between behavioral and physiological responses.

5. References

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Pure-tone auditory thresholds are decreased in depressed people with post-traumatic stress disorder

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Background: *Depression has been related to sensory modulation and notably to auditory modifications such as alterations in auditory event-related potentials, abnormal patterns of auditory habituation, increased activation of primary and secondary auditory cortex, and higher bilateral auditory thresholds. However, few experiments have considered the exploration of the auditory system in depression. The aim of the experiment is to further explore auditory thresholds across a higher number of frequencies than has previously been undertaken in depressed subjects, to determine whether thresholds are modified as compared to controls, and if so, at which frequencies.*

Methods: *25 pure-tones covering a large range of frequencies from 125 Hz to 8 kHz were used to measure both air and bone conduction (AC and BC respectively) hearing thresholds. 13 patients with depression and post-traumatic disorder matched for age, sex and education level with 13 healthy subjects were tested.*

Results: *Hearing thresholds were found to be significantly poorer in depressed participants than in controls for frequencies from 2.75 Hz to 8 kHz in BC, and for 0.5, 0.75, 0.875 and 2.0– 8.0 kHz pure-tone frequencies in AC.*

Limitations*: Given that the depressed patients also had comorbid post-traumatic disorder, it should be verified whether their modified pure-tone audiometry is only related to depression.*

Conclusions*: The AC and BC pure-tone auditory threshold measurement may provide new and different insights into the aetiology and evolution of depression.*